EQPH-2021
V. International Symposium

ÓBUDA UNIVERSITY
REJTŐ SÁNDOR FACULTY OF LIGHT INDUSTRY AND ENVIRONMENTAL ENGINEERING

ICEEE

Proceedings Book

"Environmental Quality and Public Health"

The Vth International Symposium-2021

May 20, 2021
Óbuda University
Budapest, Hungary

EQPH-2021
V. International Symposium

ÓBUDA UNIVERSITY
REJTŐ SÁNDOR FACULTY OF LIGHT INDUSTRY AND ENVIRONMENTAL ENGINEERING

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The Vth International Symposium-2021 "Environmental Quality & Public Health"

In light of the unprecedented circumstances, and the uncertainty due to the travel restrictions imposed by different countries, the Organizing Committee has made the decision to hold the Symposium virtually.

Online
May 20th, 2021
RKK – Óbuda University
Budapest, Hungary

Content

Introduction	3
Bibliographic information	6
ICEEE: Organization, Scope and Opportunities	7
Symposium: Organization and Committees	8
Acknowledgment	10
Impressum	12
Awards of Symposium	13
Main Themes	16
Program of the Symposium	18
Table of Publishing Contents	29
Abstracts of the Accepted Papers	33
Manuscripts of the Accepted Papers	81
Manuscripts of the Plenary Lectures	81
Manuscripts of the Keynote Lectures	102
Manuscripts of the Poster Presentationss	149
Manuscripts of the Technical Lecturers	214
Invitation for the 12th ICEEE-2021 Conference	453

INTRODUCTION

Why Environmental Engineering and Natural Sciences are vital for our future
Relation between Environmental Quality & Public Health!

The purpose of the Vth International Symposium is to analyse the issues of the global environmental quality and public health. The authors try to answer the questions connecting to the above mentioned phenomenon on the basis of their experiences of selected global countries.

Our planet has a natural environment, known as 'Ecosystem' which includes life of all microbiotas, humans, plant and animal, and non-living components such as atmosphere, hydrosphere and lithosphere including mountains, glaciers, rocks, galaxy, massive oceans and seas, etc. It also includes natural resources such as water, electric charge, fire, magnetism, and waste, etc.

Environmental quality has effects on human health and ecosystems quality, requiring cooperation across different sectors to determine effective responses. By increasing the global population is rising at a staggering rate and demands ever more resources, while the incorrect management of environmental quality is making public health worse.

Determination of environmental quality is a major area of interest when considering the public and ecosystem health. Achieving high-quality environmental development through environmental regulations and thus enhancing public health is a goal of the V. International Symposium.

The environment plays a crucial role in people's physical, mental and social well-being. Environmental degradation poses a significant threat to human health worldwide. Harmful consequences of this degradation to human health are already being felt and could grow significantly worse over the next 30 years.

The environment affects our health in a variety of ways. The interaction between human health and the environment has been extensively studied and environmental risks have been proven to significantly impact human health, either directly by exposing people to harmful agents, or indirectly, by disrupting life-sustaining ecosystems.

The degradation of the environment, through air pollution, noise, chemicals, poor quality water and loss of natural areas, combined with lifestyle changes, may be contributing to substantial increases in rates of obesity, diabetes, diseases of the cardiovascular and nervous systems and cancer — all of which are major public health problems for global population. Reproductive and mental health problems are also on the rise. Asthma, allergies, and some types of cancer related to environmental pressures are of particular concern for children.

Engineering developments are resulting in resource depletion and environmental destruction. Modern technologies used in the engineering and manufacturing industry have a major impact on our life in past and present as well as the future years. Due to the rapid changes in the engineering and manufacturing industry have been drastic changes in the environment.

The pollutions of environment are widespread across the planet Earth and frequently contaminate air, water and soil used for continuing the life on the Earth and distribution for human consumptions, and for irrigating crops.

Sanitation is a large part of our civil evolution; without it, we would yield more often to water-borne disease and illness - raising our mortality rates and lowering our quality of life. Therefore, we've always needed to find bigger and better ways of taking away our sewage for example, cleaning our water and harnessing natural or artificial water supplies for our health and environment.

Since the industrial revolution in the 19th century we have needed to prevent businesses and individuals from polluting the environment with harmful substances. The modern environmental engineer is dedicated to keeping our air, soil and water clean of pollutants and wastes and promoting good health for human, animal and plant and these days, protection against radioactive and toxic materials too; researchers also study the potential impacts of climate change and other environmental factors and pollution on the infrastructure and environmental health.

Couple this with environmental awareness in the general population and the growing understanding of the impacts of environmental change meant that environmental engineering was born in this era. Environmental legislation has sought to define environmental standards on clean water, air quality, solid waste disposal and pollution management — at state and national level, and to define international standards. We are using an ever-increasing number of chemicals with toxic waste and the remit of the environmental engineer is to keep the environment safe for humans and for other forms of life.

The terms climate change and global warming are often used interchangeably, but climate change refers to both the rise in global temperatures due to human activities which frequently cause droughts storms, melting glaciers and ice sheets, rising sea levels, warming seas, etc.

Since 2005, several global processes have called for an integrated approach to climate change adaptation (CCA) and disaster risk reduction (DRR). Calls to pursue this integration were intensified, with the adoption of three main and interrelated agendas, namely the 2030 Agenda for Sustainable Development, the Sendai Framework for Disaster Risk Reduction (2015-2030) and the Paris Agreement on Climate Change. While the CCA and DRR communities follow separate paths, bridging the gap between them entails both opportunities and challenges. Similarities between the two communities need to be exploited and differences investigated in order to achieve synergies in dealing with all aspects of weather-related hazards and disasters, assessment tools, institutional arrangements and means of implementation to achieve synergy between the two agendas.

Water and water security is a dynamic resource varying in quantity and quality across temporal and spatial scales. Global change drivers such as population growth, land-use change, environmental pollution, and climate change affect the availability of water resources. Microbe contamination of groundwater due to sewage outfalls and high concentration of nutrients in marine and coastal waters due to agricultural runoff are among the most serious threats. Although epidemiological studies have provided evidence of severe morbidity attributed to polluted water the issue has received limited attention in terms of valuation studies.

Today, people around the world can connect together online, and can access and disseminate vast amounts of knowledge and information quickly and easily. At the same time, increasing the availability of information & openness between these institutions has allowed greater understanding of the challenges facing the future of our global society with the increasing rate of the human population during the period from 2010 to 2050.

The aim of the Vth International Symposium is to facilitate interactions within the research community to discuss latest developments in this rapidly advancing field and find ways to respond to increasing demands of professionals, communities and industries across the world. It allowed the participants to have different issues addressed on *Environmental Quality and Public Health* by recognized global experts who are up-to-date with the latest developments in this field. This scientific meeting offers a great opportunity for students, researchers, industrialists and academic professionals to share latest research results in Environmental Quality and Public Health, network with their peers from around the globe and foster new connections that strengthen research and development activities in field of environmental health. The Symposium provided a platform for all the participants to voice their opinions and concerns as well as promoting discussions for collaborate in future.

The research papers presented in this Proceedings Book cover the latest developments and findings in the fields of environmental health, safety, energy, food staff, soil safety, waste management, reclamation and rehabilitation and environmental economic and quality.
Authors from over 12 countries with backgrounds in (bio)chemistry, (bio)engineering, (bio)technology and waste management, human and environmental health as well as quality and hailing from the government, industry and academy, have contributed to this Proceedings Book. The contents of this Proceedings Book will be of interest to scientists, engineers, consultants and government personnel who are responsible for the development and implementation of innovative approaches, techniques and technologies in the environmental industries. It will also benefit academic researchers, as it addresses the latest advances in fundamental research.

Participants in the Symposium are contributed to the development of this Proceedings Book agreed on different key conclusions from their deliberations:
Information that defines the scope of Environmental Quality and Public Health due to the human activities, their transport in the environment and exposure to pollutants for example is available in some detail. Intervention and prevention approaches for reducing exposures to hazard materials. Of the methods for identifying sources of contamination, currently comparative risk assessment provides the most reliable tool for ranking risk and making judgments about where significant economic benefits might be realized. Methods and procedures for benefit: cost and environmental health effectiveness: cost analysis exists to support decision making taking economic benefits into account.

This Proceedings Book describes the latest advances, innovations and applications in the field of environmental quality and public health as presented by leading researchers, engineers and practitioners at the Vth International Symposium on *Environmental Quality and Public Health*, held now in the 20th of May 2021, Budapest, Hungary. It providing a unique overview of new directions and opportunities for sustainable and resilient design approaches to protect the environment, it discusses diverse topics related to environmental quality and public health, through the eco-friendly re-use and processing of waste materials, the management and disposal of residual wastes, to water treatments and technologies as well as the human health. It also encompasses strategies for reducing pollution through better design, improved recovery, re-use, more efficient resource management and the performance of materials recovered from wastes. The contributions were selected by means of a rigorous peer-review process and highlight many exciting ideas that will spur novel research directions and foster multidisciplinary collaboration among different waste management specialists.

Budapest, May, 2021

Editor

BIBLIOGRAPHIC INFORMATION

Editor and affiliation	Prof. Dr. Hosam E.A.F. Bayoumi Hamuda President of ICEEE Institute of Environmental Engineering & Natural Sciences Óbuda University
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Organization, Scope and Opportunities of International Council of Environmental Engineering Education (ICEEE)

ICEEE is a non-profit organization that promotes the Engineering, innovation and Technology, related latest developments and issues to be discussed and experimented through interactions amongst the researchers and academician across the World at a common scientific platform.

ICEEE is leading event in the Environmental Engineering Education sector, sharing the latest research results, developments and innovative environmental protection and applications from industry and the policy context and also the environmental sustainability and environmental health. It is clear that, for the mitigation of climate change, urbanization and with rapid industrialization all over the world, pollution is on the increase, we need a transformation of the education, health, economy and environment, not a marginal change.

During the closing celemony of the International Annual Conference (11^{th} ICEEE-2020), it was decided that **Dr. Lyudmila SYMOCHKO** from Uzhgorod National University, Ukraine is the **General Secretory of the ICEEE**. The decision was built on her scientific activities and development as well as her activities and the cooperation with the presidency of the ICEEE.

Budapest, 20^{th} of May, 2021

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The Vth Symposium
Organization and Committees

The Vth International Symposium on
Environmental Quality and Public Health
is carrying out under the auspices of:

Prof. Dr. Levente KOVÁCS
Rector
Óbuda University (ÓU)

Presidency of the Symposium:

Dr. László KOLTAI
Dean
Rejtő Sándor Faculty of Light Industry & Environmental Engineering (RKK)

Dr. Rita BODÁNÉ-KENDROVICS
Director
Institute of Environmental Engineering & Natural Sciences (KTI)

Prof. Dr. Hosam BAYOUMI HAMUDA
President
International Council of Environmental Engineering Education (ICEEE)
Symposium Chair

Dr. Lyudmila SYMOCHKO
Uzhgorod National University, Ukraine
ICEEE AND Symposium General Secretory

Organising Committee

- Prof. Dr. Hosam **BAYOUMI HAMUDA**	(Óbuda University, Hungary)
- Dr. Rita **BODÁNÉ-KENDROVICS**	(Óbuda University, Hungary)
- Dr. Krisztina **DEMÉNY**	(Óbuda University, Hungary)
- Ms. Júlia **KASZÁS**	(Óbuda University, Hungary)
- Mr. András **SZEDER**	(Óbuda University, Hungary)
- Ms. Márta **SOÓSNÉ BERECZ**	(Óbuda University, Hungary)
- Mr. József **VÁRKÖVI**	(Óbuda University, Hungary)
- Ms. Zsuzsa **TAMÁSSY**	(Óbuda University, Hungary)

International Scientific Committee

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- Prof. Dr. István **PATKÓ**	(*Óbuda University, Hungary*)
- Prof. Dr. Borbála **BIRÓ**	(*Hungarian University of Agriculture and Life Sciences, Hungary*)
- Prof. Dr Bogdana **VUJIĆ**	(*University of Novi Sad, Serbia*)
- Prof. Dr. József **STEIER**	(*Sunwo Plc, Energy Expert, Hungary*)
- Prof. Dr. Mihájy **RÉGER**	(*Óbuda University, Hungary*)
- Prof. Dr.h.c. Miroslav **BADIDA**	(*Technical University of Košice, Slovakia*)
- Prof. Dr. Milan **PAVLOVIĆ**	(*University Business Academy, Novi Sad, Serbia*)
- Prof. Dr. Hosam **BAYOUMI HAMUDA**	(Óbuda University, Hungary)
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- Dr. Rita **BODÁNÉ-KENDROVICS**	(Óbuda University, Hungary)
- Dr. Krisztina **DEMÉNY**	(Óbuda University, Hungary)
- Dr. Ruslan **MARIYCHUK**	(*Presov University, Slovakia*)
- Dr. Lyudmila **SYMOCHKO**	(*Uzhgorod National University, Ukraine*)
- Dr. Edmond **HOXHA**	(*Polytechnic University of Tirana, Albania*)

ACKNOWLEDGMENT

Heartiest Greetings from ICEEE and Óbuda University

The International Council of Environmental Engineering Education (ICEEE) and the Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering (RKK) and Institute of Environmental Engineering and Natural Sciences have a great pleasure and cordially sincerely takes the honour & compliments to thank you to take part in the ***Vth. International Symposium on Environmental Quality and Public Health ONLINE on 20th of May, 2021*** at Óbuda University RKK, Budapest (Hungary).

The Organizing Committee of the Symposium has thought about the strong relationship between the environmental quality of Air, soil, and water and the health risk as well as the health care of the human due to the following points: Global change has resulted in a significant reduction of biodiversity on all trophic levels in our environment, often allowing for an invasion of new species, which change ecosystem properties. Changes in ecosystem properties influence ecosystem services, and in many cases human health or wellbeing is highly impacted. This strong interconnection between environmental properties and human health has promoted the so called "One Health" or "Global Health" concept.

Environmental problems, and future health effects, have changed over decades. Recently, public health was most concerned about localised environmental degradation, air and water pollution. Environmental quality has effects on human health and ecosystems quality, requiring cooperation across different sectors to determine effective responses. By increasing the global population is rising at a staggering rate and demands ever more resources, while the incorrect management of environmental quality is making public health worse. The environment affects the health of global population in a variety of ways. The interaction between human health and the environment has been extensively studied and environmental risks have been proven to significantly impact human health, either directly by exposing people to harmful agents, or indirectly, by disrupting life-sustaining ecosystems.

Environmental Quality and Public Health is an International Symposium where researchers, environmentalists, scientists, scholars and students, share their ideas, experiences, advancements, and research results. There were plenty of opportunities for organisations, projects and consortia to hold side events (meetings, seminars and workshops) on the Conference site to draw insights and encourage collaboration from many topics, disciplines, and backgrounds, promoting research and education to build a fair global community and more sustainable societies.

The purpose of the Vth International Symposium deals with Environmental Quality and Public Health due to the Climatic Change and pollution. Climate change is projected to harm human health through adverse changes in security of the life-style.

The Vth International Symposium bring together plenary, keynote, invited speakers and international researchers from academic authorities and industry, to communicate and share a wide range of highlighting potential issues and paths towards the environmental and human health due to climate change and pollution at present and future. The following core

Symposium themes reflect an integrated approach to identifying solutions to the complex global challenge of environmental quality.

After a great successful of the last four national Mini-Symposium, which brought together the professions and practitioners from different fields of applied sciences and environmental engineering, the International Council of Environmental Engineering Education (ICEEE) with the cooperation with the Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering (RKK), Institute of Environmental Engineering and Natural Sciences had the great pleasure to welcome all of you as a speakers and contributors for our Vth International Symposium on "*Environmental Quality & Public Health*" which is going online here in Budapest today May 20th 2021 in Hungary.

The main goals of the Symposium are: to promote research and developmental activities in Environmental Quality and different fields of Natural Science; and to promote scientific information interchange between researchers, developers, engineers, students, and practitioners working in and around the world.

This Symposium will provides opportunities for the delegates to exchange new ideas and application experiences face to face, to establish business or research relations and to find global partners for future collaboration.

Determination of environmental quality is a major area of interest when considering the public and ecosystem health. Achieving high-quality environmental development through environmental regulations and thus enhancing public health is a goal of this V. International Symposium.

Here, the organizing committee of the Symposium identify opportunities for international, civil society, global partners, and researchers to contribute to a high quality of global effort towards environmental health systems.

The organizing committee of the Symposium has the opportunity to thanks the contributors and the reviewers for their activities and their work to review the manuscripts of the participants.

At the end, the organizing committee of the Symposium wish all the best for all the participants and thank their attendance.

We are taking this opportunity to explore new forms of communication in Symposium publishing **Proceedings Book with ISBN: 978-963-449-238-2.**

Organizing Committee

IMPRESSUM

For the Program, Abstracts and the Proceedings Book of the papers of the V$^{\text{th}}$ International Symposium titled: "*Environmental Quality and Public Health*"

– The official language is English.
– The Program and Abstracts of the Symposium is provided to all registered participants in online (electronic) form.
– All the received papers were reviewed by two of the members of the International Committee of the Symposium.
– All reviewed papers for the V$^{\text{th}}$ International Symposium are published in the Conference Proceedings Book with the ISBN **978-963-449-238-2**.
– The Proceedings Book form with ISBN **978-963-449-238-2** in CD-ROM format and online (electronic) is present also in the website of ICEEE: www.iceee.hu
– The selected high quality manuscripts will be also published in the online journal.
– The scientific information and quality of the manuscript is due to the corresponding author of the manuscript.
– Individual authors at their manuscripts shell be responsible for any possible errors
– The Publisher of the Program, Abstracts and the Proceedings Book of the V$^{\text{th}}$ International Symposium is the ICEEE, Institute of Environmental Engineering and Natural Sciences, Sándor Rejtő Faculty of Light Industry and Environmental Engineering and Natural Sciences, Óbuda University, Budapest, Hungary.
– Publication year of the Proceedings Book is 2021.
– Important Website: www.iceee.hu

May, 2021.

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AWARDS OF SYMPOSIUM

The awards of the Vth International Symposium titled: "*Environmental Quality and Public Health"* were given to the most outstanding researchers of the conference under below four categories.

SELECTION PROCESS

CRITERIA FOR THE SESSION'S BEST PRESENTATION AWARD

Each and every presentation was evaluated by two evaluators and the average mark of both evaluators was taken as the final mark. The best presentation from sessions was selected based on the final mark received from the evaluators and the final decision was given by the Conference Chair. Below criteria were taken into consideration for this award and marks are given out of 100.

- **Value of the Content – 40%**
- **Clarity of Presentation – 20%**
- **Appropriate Audio Visual Aids- 20%**
- **Ability to Connect with the Audience 10%**
- **Proper Timing – 20%**

CRITERIA FOR THE OVERALL BEST PRESENTATION AWARD AND BEST YOUNG RESEARCHER AND STUDENT PRESENTATION AWARD

Presentations of each technical session with the highest marks were recommended for these two awards. They were evaluated by a special committee headed by the Conference Chair according to the below criteria.

- **Total Marks gained in the presentation**
- **Significance of the paper to the field**
- **Theoretical contribution**
- **The ability of practical implementation**
- **Use of appropriate methodological rigor**
- **Originality**

CRITERIA FOR THE BEST POSTER PRESENTATION AWARD

Every poster presentation is evaluated by a special evaluator based on below criteria and the presentation with the highest mark was selected as the best poster presentation award. The final mark is given out of 100.

- **Depth of Content (40%)**
- **Introduction and Abstract (15%)**
- **Content knowledge and organization (20%)**
- **Poster Design and Overall Visual Appeal (10%)**
- **Verbal Interaction (15%)**

AWARDS CEREMONY

Congratulations to all our Vth International Symposium-2021 Awards winners.

All the winners were presented with their awards during the awarding ceremony which was held on the end day of the symposium along with the symposium conclusion.

I. The Overall Best Presentations Award

These awards were provided for the most outstanding presentation of the entire conference. The two winners were:

1. ***Lyudmyla SYMOCHKO et al.*** *(Uzhhord National University, Faculty of Biology, Uzhhorod, Ukraine)* ***in the presentation:***
   SOIL RESISTOME AND PUBLIC HEALTH

2. ***Salma LATIQUE*** *(Department of Biology, Biotechnology and Plant Protection Laboratory, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco)* ***in the presentation:***
   THE USE OF MICROALGAE COMPOUNDS IN THE IMPROVEMENT OF AGRICULTURAL PRACTICES

II. THE BEST YOUNG RESEARCHER AND PHD STUDENT PRESENTATIONS AWARD

The best young researcher presentation award was given to the most outstanding presentation presented by a participant who has registered under the young researcher. The winner was:

1. ***Richárd Csaba KOVÁCS, Krisztina DEMÉNY*** **(***Óbuda University, Faculty of Light Industry and Environmental Engineering, Institute of Environmental Engineering and Nature Science, Budapest, Hungary)* ***in the presentation:***
   THE ROLE OF EDUCATIONAL PATHS IN THE ENVIRONMENTAL EDUCATION

The best PhD student’s presentation award was given to the most outstanding presentation presented by a participant who has registered under the Ph.D. student. The winner Ph.D. student was:

2. ***Abdussalam Ashour KHALIF et al.*** *(Doctoral School of Economic and Regional Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary)* ***in the presentation:***
   EMISSIONS OF GREENHOUSE GAS AND AIR POLLUTANT FROM DIFFERENT ECONOMIC SECTORS IN THE EUROPEAN UNION

III. SESSION'S BEST PRESENTATION AWARDS

These awards were provided to the presentations that have been selected to be the best in the particular sessions. They were:

1. ***Fatemeh Zarei1 et al.*** *(Food and Drug Administration, Tehran, Iran))* ***in the presentation:***
   THE EFFECT OF SUGAR BEET FIBRE AND INULIN ON THE SURVIVAL OF BIFIDOBACTERIUM BIFIDUM IN A SYNBIOTIC DRINK BASED ON GRAPE JUICE

2. ***Yara EzAl Deen SULTAN, Archana SHARMA*** *(Department of Environmental Science & Engineering, Marwadi University, Rajkot, India)* ***in the presentation:***
   APPLICATION OF CLEANER PRODUCTION IN SOAP INDUSTRY FOR A HEALTHIER WORK ENVIRONMENT

IV. THE BEST POSTER PRESENTATION AWARD

The best poster presentations were selected among all the researchers in the poster session. They wwere:

1. ***Alyona BUNAS, Yevheniia TKACH*** *(Institute of Agroecology and Environmental management of National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine)* ***in the presentation:***
   INFLUENCE OF NITROGEN FERTILIZERS ON THE BIOLOGICAL ACTIVITY OF THE SOIL OF THE RAPESEED ROOT ZONE

2. ***Beáta BARANOVÁ[1], Lenka DEMKOVÁ[2], Július ÁRVAY[3]*** *([1,2]Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Prešov, Slovakia, [3]Department of Chemistry, Faculty of Biotechnology and Food Science, University of Agriculture in Nitra, Nitra, Slovakia)* ***in the presentation:***
   ASSESMENT OF THE POTENTIAL RISK ELEMENTS AT METAL POST-MINING SPOIL HEAPS

Budapest, 20th of May, 2021

Prof. Dr. Hosam Bayoumi Hamuda
President of ICEEE
Symposium Chair

Main Themes

- Analysis of Environmental Economics
- Antimicrobial Resistance Mitigation
- Applications of Biotechnology & Bioengineering in Environment
- Applications of Civil, Electrical & Mechanical Engineering in Environmental Quality
- Climate Changes & Infectious Diseases
- Climate Changes & Landscape Conservation
- Drinking Water Quality, Treatment and Hygiene
- Ecotoxicological Risk in Wastewater Treatment
- Education Strategy in Natural & Engineering Sciences
- Environmental Contamination & Pollutions
- Epidemiological Management
- Fermentation & Food (Bio)Technology
- Global Environmental Problems & Biosafety
- Great COVID-19 Recovery
- Green Nanotechnology in Environment
- Material Science & Environmental Engineering
- Monitoring and Evaluation for Food Security and Nutrition
- Pesticides Management & Agricultural Sustainability
- Production & Consumption in Ecosystems
- Public Health & Epidemiology
- Radiological & Nuclear Protection
- Recycling Industries
- Strategic for Environmental Risk Assessment

Attention:

- The Conference will be held through **Microsoft Teams**.
- To join the Symposium:
    - There is a short guide with useful information about how to use the Microsoft Teams during the Conference
    - The participants will have the link of the Symposium
    - The time of the Conference is related to the **Hungarian time**.
- Please check the time with your time at home

Time of Oral Presentations Online (Microsoft Teams)

Presentation Type:	Total Allotted Time:
• Plenary speaker	25 min
• Keynote speaker	20 min
• Featured speaker	15 min
• Poster	10 min

Please note that:

- The time is very limited
- The official language of all the presentations including oral or poster speaker is **English**

The Vth International Symposium „Environmental Quality and Public Health”

PROGRAM

20th of May 2021 (Thursday)

09:00 a.m. – 09:30 a.m. Opening Ceremony

Prof. Dr. Hosam BAYOUMI HAMUDA
President, International Council of Environmental Engineering Education
Symposium Chair

Dr. Rita BODÁNÉ-KENDROVICS
Director, Institute of Environmental Engineering & Natural Sciences

Dr. Edit Csanák DLA
Deputy Dean, Rejtő Sándor Faculty of Light Industry & Environmental Engineering

Prof. Dr. László Gulácsi
Vice-Rector, Óbuda University

Prof. Dr. Sadhan Kumar Ghosh
Honour guest of the Symposium

09:30 – 11:10 **Plenary Session**

Chair of the Session: **Rita BODÁNÉ-KENDROVICS**

PL1 09:30 – 09:55

Sadhan Kumar GHOSH[1], Tejashwi RANA[2], Sourya Subhra CHAKRABORTY[3]
[1]Centre for Sustainable Development and Resource Efficiency Management, Mechanical Engineering Department, Jadavpur University, International Society of Waste Management, Air and Water, Kolkata, India, [2]PG Scholar, Mechanical Engineering Department, Jadavpur University, [3]PG Scholar, Civil Engineering Department, MeghnadSaha Institute of Technology, Kolkata, India

ENHANCING ENVIRONMENTAL QUALITY AND PUBLIC HEALTH BY PROMOTING RESOURCE CIRCULATION USING WASTE CO-PROCESSING IN CEMENT PLANTS

PL2 09:55 – 10:20

Borbála BIRÓ
Department of Agri-environmental Studies, Hungarian University of Agricultural and Life Sciences, Budapest, Hungary

MISSION AND POTENTIALS FOR SUSTAINABLE SOIL-ENVIRONMENTAL HEALTH – THE GREEN DEAL

PL3 10:20 – 10:45

Ruslan MARIYCHUK
Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Prešov, Slovakia

GREEN SYNTHESIS OF METAL NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

PL4 10:45 – 11:10

Jozsef STEIER
Sunwo Plc, member of the Hungarian Academy of Engineering, Budapest, Hungary

PAULOWNIA HYBRID BASED ECO-ISLANDS FOR ENHANCED URBAN HEALTH IMPROVEMENT WITH UN SDG 17 COMPATIBILITY

11:10 – 12:50 **Keynote Session**

Chair of the Session: **Krisztina DEMÉNY**

KL1 11:10 – 11:30

Lyudmyla SYMOCHKO[1,2], Hosam E.A.F. BAYOUMI HAMUDA[3], Vitaliy SYMOCHKO[1], Ruslan MARIYCHUK[4], Daniela GRULOVA[4], Jozef FEJER[4]

[1]Faculty of Biology, Uzhhorod National University, Uzhhorod, Ukraine, [2]Institute of Agroecology and Environmental Management, Kyiv, Ukraine, [3]Obuda University, Faculty of Light Industry and Environmental Engineering, Budapest, Hungary, [4]Faculty of Humanities and Natural Sciences, University of Presov, Presov, Slovakia

SOIL RESISTOME AND PUBLIC HEALTH

KL2 11:30 – 11:50

Abdelwahab TAHSIN, Rami EL-SHERBINY

Civil Engineering Department, Cairo University, Cairo, Egypt

CHARACTERIZATION OF GEOSYNTHETICS AT VARIOUS ENVIRONMENTAL CONDITIONS

KL3 11:50 – 12:10

Rehab A. ALZWAY[1], Ahmed M. MUFTAH[2], Osama R. SHALTAMI[2], Osama A. El-FALLAH[2]

[1]Department of Environmental Science and Engineering, School of Basic Science, Libyan Academy for Postgraduate Studies, Tripoli, Libya, [2]Department of Earth Sciences, Faculty of Science, Benghazi University, Libya

THE ENVIRONMENTAL IMPACT OF THE PRODUCED WATER ON THE UPPER AQUIFER IN THE WAHAT REGION, SIRT BASIN, LIBYA

KL4 12:10 – 12:30

J. Sándor ZSARNÓCZAI[1], SOMOGYI, Norbert[2]

[1]Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary, [2]Embassy of Hungary, PARIS, France

CHANGING ECONOMIC CONDITIONS OF MEDITERRANEAN REGION

KL5 12:30 – 12:50

Hosam E.A.F. BAYOUMI HAMUDA

Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary

HEALTHCARE AND TRANSMISSION OF PANADEMIC COVID-19

12:50 – 14:30 **Poster Session**

Chair of the Session: **Borbála BIRÓ**

PP1 12:50 – 13:00

Alyona BUNAS, Yevheniia TKACH
Institute of Agroecology and Environmental management of National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine
INFLUENCE OF NITROGEN FERTILIZERS ON THE BIOLOGICAL ACTIVITY OF THE SOIL OF THE RAPESEED ROOT ZONE

PP2 13:00 – 13:10

Beáta BARANOVÁ[1], Lenka DEMKOVÁ[2], Július ÁRVAY[3]
[1,2]Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Prešov, Slovakia, [3]Department of Chemistry, Faculty of Biotechnology and Food Science, University of Agriculture in Nitra, Nitra, Slovakia
ASSESMENT OF THE POTENTIAL RISK ELEMENTS AT METAL POST-MINING SPOIL HEAPS

PP3 13:10 – 13:20

Svitlana TKACHYK[1], Ivan MOSTOVIAK[2], Olena DEMYANYUK[3*]
[1]Ukrainian Institute for Plant Variety Examination; Kyiv, Ukraine, [2]Uman National University of Horticulture; Uman, Ukraine, [3]Institute of Agroecology and Environmental Management of NAAN, Kyiv, Ukraine
THE INFLUENCE OF HYDROTHERMAL FACTORS ON THE SPREAD AND DEVELOPMENT OF DISEASES IN AGROCENOSES OF CEREALS IN UKRAINE

PP4 13:20 – 13:30

Abdelwahab TAHSIN, Rami EL-SHERBINY
Civil Engineering Department, Cairo University, Cairo, Egypt
ASSESSMENT OF CREEP ON LONG-TERM PERFORMANCE AND CALIBRATION FACTOR OF GEOSYNTHETICS IN GEOENVIOR-MENTAL APPLICATIONS

PP5 13:30 – 13:40

Dominika FALVAI [1], Tivadar BALTAZÁR [2], Szilárd CZÓBEL [1]
[1]Department of Landscape Ecology & Nature Conservation, Hungarian University of Agricultural and Life Sciences, Gödöllő, Hungary, [2]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutritions, Faculty of AgriSciences, Mendel University in Brno, Czech Republic
FAKOPP 3-DIMENSIONAL ACOUSTIC TOMOGRAPHY MEASUREMENTS, IN THE EASTERN ALPS, ALONG A DESIGNATED VERTICAL TRANSECT

PP6 13:40 – 13:50

Marina NAODOVIC, Una MARCETA, Bogdana VUJIC
University of Novi Sad, Technical Faculty "Mihajlo Pupin", Zrenjanin, Serbia
TOWARDS GREENER METAL-MEDIATED SYNTHETIC METHODS

PP7 13:50 – 14:00

Ildikó JÁRDI[I], Gergely PÁPAY[1], Eszter S.-FALUSI[1], Zachar ZALÁN[1], Dénes SALÁTA[2], Károly PENKSZA[1]
[1]Department of Botany, Hungarian University of Agricultural and Life Sciences, Gödöllő, Hungary, [2]Hungarian University of Agricultural and Life Sciences, Department of Nature Conservation and Landscape Ecology, Gödöllő, Hungary
VEGETATION STUDY ON THE PASTURE ALONG THE IPOLY

PP8 14:00 – 14:10

Gergely PÁPAY[1], Norbert PÉTER[1], Zoltán BAJOR[1], Dénes SALÁTA[2], Zsuzsa LISZTES-SZABÓ[3], Zalán ZACHAR[1], Ferenc STILLING[4], Penksza KÁROLY[1]
[1]Institute Of Crop Production Sciences, Hungarian University of Agronomy and Life Sciences, Gödöllő, Hungary, [2]Institute Of Widlife Management And Nature Conservation, Hungarian University of Agronomy and Life Sciences, Gödöllő, Hungary, [3]Isotope Climatology and Environmental Research Centre, Debrecen, Hungary, [4]Hungarian Research Institute, Budapest, Hungary
LAWN REGENERATION RESULTS OF THE CONSERVATION MANAGEMENT ON THE HOMOKTÖVIS CONSERVATION AREA IN BUDAPEST

PP9 14:10 – 14:20

Una MARCETA[1], Jelena VUKOVIC[2], Bogdana VUJIC[1], Eleonora TerEcik[1]
[1]University of Novi Sad, Technical Faculty "Mihajlo Pupin", Zrenjanin, Serbia, [2]University of East Sarajevo, Faculty of Technology, Zvornik, Republic of Srpska, BiH
PUBLIC PERCEPTION, AWARENESS AND KNOWLEDGE ABOUT AIR QUALITY IN THE CITY OF ZRENJANIN

PP10 14:20 – 14:30

Hosam E.A.F. BAYOUMI HAMUDA[1], Fatemeh ZAREI[2], Lyudmyla SYMOCHKO[3,4]
[1]Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary, [2]Food and Drug Administration, Tehran, Iran, [3]Faculty of Biology, Uzhhorod National University, Uzhhorod, Ukraine, [4]Institute of Agroecology and Environmental Management, Kyiv, Ukraine,
RELATIONSHIP BETWEEN EXPOSURE TO ENVIRONMENTAL TOXICANTS AND EPIGENETICS

14:30 – 20:00 **Technical Session**

Chair of the Session: ***Hosam BAYOUMI HAMUDA***

TL1 14:30 – 14:40

Sadhan Kumar GHOSH[1], Sourya Subhra CHAKRABORTY[2], Tejashwi RANA[3]

[1]Centre for Sustainable Development and Resource Efficiency Management, Mechanical Engineering Department, Jadavpur University, International Society of Waste Management, Air and Water, Kolkata, India, [2]PG Scholar, Civil Engineering Department, MeghnadSaha Institute of Technology, Kolkata, India, [3]PG Scholar, Mechanical Engineering Department, Jadavpur University, India

PEOPLES' PERCEPTION IN PLASTICS WASTE MANAGEMENT TO PROTECT MARINE LITTERING, ENVIRONMENTAL QUALITY AND PUBLIC HEALTH AND ACTIONS THEREOF

TL2 14:40 – 14:50

Alla LEVISHKO, Iryna GUMENIUK, Olena DEMYANYUK

Institute of Agroecology and Environmental management of National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine

EFFICIENCY OF FORMATION AND FUNCTIONING OF SYMBIOTIC SOYBEAN SYSTEMS UNDER THE CONDITIONS OF PROCESSING WITH GLYPHOSATE

TL3 14:50 – 15:00

Tsend-Ayush ERDENEJARGAL[1], Enkhtuya OCHIRBAT[2], Hosam E.A.F. BAYOUMI HAMUDA[1]

[1]Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Óbuda University, Budapest, Hungary, [2]Laboratory of Flora and Plant systematics, Botanical Garden and Research Institute, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia

IMPACT ASSESSMENT OF ANTHROPOGENIC ACTIVITIES ON AIR QUALITY OF ULAANBAATAR, MONGOLIA USING LICHEN AS THE BIOINDICATOR

TL4 15:00 – 15:10

Hazim Aziz AL-ROBAI[1], Ghaith Salah AL-MAMOORI[2], Ali Akram ABDULATEEF[2], Ameer H. AL-RUBAYE[3]

[1]College of Agriculture, Al-Qasim Green University, Babylon, Iraq, [2]College of Environmental Sciences, Al-Qasim Green University, Babylon, Iraq,. [3]College of Engineering, AL-Kitab University, Kirkuk, Iraq

REMOVAL OF PB FROM CALCAREOUS AND GYPSUM SOILS CONTAMINATED WITH USED ENGINE OIL

TL5 15:10 – 15:21

Yousif F. E. IMRYED[1], Jamila A. BASHASHA[2], Fatma H. ELFALLAH[3*]

[1]*Plant Production Department, Faculty of Agriculture, Benghazi University, Libya,* [2]*Horticulture Department, Faculty of Agriculture, Benghazi University, Libya,* [3]*Botany Department, Faculty of Science, Ajdabiya University, Ajdabiya, Libya*

CYTOTOXICITY EFFECTS OF THREE PESTICIDES ON MITOTIC CHROMOSOME ON ROOT CELLS OF ALLIUM CEPA

TL6 15:20 – 15:30

Osama R. SHALTAMI[1], Ezeddin M. ELMALEKY[2], Osama A. EL-FALLAH[1], Fares F. FARES[1], Farag M. EL OSHEBI[1], Hwedi ERRISHI[3], Salah S. EL-EKHFIFI[4]

[1]*Department of Earth Sciences, Faculty of Science, Benghazi University, Libya,* [2]*Libyan General Authority of Water resources, Libya,* [3]*Department of Geography, Faculty of Arts, Benghazi University, Libya,* [4]*National Oil Corporation (NOC), Exploration Department, Libya*

GEOCHEMICAL EVALUATION OF GROUNDWATER: A CASE STUDY OF THE SIDI FARAG FARMS, BENGHAZI CITY, NE LIBYA

TL7 15:30 – 15:40

Fatemeh ZAREI[1], Leyla NATEGI[2], *Hosam E.A.F. BAYOUMI HAMUDA*[3]

[1]*Food and Drug Administration, Tehran, Iran,* [2]*Department of Food Science and Technology, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran,* [3]*Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Institute of Environmental Engineering and Natural Sciences, Budapest, Hungary*

THE EFFECT OF SUGAR BEET FIBRE AND INULIN ON THE SURVIVAL OF BIFIDOBACTERIUM BIFIDUM IN A SYNBIOTIC DRINK BASED ON GRAPE JUICE

TL8 15:40 – 15:50

Thamer Adnan ABDULLAH[1,2], Tatjana JUZSAKOVA[1], Ali Dawood SALMAN[1], Rashed Taleb RASHEED[2], Muhammed ALI MALLAH[3], Viktor SEBESTYÉN[1], Endre DOMOKOS[1]

[1]*Sustainability Solutions Research Lab, Faculty of Engineering, University of Pannonia, Veszprém, Hungary,* [2]*Chemistry Branch, Applied Sciences Department, University of Technology, Baghdad, Iraq,* [3]*National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan*

VANADIUM PENTOXIDE AND THEIR NANOCOMPOSITES FOR WATER TREATMENT

TL9 15:50 – 16:00

Richárd Csaba KOVÁCS*, Krisztina DEMÉNY

Óbuda University, Faculty of Light Industry and Environmental Engineering, Institute of Environmental Engineering and Nature Science, Budapest, Hungary

THE ROLE OF EDUCATIONAL PATHS IN THE ENVIRONMENTAL EDUCATION

TL10 16:00 – 16:10

Salma LATIQUE[1], Doha ELALAMI[1], Abdellatif BARAKAT[1,2], Abdallah OUKARROUM[1]
[1]AgroBioSciences Research Division, Mohammed VI Polytechnic University, Benguerir, Morocco, [2]IATE, Montpellier University, INRAE, Agro Institut, Montpellier, France
THE USE OF MICROALGAE COMPOUNDS IN THE IMPROVEMENT OF AGRICULTURAL PRACTICES

TL11 16:10 – 16:20
Jiang DONGZE, Edit KASZAB, Sándor SZOBOSZLAY
[1]Department of Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary
THE EVALUATION OF PESTICIDES ON OPPORTUNISTIC PSEUDOMONAS AERUGINOSA AND ITS ANTIBIOTIC RESISTANCE

TL12 16:20 – 16:30
Xinyue LIANG[1], Benjamin BÁLINT[2], Borbála SZABÓ[3]
[1]Department of Aquaculture, Hungarian University of Agriculture and Life Science, Gödöllő, Hungary, [2]Department of Zoology and Animalecology, Hungarian University of Agriculture and Life Science, Gödöllő, Hungary, [3]Centre for Ecological Research; Institute of Ecology and Botany. "Lendület" Landscape and Conservation Ecology Research Group, Vácrátót, Hungary
ECOTOXICOLOGICAL EVALUATION OF BORON, ARSENIC, IODINE AND SELENIUM ON REPRODUCTION, MORTALITY AND AVOIDANCE OF FOLSOMIA CANDIDA (COLLEMBOLAN)

TL13 16:30 – 16:40
Yara EzAl Deen SULTAN, Archana SHARMA
Department of Environmental Science & Engineering, Marwadi University, Rajkot, India
APPLICATION OF CLEANER PRODUCTION IN SOAP INDUSTRY FOR A HEALTHIER WORK ENVIRONMENT

TL14 16:40 – 16:50
Katalin Emese RAB GÁBORNÉ SERESS[1], Hosam E.A.F. BAYOUMI HAMUDA[2], Tünde TAKÁCS[3]
[1]CEEweb for Biodiversity, Budapest, Hungary, [2]Óbuda University Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Institute of Environmental Engineering and Natural Sciences, Budapest, Hungary. [3]Hungarian Academy of Sciences, Hungary, Institute for Soil Sciences and Agricultural Chemistry
DEVELOPING SOIL PROTECTION TRAINING MATERIALS BASED ON EXPERIMENTAL PROCEDURES FOR AGE BETWEEN 10 AND 14 IN HUNGARIAN PUBLIC EDUCATION

TL15 16:50 – 17:00

J. Sándor ZSARNÓCZAI, Zoltán ZÉMAN
Institute of Finance and Accountancy, Faculty of Economic and Social Sciences, Szent István University, Gödöllő, Hungary
OUTPUT VALUE AND PRODUCTIVITY OF AGRICULTURAL INDUSTRY IN CENTRAL-EAST EUROPE

TL16 17:00 – 17:10
Fatma H. ELFALLAH[1], Hosam E.A.F. BAYOUMI HAMUDA[2]
[1]Botany Department, Faculty of Science, Ajdabiya University., Ajdabiya, Libya, [2]Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary
IMPACT OF GLYPHOSATE HERBICIDE ON SOME SOIL BIOLOGY AND FUNCTION

TL17 17:10 – 17:20
Nikolett KOZELKA-BURES, Hosam E.A.F. BAYOUMI HAMUDA
Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary
ECOTOXICOLOGICAL EVALUATION OF THE TOLERANCE OF SOME SYMBIOTIC N2-BINDING SOIL BACTERIA TO ENVIRONMENTAL CONTAMINANTS

TL18 17:20 – 17:30
Zsanett MAGYAR, Hosam E.A.F. BAYOUMI HAMUDA
Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary
EFFECT OF SOME HERBICIDES ON SOIL MICROBIAL POPULATION, SOIL ORGANIC MATTER AND DEHYDROGENASE ACTIVITY

TL19 17:30 – 17:40
Lilla FARKAS, Hosam E.A.F. BAYOUMI HAMUDA
Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary
EFFECT OF FOUR HERBICIDES ON SOIL ENZYME ACTIVITY IN BROWN FOREST AGRICULTURAL SOIL

TL20 17:40 – 17:50
Barbara KÖVICS, Hosam E.A.F. BAYOUMI HAMUDA
Institute of Environmental Engineering and Natural Sciences, Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Engineering, Budapest, Hungary
SURVEY OF LICHENS BIODIVERSITY IN DIFFERENT AREAS OF CSÓR IN HUNGARY

TL21 17:50 – 18:00

Abdussalam Ashour KHALIF[1*], László LŐKÖS[2], Ferenc LIGETVÁRI[3]
[1]*Doctoral School of Economic and Regional Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary, e-mail:* khalif_salam@yahoo.com, *mobile: +36/20/2042061,* [2]*Doctoral School of Economic and Regional Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary,* [3]*Debrecen University, Debrecen, Hungary*
EMISSIONS OF GREENHOUSE GAS AND AIR POLLUTANT FROM DIFFERENT ECONOMIC SECTORS IN THE EUROPEAN UNION

TL22 18:00 – 18:10
Katalin FŐGLEIN*, István KÖVESDI, János DEÁK, Tibor TELEKESI
KTI Institute for Transport Sciences, Non Profit Ltd, Research Center for Sustainable Transport, Department for Air Quality and Propulsion Systems, Budapest, Hungary
POSSIBILITIES TO ENSURE SUSTAINABILITY AND A GREEN STRATEGY IN HUNGARIAN ROAD TRANSPORT

TL23 18:10 – 18:20
Vikas AHLAWAT[1], Mantavya BISHNOI[2]*, Soma DEHLAVI[3], Mina N. SHIRAZI[4], Sara SADEGHI[5], B.S. PANWA[6], Hosam E.A.F. BAYOUMI HAMUDA[7]
[1,6]*Department of Soil Science, CCS, Haryana Agricultural University, Hisar, India,* [2]*Department of Foods and Nutrition, CCS, Haryana Agricultural University, Hisar, India,* [3]*HSE Expert, Organization of Industry, Mine and Trade, Sanandaj, Kurdistan, Iran,* [4]*Department of Soil Biology and Technology, University of Tehran, Iran,* [5]*Department of Forest Ecology, University of Kurdistan, Sanandaj, Kurdistan, Iran,* [7]*Óbuda University, Budapest, Hungary*
EFFECT OF DIFFERENT CHELATING AGENTS AND BIOINOCULANTS ON FRACTIONAL DISTRIBUTION OF CADMIUM AND NICKEL IN SPIKED SOIL AFTER HARVEST OF INDIAN MUSTARD (*BRASSICA JUNCEA*)

18:20– 18:45
Certificate, Award Distribution
& Closing Ceremony

TABLE OF PUBLISHING CONTENTS

Presentation Type	Author(S)	Presentation Title	Page number	
			Abstract	Full Paper
Plenary Lectures	**PL1.** Sadhan Kumar Ghosh, Tejashwi Rana, Sourya Subhra Chakraborty	Enhancing environmental quality and public health by promoting resource circulation using waste co-processing in cement plants	34	82-93
	PL2. Borbála Biró	Mission and potentials for sustainable soil-environmental health – the green deal	36	
	PL3. Ruslan Mariychuk	Green synthesis of metal nanoparticles for biomedical applications	37	95-1000
	PL4. Jozsef Steier	Paulownia hybrid based eco-islands for enhanced urban health improvement with UN SDG 17 compatibility	38	
Keynote Lectures	**KL1.** Lyudmyla Symochko, Hosam E.A.F. Bayoumi Hamuda, Vitaliy Symochko, Ruslan Mariychuk, Daniela Grulova, Jozef Fejer	Soil resistome and public health	40	
	KL2. Abdelwahab Tahsin, Rami El-Sherbiny	Characterization of geosynthetics at various environmental conditions	41	104-113
	KL3. Rehab A. Alzway, Ahmed M. Muftah, Osama R. Shaltami, Osama A. El-Fallah	The environmental impact of the produced water on the upper aquifer in the Wahat region, Sirt basin, Libya	42	114-129
	KL4. J. Sándor Zsarnóczai, Somogyi, Norbert	Changing economic conditions of Mediterranean region	43	
	KL5. Hosam E.A.F. Bayoumi Hamuda	Healthcare and transmission of pandemic COVID-19	44	131-148
Posters Presentations	**PP1.** Alyona Bunas, Yevheniia Tkach	Influence of nitrogen fertilizers on the biological activity of the soil of the rapeseed root zone	46	150-154
	PP2. Beáta Baranová, Lenka Demková, Július Árvay	Assessment of the potential risk elements at metal post-mining spoil heaps	47	155-161
	PP3. Svitlana Tkachyk, Ivan Mostoviak, Olena Demyanyuk	The influence of hydrothermal factors on the spread and development of diseases in agrocenoses of cereals in Ukraine	48	

	PP4. Abdelwahab Tahsin, Rami El-Sherbiny	Assessment of creep on long-term performance and calibration factor of geosynthetics in geoenvior-mental applications	49	163-173
	PP5. Dominika Falvai, Tivadar Baltazár, Szilárd Czóbel	Fakopp 3-dimensional acoustic tomography measurements, in the eastern Alps, along a designated vertical transect	50	174-177
	PP6. Marina Naodovic, Una Marceta, Bogdana Vujic	Towards greener metal-mediated synthetic methods	51	178-180
	PP7. Ildikó Járdi, Gergely Pápay, Eszter S.-Falusi, Zachar Zalán, Dénes Saláta, Károly Penksza	Vegetation study on the pasture along the Ipoly	52	
	PP8. Gergely Pápay, Norbert Péter, Zoltán Bajor, Dénes Saláta, Zsuzsa Lisztes-Szabó, Zalán Zachar, Ferenc Stilling, Penksza Károly	Lawn regeneration results of the conservation management on the Homoktövis conservation area in Budapest	53	185-189
	PP9. Una Marceta, Jelena Vukovic, Bogdana Vujic, Eleonora Terecik	Public perception, awareness and knowledge about air quality in the city of Zrenjanin	54	190-196
	PP10. Hosam E.A.F. Bayoumi Hamuda, Fatemeh Zarei, Lyudmyla Symochko	Relationship between exposure to environmental toxicants and epigenetics	55	197-213
Technical Lectures	**TL1.** Sadhan Kumar Ghosh, Sourya Subhra Chakraborty, Tejashwi Rana	People's perception in plastics waste management to protect marine littering, environmental quality and public health and actions thereof	57	215-228
	TL2. Alla Levishko, Iryna Gumeniuk, Olena Demyanyuk	Efficiency of formation and functioning of symbiotic soybean systems under the conditions of processing with glyphosate	59	
	TL3. Tsend-Ayush Erdenejarga, Enkhtuya Ochirbat, Hosam E.A.F. Bayoumi Hamuda	Impact assessment of anthropogenic activities on air quality of Ulaanbaatar, Mongolia using lichen as the bioindicator	60	230-238
	TL4. Hazim Aziz Al-Robai, Ghaith Salah Al-Mamoori, Ali Akram Abdulateef,	Removal of Pb from calcareous and gypsum soils contaminated with used engine oil	61	

	Ameer H. Al-Rubaye			
	TL5. Yousif F. E. Imryed, Jamila A. Bashasha, Fatma H. Elfallah	Cytotoxicity effects of three pesticides on mitotic chromosome on root cells of allium cepa	62	240-247
	TL6. Osama R. Shaltami, Ezeddin M. Elmaleky, Osama A. El-Fallah, Fares F. Fares, Farag M. El Oshebi, Hwedi Errishi, Salah S. El-Ekhfifi	Geochemical evaluation of groundwater: a case study of the side Farag farms, Benghazi city, NE Libya	63	248-256
	TL7. Fatemeh Zarei, Leyla Nategi, Hosam E.A.F. Bayoumi Hamuda	The effect of sugar beet fibre and inulin on the survival of Bifidobacterium bifidum in a synbiotic drink based on grape juice	64	257-270
	TL8. Thamer Adnan Abdulla, Tatjana Juzsakova, Ali Dawood Salman, Rashed Taleb Rasheed, Muhammed Ali Mallah, Viktor Sebestyén, Endre Domokos	Vanadium pentoxide and their nanocomposites for water treatment	65	271-281
	TL9. Richárd Csaba Kovács, Krisztina Demény	The role of educational paths in the environmental education	66	382-292
	TL10. Salma Latique, Doha Elalami, Abdellatif Barakat, Abdallah Oukarroum	The use of microalgae compounds in the improvement of agricultural practices	67	193-303
	TL11. Jiang Dongze, Edit Kaszab, Sándor Szoboszlay	The evaluation of pesticides on opportunistic Pseudomonas aeruginosa and its antibiotic resistance	68	304-313
	TL12. Xinyue Liang, Benjamin Bálint, Borbála Szabó	Ecotoxicological evaluation of boron, arsenic, iodine and selenium on reproduction, mortality and avoidance of Folsomia candida (collembolan)	69	314-325
	TL13. Yara Ezal Deen Sultan, Archana Sharma	Application of cleaner production in soap industry for a healthier work environment	70	326-332
	TL14. Katalin Emese Rab Gáborné Seress, Hosam E.A.F. Bayoumi Hamuda,	Developing soil protection training materials based on experimental procedures for age between 10-14 in Hungarian public education	71	333-340

	Tünde Takács			
	TL15. J. Sándor Zsarnóczai, Zoltán Zéman	Output value and productivity of agricultural industry in central-east Europe	72	
	TL16. Fatma H. Elfallah, Hosam E.A.F. Bayoumi Hamuda	Impact of glyphosate herbicide on some soil biology and function	73	342-356
	TL17. Nikolett Kozelka-Bures, Hosam E.A.F. Bayoumi Hamuda	Ecotoxicological evaluation of the tolerance of some symbiotic N_2-binding soil bacteria to environmental contaminants	74	357-367
	TL18. Zsanett Magyar, Hosam E.A.F. Bayoumi Hamuda	Effect of some herbicides on soil microbial population, soil organic matter and dehydrogenase activity	75	368-385
	TL19. Lilla Farkas, Hosam E.A.F. Bayoumi Hamuda	Effect of four herbicides on soil enzyme activity in brown forest agricultural soil	76	386-399
	TL20. Barbara Kövics, Hosam E.A.F. Bayoumi Hamuda	Survey of lichens biodiversity in different areas of Csór in Hungary	77	400-417
	TL21. Abdussalam Ashour Khalif, László Lőkös, Ferenc Ligetvári	Emissions of greenhouse gas and air pollutant from different economic sectors in the European Union	78	418-430
	TL22. Katalin Főglein, István Kövesdi, János Deák, Tibor Telekesi	Possibilities to ensure sustainability and a green strategy in Hungarian road transport	79	431-440
	TL23. Vikas Ahlawat, Mantavya Bishnoi, Soma Dehlavi, Mina N. Shirazi, Sara Sadeghi, B.S. Panwa, Hosam E.A.F. Bayoumi Hamuda	Effect of different chelating agents and bioinoculants on fractional distribution of cadmium and nickel in spiked soil after harvest of Indian mustard (Brassica juncea)	80	441-452

Abstracts of Plenary Session

PL1.
ENHANCING ENVIRONMENTAL QUALITY AND PUBLIC HEALTH BY PROMOTING RESOURCE CIRCULATION USING WASTE CO-PROCESSING IN CEMENT PLANTS

Sadhan Kumar GHOSH[1,*], Tejashwi RANA[2], Sourya Subhra CHAKRABORTY[3]
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Abstract: *Since ages, countries throughout the world have been using landfill sites for dumping waste at minimum cost. Currently, an estimated 2 billion tonnes of municipal solid waste is generated globally, out of which almost 33% remain uncollected by municipalities (Waste Atlas 2018). Currently, the top three municipal solid waste-generating countries are the USA, i.e., 258 million metric tons, China, i.e., 220 million metric tons, and India, i.e., 169 million metric tons (Statista 2020).This daily accumulation of waste materials is increasing heavily on a daily basis with increase in population, causing scarcity of further land to be used for landfills. According to the World Bank, the generation of municipal solid waste is anticipated to rise to 3.4 billion tonnes by 2050 (The World Bank 2020a). These landfills are generally without any scientific treatment or segregation in developing countries including India. Treatment capacities are not sufficient causing local harm, release of greenhouse gases and other pollution. From the current world population of 7.6 billion (US Census Bureau 2020), nearly 3.5 billion people are deprived of basic waste management facilities (Waste Atlas 2018). As a result these landfill sites have become a breeding ground for contamination by flies, rodents, mosquitoes and causing diseases like dermatological, respiratory, genetic, and several other kind of infectious diseases. Among these wastes accumulated, various materials have been proven to be useful resources which can be used again as an input in various industries. A major portion of these wastes may be utilized and resources can be recirculating by various treatment and management processes by the implementation of 3Rs and circular economy concepts. Co-processing of wastes in cement plant is one of the options for this solution. This paper deals with enhancing the environmental quality and public health by promoting the process of co-processing of waste materials in cement plants thereby reducing the path of waste materials to the landfill sites. Co-processing is defined as the process where waste materials are used as a source of energy or raw materials. By applying this circular economy concept, usage of conventional fuel can also be greatly reduced. A case study has also been done to enquire whether co-processing is the best option to be implemented on cement plants. If the waste materials are co-processed in cement plants effectively, landfill sites will no longer overflow with waste and quality of health and environment can also be improved significantly. Moreover, it will help to obstruct the flow of waste materials to ocean, hence preventing marine littering and creating micro plastics too.*

Keywords: *Co-processing, Circular economy, GHGs. landfill, municipal solid waste, waste management.*

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Biography

Prof. Sadhan K Ghosh completed his PhD from Jadavpur University. He is the Professor in mechanical engineering and Ex-Dean of faculty of engineering. & Tech., at Jadavpur University & served as the Director, CBWE, Ministry of L&E, Govt. of India. He is internationally well-known researcher on waste management, Green Manufacturing, SDG, ISO Standards and sustainable SME. He has published more than 250 papers in reputed journals, books and proceedings. He serves as Associate Editor of the journal, Waste Management, Int. Journal of Materials Cycles and Waste Management and Editor-in-Chief of ISWMAW-IconSWM Publication Secretariat. He is an editorial board member of repute. Research interest briefly: He is *the PI of more than 20 International and 25 national Research Projects funded by different agencies, like, EU, GCRF, DST, DBT, UKIERI, Royal Acad. of Engg, SINTEF Norway, Hungarian Govt., Georgia Govt., and many others. He is international expert of UNCRD/UN DESA, SACEP, IJES and APO having research collaboration with 40 countries. His web: www.sadhankghosh.com and available at: sadhankghosh9@gmail.com*

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PL2.
MISSION AND POTENTIALS FOR SUSTAINABLE SOIL-ENVIRONMENTAL HEALTH – THE GREEN DEAL

Borbála BIRÓ

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Abstract*:* ***Motivation/Background****: New way of thinking is necessary nowadays in connection with the sustainable management of our environment, including of the soils in the Earth. Soils are potentially renewable energy sources, however, the way and techniques for those technologies are highly dependent on several biotic and abiotic environmental factors.* ***Method****: The European Commission has created 5 different "Missions" regarding the sectors, where needed crucial change in the "Green Deal" processes. Among those missions, main focus is given for the "Soil health and Food". The soil beneath our feed deserves more attention. There are various parameters suggested for the monitoring and for data-handling. The aim is to connect also for the "Sustainable developing goals (SDG)".* ***Results****: Among the suggested eight parameters of the "Soil Mission", the most important thing is to reduce the soil-pollution, created mainly by the industry. Among agri-horti-viti-cultural situation the conservation of soil-organic-matter (SOM) is suggested as a crucial management option. Soil can be a Carbon-source, so the mitigation of Global Change Processes is also highly dependent on the soil. The biodiversity of soil can offer solutions for almost all environmental- and societal problems. Up till now, no more than the 10% of soil-biota is known by the scientists. Beside the soil-quality parameters, the soil-health is providing the better food-quality and food-safety options, especially when the alternative organics (as sewage sludge composts, other biosolids are applied.* ***Conclusions****: Soil can offer several options for the solution of environmental problems nowadays. The paradigm-shift of considering its importance is needed for let the soil to providing main ecosystem services. The aim of the lecture to outline the potentials and solutions how those objectives might be possible.*

Keywords: *Soil-environmental health, monitoring, sustainability, ecosystem services*

Biography:

Borbála Biró has completed his PhD at the University of Kossuth Lajos (Debrecen) and later she was invited as postdoctoral scientist to the University of London (the Wye College) and also to the University of Verona, Department of Soil-Biology and Biochemistry. She was working for 30 years at the Research Institute for Soil Science and Agrochemistry of Hungarian Academy of Sciences. Finally, she has become a professor, at Szent István University. She was the Hungarian leader of several bi- and multilateral projects. She has published more than 200 papers in peer-reviewed journals. Recently she has been selected as a top-expert from Hungary of the EU Horizon 2020 "Mission Board for Soil Health and Food".

PL3.
GREEN SYNTHESIS OF METAL NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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Abstract: *The synthesis and application of new nanomaterials have become a key technology for many fields, like electronics, engineering, optics, computer science, sensorics, and biomedicine. Therefore, there is an interest in new cost-, energy-, and time-effective methods for preparation of new nanomaterials. Biomedical application of nanomaterials is limited not only by control over the size and morphology of nanoparticles but also by their biocompatibility. However, the trusted protocols normally involve the application of reducing agents (sodium borohydride, methoxy polyethylene glycol, potassium tartrate, etc.) and capping agents (sodium dodecyl benzyl sulphate, polyvinyl-pyrrolidone, etc.) which are usually toxic. The unavoidable presence of toxic compounds in nanocolloid systems limits the applications of the nanoparticles for biological systems. The aim of this study is the analysis of recent reports and evaluation of perspectives for the development of green methods of metal nanoparticles for biomedical applications. Numerous physical-chemical methods are in use for the characterization of nanoparticles: ultraviolet and visible spectroscopy for characterization of surface plasmon resonance, infrared spectroscopy for characterization of capping agents, surface and transmission electron microscopy and atomic force microscopy for size and shape studies, energy-dispersive X-ray spectroscopy for chemical composition determination and dynamic light scattering for size determination. Analysis of recent studies have shown that the extracts of selected plants (juniper, goldenrod, spearmint, lemon balm etc.) can be successfully used for preparation of biocompatible plasmonic materials with response in the infrared region, which make them attractive to biomedical applications.*

Keywords: *nanomaterials, nanoparticles, phytosynthesis, plasmonic materials, spectroscopy*

Biography:

Dr. Ruslan Mariychuk has obtained his PhD degree in Inorganic chemistry in 2000 at the Taras Shevchenko National University of Kyiv, Ukraine and Postdoctoral Studies on 2003-2007 at Departments of Inorganic chemistry, Regensburg University and Bayreuth University, Germany. In 2009, he obtained his Associate Professor diploma in Ecology and Environment Protection at Uzhhorod National University, Ukraine. He is the Head of Department of Ecology at the Faculty of Humanities and Natural Sciences, University of Prešov, Slovakia. He has published more than 40 papers in reputed journals and co-author of 3 patents. Research interests are green technologies, green chemistry, and nanomaterials.

PL4.
PAULOWNIA HYBRID BASED ECO-ISLANDS FOR ENHANCED URBAN HEALTH IMPROVEMENT WITH UN SDG 17 COMPATIBILITY

Jozsef STEIER

Honorary professor of University Abomey Calavy (UAC), Bénin and managing director of Sunwo Plc, member of the Hungarian Academy of Engineering

Abstract: *The research work has aimed to find an eco-friendly green solution to reduce urban health risks and refer to the everyday environmental challenges such as micro dust, smoke, pollen pollution and heat islands phenomena. These environmental dangers are to limit human life and decrease quality of living standard in urban settlements. Professor Steier has developed and successfully tested a multi-functional Paulownia hybrid (the Smaragdfa^R) which has 27 valuable qualities beside that it can be planted among different weather conditions. During the test it has become obvious that some functions can efficiently improve urban living condition. The large leafs are collecting pollens, while increased aspiration capacity collects dust. Since Smaragdfa^R is a C4 type hybrid it enjoys and consumes carbon rich smog, too. The evaporation of the tree is creating a micro climate in its surrounding and reduces heat island phenomena. Such we have achieved an eco-friendly green solutions, the so called* ***eco-island,*** *which was paralelly established in Budapest and Füzesgyarmat. The result has been exceeding our expectations beside that there are supporting several targets points of UN SDG 17 goals. The eco-friendly urban planning has won a new tool with Smaragdfa^R and eco-island solution to develop a green living settlement for the future and decrease health risks on an efficient way.*

Keywords: environmental dangers, urban settlements, multi-functional Paulownia hybrid, Smaragdfa^R which has 27 valuable qualities, eco-friendly green solutions, eco-island solution

Biography:

Prof. Dr. József Steier (Hungary, 22.11.1954) is economist- energy specialist - agricultural engineer, Prof of UAC (Benin). Graduation: University of Economics Corvinus (1974-77), University of Agriculture Saint Stephen (SZIE, Gödöllő 2013). COP 22 labelled important innovation holder (Morocco, 2016).He was chairman of Sahara Scientists Summits & Green Sahara initiatives. He is Initiator of the open field CO_2 fertilisation program for progressive climate mitigation. He was a speaker on the ***5th Crans Montana Forum in Dakhla in April 2019*** *and spent 5 years in Morocco (2015-2020) on different projects. Global Green Solution Ltd, his own company in Morocco has strong engagement in water saving technologies, bio-gas, Smaragdfa^R, carbon climate farming with research for Green Morocco Plan and for the Sahara greening. In 2019 he inaugurated his first* ***6 years old carbon climate farm in Hungary*** *and introduced his hybrid Smaragdfa^R also in Morocco with success. November 2019 he was elected Co-President of Energy and Environment Protection Cabinet of Budapest Chamber of Commerce and Industry. A* ***European pioneer village*** *in green technologies Nagypáli elected him for Honorary Citizen in 2019. On 17th February, 2021 the* ***Hungarian Academy of Engineering*** *elected him for its member.*

Abstracts of Keynote Session

KL1.
SOIL RESISTOME AND PUBLIC HEALTH

Lyudmyla SYMOCHKO[1,2], Hosam E.A.F. BAYOUMI HAMUDA[3], Vitaliy SYMOCHKO[1], Ruslan MARIYCHUK[4], Daniela GRULOVA[4], Jozef FEJER[4]

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Abstract: *Terrestrial ecosystems may provide an ideal setting for the acquisition and dissemination of antibiotic resistance because they are frequently impacted by anthropogenic activities. The soil resistome plays an important role in the development and spread of antibiotic resistance in humans. The aim of our study was to detect the antibiotic-resistant soil bacteria in different ecosystems: natural ecosystems, agroecosystems and urboecosystems. The 468 dominating bacteria have been isolated, including 79 antibiotic resistant bacteria. All isolates were multi-drug resistant, of which greater than 74.5% were resistant to 9 antibiotics. A study of soil samples from the primeval forests showed that the microbial community was characterized by a low content of antibiotic-resistant microorganisms. Among 78 isolated bacteria, only two of them, Bacillus cereus, and Pantoea agglomerans, demonstrated a high level of resistance to antibiotics. Totally 106 strains were isolated from the soil of medicinal plants, 13 of them were antibiotic-resistant. The greatest numbers of antibiotic-resistant bacteria have been isolated from soil of urboecosystems and agroecosystems contaminated by enrofloxacin. Among the 284 tested bacteria, 64 were antibiotic resistant. Multi-resistant were such pathogenic and conditionally pathogenic bacteria as: Enterococcus faecium, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli, Bacillus licheniformis, Serratia fonticola, Hafnia alvei, Bacillus cereus, Bacillus megaterium and Clostridium difficile. Management* of soil *microbiota is vitally important for safeguarding public* health.

Keywords: *Ecosystem, Resistome, Soil, Microbiome, Antibiotic resistance.*

***Biography**:*

Lyudmyla Symochko got her Master's degree in Ecology and Environment Protection in 2000. She got her Doctor's degree (Ph.D.) by Specialty - 03.00.16 Ecology in 2005. She is Associate Professor since 2008. Professional Career: a lecturer at the Faculty of Biology, Uzhhorod National University, Ukraine. Symochko Lyudmyla – a specialist in environmental microbiology and ecology. Since 2008 she has focused on autecology and synecology researches of soil and water microbiota. Dr. Lyudmyla is explores the soil resistome and the role of natural and transformed ecosystems as a reservoir of antibiotic-resistant microorganisms. She is developing new and improving existing methods of bioindication and bioremediation. She is working with microbiological monitoring in different types of ecosystems. Detects antibiotic-resistant opportunistic pathogens in the environment and provide they risk assessment to human health. She is author of over 150 scientific publications, including 5 books.

KL2.
CHARACTERIZATION OF GEOSYNTHETICS AT VARIOUS ENVIRONMENTAL CONDITIONS

Abdelwahab TAHSIN*, Rami EL-SHERBINY

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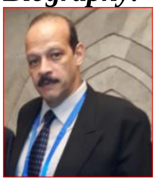
Abstract: *In the last two decades, geosynthetics became a common application in Geoenvironmental engineering. Use of geosynthetics as a sustainable construction solution in civil engineering was re-ported by the Waste and Resources Action Program (WRAP, 2010), presenting case studies of sludge lagoon, tailing ponds and soil waste systems, wherein geosynthetic introduced reduction in cost and CO_2 emission. Geosynthetics enhance the soil quality, performance, strength parameters, and regularly pragmatic as a soil reinforcement in the exhibition of high loads while strengthening of tailing impoundments and landfills. It is an appropriate soil improvement system with unique advantages compared to other conventional ones. This manuscript introduces analyses and results of laboratory constant strain rate (CSR) test of 20%, 10%, 6%, 3%, 1% and 0.05% strain/min conducted on five specimens of geosynthetics polyester (PET) geogrids. Tests were performed according to the Multi-Rib tensile method of ASTM D6637. Main output acceptance features of tensile ultimate strength and strain at break was justified. Stiffnesses at strain of 1% and 2% were derived. Sensitivity of strain rate of loading on strength, stiffness and non-linear properties of geogrid was inspected. Results of the measured tensile load- strain relationship for working loads (strain $\leq$ 2%) are considerably acknowledged of linear simulation. The larger ultimate strength, the higher anticipated stiffness irrespective to loading strain rate. The derived stiffness at 1% is larger than corresponding for 2% strain, which reflects phenomenon of non-linearity and stiffness relaxation with progressive elongation. Behaviour of reinforced soil is sensitive to stiffness rather than strength of geogrid.*

Keywords: *constant strain rate test, geosynthetics, non-linear, soil improvement, stiffness, strength*

Biography:



***Abdelwahab Tahsin** has completed his PhD in Civil engineering at the age of 51 years on July 2020 from Faculty of Engineering, Cairo University, Egypt. He is a professional Consultant engineer, certified PMP and design director at ACE consulting office, Egypt. Participating and managing large complicated civil engineering projects across multiple asset classes in Egypt, Gulf, Middle East, and Africa. Strong experience with international standards with interactive collaboration with International Managed Joint Ventures. He is an Instructor at the Egyptian Russian and 6th of October Universities, Egypt. Supervising graduation projects, was delegated to this post based on his academic background and large practical expertise. He has published 4 papers in reputed scientific journals, has been serving as a reviewer of reputable research journal "Innovative Infrastructure Solutions" (IISS). He is sharing at many conference, workshops and invited talks. He is an organizing member of GEOAFRIAC 2023 international conference.*

KL3.
THE ENVIRONMENTAL IMPACT OF THE PRODUCED WATER ON THE UPPER AQUIFER IN THE WAHAT REGION, SIRT BASIN, LIBYA

Rehab A. ALZWAY[1], Ahmed M. MUFTAH[2], Osama R. SHALTAMI[2], Osama A. EL-FALLAH[2]*

[1]*Department of Environmental Science and Engineering, School of Basic Science, Libyan Academy for Postgraduate Studies, Tripoli, Libya*
[2]*Department of Earth Sciences, Faculty of Science, Benghazi University, Benghazi, Libya*

Abstract: *This paper based on data analysis by the Waha Oil Company of water samples collected from groundwater samples and produced water from Wahat region, Sirt Basin in Libya. This study provides a new pattern in the analysis of geochemical data which are in the form of a set of major ions and parameters such as acidity (pH), Total Solids Dissolved (TDS), total hardness of water (TH), the Residual Sodium Carbonate (RSC), Electrical Conductivity (EC), Magnesium Adsorption Ratio (MAR), the Kelly Ratio (KR), the permeability Index (PI), the Corrosivity Ratio (CR) and Total Organic Carbon (TOC). All that was performed using the statistical and analytical methods of data, and linking the results and information with each other will provide information on the sources of these elements and the water, also evaluate the water quality and suitability for drinking or irrigation. Based on the concentrations of the measure ions in the studied waters were ranged between desired range and the permissive range according to the World Health Organization. It appears that pH values of the groundwater are located within the safe range for drinking. To keep track of the suitability of the water for irrigation and drinking some of the important parameters and indicators used such as (RSC), (EC), (MAR), (KR), (PI), and (CR). Groundwater has been classified and showed that the water samples are classified as type (CaMgCl), while the produced water samples were classified as (NaCl).*

Keywords: *Geochemical analysis, Groundwater, Libya, Pollution, Produced water, Wahat Region.*

Biography:

Osama A. El-Fallah, (Born in Benghazi-1972), Libyan nationality. I have completed M.Sc. Degree in Hydrogeology and Water Management, at Faculty of Agricultural and Environmental Sciences, Szent István University in Gödöllő Hungary, 2007. My first employment was as Mud-logger geologist in Jowfe Oil Technology Company (1996-2004), and then joined the department of Geology at Omar Al-Mokhtar University, Al Bayda Libya (2008-2019). Recently I am a staff member at earth science department, faculty of science, University of Benghazi Libya. My field of interest in Hydrogeology and Water Quality published some papers in the field of Hydrogeology and Stratigraphy on Al Jabal al Akhdar region eastern Libya and Tazerbo water wellfield south-eastern Sirt Basin in Libya as joint papers with other colleagues. Currently, I have enrolled as PhD candidate at Department of Geology, Cairo University.

KL4.
CHANGING ECONOMIC CONDITIONS OF MEDITERRANEAN REGION

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Abstract: *At recent time the Mediterranean region became more important in the world economy, as important centre for the international trade, international economic development, therefore, the economies of surrounding areas have got wider side relations either among themselves in this region or with other economies in different farer regions. In this region the EU member states have wide side economic and cultural connections and relations with developing world and some other developed regions. Study analyses main EU-member states, some developing and developed economies in Mediterranean region. Study analyse 21 countries, as Germany, France, Italy, Austria, Hungary, Spain, Portugal, Greece, UK, Israel, Algeria, Morocco, Tunisia, Egypt, Turkey, Iran, Saudi Arabia, Nigeria, China, India, South-Africa. Study analyses economic conditions of economies in main fields of GDP growth, FDI net inflows, Gross capital formation, High-technology exports, Investment in agriculture, Share of gross fixed capital formation of agricultural sector in Total one, Agriculture in share of GDP. Based on SPSS statistical system seven economic variances are classified into three components.*
The study provided proof in cases of 21 selected countries in 2010-2018 that the FDI net inflow has such influences on the investment in value added agriculture and share of gross fixed capital formation of agricultural sector in total gross fixed capital formation. But this correlation of the FDI net inflow is not strong with these other two economic variances. Solution is to strengthen cooperation to stimulate FDI net inflow among countries to develop investment in agricultural sector and increase high-technology exports.

Keywords: *Agricultural sector, Cooperation, FDI inflow, Germany, Investment, Statistical analyse*

Biography:
JEL code: E00, E01, E22, F00, F02, F20, O40, Q10
Economics & Working Capital, Year 2020. 3-4. issues pp, 31-41
http://eworkcapital.com/changing-economic-conditions-of-mediterranean-region/

CSc, economic sciences, Hungarian Academy of Sciences, Scientific Qualified Committee, Budapest in 1991 and Dr. of University, World Economics, Budapest Corvinus University of Economics in 1991. Habilitate Doctor, in social sciences, in Management and Business Administration, Kaposvár University, in Kaposvár in 2017. Between 1987-2017 work at University of Agricultural Sciences, then at Szent István University in Gödöllő. From 2017 work at Óbuda University. Participation at Doctoral School of Economics and Regional Sciences at Hungarian University of Agriculture and Life Sciences in Gödöllő. The research areas: Economics, Business and Management, International Regional Economic Integration, EU Study, Regional economics, Rural Development, Environmental economics. He published 207 publications with 257 independent citation count, of which 189 foreign language citations. His publications were published in Arab, English, Spanish and Russian foreign languages. International scientific conferences in Turkey, Canada, Moldavia, Czech Republic, Slovakia, Lithuania, Romania and Russia. Research project in Finland, Sweden, Denmark, Italy, Spain, France.

KL5.
HEALTHCARE AND TRANSMISSION OF PANADEMIC COVID-19

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Abstract: *The severe acute respiratory syndrome COVID-19 pandemic had a significant economic and social impact on the world. Since there is no effective medical treatment for COVID-19 at present, the international collaborative efforts are more focused on developing a safe and effective vaccine against COVID-19. This review explored various factors influencing the COVID-19 vaccination intention and the applicability of the health belief model to explore vaccination intention among the population of world. Increasing COVID-19 vaccine uptake among a larger section of the population is an important public health priority at the moment as many vaccine candidates approved for use have shown efficacy in preventing COVID-19 infection and associated complications. The COVID-19 pandemic has been a major challenge for healthcare systems throughout the world.* Transmission of *COVID-19* can occur through direct, indirect, or close contact with infected people through infected secretions such as saliva and respiratory secretions or their respiratory droplets, which are expelled when an infected person coughs, sneezes, talks or sings. The UNs WHO has described various levels of COVID-19 transmission. *There is some evidence that the COVID-19 virus may lead to intestinal infection and be present in faeces. Approximately 2−10% of cases of confirmed COVID-19 disease presented with diarrhoea. I*n this difficult period it is best to meet virtually but if you have to meet others, do it carefully and with the right precautions. *Further study should include the impact of COVID vaccine uptake on these rates. Recent emergence and spread of variants have been reported with some concern regarding possible increased transmission and impact on immune* responses. There is a real need for more studies regarding the public perception and acceptance of COVID-19 vaccines in the world.

Keywords: *vaccine, vaccination, immune response, health care system, environmental factors*

Biography

Prof. Dr. Hosam Bayoumi Hamuda is working at Óbuda University. He is Environmental Microbiologist and Soil Biotechnologist dealing with the interactions between the microbiomes and the environment for increasing soil quality and saving the soil from pollutants as well as how benefits to use the wastewater sludge in the agriculture. His investigations are on the role of waste management, on soil quality, fertility, the crop production and environmental impacts related to the application of organic wastes to soil to assess: Soil quality, microbial inoculants; nitrification inhibitors and crop quality; Monitorization of organic matter; measurements of rhizosphere and soil microbial biomass and enzymatic activities in wastewater sludge amended soils; root systems and microbial composition in the polluted environment and roles of engineered metal oxide nanoparticles in biosphere.. Also he is interested in the role of environmental quality and relation between gut microbiomes on human health. ***Research Interest****: Waste management; Soil: Biotechnology; Protection; Sustainable; Biodiversity, Plant Growth-Promoting Rhizomicrobiota (PGPR); Colonization of PGPR; Microbial inoculants in rhizosphere; Econanotoxicology; environmental quality and relation between gut microbiomes on human health as well as the modern biology topics.*

Abstracts of Poster Session

PP1.
INFLUENCE OF NITROGEN FERTILIZERS ON THE BIOLOGICAL ACTIVITY OF THE SOIL OF THE RAPESEED ROOT ZONE

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Abstract: *Soil microbiocenosis is a dynamic, well-balanced system with a complex spatial and trophic organization. The main biosphere function of soil microbiocenosis is the destruction of organic matter. The polyfunctionality of microorganisms in the rhizosphere gives them the opportunity to participate in opposite biochemical reactions of the soil, which underlie the preservation of metabolic equilibrium in nature. Agrotechnological methods of growing rapeseed require high doses of mineral fertilizers, especially nitrogen fertilizers. The use of high doses of nitrogen mineral fertilizers leads to changes in the balance of the chains in the ecosystem, including microbiological activity. It is microbiological activity that is the reactionary force that quickly reacts to external factors.* ***Method****: In our work, a study of the biological activity of the soil of the root zone of rapeseed for the action of nitrogen fertilizers is presented. The study was carried out in the soil of temporary field experiments on agrocenosis of winter rapeseed, variety Black Velikan. The conditions of the experiment assumed the introduction of nitrogen fertilizers – 120, 150 and 180 kg/ha. The control option did not include fertilization. Determination of the biological activity of the soil in the root zone of rapeseed plants included the determination of the microbial biomass of carbon, the intensity of carbon dioxide emission, antifungal and cellulosolytic activity, and the phytotoxicity of the soil.* ***Results*** *The study of the soil of the root zone of rapeseed plants showed a directly proportional relationship between the dose of nitrogen fertilizer applied and biological activity. It was revealed that the fungistatic status of the soil of the root zone of rapeseed plants under the condition of applying nitrogen fertilizers in comparison with the control grew by 2.3–5.5 times; cellulosic activity by 1.5, the intensity of carbon dioxide emission increased by 2.1–3.6 times.* ***Conclusions****: The content of microbial biomass was recorded at a high level, studies have shown that the content did not depend on the amount of fertilizers applied.Thus, the indicators of the biological activity of the soil of the root zone of rapeseed plants in the flowering phase indicate that the physiological and metabolic activity of microbial communities is at a high level and depends on exogenous sources of nitrogen.*

Keywords: *biomass, carbon dioxide emission, phytotoxicity, antifungal soil activity, cellulolytic activity, nitrogen fertilizers, rapeseed*

Biography:

Alyona Bunas – PhD in Biological Sciences, Senior explorer, Senior Researcher at the Laboratory of Ecology of Microorganisms, Institute of Agroecology and Environmental management of National Academy of Agrarian Sciences of Ukraine, Metrolohichna str., 12, Kyiv.
Yevheniia Tkach – PhD in Biological Sciences, Senior explorer, Deputy of the head of department of Agroecology and Biosafety, Institute of Agroecology and Environmental management of National Academy of Agrarian Sciences of Ukraine, Metrolohichna str., 12, Kyiv.

PP2.
ASSESMENT OF THE POTENTIAL RISK ELEMENTS AT METAL POST-MINING SPOIL HEAPS

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Abstract Motivation/Background: *Metallic minerals mining and smelting are connected with the environment degradation, including spoil heaps arising. Even after processing, spoil still contains high levels of the metals, metalloids as well as other potential risk elements and became a potential source of air, water and soil metalliferous contamination for dozens of years. Eastern Slovakia is well known for its mining history and presence of old mining excavation, yet the spoil heaps represent almost the third of them.*
***Method**: We surveyed potential risk elements**,** including metals and metalloids in the substratum from overall nine spoil heaps (study sites) within the metal post-mining areas of Krompachy (KR) and Zlatá Baňa (ZB), located in Eastern Slovakia. The paper deals with characterization of substratum metalliferous contamination, with lay emphasis on the environmentally most relevant pollutants.*
***Results**: At all study sites, iron and aluminium were in the highest content, at both localities, Fe exceeded permissible limits and background values almost 43-times. Overloading of permissible limits as well as background levels of arsenic, cadmium, lead and mercury was noticed too. Clustering indicates the identical origin of risk elements, since iron and aluminium are supposed to be purely lithogenic and the rest of them are in increased concentrations as a consequence of mining activities.*
***Conclusions**: On the basis of results obtained, both of the areas could be characterized as highly polluted by environmentally relevant elements including arsenic, cadmium, copper, lead and especially mercury, i.e. heavy metals and metalloids found to be highly toxic even at low concentrations.*

***Keywords:** heavy metals, mining activities, pollution*

Biography

Dr. Beáta Baranová is assistant professor at Department of Ecology, FHNS of University of Prešov, Slovakia, working on research of epigeal macrofauna with the lay emphasis on the ground beetles (Coleoptera: Carabidae within the environmentally differently loaded and man-made sites.

Dr. Lenka Demková is assistant professor at Department of Ecology, FHNS of University of Prešov, Slovakia, working on research of risk elements in environment with lay emphasis on heavy metals at post-mining areas.

Dr. Július Árvay is scientific researcher – Department of Chemistry (FBFS) Slovak University of Agriculture in Nitra working on research of heavy metals in the environment.

PP3.
THE INFLUENCE OF HYDROTHERMAL FACTORS ON THE SPREAD AND DEVELOPMENT OF DISEASES IN AGROCENOSES OF CEREALS IN UKRAINE

Svitlana TKACHYK[1], Ivan MOSTOVIAK[2], Olena DEMYANYUK[3]

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Abstract: *Based on a comprehensive analysis of agrotechnical and environmental factors, the main factors of destabilization of phytosanitary condition of grain ear-forming crops sowings are identified, which consist in high level of plowing, violation of scientifically based structure of sown areas, cultivation of intensive and semi-intensive species with a high ability to growth stimulation and phytopathogenic mycobiota accumulation, as well as a significant pesticide load. Among the environmental factors changes in hydrothermal conditions over the past 16 years are defined, namely an increase in average annual air temperature by $1.8^{o}C$, a decrease in annual rainfall by an average of 22% and changes in rainfall in the middle of the year. The relationship between hydrothermal conditions of the growing season and development of pathogens in crops was studied. With increasing values of HTI (hydrothermal index) we recorded an increase in the share of winter and spring wheat crops affected by root rot (from 25 to 44%), leaf septoria (from 6 to 28%), ear fusariosis (from 9 to 21%), while growing spring barley, the areas affected by ear fusariosis increased (from 11 to 17%). It was found that under conditions of severe drought and moisture deficit, the share of affected crops was minimal. The lowest expansion and development of root rot during wheat cultivation occurred in arid conditions (HTI 0.8–0.9), barley under moderate moisture (HTI 1.3–1.6). The lowest damage to wheat and barley plants with leaf and stem diseases was under HTI 1.0–1.2, ear diseases for wheat – under HTI 1.3–1.6, and for barley diseases under HTI 1.0–1.2.*

Keywords: *biological pollution of agroecosystems, hydrothermal factors, cereals, pests, phytosanitary monitoring*

Biography:

Mrs. Svitlana TKACHYK has the degree of Candidate of Sciences (PhD), specialty Breeding and Seed Production (06.01.05) in 2004. For more than 20 years she has been dealing with issues of plant variety protection, examination of plant varieties as objects of intellectual property. Her research interests: study of prohibitive criteria for dissemination of plant varieties in Ukraine, study of plant variety resources in the context of climate change, ecological safety of agricultural production; the quality and safety of agricultural products; food security in the context of climate changes; study of conditions and selection of varieties for organic production of agricultural products. She is the author of 60 scientific publications, including Test Recommendations for State Examination of Plant Varieties. She teaches the specialty "State qualification examination of plant varieties" at the National University of Life and Environmental Sciences of Ukraine

PP4.
ASSESSMENT OF CREEP ON LONG-TERM PERFORMANCE AND CALIBRATION FACTOR OF GEOSYNTHETICS IN GEOENVIOR-MENTAL APPLICATIONS

Abdelwahab TAHSIN*, Rami EL-SHERBINY

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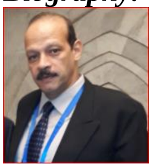
Abstract: *Geosynthetics became one of the outstanding innovations with ever-growing significance for geotechnical and geo-environmental engineering industry. Earthwork construction over soft polluted sludge lagoons and tailing impoundments is mandatory to cap various waste materials. Low shear strength and high compressibility of such soil deposits expose short and long-term geotechnical challenges. Geosynthetics provide strengthening and allow accessibility over these deposits, which save money and reduce carbon footprint. Like all polymeric products, geosynthetics are susceptible to creep. However, creep data is not published by manufactures for com-munity to disclose stiffness modulus degradation with elapsed time, which is necessary for finite element numerical modelling. This manuscript aims to assess creep effect and derive calibration factor between global to local strains. Field monitoring strain readings of high-elongation gauges glued to geosynthetics must be calibrated against "true" global strain. Geosynthetics polyester (PET) geogrid types Fortrac 35T, 55T, 80T, 110T and 150T were examined at the machine direction under constant strain load ranged from 20% to 0.05% strain/min using the multi-rib tensile method. Correlation charts were plotted to predict stiffness reduction versus logarithm of time. The long-term stiffness value decreases in non-linear manner with increasing logarithm of time but after 1000 hours, is considerably constant of 75% of the secant modulus at 2% strain (working loads). Calibration factor between global to local strains ranged from 2.08 to 1.99 was measured at 2% strain, in agreement with comparable factor of 2.2, which was introduced by Allen et al. (2002). Calibration Factor values slightly increased as strain rate decreased.*

Keywords: *creep effect, calibration factor, constant strain rate test, geosynthetics, stiffness degradation, global/local strain.*

Biography:



***Abdelwahab Tahsin** has completed his PhD in Civil engineering at the age of 51 years on July 2020 from Faculty of Engineering, Cairo University, Egypt. He is a professional Consultant engineer, certified PMP and design director at ACE consulting office, Egypt. Participating and managing large complicated civil engineering projects across multiple asset classes in Egypt, Gulf, Middle East, and Africa. Strong experience of international standards through interactive collaboration with International Managed Joint Ventures. He is an Instructor at the Egyptian Russian and 6th of October Universities, Egypt. Supervising graduation projects was delegated to this post based on his academic background and large practical expertise. He has published 4 papers in reputed scientific journals, has been serving as a reviewer of reputable research journal "Innovative Infrastructure Solutions" (IISS). He is sharing at many conferences, workshops and invited talks. He is an organizing member of GEOAFRIAC 2023 international conference, the International Geosynthetics Society IGS, Egypt chapter.*

PP5.
FAKOPP 3-DIMENSIONAL ACOUSTIC TOMOGRAPHY MEASUREMENTS, IN THE EASTERN ALPS, ALONG A DESIGNATED VERTICAL TRANSECT

Falvai DOMINIKA[1], Baltazár TIVADAR[2], Czóbel SZILÁRD[1]

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Abstract: *The global temperature increase in high mountain areas is higher than average, which can significantly affect the physiognomy of the vegetation, can change the dominant species of different alpine zones, or lead to the shift of vegetation belts. During our research, we focused on how the state of health of the dominant conifers in the montane belt and subalpine belt regions of Central Europe's mountainous areas changes along an elevation transect. However, the number of field measurements focusing on the health status of dominant trees in temperate mountains is limited. Our measurements were carried out in the Stuhleck Mountains along an elevation gradient from 850 to 1750 metres. Health status analysis of Picea abies and Pinus mugo have been completed by using FAKOPP 3D acoustic tomography, which is able to detect the size and location of decayed regions in the trunk non-destructively. For modeling, the relationship between the decay of trees and other factors simple linear regression models were used. The results showed that the individuals of Picea abies and Pinus mugo had the worst health status in the lowest and uppermost range of the taxa in the studied area. It could be a sign of the upward shift of their range. A positive significant correlation was found between the decay and the ratio of whole trunk/healthy wood both in the case of Picea abies and Pinus mugo. It seems that acoustic tomography measurements are adequate to indicate non-destructively the altitudinal optimum and upward shift of different taxa.*

Keywords: *climate change, conifers, vertical vegetation belts, ArborSonic FAKOPP 3D acoustic tomograph, health status.*

Biography:

Dominika Falvai will finish her PhD. in 2021. She is doing her studies and researches at the Department of Landscape Ecology & Nature Conservation, Szent István University, Hungary. She has many journals in Hungarian and in English languages. She is interested in climate changing, smart solutions, renewable energy, and everything related to a sustainable future.

PP6.
TOWARDS GREENER METAL-MEDIATED SYNTHETIC METHODS

Marina NAODOVIC*, Una MARCETA, Bogdana VUJIC

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Abstract: *Metal-catalyzed cross-coupling reactions represent an important class of organic transformation used routinely in academic and industrial laboratories. A subset of those reaction, reductive cross-electrophile coupling of organic halides (R-X, X=Cl, Br, I), are traditionally carried out in the presence of stoichiometric amounts of metallic reducing reagents, and require amide solvents. In this short communication, a new methodology, based on Ni-catalyzed reductive coupling, for greener approach towards diarylmethanes is described. Improved reaction was achieved by applying some of the principles of green chemistry. While the reaction still requires metal catalyst, the protocol is more environmentally benign, and it allows for synthesis of this important class of compounds in an operationally simplified one-pot procedure.*

Keywords: *metal-catalyzed synthesis, cross-coupling reactions, green chemistry*

Biography:

Marina Naodovic has completed her PhD in 2009 at the University of Chicago (IL, USA) and Postdoctoral Studies at the University of Rochester (NY, USA). She is a Research Assistant at Technical Faculty "Mihajlo Pupin" (Zrenjanin, Serbia). She has published 7 papers in international journals. Her research interests are focused on green synthetic procedures and new reactions in organic chemistry.

PP7.
VEGETATION STUDY ON THE PASTURE ALONG THE IPOLY

Ildikó JÁRDI[1*], Gergely PÁPAY[2], Eszter S.-FALUSI[2], Zachar ZALÁN[2], Dénes SALÁTA[3], Károly PENKSZA[2]

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Abstract *The vegetation of two different characters of cattle pastures in the middle Ipoly Valley was examined in this research. One of them was wet vegetation beef cattle pasture while the other area was a dry vegetation cattle pasture. The wet pasture was mowed before 2000. Here one sours sandy vegetation and a lower-lying, characterless, Elymus repens dominated grassland area, with fresh and dry patches were examined. There were also two types of vegetation analysed on the dry cattle pasture. One of them was a drier steppe under less pressure grazing, and the other one was a heavily used, degraded steppe which has been used serving as a resting place. There was a significant amount of species adapted to disturbance in each plot, but their proportions were different. The lowest rate was observed in the quadrats of pastures of the dry area cattle under smaller grazing pressure. On the basis of the recordings, on the wet area pasture the sour sandy lawn was more sensitive, where the grazing pressure should be monitored in order to preserve the characteristics of the vegetation. On the new area of wet pasture grazing after mowing favoured the appearance of species characteristic of natural vegetation. Among the examined areas, the dry cattle pasture under grazing pressure was found as be the most favourable in maintaining the natural vegetation. Note: The research was supported by the FEKUTSTRAT 2018 project and the OTKA K125423 application.*

Keywords: *grassland management, nature conservation, habitat change*

Biography

Ildikó T.-Járdi has working on her PhD thesis from Szent István University from School of Environmental Science, Gödöllő, Hungary. She has published more than 10 papers in journals. She is working on currently involved in the management and conservation of domestic grasslands.

PP8.
LAWN REGENERATION RESULTS OF THE CONSERVATION MANAGEMENT ON THE HOMOKTÖVIS CONSERVATION AREA IN BUDAPEST

Gergely PÁPAY[1*], Norbert PÉTER[1], Zoltán BAJOR[1], Dénes SALÁTA[2], Zsuzsa LISZTES-SZABÓ[3], Zalán ZACHAR[1], Ferenc STILLING[4], Penksza KÁROLY[1]

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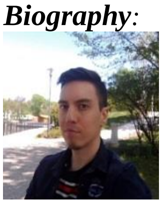
Abstract Background*: In Budapest, despite the density of the population, precious plant communities still remain in a lot of mostly isolated fragments of habitats which – especially sandy lawns – are outstanding in the diversity of species and are rare inhabitants of endemic species. On the examined territory, since 2006 long term reconstructions of the habitats have been ongoing which strive for the decrease of the invasive woody species on the territory and the insurance of the habitat of the sandy lawn, as well as the long term conservation of the fragments of the lawn and the creation of the natural sandy lawn.* ***Method****: The effects of the interventions on the vegetation were conducted on 7 sample areas, on 10-10 quadrats by examining the coenological entries; therefore we were able to provide the effects of the reconstruction of the habitats 14 years retrospectively.* ***Results:*** *During the past years, due to the systematic planning on the fragments of the habitats, 9 hectares of new surface could be opened. Due to this, more than 40% of the entire protected area could become an area of lawn. Changes in the vegetation in the examined area were clearly observable during a 7-year-long period; species of the sandy grassland has become dominant, which can generally be regarded as positive from the nature conservation point of view.* ***Conclusions****: As it has been confirmed by several authors the open sandy vegetation is more tolerant to degradation in well-formed grassland.*

Keywords: *Sandy grasslands, Nature conservation, habitat regeneration, open sandy vegetation*

Biography*:*



Dr. Gergely Pápay has expertise in botany, especially taxonomy. He has studied Natural Conservation and has a BSc and MSc in this topic. He currently works as a researcher and lecturer at the Hungarian University of Agronomy and Life Sciences, and has a PhD in environmental studies. For years he has been examined the morphology and taxonomy of the genus Festuca and had been participationing in a Hungarian application in the topic (OTKA K-125423) under the direction of Dr. Károly Penksza PhD. He had participated as an author in several papers in international journals with considerable IF in the topic.

PP9.
PUBLIC PERCEPTION, AWARENESS AND KNOWLEDGE ABOUT AIR QUALITY IN THE CITY OF ZRENJANIN

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Abstract: *Air pollution is one of the most important factors that can affect the quality of life in urban areas, given the fact that exposure to air pollution has harmful consequences for human health. The problem of air quality in the Republic of Serbia is currently attracting much more public attention than in previous years due to the appearance of many individually installed sensors for monitoring air quality, as well as the development of new applications easily accessible to the general population. To determine the perception, personal attitudes, and knowledge of citizens about air pollution, as well as ways of informing and reliability of data, a survey was conducted in the form of a questionnaire. The results showed that most citizens believe that the air they breathe is polluted or occasionally polluted; while as many as 80% of respondents do not feel sufficiently informed about air quality. In addition, the distrust of the citizens towards the official data on air quality is noticeable, as well as the reliance on the information obtained by measurements with low budget sensors of questionable reliability. Given these results, it is inevitable that more attention must be paid not only to improving the way of informing about air quality but also to educating the population.*

Keywords: *Air quality perception, Air pollution, Public concern*

PP10.
RELATIONSHIP BETWEEN EXPOSURE TO ENVIRONMENTAL TOXICANTS AND EPIGENETICS

Hosam E.A.F. BAYOUMI HAMUDA[1]*, Fatemeh ZAREI[2], Lyudmyla SYMOCHKO[3,4]

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Abstract: *World-wide, people are exposed to a huge number of environmental factors that have impacts on epigenetic mechanisms. Epigenetics is a field in modern biology that focuses on inherited changes in the gene expression caused by different mechanisms other than changes in the DNA sequence. Environmental epigenetics describes how environmental factors affect the cellular epigenetics and, in consequence, the human health. A foundational goal for the people is to improve their health, which typically includes positive changes in lifestyle and especially diet. The aim of this review is to explain* the *fundamentals of epigenetics, the* relationship between the environmental toxicants and the health care aspects of epigenetics with a focus on human systems, *environmental epigenetics and explains the influences of diet and environmental epigenetics on human health and disease. Epigenetic change is a regular and natural occurrence but can be influenced by several biotic and abiotic factors such as human age, parenting, the environment, diseases and disorders, drugs and addiction, lifestyle, diet and sport exercise. How the same genotype can give rise to different phenotypes under different environmental conditions. Identification of the fundamental epigenetic modifications and markers help to recognize the health disorders at earlier stages of the diseases, improving treatment outcomes and quality of life for many people. Epigenetics could explain why* COVID-*19 affects people differently. Extensive research in this field would do a great deal to protect public health, both now and in future generations.*

Keywords: *environmental toxicants, fundamentals of epigenetics, gene expression, health care, human systems*

Biography

Prof. Dr. Hosam Bayoumi Hamuda is working at Óbuda University. He is Environmental Microbiologist and Soil Biotechnologist dealing with the interactions between the microbiomes and the environment for increasing soil quality and saving the soil from pollutants as well as how benefits to use the wastewater sludge in the agriculture. His investigations are on the role of waste management, on soil quality, fertility, the crop production and environmental impacts related to the application of organic wastes to soil to assess: Soil quality, microbial inoculants; nitrification inhibitors and crop quality; Monitorization of organic matter; measurements of rhizosphere and soil microbial biomass and enzymatic activities in wastewater sludge amended soils; root systems and microbial composition in the polluted environment and roles of engineered metal oxide nanoparticles in biosphere.. Also he is interested in the role of environmental quality and relation between gut microbiomes on human health. ***Research Interest****: Waste management; Soil: Biotechnology; Protection; Sustainable; Biodiversity, Plant Growth-Promoting Rhizomicrobiota (PGPR); Colonization of PGPR; Microbial inoculants in rhizosphere; Econanotoxicology; environmental quality and relation between gut microbiomes on human health as well as the modern biology topics.*

Abstracts of Technical Session

TL1.
PEOPLES' PERCEPTION IN PLASTICS WASTE MANAGEMENT TO PROTECT MARINE LITTERING, ENVIRONMENTAL QUALITY AND PUBLIC HEALTH AND ACTIONS THEREOF

Sadhan Kumar GHOSH[1,*], Sourya Subhra CHAKRABORTY[2], Tejashwi RANA[3]

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[2]PG Scholar, Civil Engineering Department, Meghnad Saha Institute of Technology, OPTOCE scholar at MED, JU, Kolkata, India,
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Abstract: *Plastic waste, takes longtime to decompose, are dumped into landfills, water bodies and a portion leads to marine environment creating threat to environment and human health. Plastics turn into micro and nano-plastics which are consumed by animals& birds and, mammals & fishes in marine environment. Micro plastics find its way to the human body through eco-systems following different routes. Before a plastic waste gradually fills up our oceans and landfill sites, there is an immediate need to address this cause in order to reduce generation of plastic wastes. This study assesses the awareness of people regarding plastic waste management and its impact on environmental and health through survey. The survey is carried out both online questionnaire and in situ visits and interviews in several sectors, namely, educational institutes, health care units, industrial units and residents. Studies are carried out for efficient usage of non-recyclable plastics wastes as a fuel for effective waste management in our society by the method of co-processing in cement plants, leading to implementation of circular economy to reduce marine litteringy. This study finds that the awareness of the citizens are still have to improve to enhance the efficacy of waste management and treatment efficiency which will help in determining the future course of actions for co-processing in cement plants and other potential use, assessing the amount that leads to marine environment. The study has also mapping the behavioural pattern of citizen on use and disposal of non-recyclable plastics.*

Keywords: *Co-processing, Environmental pollution. Marine littering, Nonrecyclable plastics, Plastic waste, recycling*

Acknowledgement: *The authors gratefully acknowledge the support received from "Ocean Plastic Turned into an Opportunity in Circular Economy (OPTOCE)" research project funded by SINTEF, Norway at mechanical department at Jadavpur University and the support of International society of waste management Air and Water (ISWMAW) and IconSWM-CE, 2020.*

Biography

Prof. Sadhan K Ghosh completed his PhD from Jadavpur University. He is the Professor in mechanical engineering and Ex-Dean of faculty of engineering. & Tech., at Jadavpur University & served as the Director, CBWE, Ministry of L&E, Govt. of India. He is internationally well-known researcher on waste management, Green Manufacturing, SDG, ISO Standards and sustainable SME. He has published more than 250 papers in reputed journals, books and proceedings. He serves as Associate Editor of the journal, Waste Management, Int. Journal of Materials Cycles and Waste Management and Editor-in-Chief of ISWMAW-IconSWM Publication Secretariat. He is an editorial board member of repute. Research interest briefly: He is the PI of more than 20 International and 25 national Research Projects funded by different agencies, like, EU, GCRF, DST, DBT, UKIERI, Royal Acad. of Engg, SINTEF Norway, Hungarian Govt., Georgia Govt., and many others. He is international expert of UNCRD/UN DESA, SACEP, IJES and APO having research collaboration with 40 countries. His web:www.sadhankghosh.com and available at: sadhankghosh9@gmail.com

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TL2.
EFFICIENCY OF FORMATION AND FUNCTIONING OF SYMBIOTIC SOYBEAN SYSTEMS UNDER THE CONDITIONS OF PROCESSING WITH GLYPHOSATE

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Abstract: *The efficiency of formation and functioning of symbiotic soybean systems under the conditions of herbicide treatment during inoculation with different rhizobia strains was investigated in the field experiment. Glyphosate-containing herbicides affect symbiotic nitrogen fixation both through direct action on rhizobia and symbiotic formations. Samples of plants for analysis were taken four weeks after each herbicide treatment. The aboveground mass of plants was determined; the formation of the symbiotic apparatus was evaluated by the number, mass of nodules on their roots and N_2-fixation activity. N_2-fixation activity was determined by acetylene-reductase method. Late herbicide treatment leads to severe weeding, which in turn significantly inhibits plant development by reducing both vegetative and root mass. Treatment of plants with herbicide to form a symbiotic apparatus reduces N_2-fixation activity, but this does not significantly affect the formation of soybean yields. The obtained results confirmed our assumption about the intensification of the nitrogenase complex during late treatment of plants with glyphosate-containing herbicide in plants inoculated with strain B. japonicum EL-35 and a mix of strains EM-24+EL-35. The most effective for inoculation of soybean plants was a mix of studied strains of B. japonicumEM-21 and EL-35, which provides high N_2-fixation activity and forms a good yield. Thus, to reduce the negative impact of glyphosate on the N_2-fixation activity of symbiotic systems and to obtain high yields of soybean grain, it is necessary to select rhizobia strains that can form symbiosis faster, as even a slight decrease in N_2-fixation can have long-term consequences.*

Keywords: *N_2-fixation, root nodule bacteria, inoculation, Bradyhizobium, soybean.*

Biography:

Iryna Gumeniuk has completed her PhD at the age of 26 years (Institute of Agroecology and Environmental Management of NAAS) in Biological Sciences (Ecology). She is Senior Researcher in Laboratory of ecology of microorganisms (Institute of Agroecology and Environmental Management of NAAS). She has published more than 15 papers in scientific journals.

TL3.
IMPACT ASSESSMENT OF ANTHROPOGENIC ACTIVITIES ON AIR QUALITY OF ULAANBAATAR, MONGOLIA USING LICHEN AS THE BIOINDICATOR

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Abstract: *Lichen is a composite organism that arises from green algae or cyanobacterium (phycobiont) and fungal partner belongs to the Ascomycota and Basidiomycota (mycobiont), living together in symbiotic relationship. The interaction between lichens and air pollution has been used as a means of monitoring air quality since 1859. The most important pollutant was sulphur dioxide produced by coal burning industry and power stations. City of Ulaanbaatar, the capital of Mongolia was initially designed for a half million residents. Due to intense rural-to-urban migration after transition to market driven economy, the population of the capital is nearly tripled, which resulted in huge area of informal settlements and elevated number of vehicles. Tents and small buildings in above settlements heated by conventional stoves by burning coal and wood, while the most vehicles on the road are imported second-hand cars. During last two decades, air quality of capital city Ulaanbaatar is considered as an emerging issue and above two are primary sources of outdoor air pollution.*
The most abundant air pollutants are nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and carbon monoxide (CO) which have disastrous effects on health when inhaled over prolonged periods of time. Due to the quantities of these pollutants would be far more abundant and thus cause the catastrophic effects to the air quality that is seen in Ulaanbaatar. Various techniques have been developed to monitor air quality, including biomonitoring using lichens. In this study, we monitored types of epiphytic lichens covering sensitive to air pollution fruticose type and relatively tolerant foliose type of lichens. Unlike air quality index, lichens clearly show negative impact of poor air quality on surrounding ecosystems. We examined lichens on Larix sibirica, the most abundant coniferous tree of the area. Both foliose and fruticose types of lichens are abundant in Larix sibirica dominated forests located to the northern areas of Ulaanbaatar. It indicates the area is free of pollution. In contrary, fruticose type lichens, especially representatives from the genera Usnea, Cladonia and Vulpicidia are absent in coniferous forest to the southern areas of Ulaanbaatar. These results clearly show downwind ecosystems have been badly affected by poor quality air of the capital. Additionally, there is none of those bark dwelling lichens are observed on the three along the crowded roads, parks and residential areas which indicates the extreme pollution of the air. Finally, biomonitoring using lichen as the bioindicator is discussed, and future recommendation is provided in the end of the study.

Keywords: *Lichens, air quality, distribution, Mongolia, anthropogenic activities*

TL4.
REMOVAL OF PB FROM CALCAREOUS AND GYPSUM SOILS CONTAMINATED WITH USED ENGINE OIL

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Abstract: *The soil washing technique is considered one of the quick and effective techniques for treating soil contaminated with heavy metals. In this study, EDTA-Na_2 and oxalic acid were used as washing solution to removing Pb from calcareous and gypsum soils (CS and GS) contaminated with Pb only and with Pb in addition to the used engine oil (UEO). The soil samples were contaminated with different concentrations of Pb only using $Pb(NO_3)_2$ to soils sample for each concentration separately with continuous homogenization and left to air-dried then divide the sample into two equal parts. The first part represents the calcareous and gypsum soil samples contaminated with Pb only. The second part was taken after air-dried, then the used engine oil (50 $ml.kg^-$) was added and homogenized well. Both parts were kept in polyethylene containers and incubated for 20 days. Five parameters were applied to test their effect on Pb removal. Which were pH, contact time, Pb concentrations, temperatures, and liquid/solid (L/S) ratio. All these parameters were applied at 180 rpm of shaking speed. When applied one parameter with different values, the other parameters would have remained constant. The results showed that the pH, contact time, Pb concentrations, temperatures, liquid/solid (L/S) ratio parameters have a significant role in removing Pb from soil samples. Sequential washing was more effective for removing Pb than single washing because Pb is difficult to remove from the soil. Mixed washing solution (EDTA-Na_2 and oxalic acid) could achieve high efficiency for removing Pb with a lower concentration of washing solution, which reduce cost. Used engine oil reduced washing solution removal from calcareous and gypsum soil samples contaminated with used engine oil.*

Keywords: *EDTA-Na_2, engine oil, oxalic acid, $Pb(NO_3)_2$, Sequential technique, Soil*

Biography:

Prof. Dr. Hazim Aziz Hamza al-Robai has completed his PhD at the age of 49 years from Babylon University-Iraq in the field of environment and pollution from the Faculty of Science - Department of Biology. He contributed to the establishment of the Faculty of Environmental Sciences at Al-Qasim Green University and the Environmental Advisory Office. He is a member of the editorial board of two international journals, also chairing many local and international conferences. He published more than 41 researches in local and international journals. He has participated in more than 40 international and local seminars and conferences. He supervised many postgraduate students. He prepared curricula for the environmental departments, the environmental pollution department and the environmental health department in the Faculty of Environmental Sciences at Al-Qasim Green University. His research and teaching interests are in the fields of environmental chemistry, air pollution, soil and water pollution, and environmental treatments.

TL5.
CYTOTOXICITY EFFECTS OF THREE PESTICIDES ON MITOTIC CHROMOSOME ON ROOT CELLS OF *ALLIUM CEPA.*

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Abstract: **Motivation/Background:** *Pesticides constitute a heterogeneous category of chemical specifically designed for the control of plant diseases. The wide production and use of chemical pesticides causes a serious pollution to the surrounding environment. These chemicals, a part from affecting the target pest, also affect plants, animals and human.* ***Method****: After growing roots of Allium cepa till they reached 2–3 cm in length, they were treated with three concentrations (0.001, 0.0025, and 0.005 mg/ml) of mancozeb (fungicide), dimethoate 40 and lanate 90 (insecticides), and distilled water was used as control, for 24hour exposure.* ***Results:*** *The tested concentrations decreased the mitotic index compared to the control; the mitotic index values were significantly decreased with an increase in the concentration of the pesticides. In addition, the different treatments caused diverse types of chromosomal abnormalities during cell division. The proportion of chromosomal aberrations was significantly increased from a control value. The chromosomal abnormalities that were observed during the study are stickiness, early chromatin condensation at prophase, chromosome multipolarity, fragmentation of chromosomes, c-metaphase and micronuclei appearing in interphase cells were.* ***Conclusions****: The result indicates that three pesticides had cytotoxic activities on mitotic index and chromosomal aberration. Hence, the use of these pesticides should be under control in agricultural fields.*

Keywords*: Allium cepa, Chromosomal aberration, Environment, Mitotic index, Pesticides and Pollution.*

Biography:

Fatma H. El-Fallah was born in Benghazi, Libyan nationality. I have completed M.Sc. Degree in Biotechnology and Microbiological Sciences, Faculty of Agricultural and Environmental Sciences, Szent István University in Gödöllő Hungary, 2007. My first employment was as sciences teacher (1999-2004), and then joined the department of Botany, Ajdabiya University, Libya (2016-now). Recently I am a staff member at Botany department, faculty of science, University of Ajdabiya Libya. My field of interest is Biotechnology, Genetic and Molecular Biology.

TL6.
GEOCHEMICAL EVALUATION OF GROUNDWATER: A CASE STUDY OF THE SIDI FARAG FARMS, BENGHAZI CITY, NE LIBYA.

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[3]*Department of Geography, Faculty of Arts, Benghazi University, Benghazi, Libya*
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Abstract: *In this work, we conducted a geochemical assessment of groundwater in the Sidi Farag farms to determine the possibility of use in drinking and irrigation. The results showed that the water contains high Total Dissolved Soiled TDS contents (brackish water). The water is characterized by the dominance of Sodium Na and Chloride Cl (Sodium Chloride type) as a result of evaporation, seawater intrusion and weathering of rocks. The water is supersaturated with halite, gypsum, calcite and dolomite. Moreover, the water is seriously affected by Sodium Na, Chloride Cl, Lead Pb and Mercury Hg and therefore is not drinkable. In addition, the water is not suitable for irrigation.*

Keywords: *Drinking Water, Hydrochemistry, Irrigation, Libya, Sidi Farag, Water Quality.*

Biography:

Osama A. El-Fallah, (Born in Benghazi-1972), Libyan nationality. I have completed M.Sc. Degree in Hydrogeology and Water Management, at Faculty of Agricultural and Environmental Sciences, Szent István University in Gödöllő Hungary, 2007. My first employment was as Mud-logger geologist in Jowfe Oil Technology Company (1996-2004), and then joined the department of Geology at Omar Al-Mokhtar University, Al Bayda Libya (2008-2019). Recently I am a staff member at earth science department, faculty of science, University of Benghazi Libya. My field of interest in Hydrogeology and Water Quality published some papers in the field of Hydrogeology and Stratigraphy on Al Jabal al Akhdar region eastern Libya and Tazerbo water wellfield south-eastern Sirt Basin in Libya as joint papers with other colleagues. Currently, I have enrolled as PhD candidate at Department of Geology, Cairo University.

TL7.
THE EFFECT OF SUGAR BEET FIBRE AND INULIN ON THE SURVIVAL OF BIFIDOBACTERIUM BIFIDUM IN A SYNBIOTIC DRINK BASED ON GRAPE JUICE

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Abstract: ***Motivation/Background****: This research was studying the effect of inulin, fibrex on the Bifidobacterium bifidum on red grape juice.* ***Method:*** *For this, after preparation of grape juice 1, 2, 3% ratios of inulin and 0.7, 1.5 and 2% in fibrex with 10^6 cfu/ml from Bifidobacterium bifidum prepared and in 25 day preservation, pH, Brix, colour measurement (lightness, redness and yellowness indexes) and sensory evaluation (flavour, texture, oral sense and overall acceptability) and Bifidobacterium bifidum were measured in zero, 5th, 10th, 15th, 20th and 25th.* ***Results:*** *It was shown that 1% inulin and 0.7% fibre increased Bifidobacterium bifidum count significantly but in high ratios pH decreased and brix and sensory evaluation was changed and finally treatments encountered with yellowness, redness and darkness and sensory evaluation scores were diminished. Preservation time also decreased sensory properties, survival of Bifidobacterium bifidum and lightness index of grape treatments. Flavour of treatments also falled due to creaminess properties of inulin and in short treatments (0.1 inulin and 0.7 fibre) was proposed.* ***Conclusions****:* The study of the effects of time on Bifidobacterium bifidum also showed that with increasing time, the survival rate of probiotic bacteria is significantly reduced, which is in the control treatment due to nutrient loss and increased accumulation of peripheral cellular metabolites of the microorganism. It seems that the polyphenolic compounds of grape juice have significant effects on the survival of bifidobacteria, which also reduces the survival rate of Bifidobacterium bifidum by reducing intracellular sugar sources.

Keywords*: Bifidobacterium bifidum, Fibre, Grape juice, Inulin*

Biography

I am Fatemeh Zarei from Iran; I got my PhD in food science in 2019 at the age of 39 years from Azad University and now working in Food and Drug Administration (FDA). I have published more than 14 papers in reputed journals.

TL8.
VANADIUM PENTOXIDE AND THEIR NANOCOMPOSITES FOR WATER TREATMENT

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Abstract: *The access to fresh water for world population is limited which is further debilitated by water pollution. To improve water purification, nanomaterials have been successfully utilized for efficient and complete removal of variety of pollutants from water. Nanomaterials in the form of nanoparticles, nanowires, nanorods, nanosheets and nanocomposites can easily be prepared in desired size and morphology with specific required physical and chemical properties. The hybrid methods such as combination of nano adsorbent with metal oxide nanoparticles have been the first hand choice for researchers as adsorbent material for water purification applications. The unique characteristics exhibited by nanocomposites include strong solution solubility, high reactivity, greater mechanical strength, dispersibility, hydrophobicity, hydrophilicity and high working efficiency. This review is meant to evaluate multiple ways to synthesize numerous vanadium pentoxides (V_2O_5) nanocomposites and their applications in removing water pollutants. Simple nanocomposites and bimetallic nanocomposites have been the top choice for researchers for removal of water pollutants such as oil, hydrocarbons, dyes, pesticides, antibiotics etc. The advanced adsorption techniques coupled with nanocomposites have proved to be exhibited efficient, affordable and rapid for water purification. Similarly, the surface modification of nanocomposites is instrumental in achieving characteristic features including high adsorption capacity, low bulk density, and significant reusability.*

Keywords: *Nanocomposite, Pollutant, Vanadium Pentaoxide, Water treatment, Dye removal*

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Biography:

Thamer Adnan Abdullah completed his Master of Chemical Engineering from Guru Gobined Singh Indraprastha University New Delhi., since 2008 he is working as assistant lecturer in the University of Technology, Baghdad, in Applied Science Department, Chemistry Branch Group. Currently he is doing his PhD and he is researcher in Sustainability Solutions Research Lab, Faculty of Engineering, University of Pannonia, Veszprém, Hungary. He has several articles published in sciencediret reputed journals and has participated in many international conferences in the field of environmental chemistry and nanoresearch.

TL9.
THE ROLE OF EDUCATIONAL PATHS IN THE ENVIRONMENTAL EDUCATION

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Abstract: *The Óbuda University decided to make an educational path in the year of 2019. The financial background was provided by the III. district of Budapest from a European Union source. In the planning phase, the educational path originally imagined as a mixed type observational trail both physical and online stations to present the natural and cultural sights of the Kiscelli-plateau. The primary aim of the trail was to use as an option of the environmental education of the 10-18 years old age group, however the applicability of the BSc environmental engineering education was also examined. The environmental engineering training is also educated in English in the University. For this reason, every station's website has its own English version. To draw the aim age group's attention, we designed websites for half of the stations, but the scalability requires making online background surface in every station of the pathway. This is also important for the future developmental possibilities.*
It is important to emotionally affect for the visitors in the environmental education. The educational path could fulfill its role, because in every possible station, we determine the age of the cultural sights and search maps from the same era. We use an actual satellite image for the opportunity to comparing the actual condition with an older state. Visitors could also see the slow geological events affect and the relatively fast anthropogenic transformation of the area. This illustrative display could form the mind of the audience.

Keywords: *Environmental education, educational path, online observational trail, interactivity*

Biography:

Richárd Csaba Kovács was completed his BSc environmental engineering studies in Óbuda University, Faculty of Light Industry and Environmental Engineering in 2020. He is working as a demonstrator in the Institute of Environmental Engineering and Natural Science since 2018.

TL10.
THE USE OF MICROALGAE COMPOUNDS IN THE IMPROVEMENT OF AGRICULTURAL PRACTICES

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Abstract: *The increase in worldwide population observed within the last decades has contributed to an accrued demand for food supplies, which might solely be attained through an improvement in agricultural productivities. Moreover, agricultural practices should become more sustainable, because the overuse of chemically-based fertilisers, pesticides and growth stimulants will cause serious environmental problems (Gonçalves, 2021). One new approach for the development and extremely effective agriculture is the use of biological compounds like the extract of microalgae biomass. Currently, microalgae are attracting the interest of agrochemical industries and farmers, because of their biostimulant (MBS) and biofertiliser (MBF) properties (Ronga et al., 2019). Until recently, microalgae were mostly investigated as a practical approach for the production of lipids and for environmental purposes, like the mitigation of greenhouse gas (CO_2) emitted by industrial processes, and for effluent treatments (Oancea et al.,2013; Gonçalves,2021). On the opposite hand, investigations of microalgal product appropriate for crop productions stay largely unexploited. In the light of this point, this study highlights a number of the present researches and future development priorities and examining the factors supporting the employment of MBS and MBF for managing crop productions and abiotic stresses.*

Keywords: *Abiotic stresses, Biofertiliser, Biostimulant, Microalgae, Crop productions.*

Biography:

Researcher in Marine Biotechnology especially on plant Physiology and Seaweed Valorization; Ph D Student from Ibn Tofail University. Currently, Engineer in Biomass Valorization in the AgroBioSciences (AgBS) research program of the Mohammed VI polytechnic University (UM6P).
Engineer in Industry and Safety Food since 2010; Engineer in Biotechnology Applied to Plant Improvement since 2008 from the Faculty of Sciences and Techniques at Cadi Ayyad University. My Research focus on the importance of seaweed liquid extracts in improving the agronomic performance of crops especially Bean and wheat plants cultivated on soil or in hydroponic systems and under abiotic stress.

TL11.
THE EVALUATION OF PESTICIDES ON OPPORTUNISTIC *PSEUDOMONAS AERUGINOSA* AND ITS ANTIBIOTIC RESISTANCE

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Abstract: ***Background****: Nowadays, researchers have pay attention to the various effects of micro-pollutants in the environment. In the present thesis, I focused on the effect of pesticides on the growth and antibiotic resistance profile of Pseudomonas aeruginosa, an emerging opportunistic pathogen microorganism.* ***Method****: 5 ppm concentrations of three pesticides (glyphosate, S-metolachlor, and terbuthylazine) were used for the treatment of 5 clinical and environmental strains of Pseudomonas aeruginosa. Optical density of the treated bacterial cultures was detected after 1, 3, 24, 48, and 120 h to determine growth curves. Antibiotic resistance against 10 chosen agents was tested by MIC test strips (Liofilchem) containing a quantitative gradient of antibacterial drugs. Pesticide degradation rate of the examined strains was determined by gas chromatography.* ***Results****: Glyphosate and S-metolachlor both could be utilized as nutrients by the examined strains; therefore in the examined concentration they did not inhibit the growth of P. aeruginosa irrespectively to the origin of the strain (clinical or non-clinical). By contrast, terbuthylazine might have a negative effect on P. aeruginosa and limits its growth, but the differences were not significant. P. aeruginosa strains could degrade the S-metolachlor around 34-54% and terbuthylazine around 21-54% in 5 days. In addition, glyphosate caused a significant increase of MIC values on cefepime and ticarcillin and decreased the MIC values on tigecycline. S-metholachlor and terbutylazine have slightly increased the resistance of P. aeruginosa to ticarcillin, too but no other changes were observed.*

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Keywords: *Antibiotic resistance, micro-pollutants, pesticides, Pseudomonas aeruginosa*

Biography:

Jiang Dongze is a Ph.D. student at the Doctoral School of Environmental Sciences in the Hungarian University of Agriculture and Life Sciences at the age of 26 years. He is the member of the Department of Environmental Safety.

TL12.
ECOTOXICOLOGICAL EVALUATION OF BORON, ARSENIC, IODINE AND SELENIUM ON REPRODUCTION, MORTALITY AND AVOIDANCE OF *FOLSOMIA CANDIDA* (COLLEMBOLAN)

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Abstract: *Soil plays a significant role in the ecosystem. Soil pollutants have increased largely from a wide range of sources in decades. Trace elements accumulated in soil because of anthropogenic activities (e.g. industry, agricultural production, and health products) may pose a potential risk to environmental safety. Therefore, it is necessary to evaluate the ecotoxicological effects of trace elements on soil model animals. To evaluate the ecotoxicological impacts of four trace elements (As, B, Se, and I), the 28-days long tests based on the OECD guideline 232 had been executed with Folsomia candida (Collembola). The chosen trace elements were mixed with OECD artificial soil in the following concentrations [mg/kg]: 0, 0.0023, 0.0069, 0.0205, 0.0617, 0.185, 0.555, 1.66, and 5 for selenium; 0, 0.5, 7.5, 11.25, 16.875, 25.312, 37.969, and 56.953 for iodine; 0, 0.0002, 0.001, 0.005, 0.025, 0.125, 0.625, 3.125, and 15.625 for boron; 0, 0.01, 0.03, 0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 for arsenic. Meanwhile, 48-hours sublethal test (avoidance tests) had been carried out with Folsomia candida. Survival, reproduction, and avoidance behaviour of F. candida were regarded as endpoints with the main ecotoxicological values (NOEC, LOEC, EC_{50}, and LC_{50}). The four trace elements all showed toxic effects on F. candida in different degrees and they had similar toxicity on the survival of F. candida, while boron showed higher toxicity on reproduction than the others. The survival of F. candida was more sensitive than reproduction to each element. Avoidance behaviour of F. candida was the most sensitive to boron, followed by arsenic and iodine, and finally selenium. In this study, boron and arsenic appeared to be potential risks in the natural fields.*

Keywords: *Environmental protection, trace elements, Folsomia candida, soil pollution, ecotoxicological toxicity*

Biography

Liang Xinyue has completed her bachelor's degree as Environmental Engineer at the age of 23 from Óbuda University, Budapest, Hungary. She has completed her master's degree as Environmental Engineer at the age of 25 from Hungarian University of Agriculture and Life Sciences and she continued Ph.D. studies at the department of Aquaculture, Hungarian University of Agriculture and Life Sciences since 2020.

TL13.
APPLICATION OF CLEANER PRODUCTION IN SOAP INDUSTRY FOR A HEALTHIER WORK ENVIRONMENT

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Abstract: *In the light of the increasing industrial impact on the environment, and since small industries have a cumulative effect that increases with time, the study examines the employment of Cleaner production in soap industry to achieve two goals: the first cutting back on waste produced, and the second reducing the use of toxic materials. Thus, it can contribute in the solution of chronic problems concerning the health and safety of workers and the environment as well. The study has taken place in two different developing countries, Syria and India. The current situation in every factory was evaluated considering the occupational health and safety of workers, and the influence on the environment. This includes the evaluation of energy consumption and the use of Cleaner Production alternatives. The required data is obtained originally from the direct observation of raw materials, production stages, production quantities and unsold products and from interviews with owners, engineers, and workers in the second place. It was found that waste from soap factories are not generated in large quantities, but their accumulation creates environmental and health troubles which can be alleviated by replacing chemical materials with natural ones, using hand storage and implementing cleaner production alternatives in the soap industry. This strategy can reduce waste created and energy used through benefiting from the sunlight, natural drying, reusing byproducts, which can rise the efficiency of production and management process. It also has a significant positive impact on the work environment, health and safety of workers in general.*

Keywords: *Byproducts, Cleaner Production alternatives, India, Occupational Safety Soap Industry, Syria*

Biography

Yara EzAl Deen Sultan from Syria I have a degree in Geography, Tjshreen University, Latakia. I studied Education Habilitation Diploma. I have finished a Master degree in Environmental Science in India, and I Will complete my education to get a PhD soon. I have 11 years' experience as Geography teacher using modern techniques. I am leader in the Syrian Scouts since 2007.

TL14.
DEVELOPING SOIL PROTECTION TRAINING MATERIALS BASED ON EXPERIMENTAL PROCEDURES FOR AGE BETWEEN 10-14 IN HUNGARIAN PUBLIC EDUCATION

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***Abstract**: The choice of this study was inspired by recognition of gaps around the general knowledge and protection of soil. The poor knowledge of society today about soil protection puts the livelihoods of all life forms on Earth at risk. Loss of soil natural functions could have serious consequences in the future. Regeneration of soil as a non-renewable resource is too long to be defined on a human scale. The Food and Agriculture Organization of United Nations raised the awareness of the importance of healthy soil in connection of healthy, nutritious food production and importance of public awareness of soil protection. Therefore, it is necessary to integrate the basic theory and practice of soil protection into public consciousness, general and public education, and higher education too. Further generations will grow up without tangible knowledge and voluntarily or unintentionally destroy the essential foundation of our existence, the fertile soil. For this reason, it is important to thoroughly investigate the existing materials in the topic of soil protection in the National Core Curriculum and teaching materials. After a thorough research in the Hungarian Core Curriculum, it is clearly recognizable, that the topic of healthy soil is currently insufficient. The main question is, how to integrate more thoroughly the protection of this essential natural resource into education? My answer is in a precisely planned, practice oriented, age-appropriate educational material based on previously performed experiments, which experiments meant to provide the framework of the compiled teaching aid.*

***Keywords:** Awareness raising, Education, Healthy food, Healthy life, Soil protection*

***Biography**:*

Katalin Emese Rab Gáborné Seress was born on 17th August 1984 in Eger, Hungary. She grew up in a village, called Parád in Mátra Mountain at North-East Hungary. She was graduated in 2008 as a Special Needs Teacher in Behavioural Disorders and Special Needs Therapist in Learning Difficulties at Eötvös Lóránt University Bárczi Gusztáv Faculty of Special Education in Budapest. After a year of work experience as a Special Needs Teacher in Hungary she moved to the United Kingdom, Bristol and worked in St. Christopher's School until 2016, when she moved back to Budapest. She graduated as an Environmental Engineer in 2021 at Óbuda University Rejtő Sándor Faculty of Light Industry and Environmental Engineering with grade outstanding. She works at CEEweb for Biodiversity since May 2019 as an Office Manager, Project assistant and Internship Coordinator. She has a professional field in the protection of natural resources especially soil protection.

TL15.
OUTPUT VALUE AND PRODUCTIVITY OF AGRICULTURAL INDUSTRY IN CENTRAL-EAST EUROPE

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Abstract: *The study analyses the correlations among different economies of selected EU-12 member states based on the comparing the agricultural economic variances, namely the output value of the agricultural industry, productivity of input, agricultural gross value added, subsidies on production, agricultural labour input and agricultural income per annual working unit in period of 2010 and 2016 based on the Special Program for Social Sciences, as statistical methods. The EU-12 has achieved more increase in productivity of input, output value of agricultural industry, agricultural gross valued added, also per agricultural annual working unit than average results of EU-28 for 2010–2016. The output value of agricultural industry and agricultural gross valued added per intermediate consumption have decreased by 3.3%, but factor income – net value added at factor cost – per annual working unit increased by 0.1%, because of the subsidies on production increased by 3.4% for 2010–2016. The factor income – as net value added at factor cost – per annual working unit has increased by 21% for the same period, but most of this income was for developing agricultural production technology.*

Keywords*: annual working unit; gross value added; intermediate consumption; labour input; subsidies*

Biography:

CSc, economic sciences, Hungarian Academy of Sciences, Scientific Qualified Committee, Budapest in 1991 and Dr. of University, World Economics, Budapest Corvinus University of Economics in 1991. Habilitate Doctor, in social sciences, in Management and Business Administration, Kaposvár University, in Kaposvár in 2017. Between 1987-2017 work at University of Agricultural Sciences, then at Szent István University in Gödöllő. From 2017 work at Óbuda University. Participation at Doctoral School of Economics and Regional Sciences at Hungarian University of Agriculture and Life Sciences in Gödöllő. The research areas: Economics, Business and Management, International Regional Economic Integration, EU Study, Regional economics, Rural Development, Environmental economics. He published 207 publications with 257 independent citation count, of which 189 foreign language citations. His publications were published in Arab, English, Spanish and Russian foreign languages. International scientific conferences in Turkey, Canada, Moldavia, Czech Republic, Slovakia, Lithuania, Romania and Russia. Research project in Finland, Sweden, Denmark, Italy, Spain, France.

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TL16.
IMPACT OF GLYPHOSATE HERBICIDE ON SOME SOIL BIOLOGY AND FUNCTION

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Abstract: *Soil microbiotas regulate the bioprocesses in soil that are essential for plant growth, soil health and sustained productivity. Despite an increasing concern of consequences of using vast amounts of glyphosate-based herbicides in agroecosystems, their potential effects on non-target soil organisms and soil functioning are mostly unknown. Application of herbicides may not affect the overall size of the soil microbiotas but selectively can effect on specific microbial groups which may result in changing the balance of bioactivity and consequently nutrient availability, disease incidence and plant growth. Glyphosate, or N-(phosphonomethyl)glycine, is a broad-spectrum, non-selective and post-emergence herbicide, used as an active ingredient in several weed killing products since 1970s. Glyphosate is also vulnerable to microbial degradation and its main degradation product, aminomethylphosphonic acid, is strongly adsorbed to soil solids. For these reasons, glyphosate has generally been regarded as an environmentally safe herbicide. In the current study, N_2-fixing soil microbial populations and their activities were determined in microcosms under the stress of glyphosate herbicide were applied at 3 doses based on the active ingredients and incubated for 10 weeks at 28°C. Results indicated that: Application of glyphosate had negative impact on symbiotic N_2-fixation by legumes in rotation and application of a post-emergent herbicide caused significant changes in the microbial community and the activities of some hydrolytic enzymes in the rhizosphere. Impacts of glyphosate on rhizosphere microorganisms and activities were investigated. Information on the effects of herbicide application on useful soil microbiotas and their bioprocesses is necessary for the successful crop production. A particular practices may have the desired result in one situation but have little effect in another because biological communities respond to the interaction of multiple factors including food sources, physical habitat, moisture, and impacts of historical land use. Therefore, before a new product is applied to a field soil, it should be tested on a limited area and the results should be monitored in comparison to an untreated one.*

Keywords: *Herbicides, glyphosate, soil bioprocesses, alfalfa rhizosphere*

Biography:

Fatma H. El-Fallah was born in Benghazi, Libyan nationality. I have completed M.Sc. Degree in Biotechnology and Microbiological Sciences, Faculty of Agricultural and Environmental Sciences, Szent István University in Gödöllő Hungary, 2007. My first employment was as sciences teacher (1999-2004), and then joined the department of Botany, Ajdabiya University, Libya (2016-now). Recently I am a staff member at Botany department, faculty of science, University of Ajdabiya Libya. My field of interest is Biotechnology, Genetic and Molecular Biology.

TL17.
ECOTOXICOLOGICAL EVALUATION OF THE TOLERANCE OF SOME SYMBIOTIC N_2-BINDING SOIL BACTERIA TO ENVIRONMENTAL CONTAMINANTS

Nikolett KOZELKA-BURES, Hosam E.A.F. BAYOUMI HAMUDA*

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Abstract: *The major N_2-fixing systems can play a significant role in improving the fertility and productivity of low-N soils. The plant growth-promoting rhizobacteria (PGPR) have received the most attention and have been examined extensively. Under heavy metal stress, soil microorganisms including PGPR have developed many strategies to evade the toxicity generated by the various heavy metals. In the present study, some environmental factors such as heavy metals (Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}), Al^{3+} were studied at different concentrations (0, 20, 40, 80 and 160 µm) on the ecophysiological properties and their growth using microfermentor technique as well as their tolerances to various hydrogen ion concentration. The results showed that the stress factor suppresses the growth characteristics of the studied PGPR including symbiotic Sinohizobium strains related to R. leguminosarum, R. phaseoli, R. trifolii, and R. loti as well as Sinorhizobium meliloti in comparison with non-symbiotic N_2-fixer Azotobacter and Pseudomonas as PGPR strains which are differentially react with the stress factors at high concentrations. However, the tolerance of several investigated strains, distributed among Sinorhizobium meliloti strains: GH-130, GHR-94, GH-97 and GH-07 are sensitive to the stress of 160 µm for heavy metals effects in comparison with Azotobacter and Pseudomonas strain. None of the strains can tolerate 160 µm of Cd^{2+}, Pb^{2+} and Cu^{2+}. The most toxic metal was Cd^{2+}, followed by Pb^{2+} and Cu^{2+}. The ecophysiological tolerant Sinorhizobium and Azotobacter as well as Pseudomonas putida strains are suggested to be the ideal solution for the improvement of soil fertility and the rehabilitation of reclaimed soils and are an important direction for future soil protection and quality research. Hence, a better understanding of N_2-fixing bacteria ecophysiological responses to different intrinsic stresses factor is very important to improve crop production by harnessing the growth of the N_2-fixing bacteria process.*

Keywords: *heavy metals, relative growth rate, tolerance, N_2-fixing bacteria, Azotobacter, Pseudomonas*

Biography

Prof. Dr. Hosam Bayoumi Hamuda is working at Óbuda University. He is Environmental Microbiologist and Soil Biotechnologist dealing with the interactions between the microbiomes and the environment for increasing soil quality and saving the soil from pollutants as well as how benefits to use the wastewater sludge in the agriculture. His investigations are on the role of waste management, on soil quality, fertility, the crop production and environmental impacts related to the application of organic wastes to soil to assess: Soil quality, microbial inoculants; nitrification inhibitors and crop quality; Monitorization of organic matter; measurements of rhizosphere and soil microbial biomass and enzymatic activities in wastewater sludge amended soils; root systems and microbial composition in the polluted environment and roles of engineered metal oxide nanoparticles in biosphere.. Also he is interested in the role of environmental quality and relation between gut microbiomes on human health. ***Research Interest****: Waste management; Soil: Biotechnology; Protection; Sustainable; Biodiversity, Plant Growth-Promoting Rhizomicrobiota (PGPR); Colonization of PGPR; Microbial inoculants in rhizosphere; Econanotoxicology; environmental quality and relation between gut microbiomes on human health as well as the modern biology topics.*

TL18.
EFFECT OF SOME HERBICIDES ON SOIL MICROBIAL POPULATION, SOIL ORGANIC MATTER AND DEHYDROGENASE ACTIVITY

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Abstract: *A balanced agroecosystem is dependent on soil microbial biomass and population which are integral parts of the soil environment. The effect of five herbicides (bromoxynil, paraquat, glyphosate, linuron, and acetochlor) on soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), soil microbial population, soil organic matter, and dehydrogenase activity was assessed over a period of four weeks. The herbicides were applied at recommended rates to brown forest soil which obtained from the garden of RKK and contained in 250 g plastic pots. The results demonstrated that bacterial, fungal, actinobacterial; phosphate solubilizing, and cellulose-decomposing populations were decreased upon treatment with herbicides when compared to the control. The study showed that the herbicide bromoxynil caused suppression in microbial activity and biomass by exerting a toxic effect on them. The result illustrated a significant reduction in the percentage of soil organic matter after the application of the herbicide to soil samples. Soil organic matter then increased after continuous application from the second to the fourth week of treatment. Herbicide treatment caused a significant decrease in dehydrogenase activity when compared to the control soil samples. The obtained results indicated that soil samples treated with bromoxynil and paraquat had the lowest dehydrogenase activity after the 4th week of treatment, while soil samples treated with glyphosate followed by linuron and acetochlor had the highest dehydrogenase activity when compared to control treatment. This study indicated a significant response of soil microbial activity to herbicide treatment and increased adaptation of the microbial community to the stress caused by an increase in the concentration of the herbicides over weeks of treatment.*

Keywords: *soil microbial biomass, microbial population, herbicides, dehydrogenase activity, soil C and N*

Biography

Prof. Dr. Hosam Bayoumi Hamuda is working at Óbuda University. He is Environmental Microbiologist and Soil Biotechnologist dealing with the interactions between the microbiomes and the environment for increasing soil quality and saving the soil from pollutants as well as how benefits to use the wastewater sludge in the agriculture. His investigations are on the role of waste management, on soil quality, fertility, the crop production and environmental impacts related to the application of organic wastes to soil to assess: Soil quality, microbial inoculants; nitrification inhibitors and crop quality; Monitorization of organic matter; measurements of rhizosphere and soil microbial biomass and enzymatic activities in wastewater sludge amended soils; root systems and microbial composition in the polluted environment and roles of engineered metal oxide nanoparticles in biosphere.. Also he is interested in the role of environmental quality and relation between gut microbiomes on human health. ***Research Interest****: Waste management; Soil: Biotechnology; Protection; Sustainable; Biodiversity, Plant Growth-Promoting Rhizomicrobiota (PGPR); Colonization of PGPR; Microbial inoculants in rhizosphere; Econanotoxicology; environmental quality and relation between gut microbiomes on human health as well as the modern biology topics.*

TL19.
EFFECT OF FOUR HERBICIDES ON SOIL ENZYME ACTIVITY IN BROWN FOREST AGRICULTURAL SOIL

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Abstract: *Soil enzymatic activity assays are only one way to measure the soil ecosystem status. The technique is quite simple and produces reproducible results, and is nowadays of practical importance because the influence of herbicides, as well as soil fertility management, can be measured. Especially the search for urease inhibitor is of particular interest in order to reduce ammonia losses from soils. Soil enzymes have been reported as useful soil quality indicators due to their relationship to soil biology, being operationally practical, sensitive, integrative, easy to measure, and described as "biological fingerprints" of past soil management. Herbicides are biologically active compounds, and an unintended consequence of their application may lead to significant changes in biological activities influencing microbial ecological balance affecting soil fertility. The fate of herbicides applied in agricultural ecosystems is governed by the transfer and degradation processes, and their interaction with soil microorganisms. The increasing reliance of sustainable agriculture on herbicides has led to concern about their ecotoxicological effects influencing enzyme activities, which may serve as indicators of soil quality. The effects of herbicides (glyphosate, paraquat, trifluralin, and 2,4-D) on soil enzyme activities (β-glucosidase, amylase, invertase, cellulase, protease, and urease as well as phosphatase and arylsulfatase) were assessed over a period of four weeks. There was a significant reduction in organic carbon with time. Herbicide treatments resulted from variation in enzyme activities, while the highest activity was recorded for control soil. Only paraquat and glyphosate at field recommended dose were found to be more inhibit the enzyme activities and 2,4-D was the least inhibited herbicide. The study suggested that the herbicides cause a transient impact on enzyme activities associated with the type of herbicides at recommended field application rate.*

Keywords: *Soil enzymatic activity, herbicides, sustainable agriculture, brown forest soil*

Biography

Prof. Dr. Hosam Bayoumi Hamuda is working at Óbuda University. He is Environmental Microbiologist and Soil Biotechnologist dealing with the interactions between the microbiomes and the environment for increasing soil quality and saving the soil from pollutants as well as how benefits to use the wastewater sludge in the agriculture. His investigations are on the role of waste management, on soil quality, fertility, the crop production and environmental impacts related to the application of organic wastes to soil to assess: Soil quality, microbial inoculants; nitrification inhibitors and crop quality; Monitorization of organic matter; measurements of rhizosphere and soil microbial biomass and enzymatic activities in wastewater sludge amended soils; root systems and microbial composition in the polluted environment and roles of engineered metal oxide nanoparticles in biosphere.. Also he is interested in the role of environmental quality and relation between gut microbiomes on human health. Research Interest: Waste management; Soil: Biotechnology; Protection; Sustainable; Biodiversity, Plant Growth-Promoting Rhizomicrobiota (PGPR); Colonization of PGPR; Microbial inoculants in rhizosphere; Econanotoxicology; environmental quality and relation between gut microbiomes on human health as well as the modern biology topics.

TL20.
SURVEY OF LICHENS BIODIVERSITY IN DIFFERENT AREAS OF CSÓR IN HUNGARY

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Abstract: *The bioindication is an important agent today and in the future, as what could give a clearer picture of the atmosphere around us. Lichens play as bioindicator. Lichens do not tolerate the sulfur dioxide content of the air. This urban or industrial pollution is polluted air are often called "lichen deserts". So where the lichens absence, there is SO_2 in the air. For this purpose, 58pictures of lichens were taken in 4 different areas of Csór (Mátyás Király utca, Hadi út, Vízmű telep, Dózsa György köz). Study areas were selected to include a wooded, green area, a less populated, not very busy area, and parts with more traffic in the city. First location was the garden of a family house, where most varieties of fruit trees are found are full with lichens. Second area was examined a small part of a wooded area were one or two or many lichen colonies are found. Third location was a part of the waterworks site is already a wooded area. The fourth area was the garden of another family house. There are no fruit trees in this area, only two pieces of ornamental trees. It was the first area where the lichens found on the fence. Based on the photos, morphology, color, diameter, and extent of the lichens were examined. In addition, most dominant types were investigated. Lichens of different shapes and sizes in the four areas were examined. The investigations showed that mostly yellow and white leafy lichen are there. Yellow plate lichen and map lichen were also found. Exceptions are the third and fourth locations, where the yellow-leafed lichen outside the trunk of the tree on the stones on the side of the building and on the boards of the bench and fence. The sizes of the lichens are different. Their surface area is very varied. Because Csóron does not contain SO_2 in the air, lichens are present everywhere in an undamaged state. The most dominant species in the studied areas was the yellow-leafed lichen. Lichens are used to study global climate change, air quality. Attention must be paid to its use in the pharmaceutical industry due to its antibiotic and antiseptic effect.*

Keywords: *Lichens, air quality, Csóron, morphological investigation*

Biography

Prof. Dr. Hosam Bayoumi Hamuda is working at Óbuda University. He is Environmental Microbiologist and Soil Biotechnologist dealing with the interactions between the microbiomes and the environment for increasing soil quality and saving the soil from pollutants as well as how benefits to use the wastewater sludge in the agriculture. His investigations are on the role of waste management, on soil quality, fertility, the crop production and environmental impacts related to the application of organic wastes to soil to assess: Soil quality, microbial inoculants; nitrification inhibitors and crop quality; Monitorization of organic matter; measurements of rhizosphere and soil microbial biomass and enzymatic activities in wastewater sludge amended soils; root systems and microbial composition in the polluted environment and roles of engineered metal oxide nanoparticles in biosphere.. Also he is interested in the role of environmental quality and relation between gut microbiomes on human health. ***Research Interest****: Waste management; Soil: Biotechnology; Protection; Sustainable; Biodiversity, Plant Growth-Promoting Rhizomicrobiota (PGPR); Colonization of PGPR; Microbial inoculants in rhizosphere; Econanotoxicology; environmental quality and relation between gut microbiomes on human health as well as the modern biology topics.*

TL21.
EMISSIONS OF GREENHOUSE GAS AND AIR POLLUTANT FROM DIFFERENT ECONOMIC SECTORS IN THE EUROPEAN UNION

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Abstract: *The article focuses on influences of economic development on gas emissions and air pollutants effects in EU-28. The study analyses emissions of different kinds of greenhouse gases (CO_2, CH_4, N_2O, HFC, PFC, NF_3_SF_6), acidifying gas (SO_X, NO_X, NH_3), ozone precursors (CO, NMVOC) and particulate matter (PM_{10}, $PM_{2.5}$) resulted by economic activities of different economic sectors including the household during period of 2010-2018. Total emissions of some selected economic sectors plus households issuing greenhouse gas and acidifying gas concentrations significantly respectively reduced by 10.6% and 15.2% in EU-28. In spite that the trends of SO_X and CO emissions have sharply respectively decreased by about 38.4% and 21.1%, but the NF_3_SF_6 has increased by 18.8% for the period of 2010-2018 in EU-28. Also, household activities have respectively increased N_2O and HFC emissions by 5.8% and 6.6% for the same period in EU-28, which emphasizes the importance of the considerable force to decrease gas emissions and air pollutions in order to follow the sustainable economic development accompanying with sustainable environment as well as remaining and protecting the natural environment. The database in this article was selected from the Eurostat online database published in 2020. The paper focuses on analysing the different economic sectors, creating gas emissions and air pollutions. Therefore, the main object of this study is to give an overview about the gas emissions and air pollutions cumulative pressures deriving from growth and change of economic sectors, which influenced on the EU-28 environment. The environmental conservation needs for introducing new technologies and strategies to mitigate the gas emissions and air pollutants in order to avoid danger air emissions causing global warming and air pollution as well as to maintaining the environmental conservation, protection and management.*

Keywords: *Environmental conservation, GDP growth, Global warming, Household, Particulate matter, Sustainable development*

TL22.
POSSIBILITIES TO ENSURE SUSTAINABILITY AND A GREEN STRATEGY IN HUNGARIAN ROAD TRANSPORT

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Abstract: *Greening transport plays a key role in fulfilling climate goals and carbon neutrality. The sector is responsible for one fifth of greenhouse gas emissions in Hungary. Electric transport creates the possibility of zero emissions, so the Green Strategy can be implemented by supporting the spread of electric cars and implementing the Green Bus program. Our goal is to realize climate strategy, green transport, sustainability by taking into account the financial possibilities. Financial support is needed to put transport on less polluting fundaments. It can involve introducing a new type of tax or supplementing existing taxes. Based on the polluter pays principle, this can be mileage or consumption based. At the same time, the increase in fuel costs for heavy duty vehicles reduces competitiveness, so compensation is needed for the sustainable operation of hauliers and delivery companies.*

Keywords*: Ensure Sustainability, Green Strategy, Hungarian, Road Transport*

TL23.
EFFECT OF DIFFERENT CHELATING AGENTS AND BIOINOCULANTS ON FRACTIONAL DISTRIBUTION OF CADMIUM AND NICKEL IN SPIKED SOIL AFTER HARVEST OF INDIAN MUSTARD (*BRASSICA JUNCEA*)

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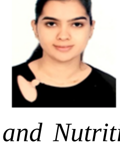
Abstract: *Screen house experiment was conducted to study the different forms of Cadmium (Cd) and Nickel (Ni) in the soil after harvest of Indian mustard (Brassica juncea L.) as affected by chelating agents and bioinoculants from metal enriched soil. A seven step sequential extraction procedure was applied to evaluate the association of metals with soil constituents. The different chemical forms of fraction of Cd extracted were exchangeable + water soluble carbonate, organic matter complexed, Mn-oxide bound, occluded in amorphous and crystalline Fe-oxide and residual mineral fraction. The present study revealed that dominant Cd fraction was organic followed by carbonate, Manganese oxide, exchangeable, crystalline Fe-oxide, amorphous Fe-oxide and residual Cd fraction. Application of chelating agents increased the exchangeable fraction causing an increase in its availability in the soils. It may be ascribed that application of chelating agent caused an increase in the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, Mn-oxide, AFe-oxide and CFe-oxide bound fractions. Highest amount of exchangeable fraction was observed in EDTA treated soil. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd (greater than non bioinoculants treated soil). The results are in conformity with those of Xian (1989) and Shuman (1998). Application of chelating agent (EDTA) significantly increased the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, MnOX, AFeOX, bound fractions. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd. Fractionation of soil Cd after the harvest of Brassica juncea crop showed that the application of chelating agent (EDTA) excellently improved the exchangeable pool of Cd.*

Keywords: *Cadmium, Nickel, different fractions, Indian mustard, chelating agents, bioinoculant*

Biography



Mantavya Bishnoi, currently, she perusing Ph.D. in Foods and Nutrition, Department of Foods and Nutrition, CCS HAU Hisar. PROFESSIONAL OBJECTIVES: To pursue Doctorate degree *with financial assistance in Foods and Nutrition Science enabling proven in this exciting field as a career to conduct high quality research and effectively utilizing then meet the challenges of Foods and Nutrition Science for food safety, quality, nutritional value and security by the industries as well as consumers. Aspire to take a challenging position and be part of efficient, winning team and serve in an organization, which offers challenging tasks and where a constant up gradation of my knowledge is possible. PROFESSIONAL QUALIFICATIONS: Pursuing MSc. in Foods and Nutrition from Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, Haryana, India 125004. She was selected for Masters in Research Dual Degree Award by CCS,HAU, HISAR- INDIA and WSU, SYDNEY- AUSTRALIA under the project " Characterization of Stingless Bee Honey Derived from Different Locations in Australia in terms of Nutrition and Quality".*

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PL1.

ENHANCING ENVIRONMENTAL QUALITY AND PUBLIC HEALTH BY PROMOTING RESOURCE CIRCULATION USING WASTE CO-PROCESSING IN CEMENT PLANTS

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Abstract: *Since ages, countries throughout the world have been using landfill sites for dumping waste at minimum cost. Currently, an estimated 2 billion tonnes of municipal solid waste is generated globally, out of which almost 33% remain uncollected by municipalities (Waste Atlas 2018). Currently, the top three municipal solid waste-generating countries are the USA, i.e., 258 million metric tons, China, i.e., 220 million metric tons, and India, i.e., 169 million metric tons (Statista 2020).This daily accumulation of waste materials is increasing heavily on a daily basis with increase in population, causing scarcity of further land to be used for landfills. According to the World Bank, the generation of municipal solid waste is anticipated to rise to 3.4 billion tonnes by 2050 (The World Bank 2020a). These landfills are generally without any scientific treatment or segregation in developing countries including India. Treatment capacities are not sufficient causing local harm, release of greenhouse gases and other pollution. From the current world population of 7.6 billion (US Census Bureau 2020), nearly 3.5 billion people are deprived of basic waste management facilities (Waste Atlas 2018). As a result these landfill sites have become a breeding ground for contamination by flies, rodents, mosquitoes and causing diseases like dermatological, respiratory, genetic, and several other kind of infectious diseases. Among these wastes accumulated, various materials have been proven to be useful resources which can be used again as an input in various industries. A major portion of these wastes may be utilized and resources can be recirculating by various treatment and management processes by the implementation of 3Rs and circular economy concepts. Co-processing of wastes in cement plant is one of the options for this solution. This paper deals with enhancing the environmental quality and public health by promoting the process of co-processing of waste materials in cement plants thereby reducing the path of waste materials to the landfill sites. Co-processing is defined as the process where waste materials are used as a source of energy or raw materials. By applying this circular economy concept, usage of conventional fuel can also be greatly reduced. A case study has also been done to enquire whether co-processing is the best option to be implemented on cement plants. If the waste materials are co-processed in cement plants effectively, landfill sites will no longer overflow with waste and quality of health and environment can also be improved significantly. Moreover, it will help to obstruct the flow of waste materials to ocean, hence preventing marine littering and creating micro plastics too.*

Keywords: *Co-processing, Circular economy, GHGs. landfill, municipal solid waste, waste management.*

INTRODUCTION

Landfills have been extensively utilized for solid waste disposal throughout the globe. Developing countries in particular, such as China and Mexico, India treat landfills as the primary method for disposing solid waste in landfills due to several advantages, including economic efficiency and low technological barriers (Zacarias-Farah and Geyer-Allely, 2003; Rodrigo et al., 2016).

Presently, solid waste landfilling is considered to be one of the major environmental concerns due to its significant methane (CH_4) production. Although selecting landfill as a viable option for solid waste management should be given the last priority due to considerable greenhouse gas (GHG) emissions in comparison to other waste management technologies. (Majdinasab, A.; 2017)

The most significant environmental nuisance of the landfill approach is the landfill gas (LFG) production resulting in thousands of tons of the greenhouse gas (GHG) to be vented into the global atmosphere. The LFG is mainly composed of CH_4 (50–55%) and CO_2 (40–45%), which are generated during the anaerobic digestion of the solid waste through landfill sites (Ayalon et al., 2001; Scheutz et al., 2009a)

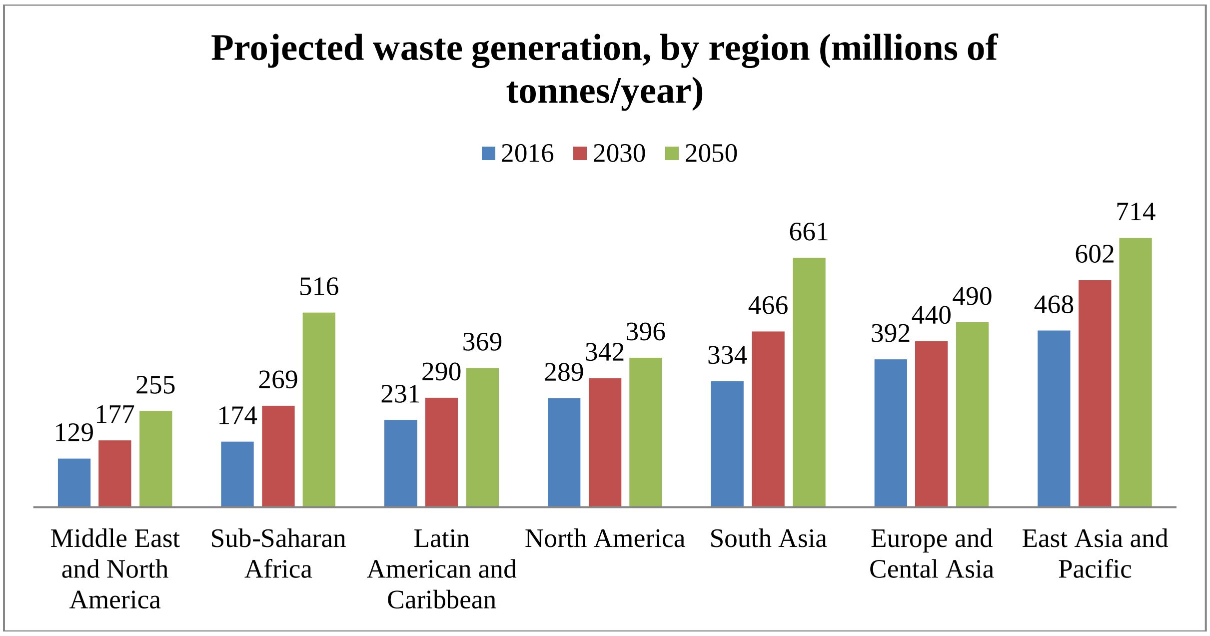

Figure (1): Projected Solid Waste Generation in different region of the world from 2016 to 2030 (The World Bank; 2021)

The landfill wastes include both organic and inorganic matter. The organic matter covers more than 50% of the total waste material. Due to improper management of landfill garbage, it causes serious risks to human health directly by the emission of toxic gasses. On the other hand, landfill sites are the natural habitat of several microbes and arthropods. Moreover, the population of insect vectors of various diseases, insect scavengers as well as pollinators is thriving in landfill sites. It shows that landfill sites and adjacent areas are hotspots for a wide variety of arthropods.

Landfill sites are also likely to generate other non-methane volatile organic compounds (VOCs) including hydro-chlorofluorocarbons (HCFCs) and chlorofluorocarbons (CFCs) which are typically generated at low scales (Bogner et al., 2010).

Uncontrolled waste management degrades land, ground water and air quality, leading to health risks to humans, animals and the ecosystem. Presently, industrial waste in most cases is disposed to landfills after incineration, without utilizing the full potential of the wastes through recirculation. (Sadala; 2019)

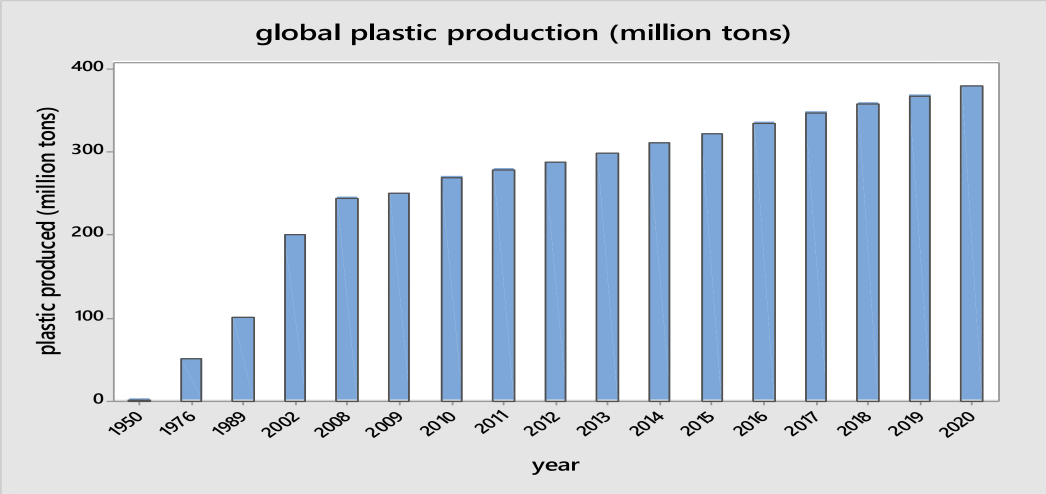

Figure (2): Production of plastics worldwide 1950-2019
(Statista; 2021)

In kolkata, a city of India, there is a dumpsite named Dhapa, whose area is around 21.1hectares where 3500 tons of solid wastes are accumulated on a daily basis, causing giant mountains of waste materials. Kolkata has an area of about 187.33 km^2 and a population of about 10 million (including floating population). Kolkata Municipal Corporation (KMC) comprises 15 “boroughs” and 141 electoral “wards”; each borough consisting of a cluster of wards. (WBPCB)
Garbage is repulsive and malodorous material attracting insects and eaten by different animals and scavengers (Jofre et al., 2011). Garbage destroys natural sights, produce a bad smell, provide a breeding site to insects, spread disease and pollute the environment, leading to problems for natural activities.
So it has become an evident warning that alternatives should be found out in order to reduce this huge heaves of waste material, piling up in landfill sites and causing scarcity of land.
Several alternative options have been implemented to manage accumulated solid waste to reduce the necessity of landfilling. However, recent investigations in the US show that over 50% of the MSW produced is currently decomposed through landfill sites. This clarifies that landfilling is the most common approach to solid waste management (USEPA, 2012; Weitz et al., 2002).
The proper management of landfill sites could reduce the population dynamics of various insect pests, and health risks could be decreased in low and middle-income countries. (Muhammad Qasim et al, 2020)
Co-processing of this waste for energy recovery and as an alternative raw material in cement kiln can be an effective management methodology for this waste stream. This is being practiced sustainably in number of countries but in India the process lacks proper implementation. The auxiliary technological requirement is less and the process is highly economical and effective. (S K Ghosh et al, 2016)
Indian cement industry is rated as one of the best performing industry across various industrial sectors in terms of energy efficiency, quality control, and environmental sustainability and adaptive in venturing into new technology. Indian cement industry is contributing to circular economy primarily by (i) Circular Supply Chain, (ii) Recovery and Recycling.
Waste from various industries is being utilized by the cement industry as alternative fuels and raw materials (AFR). As cement manufacturing process itself supports the environmentally sustainable waste utilization due to high temperature incineration without leaving any residue, hence it is acting as backbone for waste generating industries. (Kapil Kukreja et al, 2020)

LITERATURE REVIEW

Co-processing

Co-processing is defined as the process where wastes are utilized as a source of energy or raw material, or both to replace natural mineral resources and conventional fuels such as coal, petroleum coke etc. in the manufacturing process. Basel and Stockholm Conventions highlighted the suitability of cement kiln for co-processing of hazardous and other wastes. Co-processing in cement kiln is scientific, sustainable, proven and established technology for disposing hazardous and non- recyclable waste in environmentally sound manner. This practice can be extremely attractive and cost-efficient, especially for emerging economies having insufficient waste treatment capacity, like India. (Saha et al, 2017). There are several benefits of co-processing when compared to other recycling processes. When waste is co-processed in a cement kiln, it leads to 100% energy and material recovery and does not leave behind any byproducts like ash, etc. Due to its economic, environmental and social benefits, it has been proved as one of the five important pillars for large scale reduction of CO2 emissions from Indian cement industry. The traditional linear extract-produce-use-dump material and energy flow model implemented so far in the modern economic system has been proved unsustainable at long term (Korhonen et al., 2018).

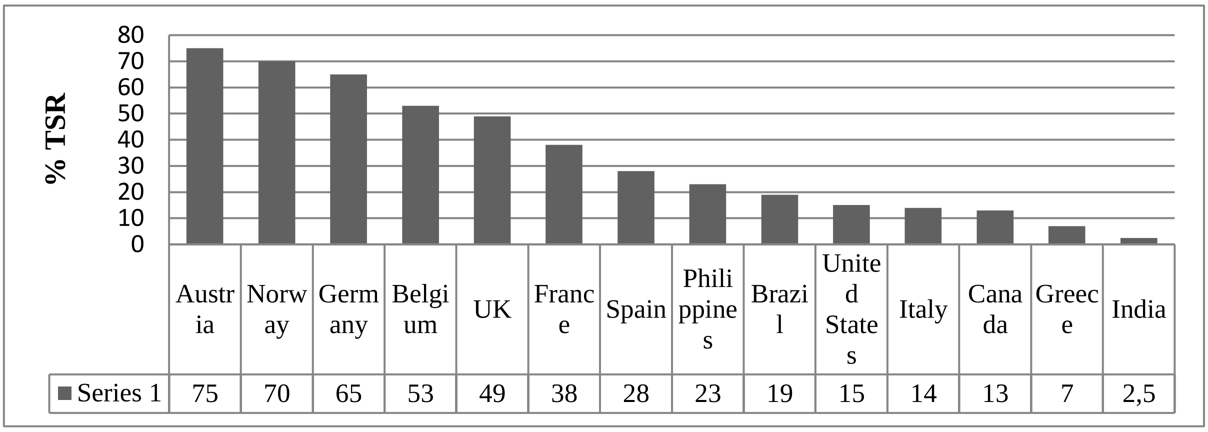

Figure (3): Countries and their Thermal Substitution Rates (TSR)
(Saha et al., 2017; Karstensen et al., 2018)

In contrast, the circular economy provides the economic system with an alternative that fosters to reduce negative environmental impacts and stimulate new business opportunities. In this line, most materials (including biomass and waste-derived fuels such as tires, sewage sludge (SS) and municipal solid waste (MSW), plastic waste, E wastes) contain some potential energies that can be utilized by cement industries in order to meet the requirements of the thermal energy, (Malinauskaite et al., 2017). Therefore, the use of those materials as a source of energy in cement plants contributes towards overcoming challenges such as climate change, waste management and fossil fueldepletion, while utilizing principles of circular economy (ECOFYS, 2016; Malinauskaite et al., 2017). On the other hand, unsuitable technical conditions in co-processing cement plants may lead to the emission of some of the most hazardous pollutants, such as heavy metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) (Mari et al., 2009). Consequently, a number of recent studies have been aimed at evaluating the environmental impact of co-processing cement plants (Georgiopoulou and Lyberatos, 2018; Mari et al., 2017; Richards and Agranovski, 2017; Rovira et al., 2014, 2015, 2016).

TYPES OF WASTE IN CO-PROCESSING

Use of MSW in cement kiln

In Vietnam, a study showed that utilizing cement kiln to dispose the municipal solid wastes is good way to choose. In Vietnam, there are more than 76 cement kilns, if it is assumed that each cement kiln could dispose 70 000 tons wastes per year (200 tons per day), the cement industry can dispose 5.3 million tons wastes per year, contributed for handling 23% of total MSW generation in Vietnam. The waste could turn into the components of cement and will not pollute again or leave any byproduct. The cement kiln can dispose different states wastes (Solid, Semi-solid or liquid). It is cheaper way of disposing MSW than building the incinerator plants (Lê Cao Chiến, 2016).

Use of AFR in cement kiln

The substitution of fossil fuels by alternative fuels (AF) in the production of cement clinker is ofgreat importance both for cement producers and for society because it conserves fossil fuels and, in the case of biogenic wastes, reduces greenhouse gas emissions. In addition, the use of alternative fuels can help to reduce the costs of cement production. Energy costs and environmental concerns have encouraged cement companies worldwide to evaluate to what extent conventional fuels can be replaced by waste materials, such as waste oils, mixtures of non-recycled plastics and paper, used tires, biomass wastes, and even wastewater sludge (D. Amsaveni et al, 2020).

Use of plastic waste in cement kiln

Various studies in a paper showed that there was no unfavorable impact on the environment, clinker and cement properties when plastic waste was used a fuel in cement production. Hence co-processing of plastic waste is one of the best alternatives for its disposal, saving of energy resource, in ecological sustainable and environmental friendly manner. The total cost saving per day was Rs. 9705 (Rs. 542,325 per annum) and 9 tons coal save per day. The co-processing of waste plastics reduces the overall CO2 emissions, after replacement of coal by plastic waste the total reduction of CO2 is17.81 tons/day or 6500 tons/annum. The cost of collection and treatment may limit the use of waste plastics‟ Interactions between coal and waste plastics can improve combustion efficiency (Ghosh, 2019).

Waste materials in cement production

it was found out that Various waste materials like Coating residues, aluminium recycling sludge, Industrial lime, Lime sludge, Fly ashes, Crushed sand, Roasted pyrite, Mechanical sludge, Red sludge contained compounds with cementious value and they could be also used as a raw material in production of cement. The goal was the ecologically beneficial and economically viable co-processing of high calorific waste materials as a contribution to the conservation of non-renewable primary energy resources. It is generally agreed that co-processing of waste in cement kilns can be a valid option for solving waste problems in developing and emerging countries, provided basic rules and principles are observed. (Dieter Mutz.2017)

CO-PROCESSING TECHNOLOGY

Co-processing is an industrial technique of using wastes as a substitute for raw materials and/or fuels, as a way to minimize the consumption of minerals and non-renewable fossil fuels (Stafford, 2015).Co-processing is a more environmentally friendly and sustainable method of waste disposal in comparison of land filling and incineration because of reduced emissions and no residue after the treatment (Geocycle). Wastes occur in different forms and qualities. The transformation of waste to AFR requires certain standards. Some types of waste cannot be used directly as AFR, but must undergo a preparation process. This step produces a waste product with defined characteristics that complies with the technical specifications of cement production and guarantees that environmental standards are met (GTZ, 2006).

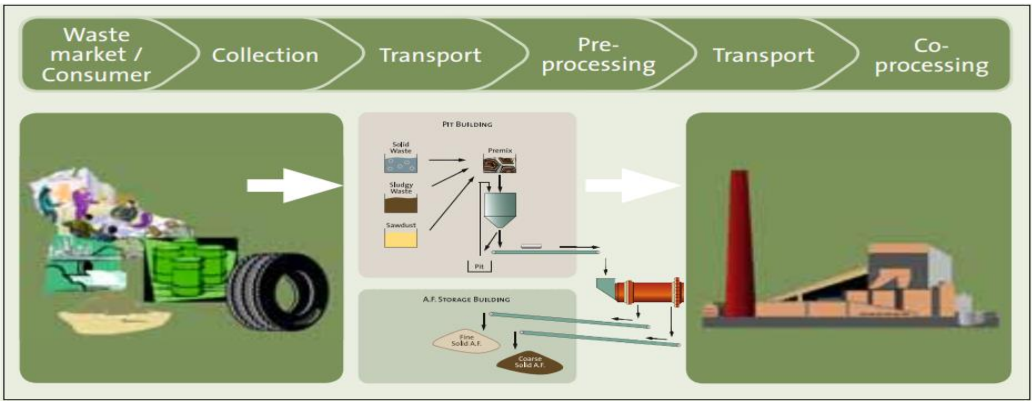

Figure (4): Pre-processing technology from waste to alternative fuel (GTZ, 2006)

Due to the high temperature (1450ºC) in cement kiln, different types of wastes can be successfully disposed without harmful emissions. As per the Basal Convention, wide variety of wastes including hazardous wastes, get disposed in an environmentally safe and sound manner through the technology of co-processing in cement kiln. Disposal of non-recyclable fraction of plastic wastes through co-processing is practiced in many countries as a regular method for their environmentally sound disposal. Different feed points can be used to insert AFR into the cement production process. The most common ones are:

- via the main burner at the rotary kiln outlet end
- via a feed chute at the transition chamber at the rotary kiln inlet end (for lump fuel)
- via secondary burners to the riser duct
- via pre-calciner burners to the pre-calciner
- via a feed chute to the pre-calciner (for lump fuel)
- Via a mid-kiln valve in the case of long wet and dry kilns (for lump fuel) (GTZ, 2006).

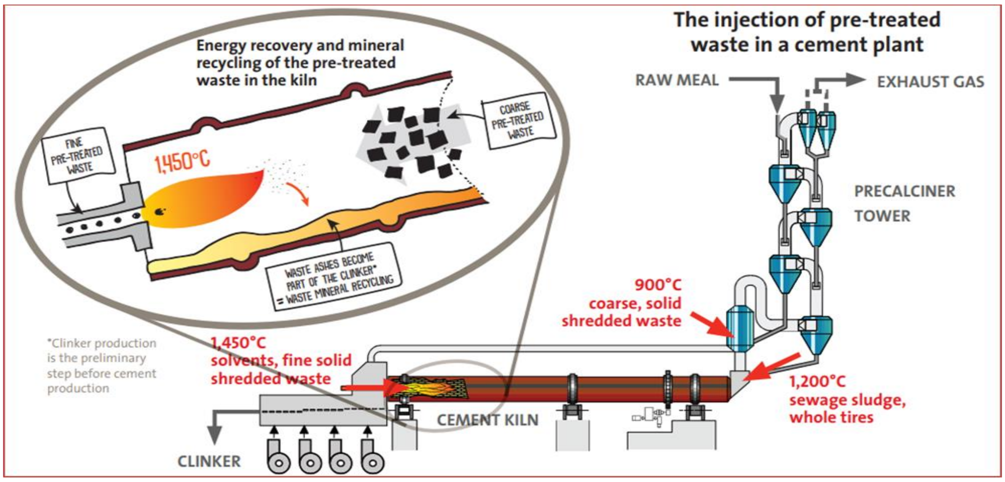

Figure (5): Co-processing technology in cement kiln (Geocycle)

EMISSION IN CO-PROCESSING

Carbon dioxide (CO_2) is the major oxide produced from the combustion of most fuels. Carbon monoxide (CO) is formed in the case of incomplete combustion as an intermediate product, and converted to CO_2 in the presence of oxygen. Potentially harmful acid gases, particulate matter and VOC are also associated with the combustion of fuels in cement kilns. A substantial amount of these emissions are due to the organic content of raw materials introduced into the kiln.

Emission of sulphur dioxide

Sulphur dioxide (SO_2) is generated from the combustion of fuels containing sulphur. Its formation and abundance are governed by the sulphur content of the fuel. Low sulphur coals have occasionally been blended with traditional coals to reduce SO_2 emissions. Several major utility providers and the U.S Department of Energy have also considered the co-firing of biomass with coal in boilers for the same purpose (Tillman.D.A, 2000). SO_2 can be removed, post-combustion, from the exhaust going through the kiln stack, by the use of scrubbers and adsorbents.

Emission of oxides of nitrogen

Formation of oxides of nitrogen (NO_x) increases exponentially with increasing temperature and excess air because air contains N_2 and O_2, which react to form thermal NO_x. NO_x formed by the oxidation of organo-nitrogen compounds found in the fuel is dependent on the reactivity of the fuel. Highly reactive fuels promote early release of nitrogen in the fuel mass. The nitrogen content in fuels is generally low; therefore, fuel derived NOx for cement kilns forms a small fraction of total NO_x expected after combustion in excess air (79% N_2) (Cembureau, 1999).

Emission of particulate matter

Particulate matter (PM) emissions are influenced by fuel composition and extent of combustion, especially PM with a median aerodynamic diameter ≤ 10 µm (PM10) or ≤ 2.5 µm (PM2.5) (Gibson, 2009). Total suspended particulates (TSP) which include all airborne PM (~ < 60 µm) are a function of the percentage of ash or mineral matter in the fuel, coupled with the unburned carbon resulting from incomplete combustion. It is also expected that a higher volatile content will reduce unburned carbon in the products of combustion leading to reduced TSP. Gases such as SO_2, NOx, and VOC's can also transform in the atmosphere to form secondary fine PM and ground-level ozone (O_3) by a number of chemical reactions (Tillman, 2012).

Emission of volatile organic compounds

The extent of thermal degradation of produced VOC's, such as benzene, xylene and toluene, is largely dependent on the prevailing temperature and residence time. At temperatures above 1000°C, and favorable retention time within this zone, the mixture of organic vapour from devolatilization of the solid fuel and air is oxidized to form CO, CO_2, NOx and H_2O, thereby removing harmful VOC's in emissions. Emissions of VOC's from the kiln stack are therefore more attributable to the release of the organic content of raw materials before reaching the high temperature zone of the kiln than to incomplete combustion of the fuel (Cembureau, 1999).

IMPROVEMENT OF ENVIRONMENTAL QUALITY BY USING ALTERNATIVE FUELS

The usage of Alternative fuels in cement manufacturing helps in reducing the emission, along with it plays role in significant ecological benefits of conserving non-renewable resources (Rahman, et al; 2013). The primary benefit of using alternative fuel is potential reduction in CO_2 emissions at plant level, as alternative fuels have lower carbon contents, on an energy basis, than fossil fuels. Co-processing in the European cement industry resulted in an approximately 18 percent reduction in conventional fuel (mostly coal) use, a reduction of about 8Mt of CO_2 emissions each year, and a savings of about 5Mt of coal (Cembureau, 2009). The CHG emissions can be reduced as for prevention of methane gas. Usage of alternative fuels also which will, reduce the environmental impacts of the extraction (mining or quarrying), transporting, and processing of fossil fuels by reducing of coal imports and conservation of fossil fuels (CII, 2016). Due to its advantages in terms of combustion of waste & no residual left over, hazardous wastes in alternative fuel can be disposed safely. Flame temperatures of up to 2000°C and high residence times provide good conditions for destruction of organic compounds also (Ghosh, et al; 2019). Considering the morphology of the waste used as alternative fuel, the environmental potential was analyzed not only for the waste sector but also for clinker production, which allows achieving the task – to estimate the environmental potential for co-processing of waste implementation (Kleshchov, et al; 2019).

IMPACT OF CO-PROCESSING ON PUBLIC HEALTH

Co-processing of alternative fuel in cement production can successfully convert the hazardous and other toxic materials into clinker, without producing any ash. Those hazardous and toxic materials dumped in the landfill site can cause various adverse effects on humans residing nearby the landfill sites. The various wastes in landfill sites generates foul smelling, which makes various contaminations, produces hygiene related issues and also spreads to the farming lands by leakage. The foul smelling of various toxic waste materials can also cause different health related problems for the people of near residence has been observed. An increased occurrence of health symptoms such as sleepiness, fatigue, headaches etc among residents near waste sites has consistently (Vrijheid; 2000). Leachate problem, forming from rain falling on top of the landfill or from groundwater addition, can also cause severe concern to the environment through producing toxins like Methane, Carbon Dioxide Organic Acids, Alcohols Aldehydes and many more and hence affecting public health . Water storage adjacent to the dump sites becomes contaminated due to dumping of solid hazardous wastes. Marine litter poses a major danger to charismatic species as Plastic bags and abandoned nets are a risk to turtles, dolphins and seals. Plastic ingestion can guide to ulceration gastrointestinal blockages and internal perforation and death. About 15 % of the species affected through entanglement and ingestion are on the IUCN Red List (Ten Brink et al, 2016). Plastic debris such as discarded or lost fishing gears are threats to coral reefs. Similarly, sea grass beds are also at risk as plastic debris pile up on them. This is how plastic debits and marine litters become a real threat to the marine environment (Das et al; 2020) .This polluted water can be accumulated to the human body through Chemicals in the food chain leached from some plastics used in food/beverage storage are harmful to human health which may cause blood infection; and reduced oxidative stress, reproductive system abnormalities, even cancer (Padgelwar et al; 2019). Co-processing in cement kiln contributes helps in efficient eliminating plastic waste disposal in open landfills, hence promotes in reduction in dumping of water sources nearby, which creates marine littering problem (UNEP; 2019) .So, utilizing these waste materials as alternative fuels in co-processing process of cement manufacturing helps in reduction of waste accumulation in dumpsites subsequently helps in blockage the flow path to the ocean and other water storage nearby and can solve the marine littering problem (Intercement, 2019).

CASE STUDY: Co-processing of NRPW in cement plant, Ariyalur, Tamil Nadu
[Visit date – Feb. 06 - 08, 2021]
A visit was organized as working under the project "OPTOCE", an initiative of Norwegian company SINTEF in collaboration with Jadavpur University, West Bengal to cement manufacturing plants at Ariyalur district, Tamil Nadu.
The project OPTOCE is about research on use of non-recyclable plastics as an alternative fuel in cement kilns results in reducing marine litter and landfills area. The objective of the visit was to provide us an insight knowledge regarding mainly the usage of AFR in the cement manufacturing process and its components, internal working of the company and opportunity to learn practically through interaction regarding the manufacturing, quality control, packaging in the plant, etc.

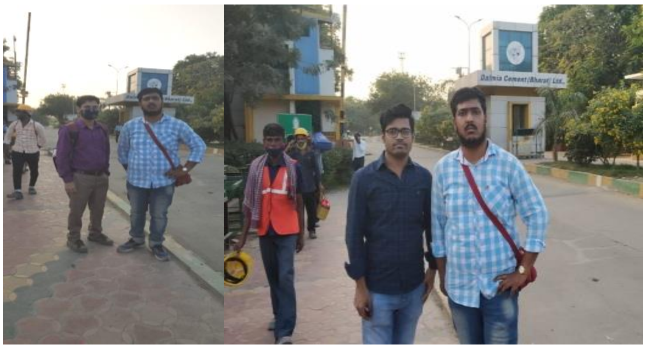

Figure (6): Picture captures at main entrance of cement plants in Ariyalur, Tamil Nadu.

Cement plants at Ariyalur in Tamilnadu take raw material (NRPW) from municipalities of Tiruchirappalli, Coimbatore, and Chennai. Private companies like Zigma segregate the plastic waste from dumpsites of mentioned places and send it to AFR plant of cement plants.
These private companies are handover the segregated waste to cement plants at zero cost and get subsidy from Government.
Cement plant take the responsibility for decomposition of these wastes without harming the environment. Dalmia also buy a huge amount of plastic waste from biscuit factories in Puducherry and paper mills near the plant. Plants receive the waste having size less than 500 mm and store it at the storage facility of AFR handling plant.
These wastes can't use directly for co-processing as an alternative fuel due to limitation of size and quality. For co-processing samples are taken from each vehicle and get tested. Dalmia cement plant has a separate lab for sampling and testing of the incoming waste from different location.

Table 1: Parameters and testing methods for sample of waste in cement plant

Sl. No.	Parameters	Unit	Test Method
1.	Ash content	%	Muffle Furnace (850^0C)
2.	Moisture content	%	Oven (188^0C)
3.	Gross calorific value (GCV)	Kcal/Kg	Bomb calorimeter
4.	Chlorine (Cl)	%	Volumetric analysis
5.	Sulphur (S)	%	Gravimetric analysis

After getting the test result, data matched with the government guidelines on co-processing of waste in cement kiln and allowed for co-processing or rejected to the respective supplier. Waste loaded to the conveyers attached with shredder and shredded up to size of 75 mm. these shredded waste passes through magnetic separator where all the ferrous part get removed. After magnetic separator it passes through a mechanical screen called screening of waster as per the required size. Screen segregates these wastes into more than 75 mm and less than 75 mm size. Waste having size of more than 75 mm get rejected and send again to the shredder through conveyors.

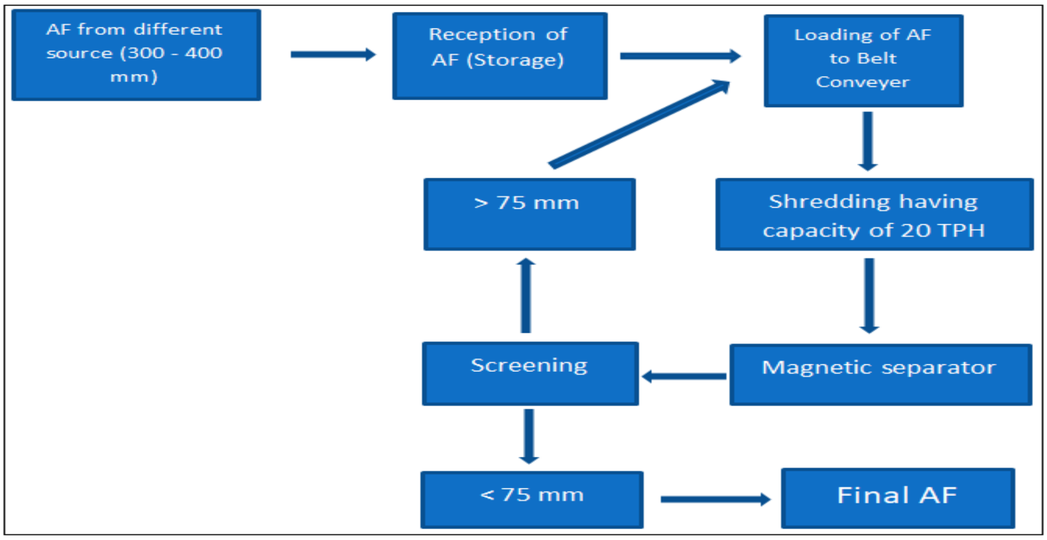

Figure (7): Flow chart for Pre-processing of Alternative Fuel in Cement plants

As per the data available in report of CPCB on plastic waste generation in 2018-19, total non-recyclable plastic waste co-processed in cement kiln is 1.38 million tons, which is around 4% of total plastic waste generated (3.36 million tons) and 38.4% of total plastic waste used for material and energy recovery in India (2018-19).

Table 2: Non-recycled plastic waste co-processed in cement kiln in India

Sl.	States & Uts	Amount of non-recycled plastic waste co-processed in cement kiln (Tons)
1.	Goa	6057.62
2.	Gujarat	86553.62
3.	Karnataka	25500
4.	Madhya Pradesh	18283
5.	Puducherry	272
6.	Telangana	1500
7.	Uttar Pradesh	21.37
Total		**1,38,188 tons**

Source: CPCB, 2019

DISCUSSION

Daily accumulation of waste materials is increasing heavily on a daily basis with increase in population, which are filling up landfill sites. We are presently running out of further land to be used as landfill sites. These waste materials are piling up, and causing heavy damage to our environment. So there is an immediate need to address this concern. Co-processing is one of the way to solve this problem. It can use up the waste materials and stop them from piling up in the landfill sites. Co-processing of alternative fuel in cement production can successfully convert the hazardous and other toxic materials into clinker, without producing any ash. Those hazardous and toxic materials dumped in the landfill site can cause various adverse effects on humans residing nearby the landfill sites which include reproductive system abnormalities, impaired brain, neurological functions, cancer, cardiovascular system damage and other sever issues. Not only, has human health, marine littering problem has impacts on marine life, it effects on Toxicology, Impacts on Inaction on Shipping and Tourism, and many more.
But further research and technology should be made in order to implement it successfully. Also, it should be economical. Standards on quality, environment, health of workers are also some concerns which needs to be addressed. It can be also analysed that if waste materials are used as fuel, amount of conventional fuel consumed daily, can also be readily reduced.

CONCLUSION

1) From the literatures and the case study on wastes from the various landfill sites, utilising as alternative fuels and raw materials (AFR), it can be evident that, alternative fuels not only minimise the overall cost of cement production and enhance the production efficiency, but also it helps to reduce the emission and upgrade the environmental quality around the cement plant.
2) Moreover the co-processing also promotes resource circularity by sustainable disposal of wastes from various landfill sites without formation any residual left over. Utilising waste as AFR also helps waste reduction in landfill sites, which will further promote waste reduction in landfill sites and helps in reduction of marine littering. In both ways
3) co-processing can help to cut down age old problem of land scarcity, as well as it will take a step forward to enhance the environmental quality to the surroundings of land filling site and the sea or river water nearby.
4) Reducing landfill volume, wastes can be collected directly from the source, like household or and market; where fresh waste are generated. So the waste quality will be enhanced as they can be collected separately and its pure form and also, cost for the waste dumping in dumpsites will be minimise.
5) So, encouraging co-processing of AFR can enhance the Thermal Substitution Rate (TSR) in Indian cement and which can help economically as well as environmental point of view.

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PL3.

GREEN SYNTHESIS OF IRREGULARLY SHAPED GOLD NANOPARTICLES

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***Abstract**: The synthesis and application of new nanomaterials have become a key technology for many fields, like electronics, engineering, optics, computer science, sensorics, and biomedicine. Therefore, there is an interest to new cost, energy, and time effective methods for preparation of new nanomaterials.*
Biomedical application of nanomaterials is limited not only by control over the size and morphology of nanoparticles but also by their biocompatibility. However, the trusted protocols normally involve the application of reducing agents (sodium borohydride, methoxy polyethylene glycol, potassium tartrate, etc.) and capping agents (sodium dodecyl benzyl sulphate, polyvinyl-pyrrolidone, etc.) which are usually toxic. The unavoidable presence of toxic compounds in nanocolloid systems limits the applications of the nanoparticles for biological systems.
The aim of this study is the analysis of recent reports and evaluation of perspectives for the development of green methods of metal nanoparticles for biomedical applications.
Numerous physical-chemical methods are in use for the characterization of nanoparticles: ultraviolet and visible spectroscopy for characterization of surface plasmon resonance, infrared spectroscopy for characterization of capping agents, surface and transmission electron microscopy and atomic force microscopy for size and shape studies, energy-dispersive X-ray spectroscopy for chemical composition determination and dynamic light scattering for size determination.
Analysis of recent studies have shown that the extracts of selected plants (juniper, goldenrod, spearmint, lemon balm etc.) can be successfully used for preparation of biocompatible plasmonic materials with response in infrared region what make them attractive for biomedical applications.

***Keywords:** nanomaterials, nanoparticles, phytosynthesis, plasmonic materials, spectroscopy*

INTRODUCTION

Nanomaterials have become an important branch of modern science due to the unique physical and chemical properties. Due to their unusual electric, optical, magnetic, and catalytic properties, nanomaterials were recognised as a new and efficient method for the creation of novel advanced materials.
Biocompatibility and optical properties (surface plasmon resonance) of noble metals nanoparticles open the possibility of its modern applications is in nanomedicine, which is currently being actively developed. Nanoparticles of noble metals are employed to deliver therapeutics and mediate heat and light to specific types of tissues [1]. The composition, structure, size, shape, and of metal nanoparticles define the optical and electronic properties which can be adjusted to utilisation for advanced photothermal therapy [2] and the controlled drug release systems [3].

Gold nanoparticles with their excellent chemical stability and biocompatibility are suitable for biomedical applications. However, gold nanoparticles with reasonably small size (< 60 nm) exhibit their surface plasmon resonance in the range of 510-560 nm, i.e., far beyond the biological transparency window of 650-1350 nm. This region is divided into two optical near-infrared ranges, at λ = 650-850 nm and 950-1350 nm, respectively. The effective utilisation of nanoparticles for photothermal therapy because of the deeper penetration of long-wave radiation requires nanoparticles with response in the second near infrared range [4]. By this reason, the significant interest of scientist is dedicated to the synthesis of gold nanoparticles with regular (spherical) and irregular shapes (non-spherical) – spherical [5, 6], nanorods [7], nanoshells [8], nanostars [9], nanoprisms [10], nanotriangles [11, 12], and nanohexagons [13].
Numerous syntheses methods for the synthesis of metal nanoparticles were developed in recent decades: hydrothermal synthesis, coprecipitation, microemulsion, inert gas condensation, ion sputtering scattering, microwave, pulse laser ablation, sol-gel, spark discharge, sonochemical, and biological synthesis. Physical methods usually require the utilisation of highly expensive equipment and do not provide the control over parameters of final nanoparticles (size and shape). Therefore, traditional wet chemistry methods were recognised as more preferable. However, there is often a need to use toxic reducing and capping agents which stay in resulting nanocollods. As result, such nanoparticles show high cytotoxicity towards living organisms and are unsuitable for application in biomedicine.
Biological methods of nanoparticles synthesis which are consist in the application of biological materials (plants, fungi and bacteria), can be considered as a possible alternative. Such methods are also cost- and time-effective and environmentally friendly. One of the routes for obtaining biocompatible non-spherical gold nanoparticles in the greenest manner is phytosynthesis - the using of plant extracts and natural surfactants [14].
Despite the extensive studies, the wide application of photosynthetic protocols is restricted due to many components presented in the plant extracts and the limited knowledge on the formation mechanism of non-spherical nanoparticles [15].
This study aims to consider the recent state in the development of the phytosynthesis protocols for preparation of gold nanoparticles with optical activity in the near infrared region.

MATERIALS AND METHODS

Chemicals

Ethanol (C_2H_5OH, 96% p.a., ITES Vranov, Slovak Republic (SR)) and acetic acid (CH_3COOH, 99% p.a., Centralchem, SR) were used for extraction of the plant material. Sodium hydroxide (NaOH) 0.1 mol/L standard solution (Normanal, Lach-Ner, s.r.o., CR), silver nitrate ($AgNO_3$, ITES Vranov, SR) and chloroauric acid hydrate ($HAuCl_4 \cdot H_2O$, Sigma-Aldrich, St. Louis, MO, USA) were used for nanoparticle synthesis. Double-distilled water was used for extract preparation, reagents preparation and nanoparticles synthesis.

Synthesis of nanoparticles

Aqueous and aqueous-ethanol leaves extracts of juniper (*Juniperus communis*) [11], peppermint (*Mentha piperita*) [12], and goldenrod (*Solidago canadensis*) [13] were applied for the synthesis of gold nanoparticles. Syntheses were performed at an ambient laboratory temperature (20-25 °C), by the direct interaction of the plant extracts with 1 mM $HAuCl_4$ aqueous solution under continuous stirring. Amounts of extracts were normalized to dry matter content, with the estimation of 0.25-1.5 mg/ml of the dry matter in the final solution. In a typical synthesis, the estimated volume of plant extract was diluted by double distilled water followed by addition of 1 mM $HAuCl_4$ solution.
Aqueous and aqueous-ethanol extracts of elderberry fruits (*Sambucus nigra*) [6] were used for preparation of silver nanoparticles by direct interaction of extract with 1-10 mM $AgNO_3$ at 55ºC for 24 hours. The pH of reaction mixture was adjusted with NaOH solution.

Characterization of nanoparticles

UV-Vis spectra were collected by Shimadzu UV-1800 spectrophotometer with matched 1-cm quartz cells. Attenuated total reflectance Fourier transform infrared spectra were acquired with a Shimadzu Prestige 21 instrument equipped with a single reflection accessory and ZnSe ATR crystal (PIKE Technologies, USA). Transmission electron microscopy imaging was conducted with a JEOL JEM-2100F microscope equipped with attachments for electron-dispersive X-ray analysis and a GIF TRIDIEM post-column energy filter for the acquisition of energy-filtered images and selected area electron diffraction. TEM specimens were prepared by dropping a sonicated aqueous suspension of AuNPs on a carbon-coated copper grid and followed with drying it under the infrared lamp. Transmission electron images of different magnifications were captured at a maximum acceleration voltage of 200 kV. A selected nanometer-sized object was imaged by atomic force microscopy with a commercial Nanoscope III instrument (Digital Instruments, Santa Barbara, CA).

RESULTS AND DISCUSSION

UV–Vis spectroscopy was applied to follow the nanoparticles formation through the observation of the position and shape of surface plasmon resonance peaks (Fig. 1). The spectrum of silver nanoparticles prepared with elderberry extract contains the single surface plasmon resonance peaks at 395 nm which confirm the formation of spherical nanoparticles. However, the spectra of gold nanoparticles prepared using different extracts are different. In some cases, the second maximum in the near infrared range occurs. For example, some extracts (clove [5], elderberry [6]) produce spherical nanoparticles (Figure 1, red). But others, support the formation of both spherical [11], peppermint [12], and goldenrod [13]) and non-spherical nanoparticles (blue). The occurrence of absorbance in the near infrared range depends not only on the selected extract, but also on the extract to Au^{3+} ions ratio. Usually, low concentrations of Au^{3+} ions are lead to the formation of spherical nanoparticles. The absorbance in the near infrared range increases with the concentration of reagents. But only to a certain limit when the formation of macroparticles occurs.

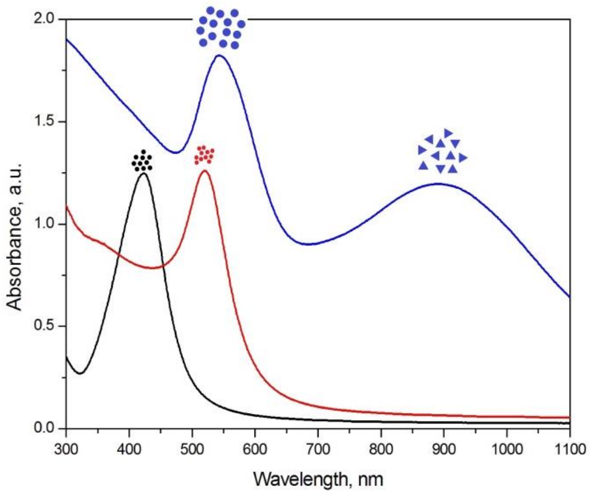

Figure (1): UV-Vis spectra of silver (black), spherical gold (red) and non-spherical (blue) gold nanoparticles prepared using plant extracts.

A simultaneous presence of highly monodisperse spherical silver (Figure 2a) and gold (Figure 2b) nanoparticles with diameter of 10 nm in nanocolloid solution are confirmed by transmission electron microscopy. As can be seen from the typical transmission electron microscopy image (Figure 2c), the average composition of gold nanocolloids shows the presence of triangular and pseudospherical particles in the nano-dimensions.

Energy dispersive X-ray analysis confirmed that the resulting morphological different nanoparticles are all gold nanoparticles (Figure 3). The smallest nanotriangles have 20–50-nm edge lengths.
Above-mentioned studies confirm the high polydispersity of nanoparticles. Phytosynthesised nanocolloids contain nanoparticles with different sizes regardless of the intensity of stirring during the preparation. This might be solved by fractionalisation of extract before the synthesis of nanoparticles. However, we need to learn the role of different components of extract more.
With this aim, the transformation of organic compounds in a reaction mixture was followed by Fourier transform infrared spectroscopy. The comparison of spectra [6, 11] suggests that reduction of Au^{3+} is followed with the oxidation of polyphenolic hydroxyl groups to carbonyl groups which play an important role in the stabilisation of nanoparticles.

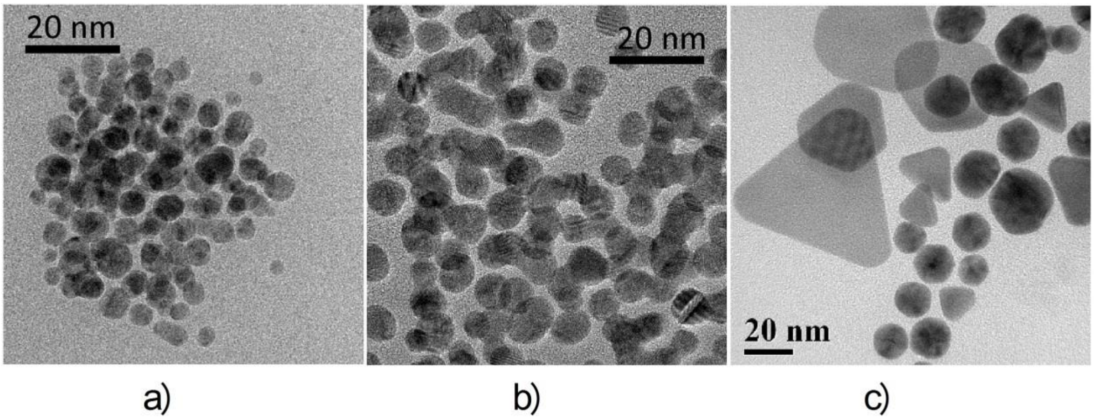

Figure (2): Transmission electron images of phytosynthesized a) silver, b) spherical gold and c) non-spherical gold nanoparticles.

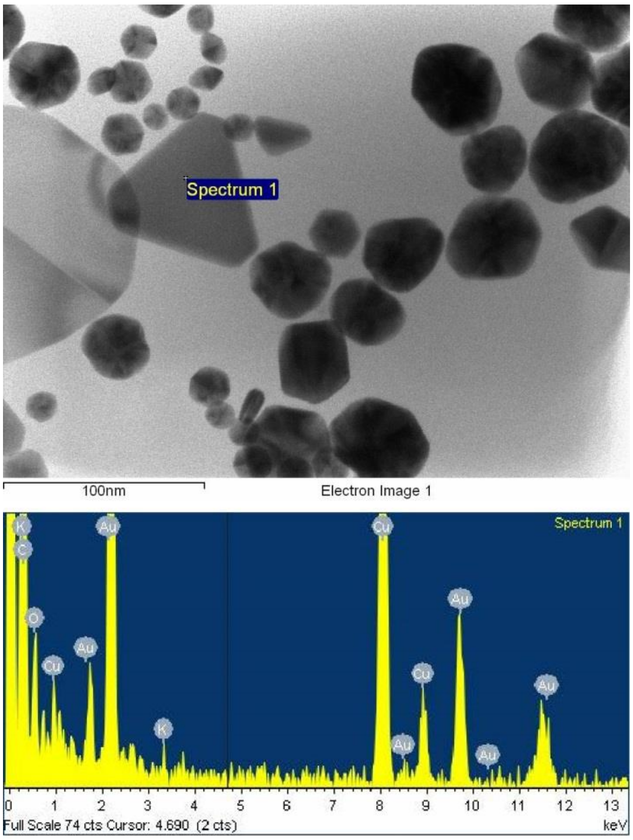

Figure (3): Transmission electron image (upper) and energy dispersive X-ray analysis (down) for gold nanoparticles prepared with goldenrod extract.

Other alternative for preparation of monodisperse irregular shape gold nanoparticles is further separation by centrifugation or electrophoresis.

CONCLUSIONS

The easy green protocols for synthesis of spherical silver and gold nanoparticles and irregular shaped gold nanoparticles are considered. It was observed that the utilisation of some plant extracts (*Syzygium aromaticum* L., *Sambucus nigra*) leads to the formation of almost perfectly spherical silver and gold nanoparticles. However, the application of the different ratios of the other aqueous and aqueous-ethanol plant extracts, like *Juniperus communis*, *Solidago canadensis*, *Mentha × piperita*, leads to preparation of the prismatic-shaped gold nanoparticles.
It was observed that the using of extracts of selected plants as reducing and capping agents lead to the formation of both spherical and non-spherical nanoparticles. The ratio between reagents (extract to Au^{3+} ions) can influence the ration of different shaped nanoparticles.
Fourier transform infrared spectroscopy analysis proves the presence of hydroxyl, carbonyl, and carboxyl groups on the surface of metallic nanoparticles. Increasing absorbance of the carbonyl bonds in the infrared spectra of nanoparticles confirms the significant role of polyphenols of the plant extracts in the self-organization and stabilization of metal nanoparticles.
The synthesis of nanoparticles is a prospective route for green preparation of biocompatible nanoparticles for biomedical applications, but there are still many questions which need to be answered.

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CHARACTERIZATION OF GEOSYNTHETICS AT VARIOUS ENVIRONMENTAL CONDITIONS

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Abstract: *In the last two decades, geosynthetics became a common application in Geoenvironmental engineering. Use of geosynthetics as a sustainable construction solution in civil engineering was reported by the Waste and Resources Action Program (WRAP, 2010), presenting case studies of sludge lagoon, tailing ponds and soil waste systems, wherein geosynthetic introduced reduction in cost and CO_2 emission. Geosynthetics enhance the soil quality, performance, strength parameters, and regularly pragmatic as a soil reinforcement in the exhibition of high loads while strengthening of tailing impoundments and landfills. It is an appropriate soil improvement system with unique advantages compared to other conventional ones. This manuscript introduces analyses and results of laboratory constant strain rate (CSR) test of 20%, 10%, 6%, 3%, 1% and 0.05% strain/min conducted on five specimens of geosynthetics polyester (PET) geogrids. Tests were performed according to the Multi-Rib tensile method of ASTM D6637. Main output acceptance features of tensile ultimate strength and the strain at break were justified. Stiffnesses at strain of 1% and 2% were derived. Sensitivity of strain rate of loading on strength, stiffness and non-linear properties of geogrid was inspected. Results of the measured tensile load-strain relationship for working are loads (strain ≤ 2%) and considerably acknowledged of linear simulation. The larger ultimate strength, the higher anticipated stiffness irrespective to loading strain rate. The derived stiffness at 1% is larger than corresponding for 2% strain, which reflects phenomenon of non-linearity and stiffness relaxation with progressive elongation. Behavior of reinforced soil is sensitive to stiffness rather than strength of geogrid.*

Keywords: *constant strain rate test, geosynthetics, non-linear, soil improvement, stiffness, strength*

INTRODUCTION

In the last two decades, geosynthetics became a common application in Geoenvironmental engineering. Use of geosynthetics as a sustainable construction solution in civil engineering was reported by the Waste and Resources Action Program (WRAP, 2010), presenting case studies of sludge lagoon, tailing ponds and soil waste systems, wherein geosynthetic introduced reduction in cost and CO_2 emission. Geosynthetics enhance the soil quality, performance, strength parameters, and regularly pragmatic as a soil reinforcement in the exhibition of high loads while strengthening of tailing impoundments and landfills. It is an appropriate soil improvement system with unique advantages compared to other conventional ones.
Hypostatically, the behavior of geosynthetic reinforced soil is complicated due to the mechanical complexity of component materials, their interactions, geosynthetic properties, and soil characteristics as well. Numerical modelling of geosynthetic reinforced soil systems is now extensively utilized for

the design, prediction of measured responses, and in research to generate synthetic data for the purpose of filling-in knowledge gaps on the behavior of these systems. (Yu and Bathurst, 2016).
In the engineering community, the available technical data sheets promoted by the geosynthetics manufacturers include only key design features of the ultimate strength and strain at break. On the contrary, the information about stiffness and secant modulus are not disclosed. These stiffness data are essential key input parameters for numerical modelling (Finite Element Method). Also, the non-linear stress-strain relationship of the geosynthetics is necessary to be investigated by conducting series of laboratory tensile test, perform date interpretation of the measured test results and identify the secant modulus at various strain domains.
In this research paper work, we are focused on conducting proper geosynthetics characterization necessary for numerical simulations that can be used to predict operational (working stress) conditions rather than incipient wall collapse. A contribution of such undertaken proper material characterization of geosynthetics is essential for numerical finite element modelling for multi- asset application of geosynthetics reinforced soils such as: (a) soil reinforcement in the exhibition of high loads while strengthening of tailing impoundments and landfills, (a) soil improvement in general applications, (c) slope stability deemed to achieve the stability of sharp slopes in the presence of low shear strength deposits, and (d) mechanically stabilized earth wall to act as an efficient retaining system in an economical design basis.

MATERIALS AND METHODS

a. OVERVIEW

The current study comprises 5 sets of nominated specimens Fortrac 35T, 55T, 80T, 110T, and 150T with varying strength and stiffness. Table 1 listed the physical properties of geogrid types as per the technical data sheet introduced by the manufacturer HUSKER. The standard raw material of these geogrids is the high-modulus polyester (PET), which of high stiffness, low creep and uniform product strength.
The geogrid specimens were tested on a tensile test machine under Constant Strain Rate (CSR) test in accordance with ASTM D6637, to investigate: (a) Tensile ultimate strength KN/m, and (b) strain at break. The secant modulus (J) KN/m at strain of 1% and 2% were derived based on the tensile force-strain relationship which is necessary for numerical modelling simulation. The CSR was varied to study the effect of strain rate of loading on the tensile strength and stiffness of the examined specimens of the PET geogrid.

Table 1: Physical properties for the tested geogrid types as per HUSKER data sheets

PROPERTY	TEST	**35T**	**55T**	**80T**	**110T**	**150T**
Mass/Unit Area (g/m^2)	ASTM D-5261	185	240	320	350	440
Aperture Size (mm)	measured	25 x 25	25 x 25	25 x 25	25 x 25	25 x 25
Percent open area	CWO 22125	70%	70%	65%	65%	62%
Ultimate wide width (MD) Tensile Strength (kN/m)	ASTM D-6637	**35**	**55**	**80**	**110**	**150**
Elongation at ultimate tensile strength –MD	ASTM D-6637	≤10%	≤10%	≤10%	≤10%	≤10%
Long term design strength- MD (kN/m)*	GRI GG4	18.8	29.5	42.9	59	81
Standard Roll Size (m)		5x100	5x100	5x100	5x100	5x100
Weight (kg)		114	141	182	192	205

* To be reduced by factors: Durability = 1.2 and Installation= 1.2.

b. TEST APPARATUS AND SPECIMEN PREPARATION

TESTOMETRIC M500- 50CT Universal Testing machine was used to perform the geogrid load-extension under Constant Strain Rate CSR. The apparatus comprises main tensile machine, pressure jack and computer control unit. The tensile machine consists mainly of clamps, sensors for recording the tensile force, and other sensors to monitor the extent of grips separation. The clamps must be attached with suitable pressure to avoid slippage. Clamping pressure must be equal for all specimens under the same test to manage consistent judgment between the anticipated results. Figure (1) presents photo of the tensile test apparatus and specimen installation prior to start of strain loadings. According to the Multi-Rib tensile method of ASTM D6637 “Standard Test Method for Determining Tensile Properties of Geogrids by the Single or Multi-Rib Tensile Method"; the specimen is schematically illustrated at Figure (2) should be a minimum of 200 mm wide and contains five ribs in the cross-test direction by at least three junctions or 300 mm long in the direction of the testing. The outermost ribs are cut prior to testing to prevent slippage from occurring within the clamps as instructed by ASTM D6637, clause 8.2.4. Thus, the width of intact ribs is 120 mm. The room ambient temperature was 20°c during test procedures to avoid adverse impact of the temperature in the test results.

Figure (1): Constant Strain Rate CSR tensile test apparatus

Figure (2): Specimen configuration; Multi-Rib tensile method -ASTM D6637

STATISTICAL ANALYSIS AND GRAPHICAL PRESENTATION

Minimum of three to five single tests were performed on the Machine Direction (MD) for each specimen of Fortrac 35T, 55T, 80T, 110T, and 150T, in accordance with ASTM D6637 using the multi- rib tensile method. Wide scatter of Constant Strain Rate CSR of 20%, 10%, 6%, 3%, 1% to slow rate loading 0.05% strain/min. were performed. The CSR was varied to study the effect of strain rate of loading on the tensile strength and stiffness of the PET geogrid.

The tensile test results are necessary to address the main output acceptance features: (a) tensile ultimate strength kN/m, and (b) strain at break. The secant modulus (J) kN/m at strain of 1% and 2% were derived based on the tensile force- strain relationship which is necessary for numerical modelling simulation. Since the geogrid CSR tests were carried out at the same in-door ambient temperature of 20°C, temperature effect was not a factor in the test results accuracy.

RESULTS AND DISCUSSION

a. Tensile load versus strain test results

In accordance with ASTM D 4595 and after Hatami and Bathurst 2005; the determination of the ultimate strength is established on the peak strength measured during CSR of 10% strain/min. Based on the test measurement results, the Tensile Strength (kN) was derived by dividing the measured Peak Force by the width of intact ribs (120 mm). The Secant modulus kN/m at strain 2% was calculated by dividing the measured tensile force (at strain 2%) by 120 mm to get the tensile stresses then divided by 0.02 strain to result in the stiffness (secant modulus $J_{2\%}$).
A range of 1.92-1.75 was derived for Factor of Safety FOS which is safely covered the deemed long term strength as well as durability and installation factors (reductions) with wide acceptable margin as shown at Table 2. This FOS is calculated by dividing the mean measured tensile strength of the CSR tests listed in Table 2 by the long term design strength listed in Table 1 “physical properties of the examined geogrid as per the technical data sheet”. For example for 110T; FOS= 112.73/59.0= 1.92. Hence, results are in good agreement with manufactures technical data sheet.
The linear approximation of tensile load- strain response is considerable accepted in the strain domain up to 2% which covers a wide scatter of geosynthetics reinforced structures conditions under service (working) loadings. Sample of the test results for five single specimens at 20% /min of 35T, 55T, 80T, and 110T are presented at Figure (3).

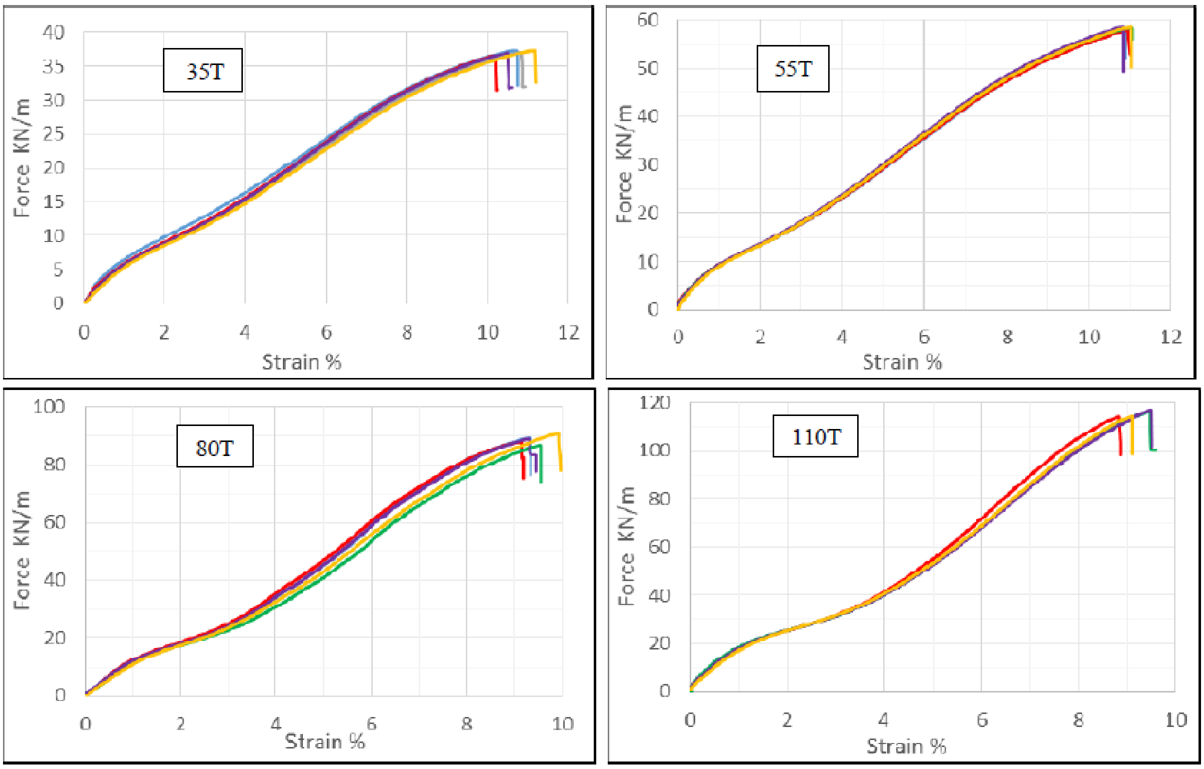

Figure (3): Force- strain response at CSR 20 %/min for the tested specimens of Fortrac 35T, 55T, 80T, and 110T

Table 2: CSR test results for 10% strain/min- ASTM D 4595

Type	Test	Peak Force (kN)	Tensile strength (kN/m)	Secant modulus kN/m (strain 2%)	Strain at peak %
35T	1	4.30	35.85	384	10.3
	2	4.41	36.74	377	10.6
	3	3.87	32.27	395	9.5
	4	4.21	35.07	389	10.3
	5	3.77	31.44	403	9.0
	mean	4.11	34.27	390	9.9
55T	1	7.23	60.28	546	11.5
	2	7.08	58.98	528	11.4
	3	6.12	51.03	512	10.7
	4	6.05	50.43	537	9.6
	5	6.10	50.83	558	10.8
	mean	6.52	54.31	536	10.8
80T	1	11.06	92.19	859	11.1
	2	9.69	80.75	822	10.0
	3	10.95	91.24	854	11.0
	4	8.81	73.42	923	9.9
	5	8.09	67.42	883	8.3
	mean	9.72	81.00	868	10.1
110T	1	15.7	130.83	1099	11.9
	2	15.08	125.67	1126	11.2
	3	14.06	117.13	1095	10.8
	4	11.48	95.67	1165	9.1
	5	11.32	94.33	1153	8.9
	mean	13.53	112.73	1128	10.4

b. Influence of strain loading rate on strength

The CSR was varied at rates 20%/min, 10%/min, 6%/min, 3%/min, 1%/min, and 0.05%/min to study the effect of strain rate of loading on the tensile strength and stiffness of the examined PET geogrid specimens. The influence of CSR on the tensile force- strain relationship and ultimate strength is illustrated in Figure 4 for the different geogrid examined specimens at rates 10%/min, 6%/min, 3%/min, and 1%/min where, the recorded tensile load increased as the CSR increased. The slow-rate rate 0.05%/min. was not conducted to the failure, as it consumed long time to reach the peak strain associated with failure. Testing was completed to approximate of 3% strain which cover wide range of the anticipated strain for service loading for various types of geosynthetic reinforced soil applications. Hence, the secant modulus at strain 2% was calculated accordingly.

Results show that, the higher CSR reveals increase of the measured secant modulus. The larger ultimate strength, the higher anticipated stiffness irrespective to loading strain rate. Based on the measured tensile load- strain relationship, the linear approximation of secant modulus is considerably acknowledged in the strain domain $\leq 2\%$ which covers a wide scatter of geosynthetics reinforced structures conditions under service (working) loadings. Behavior of geosynthetics reinforced soil is sensitive to stiffness rather than strength of the geogrid.

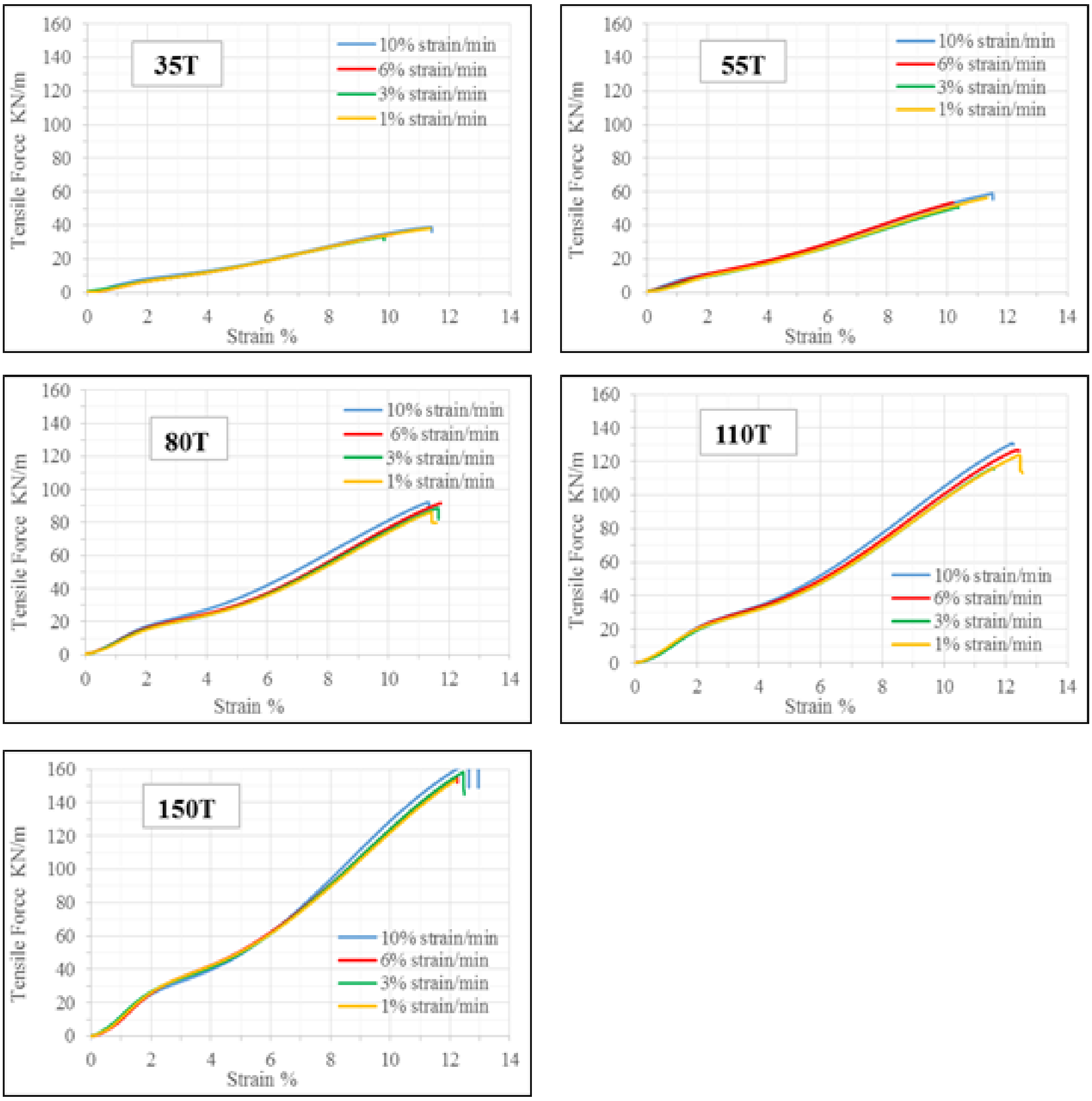

Figure (4): Influence of strain rate of 10, 6, 3 and 1%/min. on tensile force-strain relationship for Fortrac 35T, 55T, 80T, 110T, and 150T

c. Secant modulus (stiffness)

The sample total width is 200 mm, while the intact width is limited to 120 mm. According to ASTM D6637, clause 8.2.4 which stated that "within test methods A, B and C the outermost ribs are cut prior to testing to prevent slippage from occurring within the clamps. For those cases where the outermost ribs are severed, the test results shall be based on the unit of width associated with the number of intact ribs". Hence, calculation of the secant modulus ($J = EA_1$) at certain strain domain shall be the maximum tensile force divided by the total intact width per meter. The strength and stiffness of the geogrid is load, time, and temperature dependent. The room temperature is to be 20°c during testing procedure to avoid adverse influence of temperature on the test results. The literature (Boyle et al., 1996; Sawicki et al., 2002; Walters et al., 2002), stated that the geogrid is affected by strain rate of loading. After Hatami and Bathurst (2005), the determination of the ultimate strength, characteristics, and secant modulus are based on the results of CSR for 10% strain/min- ASTM D4595.
Accordingly, at CSR 10%/min., the derived secant moduli for stain domain of 2% are 390, 536, 868, 1128 and 1396 kN/m for types 35T, 55T, 80T, 110T and 150T, respectively. The resulting secant moduli for strain domain up to 1% are 521, 729, 1104, 1583, and 1583 kN/m for specimens 35T, 55T,

80T, 110T and 150T, respectively. Figure (5) demonstrates the trend between ultimate tensile strength for the examined specimens of Fortrac 35T, 55T, 80T, 110T, and 150T under CSR 10%/min. and the resultant secant modulus $J_{2\%}$ for strain domain 2% as well as secant modulus $J_{1\%}$ for strain domain 1%. For all examined geogrid specimens, the revealed modulus at domain 1% is relatively larger than corresponding for strain domain 2%, which reflects the phenomenon of the non-linearity and relaxation of geogrid stiffness with progressive elongation (strains). The higher induced strain, the relatively lower anticipated stiffness is. As reported by Hatami and Bathurst 2005, the linear approximation of tensile load- strain response is considerable accepted in the strain domain up to 2% which covers a wide scatter of geosynthetics reinforced soil/ structures conditions under service loadings rather than the incipient collapse.
It should be pointed that, the performance of geosynthetic reinforced structure is sensitive to the geogrid stiffness rather than the geogrid strength. Values for secant modulus J at strain level 1% and 2% which were derived from the CSR tensile force- strain relationship results are utilized for derivation of the brought geogrid tensile forces based on the measured strains of the instrumented geogrid layers. Secant modulus of strain 2% is a necessary key input parameter for numerical finite element modelling of geogrid properties.

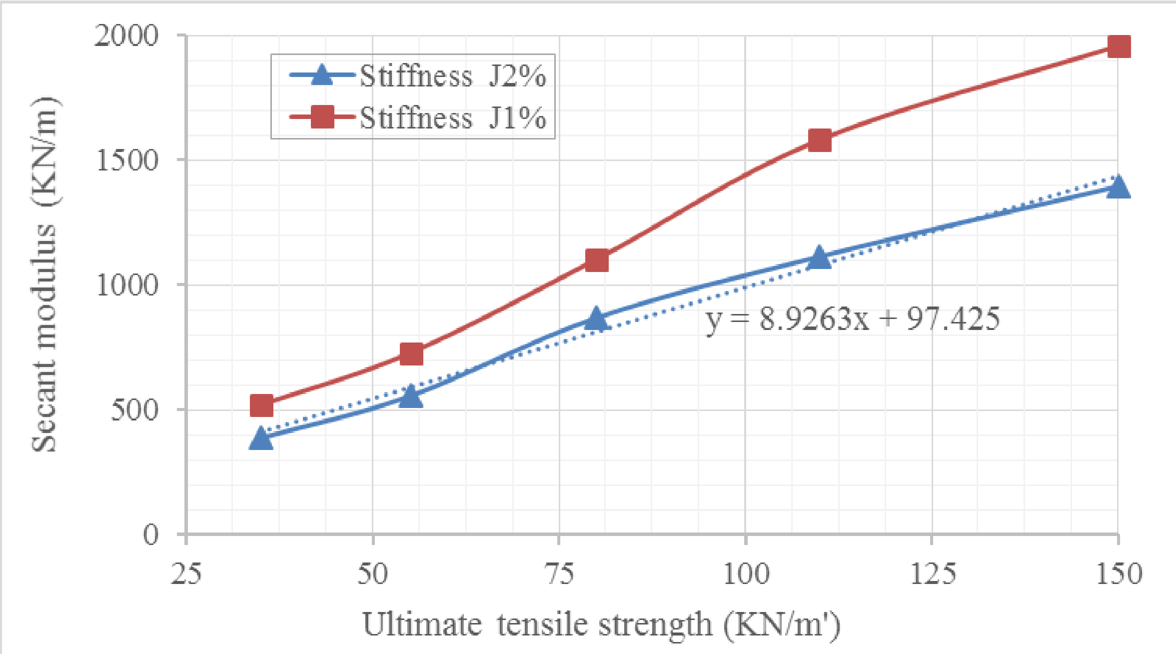

Figure (5): Trend of ultimate tensile strength versus secant modulus $J_{2\%}$ and $J_{1\%}$ for CSR 10%/min.

The performed CRS tests program did not involve testing of specimen 200T. Thus, correlated to the examined specimens CSR test results of 35T, 55T, 80T, 110T and 150T, a statistical prediction for the trend among strength, secant modulus $J_{2\%}$ at 2% strain and $J_{1\%}$ at 1% strain was used to estimate the secant modulus $J_{1\%}$ and $J_{2\%}$ of Fortrac 200 of value 2538 kN/m and 1893 kN/m, respectively. Ratios between stiffness ($J_{2\%}$) to stiffness ($J_{1\%}$) for various examined specimens and predicted stiffness values of Fortrac 200T were evaluated as presented in Table 3, where the predicted (fitted) secant modulus $J_{2\%}$ = 8.9263 x Ultimate Tensile Strength + 97.425.
From a practical stand point, such expression and chart (correlation) are simple and helpful to predict secant modulus (not published by manufactures to public engineering community) of geosynthetic PET geogrid based on nominal ultimate strength, which is essential key input parameter for numerical FE modelling purposes.

Table 3: CSR test for 10% strain/min. – secant modulus $J_{2\%}$ and $J_{1\%}$

	Tested specimens					Predicted
	35T	55T	80T	110T	150T	200T
Measured $J_{2\%}$ (kN/m)	390	536	868	1128	1396	
Fitted $J_{2\%}$ (kN/m)	410	588	812	1079	1436	1893
diff. (kN/m)	5.09	9.77	-6.51	-4.32	2.89	
Measured $J_{1\%}$ (kN/m)	521	729	1104	1583	1958	2538.9 (fitted)
$J_{1\%}$/ $J_{2\%}$ ratio	1.336	1.360	1.272	1.403	1.403	average = 1.355

The CSR was varied to assess the effect of strain rate of loading of 20%/min, 10%/min, 6%/min, 3%/min, %/min and 0.05%/min. on the secant modulus at strain 2% ($J_{2\%}$) and at strain 1% ($J_{1\%}$) as illustrated in Figures. 6 and 7, respectively.

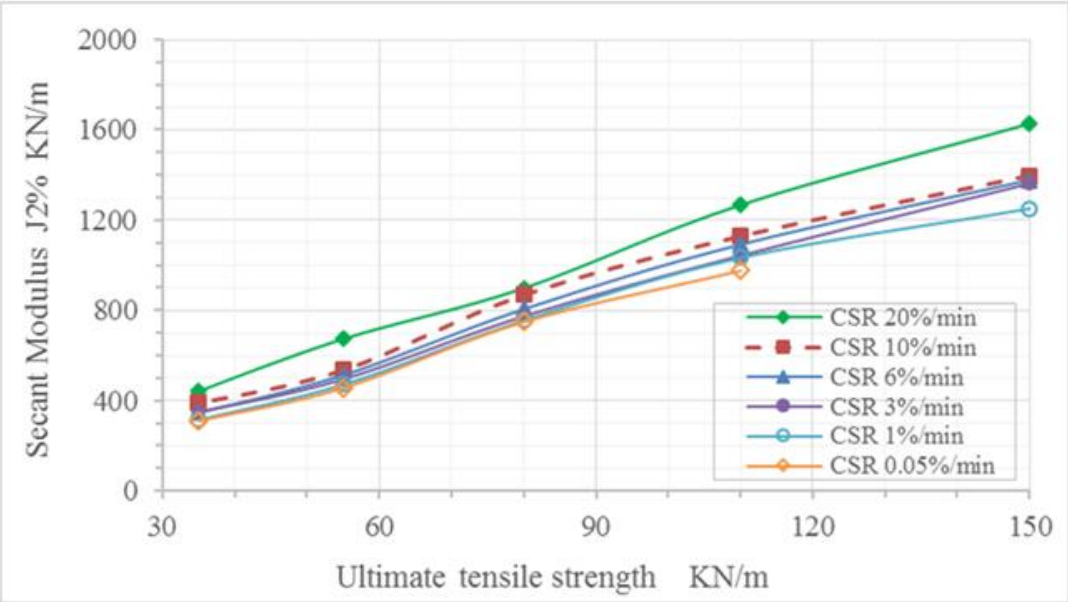

Figure (6): Influence of strain rate of 20, 10, 6, 3, 1 and 0.05 %/min. on geogrid secant modulus J2%

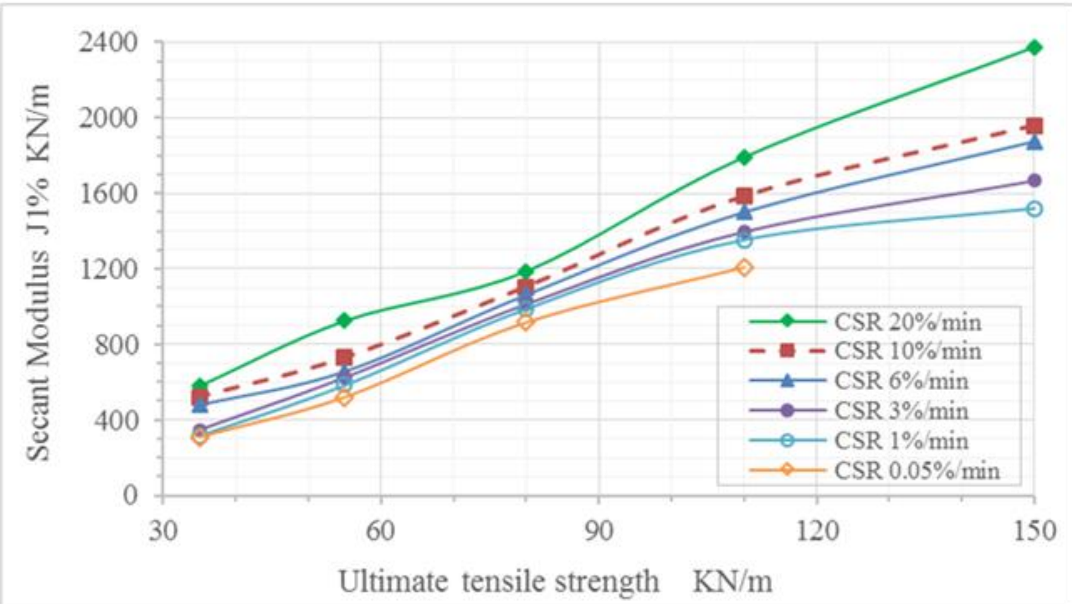

Figure (7): Influence of strain rate of 20, 10, 6, 3, 1 and 0.05 %/min. on geogrid secant modulus J1%

General trend of the test results shows that, the larger ultimate strength, the higher anticipated secant moduli, regardless of the strain rate of loading. For the different examined geogrid specimens, the higher CSR reveals an increase of the induced secant modulus J2% and J1% for the same examined specimen with variable ratios.

For practical assessment of the influence for strain rate of loading, the anticipated secant modulus for the examined specimens shall be normalized to the corresponding of the nominal representative test of rate 10%/min. of ASTM D4595 (after Hatami and Bathurst 2005).

Table 4 listed the normalized ratios of the variable strain loading rates to rate 10%/min. of $J_{2\%}$. For example; increasing rate to the upper bound (fast loading rate) 20 %/min provided an increased ratio of $J_{2\%}$ from 1.03 to 1.26. On the contrary, reducing rate to the lower bound (slow loading rate) 0.05 %/min. exposed a decreased ratio of $J_{2\%}$ from 0.80 to 0.87.

Table 4: Influence of CSR at modulus $J_{2\%}$ -normalized to 10% strain/min.

	CSR 20%/min.	CSR 6%/min	CSR 3%/min	CSR 1%/min	CSR 0.05%/min
150T	1.17	0.99	0.98	0.90	
110T	1.12	0.97	0.92	0.92	0.87
80T	1.03	0.93	0.89	0.87	0.86
55T	1.26	0.96	0.92	0.88	0.85
35T	1.13	0.89	0.89	0.81	0.80

Table 5 shows the normalized ratios of the variable strain loading rates to rate 10%/min. of secant modules $J_{1\%}$. For example; increasing rate to the upper bound (fast loading rate) 20 %/min provided an increased ratio of $J_{1\%}$ from 1.08 to 1.27. In contrast, decreasing rate to the lower bound (slow loading rate) 0.05 %/min. exposed a reduced ratio of $J_{1\%}$ ranged from 0.71 to 0.83.

Table 5: Influence of CSR at modulus J1%- normalized to 10% strain/min.

	CSR 20%/min.	CSR 6%/min	CSR 3%/min	CSR 1%/min	CSR 0.05%/min
150T	1.21	0.96	0.85	0.78	
110T	1.13	0.95	0.88	0.86	0.76
80T	1.08	0.96	0.92	0.89	0.83
55T	1.27	0.90	0.86	0.80	0.71
35T	1.11	0.92	0.88	0.76	0.76

CONCLUSIONS AND RECOMMENDATIONS

This manuscript introduces analyses, interpretation and results for series of laboratory constant strain rate (CSR) tests of 20%, 10%, 6%, 3%, 1% and 0.05% strain/ min. conducted on five sets of geosynthetics polyester (PET) geogrid specimens. Tests were performed according to the Multi-Rib tensile method of ASTM D6637. Main output acceptance features of tensile ultimate strength and strain at break was justified. Results are in good agreement with manufactures technical data sheet. Values for Secant modulus J at strain level 1% and 2% were derived from the tensile force- strain relationship results, which are necessary for numerical Finite Element modelling. Sensitivity for the influence of strain rate of loading on strength, stiffness and non-linear properties of geogrid was inspected for the different examined specimens. We are focused on simulations that can be used to predict operational (working stress) under static conditions rather than incipient collapse.

The main findings might be summarized as follows:

- The measured tensile force –strain relationship showed a non-linear behavior of the examined geosynthetics PET geogrid.

- The higher CSR reveals increase of the measured secant modulus. The larger ultimate strength, the higher anticipated stiffness irrespective to loading strain rate.
- Based on the measured tensile load- strain relationship, the linear approximation of secant modulus is considerably acknowledged in the strain domain ≤ 2% which covers a wide scatter of geosynthetics reinforced structures conditions under service (working) loadings.
- The derived stiffness at 1% is larger than corresponding for 2% strain, which reflects phenomenon of non-linearity and stiffness relaxation with progressive elongation.
- Behavior of reinforced soil is sensitive to stiffness rather than strength of geogrid.
- For numerical modelling purposes, a simple correlation chart was developed to predict secant modulus (not published to public) of geosynthetic PET geogrid based on the ultimate strength.
- For practical assessment of the influence for strain rate of loading on stiffness; tables were developed for the normalized ratios to rate of 10% strain/min. (ASTM D4595) for $J_{2\%}$ and $J_{1\%}$.

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KL3.

THE ENVIRONMENTAL IMPACT OF THE PRODUCED WATER ON THE UPPER AQUIFER IN THE WAHAT REGION, SIRT BASIN, LIBYA

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Abstract:
This paper based on data analysis by the Waha Oil Company of water samples collected from groundwater samples and produced water from Wahat region, Sirt Basin in Libya. This study provides a new pattern in the analysis of geochemical data which are in the form of a set of major ions and parameters such as acidity (pH), Total Solids Dissolved (TDS), total hardness of water (TH), the Residual Sodium Carbonate (RSC), Electrical Conductivity (EC), Magnesium Adsorption Ratio (MAR), the Kelly Ratio (KR), the permeability Index (PI), the Corrosivity Ratio (CR) and Total Organic Carbon (TOC). All that was performed using the statistical and analytical methods of data, and linking the results and information with each other will provide information on the sources of these elements and the water, also evaluate the water quality and suitability for drinking or irrigation. Based on the concentrations of the measure ions in the studied waters were ranged between desired range and the permissive range according to the World Health Organization. It appears that pH values of the groundwater are located within the safe range for drinking. To keep track of the suitability of the water for irrigation and drinking some of the important parameters and indicators used such as (RSC), (EC), (MAR), (KR), (PI), and (CR). Groundwater has been classified and showed that the water samples are classified as type (CaMgCl), while the produced water samples were classified as (NaCl).

Keywords: *Geochemical analysis, Groundwater, Libya, Pollution, Produced water, Wahat Region.*

INTRODUCTION

The environmental pollution is the interruption on environment system due to of the natural or anthropological activities, which destruct the environmental components such as air, water and soil. The pollution is one of the most serious challenges to scientists today as it has a negative impact on human and animal lives, hence this fact pushes the social and governmental organizations to focus their efforts to save the environment by education people via different sorts of public media and make possible all attempts to sustain the environment. This paper focusing on the negative impact of the surrounding oil fields to the Wahat (Oases) region, in particular Gialo, where produced water is spilled out in some places and hence polluted the surrounding environment where very sensitive desert plants are growing, which need special care to reduce any possible contamination. Similarly, the sensitive animals inhabit this arid to semiarid areas such as the study area. Due to the presence of several oil fields in the Wahat region (which covering an area of 2600 km^2) and witnesses the drilling of the first

Oil Well in Libya in 1958. Since that time the oil activities in forms of exploration, production, and development wells as well as pumping stations, oil tanks, pipe lines and produced water basins, in addition to the residual products from the oil/gas/water separators are increased in number. Which ultimately resulted in incorporate huge amount of pollutants such as hydrocarbons, heavy metals and radioactive elements? The impact of the produced water in Wahat region (i.e. Galo-Awjila- Ajkharah) is not according to what addressed by the international rule in discharging produced water.
As well as, to expose the effects of the produced water on the Wahat region which are ignoring the well-known environmental rules in the discharging or recycling operations to eliminate the produced water impact. Accordingly, the researchers should assess both the produced water and the groundwater in Wahat region in order to find the possible scientific solutions for sustaining the environment.

OBJECTIVES

1- Determine the different pollutants which are infiltrated from Oil fields to groundwater.
2- Inspect the impact of the oil pollution on groundwater of Wahat region, with particular attention on Galo.

LOCATION

The geochemical data used in this study are obtained from geochemical analyses performed in 2009 by Waha Oil Company, (6 samples from produced water samples; 3 samples from shallow groundwater; and 2 from deep groundwater) as indicated in (Fig. 1).

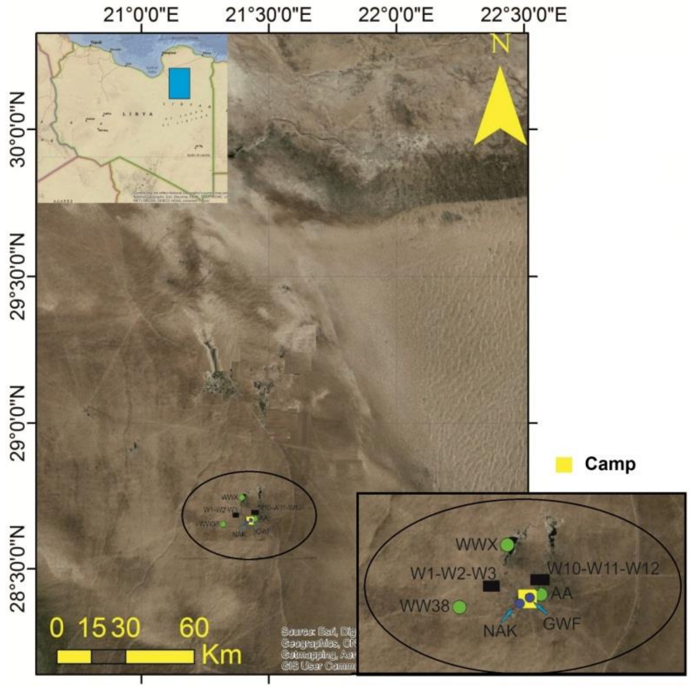

Figure 1: Location map of the analyzed water samples in 2009 from Wahat region, Libya.

GEOLOGICAL SETTINGS

The study area is located in largest sedimentary basin of Libya, which called Sirt Basin which has an area of 400.000km^2, and extending towards northwest-southeast as well as northward to the Mediterranean Sea. The Basin is architecture by faulting system with two major trends (NW-SE and E-W). The basin was formed between the beginnings of Late Cretaceous to the Early Miocene [1]. The study area is located in the northeastern part of Kalanshiyu province, which is the largest underground water region in Libya and seems to be hexagonal in shape and bound an area of 375.000 km^2, this province linked geologically with Sirt Basin via post-Eocene aquifer network, it consists of continental sands, clays in the southern part, but marine carbonates with evaporates in the north. There is large amount of stagnant and clean underground water with salty water which is partly linked to rainy seasons during (Pleistocene age) when the province was under high moisture climate.

STRATIGRAPHY OF THE STUDY AREA

Important previous studies in the study area were summarized from the results of drilled water wells and exploration wells, and reports of consultants, including [2] from the British Geological Survey (IGS, 1982), and the French Giffley [3], from which a group of geological strata was identified and acting as underground water reservoirs extending from Al-Sarir area to the north of the study area.

The penetrated sequence of this region consists of sandstones and loose sands intercalated with clay and limestone, in addition to some evaporitic rocks (Fig. 2). According to what presented in an unpublished report about the water assessment in the Galo / Awjilah region (2002) the sequence from bottom to top as follows:

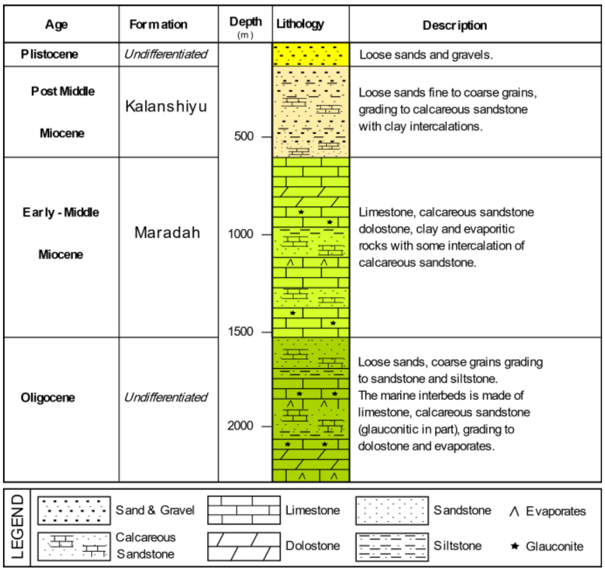

Figure 2: Lithostratigraphic Column of the Study Area.

Oligocene Interval (lower part): This interval consists of inter bedded coarse-grained sands to sandstone and siltstone reflecting the continental pulses of deposition. However, the marine intercalation of limestone and calcareous and glauconitic sandstones grading to dolostone with few evaporitic rocks are also presents. with thicknesses ranging from 242-697 meters depending on the site.

Previous studies showed that the water in this Oligocene reservoir is highly saline northwards including the oases area.

Maradah Formation (Early-middle Miocene): Maradah Formation in this study consists of limestone, sandy limestone, dolostone with a few clay and shale, which increase in importance in the southwest of the Sarir region with a thickness ranging between 121 - 697 m. The previous studies showed that there are hydraulic connections between the underground reservoirs in the area, as indicated by the leakage from bottom to top or sideways due to pressure contrast. The Groundwater flows from the south to the north / northeast in the direction of the topographical depressions of the oases following the hydraulic slope of 0.3%, and the water continues to flow northward until it intersects with subakhs in south of Jabal Al Akhdar and Sirt.

The near surface arrival of groundwater at the depressions allows the accumulation of salts as a result of evaporation and transpiration processes due to the hot dry climate in the oases region. The Oligocene reservoirs and the lower- middle Miocene reservoir contain high salinity water in several locations.

Kalanshiyu "calinchio" Formation (post- middle Miocene): It consists of medium grained sands with some fine and coarse grains locally becoming sandstones with thin intersections of clay with thicknesses up to 182m.This formation contains the first reservoir, which consists of the upper part of the Kalanshiyu Formation (upper sandstone) and the lower part represented by the formation formerly known as the Akalash Formation, whose importance lies north of the 29° line. The Institute of Geosciences and Scientific (IGS), according to According to Wright [2] reported that the post- middle Miocene reservoir increases in thickness from west to east in the study area by an amount ranging between 75 - 115m. Since the maximum recorded thickness is to the North of the Concession-103 Oil Field.

Kalanshiyu "Calinchio" Formation (the upper part): This interval consists of fine to coarse loose sands and coarse-grained surface sand interbedded with clay and sometimes sandy limestone. The thicknesses are ranging from 0 to 30m. This interval of the formation contains the Kalanshiyu reservoir (the upper part). This aquifer is usually reached in all water produced drilled wells.

PRODUCED WATER

Produced water is low in the beginning of the operation processes and gradually increases with the continuation of production over time. For example, studies conducted in the North Sea by TotalFinaElf, [4] indicated that the quality of marine water can deteriorate with an area estimated at about 10 km^2 from drainage points and produced waters usually have a greater density than marine waters because they contain metal ions and contain small quantities of hydrocarbon. However, they are self-degrading by bacteria, wave movement and sunlight, and thus their environmental impact can be considered spatially limited [4]. On the other hand, most of the oil-producing companies in the oil fields surrounding the study area "Al-Wahat" relied on dumping the produced water in the adjacent sand basins as a final destination.

GEOCHEMISTRY AND ENVIRONMENTAL GEOCHEMISTRY

Groundwater is a vital source of clean drinking and irrigation water. This is increasingly the case due to the effects of population growth and climate change, which are causing severe stress to surface water supplies in these areas [5, 6]. The chemical composition of groundwater is controlled by several factors that include composition of precipitation, anthropogenic activities, geological structure and mineralogy of the watershed and aquifers and geological processes within the aquifer medium [7]. Chemistry of groundwater is an important factor determining its use for domestic and irrigation purposes. Interaction of groundwater with aquifer minerals through which it flows greatly controls the groundwater chemistry. Hydrogeochemical processes that are responsible for altering the chemical composition of groundwater vary with respect to space and time. The hydrogeochemical processes of the groundwater system help to obtain an insight into the contributions of rock and soil–water interaction and anthropogenic influences on groundwater. These geochemical processes are responsible for the spatiotemporal variations in groundwater chemistry [8,9,10]. Groundwater chemistry, in turn, depends on a number of factors, such as general geology, degree of chemical weathering of the various rock types, quality of recharge water and inputs from sources other than water–rock interaction [10, 11,12,13]. The quality of ground water is the resultant of all the processes and reactions that have acted on the water from the moment it condensed in the atmosphere to the time it is discharged by a well. Therefore, the quality of ground water varies from place to place, with the depth of water table, and from season to season and is primarily governed by the extent and composition of dissolved solids present in it. Worldwide, aquifers are experiencing an increasing threat of pollution from urbanization, industrial development, agricultural activities and mining enterprises. In recent years, an increasing threat to ground water quality due to human activities has become of great importance [14]. The chemical analysis of the studied water samples includes a group of major ions such as Ca^+, Na^+, K^+, Fe^+, Mg^{2+}, Cl^-, HCO_3^- and SO_4^{2-} (Tables 1-3). The pH values range from 6.95 to 7.60 in the produced water, from 6.89 to 7.60 in the shallow water and from 7.44 to 7.54 in the deep water indicating acidic conditions. Differences in chemical composition of the studied water samples suggest different sources of the major ions. The sources of these ions could be natural or anthropogenic.

Table 1: Chemical analysis data (concentration in mg/l) of the studied produced water samples

Sample No.	W1	W2	W3	W10	W11	W12
pH	7.60	7.35	7.35	7.15	6.95	7.30
K	200.00	226.00	216.00	510.00	360.00	342.00
Ca	640.00	816.00	800.00	3280.00	2280.00	1840.00
Na	6000.00	9100.00	7700.00	20400.00	16000.00	13700.00
Mg	216.00	326.40	230.40	566.40	436.80	403.20
Cl	8997.20	13695.75	13525.81	39987.60	24626.00	24992.25
HCO_3	884.50	732.00	610.00	610.00	701.50	915.00
SO_4	2276.00	3392.00	386.00	1600.00	1400.00	472.00
SO_3	1897.00	2827.00	322.00	1333.00	167.00	393.00
Br	0.41	0.42	0.13	0.36	0.22	0.36
TOC	3.30	3.50	1.70	0.90	1.40	0.80
TDS	19317	27975	24438	27055	27165	43103

Table 2: Chemical analysis data (concentration in mg/l) of the studied shallow water samples

Sample No.	AA	WW38	WWX
pH	7.60	7.51	6.89
K	24.00	56.00	32.00
Ca	272.00	624.00	184.00
Na	450.00	610.00	420.00
Mg	105.60	129.60	67.00
Cl	989.69	819.75	909.72
HCO_3	122.00	152.50	30.50
SO_4	612.00	2123.00	367.00
SO_3	510.00	1769.00	305.00
Br	0.18	0.27	0.65
TOC	0.00	0.00	0.00

Schoeller [15] diagram provides a convenient way to present chemical composition of groundwater of a region. In the present study, plots of average composition in mg/l are presented in Fig. (3). The relative tendency of ions in mg/l shows Na > Ca > Mg > K and Cl > SO_4 > HCO_3. The average ionic composition of groundwater is also represented by Stiff diagram. The shape analysis of stiff diagram signifies dominance of Na-Cl, while Ca-HCO_3 and Mg-SO_4 are nearly equal in proportion.

Table 3: Chemical analysis data (concentration in mg/l) of the studied deep-water samples

Sample No.	GFW	NAK
pH	7.54	7.44
K	30.00	32.00
Ca	224.00	280.00
Na	390.00	530.00
Mg	110.40	105.60
Cl	869.73	1169.64
HCO_3	152.50	152.50
SO_4	541.00	533.00
SO_3	450.00	444.00
Br	0.04	0.09
TOC	0.00	0.00

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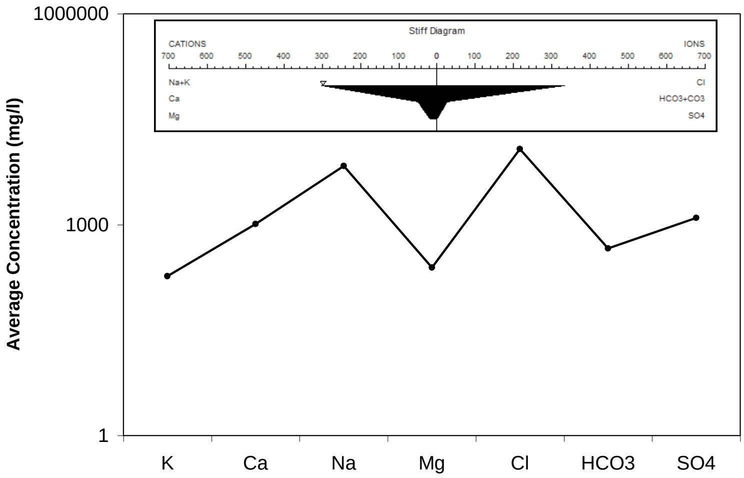

Figure 3: Schoeller diagram showing average composition of the studied water samples. Stiff diagram is shown in inset.

Statistical treatment

The statistical treatment of the obtained data involves descriptive statistics and correlation matrix using the SPSS© program (Tables 4-5). The correlation matrix suggests that the analyzed ions are possibly of common source (except SO_4).

Table 4: The descriptive statistics of the studied water samples (concentration in mg/l)

Ions	N	Min	Max	Mean	Std. Deviation
K	11	24.00	510.00	184.36	166.48
Ca	11	184.00	3280.00	1021.82	1009.87
Na	11	390.00	20400.00	6845.45	7236.51
Mg	11	67.00	566.40	245.22	165.76
Cl	11	819.75	39987.60	11871.19	13175.57
HCO_3	11	30.50	915.00	460.27	338.66
SO_4	11	367.00	3392.00	1245.64	1005.14

Table 5: Correlation matrix of the studied water samples

Ions	K	Ca	Na	Mg	Cl	HCO_3	SO_4
K	1.00						
Ca	0.95	1.00					
Na	0.99	0.96	1.00				
Mg	0.99	0.96	0.99	1.00			
Cl	0.99	0.97	0.99	0.98	1.00		
HCO_3	0.78	0.58	0.76	0.74	0.70	1.00	
SO_4	0.26	0.16	0.24	0.31	0.18	0.41	1.00

Sources

In the present study, the Cl/Na ratio is more than 1.17 in all the studied water samples (Tables 6-8). According to Shaltami [16] the calculation of none sea salt sulfate (nss-SO_4) values is based on equations as follows:

1) If Cl/Na ratio > 1.17

$$\text{nss-}SO_4 = [SO_4]_{sample} - [Na]_{sample} \times [SO_4/Na]_{seawater}$$

2) If Cl/Na equivalent ratio < 1.17

$$\text{nss-}SO_4 = [SO_4]_{sample} - [Cl]_{sample} \times [SO_4/Cl]_{seawater}$$

Table 6: Ratios of major ions in the studied produced water samples

Sample No.	W1	W2	W3	W10	W11	W12
Cl/Na	1.50	1.51	1.76	1.96	1.54	1.82
nss-SO_4	1916.00	2846.00	76.00	376.00	440.00	350.00
nss-SO_4/Na	0.32	0.31	0.01	0.02	0.03	0.03
K/Na	0.03	0.02	0.03	0.03	0.02	0.02

Table 7: Ratios of major ions in the studied shallow water samples

Sample No.	AA	WW38	WWX
Cl/Na	2.20	1.34	2.17
nss-SO_4	585.00	2086.40	341.80
nss-SO_4/Na	1.30	3.42	0.81
K/Na	0.05	0.09	0.08

Table 8: Ratios of major ions in the studied deep-water samples

Sample No.	GFW	NAK
Cl/Na	2.23	2.21
nss-SO_4	517.60	501.20
nss-SO_4/Na	1.33	0.95
K/Na	0.08	0.06

According to El-Omla and Aboulela [17] there are three main origins for dissolved solids in waters: marine, anthropogenic and terrigenic. Sodium is generally considered as a tracer of the marine source, although a small contribution of terrigenic origin arises due to the input of crustal aerosols [18, 19]. The source of sulfate is complicated, as it also has a marine origin [20, 21]. Consequently, these ions are used to identify the sources of the dissolved solids in the studied water samples.

In the studied samples, the very strong correlation between Na and Cl (r = 0.99, Fig. 4) is valuable to appraise relation with seawater. The Cl/Na ratio ranges from 1.34 to 2.23, all slightly higher than the average seawater value (Cl/Na=1.17). Since the existence of the abundant nss-SO_4 indicates an anthropogenic origin, waters with a Cl/Na ratio larger than 1.17 may have not only marine and terrigenic but also anthropogenic origins. In agreement with [16] this assumption seems to be eligible for the studied waters as confirmed by the negative weak correlation between the Cl/Na ratio and the nss-SO_4/Na ratio (r = -0.09, Figure 5).

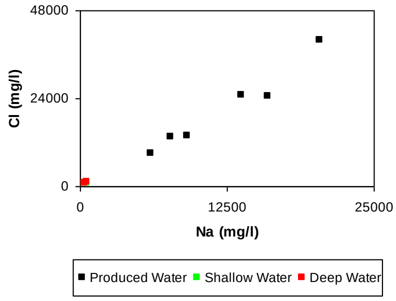

Figure 4: Relationship between Na and Cl in the studied water samples

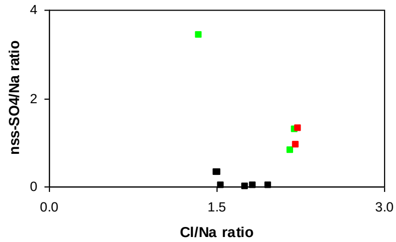

Figure 5: Relationship between Cl/Na and nss-SO_4/Na ratios in the studied water samples (For legend see figure 4).

In the present study, K is strongly correlated with Na (r = 0.99, Fig. 6), suggests the same or similar origin of K and Na, while it is weakly correlated SO_4 (r = 0.26, Fig. 7), suggesting a different origin. According to [10] the main sources of K in ground water include rain water, weathering of potash silicate minerals, use of potash fertilizers and use of surface water for irrigation.

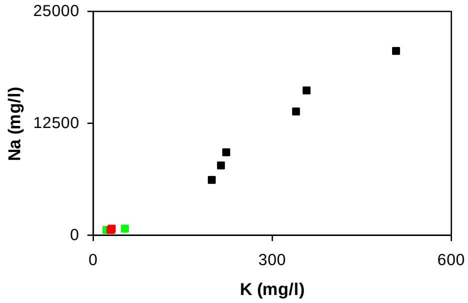

Figure 6: Relationship between K and Na in the studied water samples (For legend see figure 4).

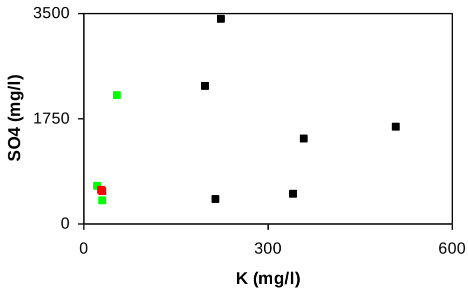

Figure 7: Relationship between K and SO_4 in the studied water samples

The molar Na/Na+Cl and Ca/Ca+SO_4 ratios are imperative to relate the genetic relationship [22]. Groundwater of the study area show that majority of the samples are in the alignment from seawater to domain of evaporation (Fig. 8). The influence of evaporation in the study area also show compatibility with [23] work, indicating high ionic Cl+SO_4 balance (13116.83 mg/l) in contrast to HCO_3 (460.27 mg/l) in the groundwater.

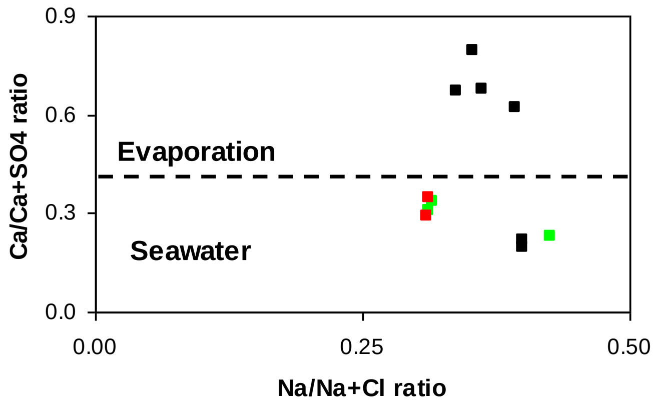

Figure 8: Molar Na/Na+Cl and Ca/Ca+SO_4 to differentiate water of different origin (fields after [22]). (For legend see Figure 4).

The concentration of SO_4 in the studied samples ranges from 367 to 3392 with a mean concentration of 1245.63 mg/l. Sulfate do not demonstrate any confident coherence to any of the analyzed major ions. Water containing more than 1000 mg/l sulfate are harmful for plants [24].

Chloride ion up to 70mg/l is considered safe [25]. It causes severe problem in the crops at concentration > 350 mg/l [26]. The content of Cl in the groundwater is best to study water-rock interaction. Groundwater having more HCO_3 and less Cl ions is the indication of natural water with no influx of pollution [27]. In the studied samples, the Cl concentration is much higher than the HCO_3 concentration. The average HCO_3/Cl ratio is valuable to discriminate either seawater intrusion or rock weathering [28]. The HCO_3/Cl ratio of study area, except the produced water samples, is much higher than the average seawater ratio (0.004). Probably the high ratio is due to the weathering of rocks. Mutual relationship of Cl/Cl+HCO_3 vs. logs TDS in form of [29] diagram signifies dominance of evaporation in the studied produced water samples (Figure 9).

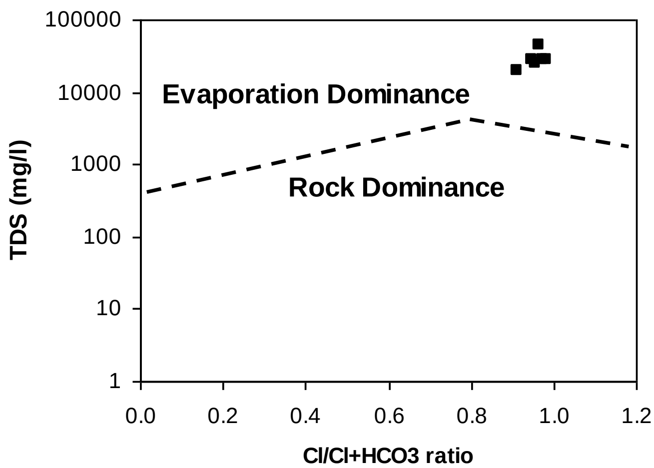

Figure 9: Dominance of rock and evaporation on Cl/Cl+HCO_3 vs. log TDS of the study area (fields after [29]) (For legend see figure 4).

The Ca and Mg ions are significant to appraise the magnitude of seawater contribution. The low Ca/Mg ratio (0.3) may indicate seawater influence [30]. In the studied water samples, Ca is strongly correlated with Mg (r = 0.96, Fig. 10), suggests the same origin of Ca and Mg. The Ca/Mg ratio ranges from 2 to 5.79, indicating, in agreement with [25], complexity in the budget of Ca and Mg of groundwater due to interaction with rocks and the arid climate of the study area. The Ca+Mg-HCO_3 in the present study range between 28.5 to 3236.4 with a mean of 938.18 indicating that Ca and Mg are driven from different sources.

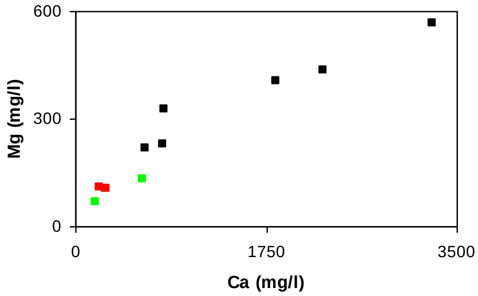

Figure 10: Relationship between Ca and Mg in the studied water samples (For legend see figure 4).

DRINKING WATER QUALITY

Power of hydrogen (pH): The pH of drinking water is an important parameter and may affect health of humans. The studied water samples display pH within a range of 6.89 to 7.60 with a mean 7.32, indicating all samples are suitable for drinking purpose as recommended by World Health Organization (WHO [31]).

Total Dissolved Salts (TDS)*:* In the produced water samples, TDS ranges between 19317 to 43103 mg/l with a mean of 28934 mg/l. All the produced water samples are over the safe limit (500 mg/l, by WHO, [31]).

Sodium (Na): The studied water samples display Na within a range of 390 to 20400 mg/l with a mean 6845.45 mg/l. All the water samples are over the safe limit (200 mg/l, by [31]). It is important to note that ~50 mg/l is desirable for human consumption [31]. High amount of Na in the drinking water has been linked to hypertension, cardiovascular disease, kidney damage, and calcium retention and bone density [32]. It regulates electrolytic balance in the human body and helps to transmit nerve impulses. Approximately 98% sodium is absorbed in the intestine, and excess sodium is excreted mainly by the kidneys with some loss occurring in sweat. In healthy adult humans at steady state conditions, urinary sodium excretion roughly equals to intake [33]

Potassium (K): The abundance of K in the waters of the study area varies between 24 to 510 mg/l with an average of 184.36 mg/l. The studied waters (except the produced water samples) are below the safe limit (100 mg/l, [31]). Potassium, the third most abundant mineral in human body, is the synonym for health insurer. Apart from acting as an electrolyte, K is required for keeping heart, brain, kidney, muscle tissues and other important organs of human body in good condition. High-K may also prevent or at least slow the progression of renal disease [34]. An increased K intake lowers urinary calcium excretion and plays an important role in the management of hypercalciuria and kidney stones and is likely to decrease the risk of osteoporosis. Low serum K is strongly related to glucose intolerance, and increasing K intake may prevent the development of diabetes that occurs with prolonged treatment with thiazide diuretics [35].

Calcium (Ca): Calcium in the waters of study area varies from 184 to 3280 mg/l with the mean of 1021.82 mg/l. The desirable and permissible limit of Ca in the drinking water is 75 and 200 mg/l [31]. All the studied water samples are over the safe limit (except samples W12 and WWX). Calcium is the most abundant metal in the human body. Calcium is present in the human body in amounts of about 1.2 kg. It is the main constituent of bones and teeth and it has key metabolic functions. It also assists the functions of nerves and muscles. Lack of calcium is one of the main causes of osteoporosis. Osteoporosis is a disease in which the bones become extremely porous, are subject to fracture. Calcium is also present in muscle tissue and in the blood. It is required for cell membrane development and cell division, and it is partially responsible for muscle contractions and blood clotting [36]. In order to stimulate these body functions a daily intake of about 1000 mg of Ca is recommended for adults [34].

Magnesium (Mg): In the present study, the lowest content of Mg is noted as 67 mg/l, while highest amount is recorded as 566.4 mg/l. Drinking water having Mg content ~30 is best and up to 150 mg/l concentration it is considered safe for human health [31]. The studied waters (except the produced water samples) are below the safe limit. Magnesium in drinking water may have a laxative effect, particularly with $MgSO_4$ on concentration above 700 mg/l [34]. However, the human body tends to adapt to this laxative effect with time. Magnesium is responsible for the activation of enzymes, maintain muscle and nerve function in human body. Early signs of Mg deficiency include loss of appetite, nausea, vomiting, fatigue, and weakness [37]. As Mg deficiency worsens, numbness, tingling, muscle contractions and cramps. Severe Mg deficiency can result in low levels of Ca and K in the blood [38]. Disease like hypertension, cholesterol level, type2 diabetes and cardiovascular disease can be control by taking high Mg [39].

Sulfate (SO_4): In the studied samples, the SO_4 values range from 367 to 3392 mg/l with a mean concentration of 1245.64 mg/l. [31] has established 200 and 600 mg/l as desirable and permissible limit in the drinking water. The studied waters (except the produced water samples and sample WW38) are below the safe limit. People not familiar to consume high levels of SO_4 ions can experience dehydration and diarrhea. Kids are often more sensitive to SO_4 than adults. Drinking high SO_4 containing water may cause laxative effect in human beings [40].

Chloride (Cl): The chloride concentration in the waters of the study area ranges from 819.75 to 39987.6 mg/l. These amounts are higher than desirable limit (250mg/l, by [31]). In humans, 88% of chloride is extracellular and contributes to the osmotic activity of body fluids. The electrolyte balance in the body is maintained by adjusting total dietary intake and by excretion via the kidneys and gastrointestinal tract [34]. Chloride is almost completely absorbed in normal individuals, mostly from the proximal half of the small intestine. Normal fluid loss amounts to about 1.5-2 liters/day, together with about 4 g of chloride per day. Most (90-95%) is excreted in the urine, with minor amounts in faeces (4-8%) and sweat (2%) [41]. Chloride toxicity has not been observed in humans except in the special case of impaired NaCl metabolism, e.g. in congestive heart failure. Little is known about the effect of prolonged intake of large amounts of chloride in the diet [42].

Bicarbonate (HCO_3): The quantity of HCO_3 ions in the water samples of study area ranges between 30.5 to 915 mg/l. [31] has established 300 and 600 mg/l as desirable and permissible limit in the drinking water. The studied waters (except the produced water samples) are below the safe limit. Bicarbonate is a major ion in our body, secreted by the stomach, it is necessary for digestion. When ingested, for example, with mineral water, it helps buffer lactic acid generated during exercise and also reduces the acidity of dietary components. Finally, it has a prevention effect on dental cavities. The most important effect of bicarbonate ingestion is the change in acid-base balance as well as blood pH and bicarbonate concentration in biological fluids. High HCO_3 bearing water is useful in the prevention of the recurrence of calcium oxalate and uric acid renal stones [43].

Total Hardness (TH): Water hardness is the measure of the ability of water to react with soap and produce froths. It is based on Ca and Mg salts and can be calculated as follows:

$$TH\ (mg/l\ CaCO_3) = 2.5\ Ca\ (mg/l) + 4.1\ Mg\ (mg/l)$$

In the studied water samples, TH ranges from 734.7 to 10522.24 with a mean of 3559.94 mg/l. [44] permit maximum 500 mg/l standard for drinking purposes. All the studied water samples have TH exceeding the maximum allowable limit. Both Ca and Mg are essential minerals for human health, deficiency or excess can result in adverse health consequences. Continuous use of hard water may cause cancer, cardiovascular disease, urolithosis and another kidney ailment [45].

IRRIGATION WATER QUALITY

Power of hydrogen (pH): The pH is one of the most important factors influencing the suitability of water for irrigation purpose. The normal pH range for irrigation water is from 6.5 to 8.4 [46]. The water samples of the study area within a range of 6.89 to 7.6 with a mean 7.32, indicating all samples are suitable for irrigation purpose.

Residue Sodium Carbonate (RSC): Residual sodium carbonate (RSC) exists in irrigation water when the carbonate (CO_3) plus bicarbonate (HCO_3) content exceeds the calcium (Ca) plus magnesium (Mg) content of the water [47]. RSC is a significant factor that has a great influence on the suitability of water for irrigation purposes. It is estimated from the following expression:

$$RSC= [(HCO_3) + CO_3)] – [Ca+Mg] \quad \text{(all concentrations are expressed in meq/l)}$$

When the RSC value is lower than 1.25meq/l, the water is considered good quality, while if the RSC value exceeds 2.5meq/l, the water is considered harmful. The samples of the study area have RSC between -177.77 to -0.09, indicating good quality water for irrigation purpose. Continuous use of waters having RSC more than 2.5 meq/l leads to salt build up which may hinder the air and water movement by clogging the soil pores [48].

Sodium Percent (Na %) and Electrical Conductivity (EC): Johnson and Zhang [49] used total salt (EC) and Na% to classify the irrigation water into six classes. Total salt concentration (i.e. salinity) is the sum of cations plus anions. The Na in irrigation waters is usually refers as Na percent and determined using the given equation [50].

$$Na\% = (Na^{+} * 100) / (Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}) \quad \text{(all concentrations are expressed in meq/l)}$$

Irrigation water with Na% > 60% may result in Na accumulation and possibly a deterioration of soil structure, infiltration, and aeration [51]. Figure (11) shows that the shallow and deep waters are classified as good, while the produced waters are classified as very poor and unsuitable.

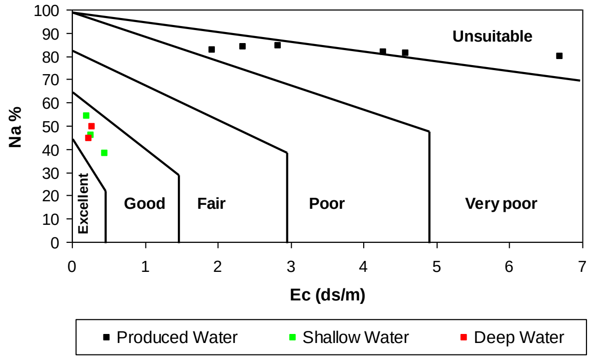

Figure 11: Classification of irrigation water on EC vs. Na% (Fields after [49])

Sodium Adsorption Ratio (SAR): The Na or alkali hazard is expressed in terms of sodium adsorption ratio [27]. Sodium absorption ratio (SAR) is the measurement of Na content relative to Ca and Mg in soil-water phase which influences soil properties and plant growth. Sodium adsorption ratio (SAR) can be determined from the following expression:

$$SAR = Na / \sqrt{(Ca+Mg)/2} \quad \text{(all concentrations are expressed in meq/l)}$$

SAR values <10 is classified as excellent for irrigation. Values in between 10-18 are good, moderate 18-26 and >26 are hazardous. The studied shallow and deep waters display SAR values <10 (6.1 in average), while the calculated SAR values of the produced waters lie between 52.17 and 86.31 (>26), indicating that the shallow and deep waters are classified as excellent for irrigation, while the produced waters are hazardous.

Magnesium Adsorption Ratio (MAR): Likewise, sodium adsorption ratio (SAR), magnesium adsorption ratio (MAR) is also considered as one of the significant parameters to evaluate irrigation water quality. The magnesium hazard (MAR) of irrigation water is proposed by Szabolcs and Darab [52] and redefined by Raghunath [53]).

$$MAR = [Mg^{2+} / (Mg^{2+} + Ca^{2+})]100 \quad \text{(all concentrations are expressed in meq/l)}$$

In the analyzed groundwater samples, the MAR values range from 22.35 to 45.1. According to Ayers and Westcot [54]) MAR values exceeding 50 is considered harmful and unsuitable for irrigation use and may causes infiltration problem.

Kelley's Ratio (KR): Kelley [55] and Paliwal [56] introduced an important parameter to evaluate irrigation water quality based on the level of Na measured against Ca and Mg. Waters with KR <1 are suitable for irrigation. The studied shallow and deep waters display KR values <1 (0.92 in average) indicating their suitability for irrigational uses, while the calculated KR values of the produced waters lie between 4.2 to 5.82.

Permeability Index (PI): Continuous use of high salt bearing irrigation water may reduce soil permeability [57](Singh and Singh, 2008). Such problem can be assessed by computing PI of the irrigation water from the expression, suggested by Doneen [58].

$$PI = [(Na^{+} + HCO_3^{-}) / (Ca^{2+} + Mg^{2+} + Na^{+})]\ 100 \quad \text{(all concentrations are expressed in meq/l)}$$

According to this classification, irrigation water with high permeability (>75%) is classified as class I; class II has permeability between 50-75%; while class III has permeability <25% and unsuitable for irrigation purpose

[59]. On the basis of PI classification, the shallow and deep-water samples are class II (PI ranges from 43.35 to 56.78), while the produced water samples belong to class I (PI ranges from 81.68 to 88.58).

Corrosivity Ratio (CR): It is the tendency of water to corrode steel pipelines during the transportation of irrigation water or deposition of scaling film on the pipes. Alkalinity, Ca, TDS, pH and temperature is mainly responsible for corrosion. According to Tripathi *et al.,* [60] the CR is expressed as:

$$CR = [135.5\ Cl + 2(SO_4/96)] / 2[(HCO_3 + CO_3)/100] \quad \text{(all concentrations are expressed in meq/l)}$$

The groundwater with corrosivity ratio < 1 is considered to be safe for transport of water in any type of pipes, whereas >1 indicate corrosive nature and hence not to be transported through metal pipes [61]. All the studied samples show CR more than 1, which suggests that the analyzed waters are corrosive in nature and need non-corrosive pipe for transporting and lifting of groundwater.

Classification of water

Piper [62] classified water types and hydrogeochemical facies on the basis of major ion chemistry in groundwater flow systems. The shallow and deep-water samples of study area show distribution of mixed-type cations, while the produced water samples have influence of Na+K-type water on piper diagram (Fig. 12). Among the major anions, the studied water samples are plotted towards Cl-type. The hydrofacies analysis of the study area reflects that water originates in the close geological environment. Majority of the shallow and deep-water samples are plotted in the CaMgCl-type of water, while the produced water samples are plotted in the NaCl-type of water.

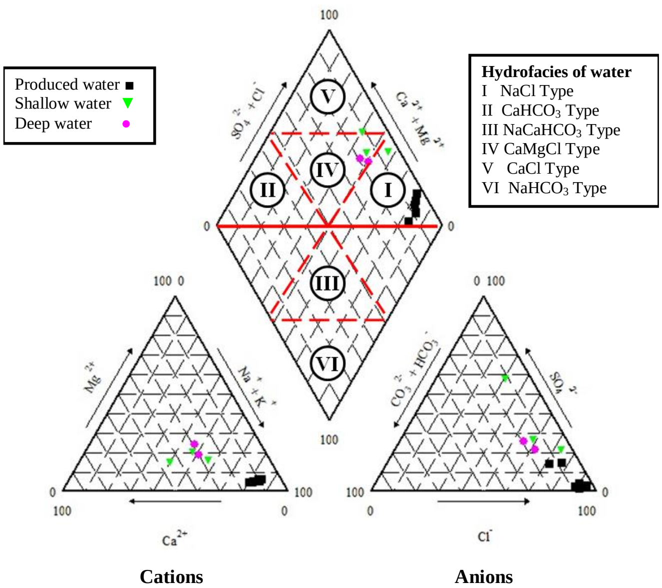

Figure 12: Piper diagram of groundwater chemistry in the study area (fields after Tweed et al., [63])

RESULTS AND DISCUSSIONS

The chemical analysis of the analyzed water samples included a group of main ions such as calcium, sodium, potassium, iron, magnesium, chlorine, bicarbonate and sulfate. The pH values range between 6.77 and 7.60 in the studied water, indicating acidic conditions. The studied water samples showed that sodium> calcium> magnesium> potassium and chlorine> sulfate> bicarbonate. Stiff's diagram indicated the dominance of (sodium-chlorine), while roughly equal in the values of the ratios of (calcium-bicarbonate) as well as magnesium-sulfate.
A strong relationship between sodium and chlorine (r = 0.99) is useful for assessing the relationship with seawater. The ratio of chlorine / sodium ranges between 0.75 and 18.51, while the average value of sea water is estimated at (1.17). The presence of an abundance of non-marine sulfate salts (nss-SO4) indicates human origin, and the chlorine / sodium ratio greater than 1.17, which may not only be marine or continental, but even anthropogenic. This hypothesis seems valid for the studied samples as confirmed by the weak correlation between the ratio of Cl/Na and the ratio of (nss-SO_4 / Na r = -0.27). It may not be of marine and continental origin only, but also the result of industrial activity, and this may qualify for the studied water as indicated by the weak negative correlation between the chlorine / sodium ratio as well as the nss-SO_4 / sodium ratio (r = -0.09).

Potassium is strongly correlated with sodium (r = 0.99), indicating that the two have roughly the same or similar source, while it is poorly correlated to sulfate (r = 0.39) indicating different sources.
Groundwater in the study area was indicated that the vast majority of samples are located along the sea water to the evaporation field. The concentration of chlorine is much higher than that of bicarbonate, while the percentage of bicarbonate / chlorine in the study area (excluding the produced water samples 2009) is much higher than the average percentage of sea water (0.004). This high percentage is probably due to the weathering of the host rocks. The plotted values of the studied samples on the Gibbs graph indicated the predominance of evaporation in the produced water samples. Calcium is strongly correlated to magnesium (r = 0.78) indicating the same source for both. The ratio of calcium / magnesium ranges between 0.22 to 5.79, and this indicates the complexity in the calcium and magnesium budget of the groundwater due to the interaction of the groundwater with rocks and the arid climate of the study area.
In general, the total solid salts, total hardness, and the ratios of sodium, calcium and chlorine were above the safe limit, while the levels of potassium, magnesium, sulfate and bicarbonate in the studied water (excluding the produced water samples) were below the safe limit. While the pH within the safe range for drinking purposes.
The assessment of groundwater quality for irrigation purposes was carried out using various indicators such as Residue of Sodium Carbonate (RSC), which ranges between -177.77 to 0.4, indicated it is good for irrigation.
Based on the values obtained in this study for Electrical Conductivity (EC), Sodium Absorption Ratio (SAR), Magnesium Absorption Ratio (MAR), Kelly Ratio (KR) and Corrosion Ratio (CR), shallow and deep water are classified as good, however, the produced water is classified as very bad and unsuitable. Shallow water and deep water are classified as excellent for irrigation, while, the produced water is hazardous. According to the Permeability Index (PI), the produced water is classified as Class I, while shallow water and deep water are classified Class II.
All the studied samples showed that the Corrosivity Ratio is <1, which indicates that the analyzed water is corrosive in nature and needs non-corrosive pipes to transport and raise the groundwater. According to the Piper diagram the shallow water samples and the deep-water samples for this study presented a distribution of mixed types of cations, while the produced water samples fall into (Na + K) type. However, anions of the studied water samples fall into (Cl) type. The hydrofacies analysis of the studied water showed that the water originates from its nearby geological environment. Most of the shallow and deep-water samples fall into (CaMgCl) type, while the produced water samples (2009) fall into (NaCl) type.

CONCLUSIONS

The current study concluded the followings:

1. The Power of Hydrogen pH, Residual Sodium Carbonate, magnesium absorption percentage and permeability coefficient indicate that all water is suitable for irrigation.
2. According to the Sodium Percent, the Electrical Conductivity and the Kelly Ratio, shallow and deep water are good for irrigation, while the produced water is not suitable for irrigation.
3. The Sodium Absorption Ratio indicates that shallow and deep waters are considered excellent, and produced waters are considered harmful.
4. The corrosivity Ratio is high in all the studied water, which indicates that this water causes corrosion of the pipes and therefore needs special pipes.
5. Pipe diagram indicated that most of the shallow and deep-water samples show the distribution of a mixture of cations, while the produced water is biased towards the type of Na+K.

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HEALTHCARE AND TRANSMISSION OF PANADEMIC COVID-19

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Abstract: *The severe acute respiratory syndrome COVID-19 pandemic had a significant economic and social impact on the world. Since there is no effective medical treatment for COVID-19 at present, the international collaborative efforts are more focused on developing a safe and effective vaccine against COVID-19. This review explored various factors influencing the COVID-19 vaccination intention and the applicability of the health belief model to explore vaccination intention among the population of world. Increasing COVID-19 vaccine uptake among a larger section of the population is an important public health priority at the moment as many vaccine candidates approved for use have shown efficacy in preventing COVID-19 infection and associated complications. The COVID-19 pandemic has been a major challenge for healthcare systems throughout the world. Transmission of COVID-19 can occur through direct, indirect, or close contact with infected people through infected secretions such as saliva and respiratory secretions or their respiratory droplets, which are expelled when an infected person coughs, sneezes, talks or sings. The UNs WHO has described various levels of COVID-19 transmission. There is some evidence that the COVID-19 virus may lead to intestinal infection and be present in faeces. Approximately 2−10% of cases of confirmed COVID-19 disease presented with diarrhoea. In this difficult period it is best to meet virtually but if you have to meet others, do it carefully and with the right precautions. Further study should include the impact of COVID-19 vaccine uptake on these rates. Recent emergence and spread of variants have been reported with some concern regarding possible increased transmission and impact on immune responses. There is a real need for more studies regarding the public perception and acceptance of COVID-19 vaccines in the world.*

Keywords: *COVID-19, transmission, healthcare system, immune response, vaccination*

INTRODUCTION

The novel coronavirion named as SARS-CoV-2 has shaken the world by bringing the disastrous pandemic of the century. The COVID-19 pandemic has become one of the most alarming global health crises of our time. On 30 December 2019, the Program for Monitoring Emerging Diseases notified the world about pneumonia of unknown cause in Wuhan, China. Coronavirion is thought to be transmitted from animals (bats or civets) into the human race and began spreading. Since then, scientists have made remarkable progress in understanding the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), its transmission, pathogenesis, and mitigation by vaccines, therapeutics, and non-pharmaceutical interventions. Yet more investigation is still needed to determine the origin of the pandemic. Theories of accidental release from a lab and zoonotic spreading both remain viable. Knowing how COVID-19 emerged is critical for informing global strategies to mitigate the risk of future outbreaks. In May 2020, the World Health Assembly requested that the

World Health Organization (WHO) director-general work closely with partners to determine the origins of SARS-CoV-2 (World Health Assembly Resolution 73.1, 2020).

Furthermore, the two theories were not given balanced consideration. Only 4 of the 313 pages of the report and its annexes addressed the possibility of a laboratory accident (WHO, 2021a). Notably, WHO Director-General Tedros Ghebreyesus commented that the report's consideration of evidence supporting a laboratory accident was insufficient and offered to provide additional resources to fully evaluate the possibility (WHO director-general's remarks, 2021). As scientists with relevant expertise, we agree with the WHO director-general (WHO, 2021b), the USs and 13 other countries (US Department of State, 2021), and the European Union (Delegation of the European Union, 2021) that greater clarity about the origins of this pandemic is necessary and feasible to achieve. It must take hypotheses about both natural and laboratory spreading seriously until sufficient data are collected. A proper investigation should be transparent, objective, data-driven, inclusive of broad expertise, subject to independent oversight, and responsibly managed to minimize the impact of conflicts of interest. Public health agencies and research laboratories alike need to open their records to the public. Investigators should document the veracity and provenance of data from which analyses are conducted and conclusions drawn, so that analyses are reproducible by independent experts.

The origin and early spread of COVID-19 remains shrouded in mystery. Here, data was identified a set containing COVID-19 sequences from early in the Wuhan epidemic that has been deleted from the NIH's Sequence Read Archive. It was recovered that the deleted files from the Google Cloud, and reconstruct partial sequences of 13 early epidemic viruses. Phylogenetic analysis of these sequences in the context of carefully annotated existing data suggests that the Huanan Seafood Market sequences that are the focus of the joint WHO-China report are not fully representative of the viruses in Wuhan early in the epidemic. Instead, the progenitor of known SARS-CoV-2 sequences likely contained three mutations relative to the market viruses that made it more similar to SARS-CoV-2's bat coronavirus relatives (Bloom, 2021).

The COVID-19 pandemic caused by a novel 2019 SARS coronavirion, known as COVID-19 which is rapidly spreading worldwide, greatly surpassing the 8,000 total cases of the 2002–2004 SARS coronavirion outbreak (SARS-CoV-1) after 1 month of the initially identified case on 31 December 2019, in Wuhan city, China (Wilder-Smith et al., 2020). As COVID-19 is human-to-human transmitted, it is a threat to the global population.

STRUCTURE OF COVID-19

The novel coronavirus disease (COVID-19) has spread worldwide, resulting in numerous cases of morbidity and death. According to transmission electron microscopy (EM) and cryo EM, the morphology of the COVID-19 particle is round, oval or pleomorphic, with a diameter of 60–140 nm.

COVID-19 virions have distinct spikes, protruding approximately 9 to 12 nm from the membrane surface that give virions the appearance of a solar corona or medieval crown and hence gave this virus family its name (Lu et al. 2020; Zhou P et al. 2020; Zhu et al. 2020).

The composition of envelope of coronavirions is reminiscent to the membrane of the former host cell, comprising membrane proteins and lipid bilayers. Klein et al. measured the lipid bilayer separation of SARS-CoV-2 virions: a phospholipid monolayer separation was 3.6 nm whereas the plasma membrane was 3.9 nm (Klein et al. 2020). The structure of the nucleocapsid is a symmetrical helix, formed by the combination of positive-strand RNA and nucleocapsid protein (N) (Peiris et al. 2004).

The single-stranded RNA genome of the COVID-19 reference sequence is 29,891 nucleotides in size and encodes 9860 amino acids. The G:C content is 38% (Chan et al. 2020). COVID-19 shows a genome organization typical for beta coronavirions: a 50 un-translated region (50-UTR), an RNA polymerase open reading frame (ORF1ab) followed by genes encoding for the spike (S) protein, the envelope (E) protein, the membrane (M) protein, the nucleocapsid (N) protein, a 30 un-translated region (30-UTR), and several non-structural open reading frames (Chan et al. 2020; Wu F et al. 2020; Zhu et al. 2020). The genome gives rise to at least 12 canonical proteins and 23 non-canonical translation products (Finkel et al. 2020).

According to GenBank, the protein lengths of these five important proteins are 7096 amino acids (ORF1ab), 1273 amino acids (S protein), 75 amino acids (E protein), 222 amino acids (M protein) and 419 amino acids (N protein).

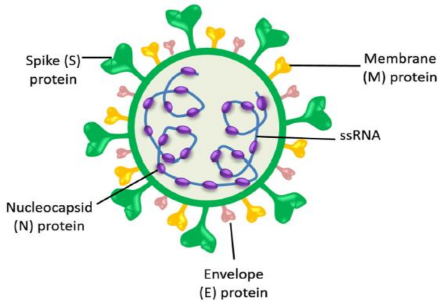

Figure (1) The genomic organization and structural features of COVID-19

The RNA polymerase gene occupies 67% of the coronavirus genome, while all other structural proteins and accessory proteins are encoded by the remainder of the genome (Chen Yu et al. 2020; Guan et al. 2003; Wu A et al. 2020). One study predicted putative functions and proteolytic cleavage sites of ORF1ab, including 16 non-structural proteins, by bioinformatics (Chan et al. 2020).

RECEPTORS OF COVID-19

The spike glycoprotein forms spikes on the surface of coronaviruses, attaching the viruses to host cell receptors and catalysing virus–cell membrane fusion. For this purpose, the receptor-binding domain (RBD) in the spike glycoprotein directly binds receptors on the surface of host cells. The amino acid sequences and predicted protein structure are highly similar between COVID-19 and SARS-CoV RBD, suggested that COVID-19 may also use human angiotensin-converting enzyme 2 (ACE2) as a cellular entry receptor, facilitating human-to-human transmission (Letko et al. 2020; Lu et al. 2020; Wan et al. 2020; Wu F et al. 2020; Zhu et al. 2020). It was found that COVID-19 can use all but mouse ACE2 as entry receptors (Zhou P et al. 2020).

It is critical to understand COVID-19 characteristics to deal with this ongoing pandemic and to develop future treatments. COVID-19 virion is an enveloped, positive-stranded RNA virion with a large genome (29.9 kb) belonging to the family Coronaviridae, order Nidovirales (De Wit et al., 2016). One of the striking genomic features of this novel virion is the presence of a novel furin-like cleavage site in the S-protein of the virion, which differs from SARS-CoV-1 and may have implications for the life cycle and pathogenicity of the novel virion (Coutard et al., 2020; Wu et al., 2020a).

Firstly, it was suggested that COVID-19 is a close relative of the RaTG13 bat-derived coronavirus (around 88% identity) rather than of SARS-CoV-1 (79% identity) or Middle East respiratory syndrome coronavirus MERS-CoV (50% identity) (Lu et al., 2020).

Due to this association with bat coronaviruses, it was argued that COVID-19 virion has the potential to spread into another species, as bat coronavirions do (Hu et al., 2018). Although bats are likely natural reservoir hosts for COVID-19, it was recently demonstrated that COVID-19 is closely related to a pangolin coronavirion (Pangolin-CoV) found in dead Malayan pangolins with a 91.02% identity, the closest relationship found so far for COVID-19 (Zhang et al., 2020). In that study, genomic analyses revealed that the S1 protein of Pangolin-CoV is related closer to COVID-19 than to RaTG13 coronavirion. Also, five key amino acid residues involved in the interaction with the human ACE2 receptor are maintained in Pangolin-CoV and COVID-19, but not in RaTG13 coronavirion. Thus, it is likely pangolins are an intermediate host in the transmission of coronaviruses between bats and humans. In this manner, it was argued COVID-19 acquired mutations needed for human transmission and will continue to evolve with novel mutations, as the pandemic evolves (Zhang & Holmes, 2020). In this scenario, it is expected that diverse signatures of viral variants spread among different populations in the world.

Recently, thousands of GenBank sequences from SARS-CoV-19 available at the NCBI virus database were trackable by region, suggesting that the transmission occurred mainly through clonal events due to clustering of the available sequences (Chen et al., 2020; Kupferschmidt, 2020). As a proof of concept, in the early beginning of the outbreak in China, sequencing the virus from nine patients from Wuhan in China revealed 99.9% similarity among samples.

That finding suggests 2019-nCoV originated from one source within a very short time, supporting clonality of spreading (Lu et al., 2020). Coronavirion Disease 2019 (COVID-19) is caused by severe acute respiratory syndrome Coronavirion 2 (SARS-CoV-2) and it was declared a public health emergency of global concern on January 30, 2020 (WHO, 2020).

The disease has spread globally and an estimated 349 million peoples are at risk of the severe form of COVID-19 (Clark et al. 2020). Globally, as of May 14, 2021, more than 160 million confirmed cases and 3.3 million deaths have been reported to the World Health Organization (WHO) and the Americas, Europe and Southeast Asia were the most affected regions. However, South Africa, Nigeria, and Ethiopia were among the countries highly burdened by the COVID-19 pandemic in Africa (WHO, 2021).

TRANSMISSION OF COVID-19

Some of the countries that have been most successful at keeping COVID-19 at very low are now seeking ways to gingerly lower the barriers that protect them while minimizing the risk that the disease will become endemic. China, Australia, New Zealand, Singapore, and Taiwan all closed their borders last year and forced the few people allowed entering into strict hotel quarantines. Internally, they quashed outbreaks early, using lockdowns and other restrictions. As a result, residents could live near-normal lifestyles most of the time.

Countries with this “zero COVID-19” strategy, as it has been dubbed have generally fared better than countries that opt for mitigation. They found the elimination cohort had lower per capita death rates, shorter and less strict lockdowns, and faster economic recoveries than the mitigation camp, including the USs and Europe, which have used vaccines and other measures to try to blunt massive waves of infection. But the spread of the highly infectious Delta variant, the economic burden of closed borders, lockdown fatigue, and increasing vaccine availability are changing the equation. “In the long-term is not really economically sustainable. Countries are going to need to test out different approaches to find the right balance between infection prevention and control and normalizing societal activities.

COVID-19 is a highly communicable disease transmitted to susceptible individuals by respiratory droplets and nasopharyngeal secretion (Acter et al., 2020). Peoples infected with COVID-19 have been diagnosed usually by taking nasopharyngeal swabs or pharyngeal aspirates. These procedures are invasive and challenging both for health-care providers and patients.

However, there is evidence which indicates that patients infected with COVID-19 can be detected from saliva (Khurshid et al., 2020). All individuals infected with COVID-19 may not show clinical manifestations of the disease (Getaneh et al., 2020). Fever, cough, fatigue and dyspnea were the common symptoms observed in people infected with COVID-19 (Gulati, et al., 2020).

However, the severe form of the disease: acute respiratory distress syndrome, organ dysfunction and death could occur in the elderly and patients who have chronic disease concomitantly (Li et al., 2020). Cardiovascular, diabetic mellitus and chronic lung diseases were associated with COVID-19 illness and death (Stokes et al., 2020).

Several vaccines have been used worldwide to prevent COVID-19 despite other prevention strategies (WHO, 2021). However, there was no effective and approved drug to cure the disease. Thus, patients have been treated symptomatically with supportive measures (WHO, 2020). However, the clinical setups and level of supportive measures employed varied across countries and it mainly depends on economic status (Bong et al., 2020).

The African economy and healthcare system was significantly affected by the COVID-19 pandemic (Kanu, 2020 and Paintsil, 2020). This pandemic showed that the capacity of the countries’ healthcare system has to be enhanced. Besides, health policies should be modified and emergency preparedness strategies have to be strengthened to prevent and control public health emergencies (Xing and Zhang, 2021). The COVID-19 public health crisis has helped countries to revise their governance structures indirectly and to make some political reforms (Zhang and Zhang, 2020).

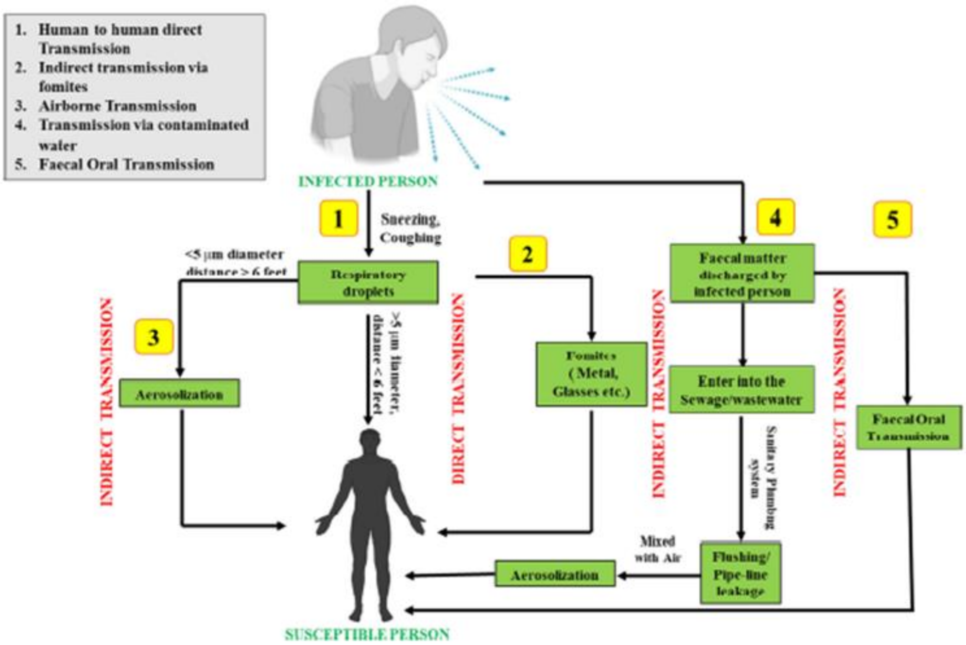

Figure (2) Different transmission pathways COVID-19 in the environment

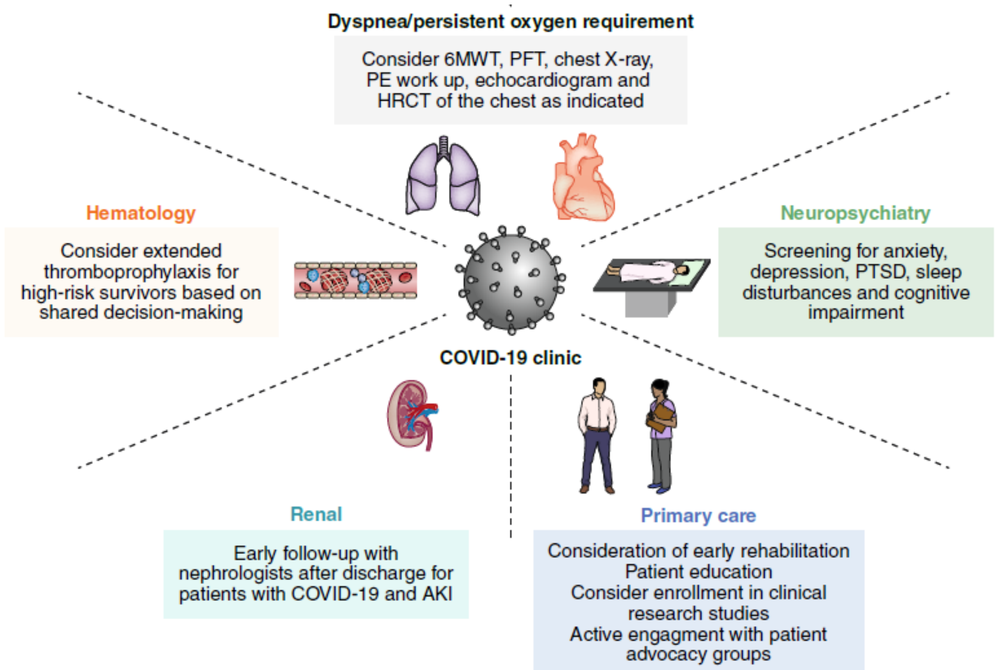

Figure (3) Interdisciplinary management in COVID-19 clinics

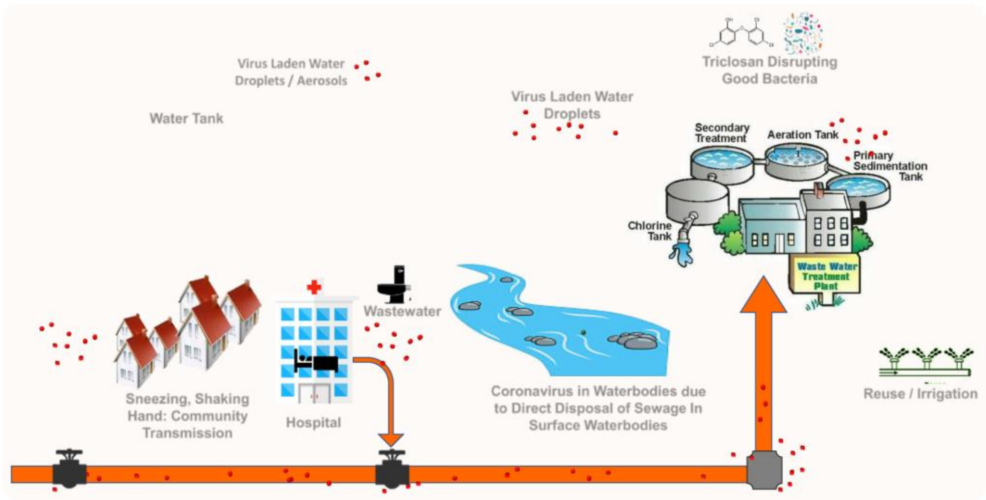

Figure (4) Occurrence, transmission, fate of Coronavirion in community

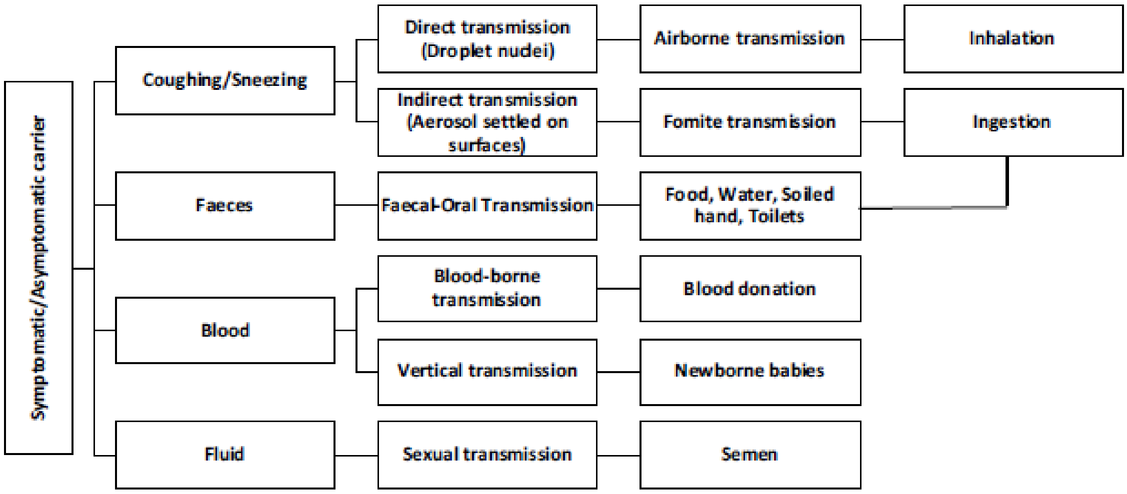

Figure (5) Flow diagram explaining different modes of transmission of SARS-CoV-2

REDUCTION OF VIRION TRANSMISSION

The COVID-19 virion will not stop entirely, unless people develop strong immunity against the virus, which would be achievable through proper vaccination that is specifically built to fight COVID-19. It is unlikely that even people with an open mind will accept a new vaccine that has not been tested for more than a year because they fear being one of the first to get vaccinated with this new vaccine.
To reduce transmission of COVID-19, primary prevention strategies are feasible and the best options in resource-limited settings (Ali and Alharbi, 2020).
Vaccination (A vaccine is a substance that stimulates your immune system to make antibodies (blood proteins produced in response to a foreign substance) as it would if you were exposed to the actual disease. After vaccination, human body develops immunity to the disease, so people are protected from getting sick if they are infected), maintaining physical distance, washing hands, staying home, and wearing face mask were the main measures recommended to prevent the disease worldwide (WHO, 2020). Globally, there are different commercially available antiviral solutions used to prevent COVID-19 infection during clinical practice in health-care settings, but many health-care providers had lack of awareness about the prevention benefit of those solutions (Imran et al., 2021).
Elderly populations are high risk groups for COVID-19 due to weakened immune status. Therefore, elderly populations are recommended to have awareness about the disease and they need to apply all the precautionary measures.

A study among the elderly population in China indicated that females had a higher level of understanding about COVID-19 than males and awareness was the main determinant factor to develop protective behaviors (Sun et al., 2020a). Perception of people about the COVID-19 pandemic was the main determinant factor to the utilization of preventive behaviors (Duan et al., 2020).
Generally, a systematic review and meta-analysis report revealed that more than two-thirds of study participants in the general population had good knowledge and favourable attitude toward the pandemic (Saadatjoo et al., 2019). However, adhering to COVID-19 prevention methods was not satisfactory despite the increment of cases and death in the country. Fear and anxiety are common experiences during a public health crisis and in the era of the COVID-19 pandemic. These problems were exacerbated in communities who lacked knowledge about the pandemic and COVID-19-related rumors and fake news contributed to the spread of the disease (Sun et al., 2020b; Orso et al., 2020).

DIAGNOSIS OF COVID-19

Diagnosis of COVID-19 needs a comprehensive analysis of epidemiological history and clinical presentation. Fever and dry cough were the common symptoms of a COVID-19 infection, while upper respiratory symptoms such as rhinorrhea were rare. Finally, nasopharyngeal swabs with positive viral RNA remain the gold standard for the diagnosis of COVID-19. In addition, RNA tests can also produce initially false negative results due to low viral load or the method of collecting nasopharyngeal swab specimens, so samples should be taken multiple times to confirm COVID-19. Besides, the serological testing of anti- COVID-19 immunoglobulin G (IgG) and/or IgM were widely used as a complement method in the diagnosis of COVID-19. As such, the eventual diagnosis of COVID-19 should be determined by a comprehensive analysis of CT imaging and blood test results.

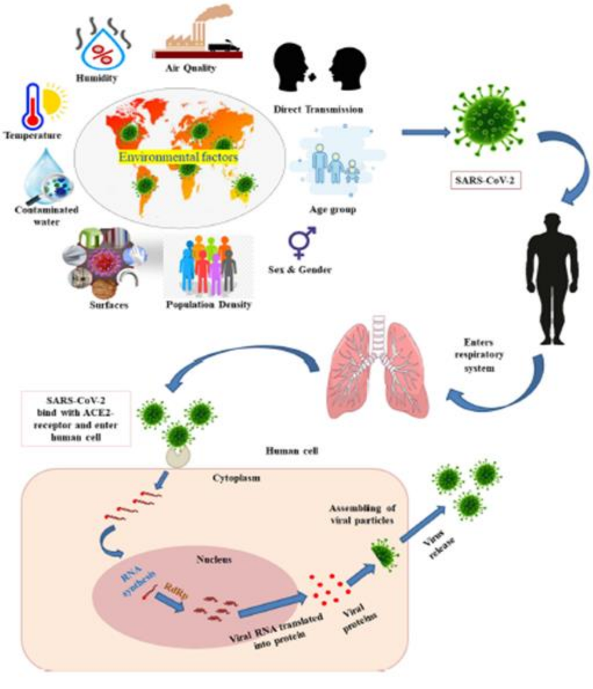

Figure (6) Influence of Environmental factors on THE infection OF COVID-19

HOW DO THE VACCINES WORK?

Two viral vectors are better than one?

Sputnik V is an adenovirus vaccine, which means that it uses an engineered adenovirus that generally cause only mild illness s a delivery mechanism for inserting the genetic code for the COVID-19 spike protein into human cells. It is similar to the Oxford–AstraZeneca and Johnson & Johnson vaccines.

But instead of using one engineered adenovirus, as those two vaccines do, Sputnik V uses different adenoviruses, called rAd26 and rAd5, for the first and second doses, respectively.
The two adenoviruses have slightly different methods of introducing their genetic material into a host cell, which would theoretically improve the success rate of getting the viral genetic material where it needs to go.
The two preliminary studies from the vaccine developers, published in September 2020[2], involved 76 healthy adults who received the two doses with different viral vectors three weeks apart. All participants produced antibodies to the COVID-19 spike protein, and adverse events reported were mainly mild pain at the injection site, fever, headache, fatigue and muscle aches — adverse events typical of other COVID-19 vaccines.

Pfizer and Moderna Vaccines

- The vaccines from Pfizer and Moderna use a technique known as messenger RNA (mRNA).
- These vaccines give instructions for our cells to make a harmless piece of what is called the 'spike protein.
- This protein is found on the surface of the coronavirus that causes COVID-19.
- Once these vaccine instructions, or mRNA, are injected, your cells use it to make the spike protein; then the instructions are broken down and eliminated.
- The protein piece is displayed on the cell surface, triggering our immune system to make antibodies against it, just as it would if it were exposed to the real coronavirus that causes COVID-19.
- In this way, the body learns how to protect itself when and if the real virus shows up.
- The mRNA vaccines don't use the live virus that causes COVID-19, nor does the mRNA get into the cell's nucleus, which is where our DNA is stored.

Sputnik V, Oxford–AstraZeneca and Johnson & Johnson Vaccine

Sputnik V is an adenovirus vaccine, which means that it uses an engineered adenovirus (a family of viruses that generally cause only mild illness) as a delivery mechanism for inserting the genetic code for the COVID-19 spike protein into human cells.
The two adenoviruses have slightly different methods of introducing their genetic material into a host cell, which would theoretically improve the success rate of getting the viral genetic material where it needs to go. It is similar to the Oxford–AstraZeneca and Johnson & Johnson vaccines. But instead of using one engineered adenovirus, as those two vaccines do, Sputnik V uses different adenoviruses, called rAd26 and rAd5, for the first and second doses, respectively.

Johnson & Johnson Vaccine

- The vaccine from Johnson & Johnson is made using a type of virus called adenovirus type 26, or Ad26.
- The Ad26 delivers a piece of the DNA, used to make the spike protein, so the person can temporarily make this protein and teach the immune system to react against the coronavirus.
- Ad26 is modified so it can't make the person sick.

How many doses do you need?

- One dose for the J&J vaccine, and
- Two doses for the vaccines from Moderna and Pfizer and the others.

Right dose

- In the earliest trial of Moderna’s mRNA-based vaccine, study participants received one of three dose levels: 25, 100 or 250 micrograms (Jackson et al., 2020).
- The top dose proved too toxic. The low dose elicited the weakest immune response. The middle dose seemed to offer the best balance: it triggered strong immunity and had acceptable side effects.
- That 100-microgram dose ultimately became the one authorized for mass use in dozens of countries. But Moderna scientists later showed that a half-dose seemed to be just as good as the standard dose at stimulating immune protection (Chu et al., 2020).

What is the interval between doses?

- For the Moderna vaccines, the two doses are given 4 to 6 weeks apart.
- For Pfizer's vaccine, the two doses are given 3 to 6 weeks apart.

What happens if you don't take the second dose?

- Protection is assumed to be less.
- In data that Moderna, its analysis suggested that the first dose provides protection from getting COVID-19, but the data did not allow for a “firm conclusion".
- Both the Pfizer and Moderna vaccines are believed to be around 50% effective after just one dose.

After the required doses, how long until it takes effect and provides protection

- That happens about a week after the second dose.
- Once the second dose kicks in, both the Pfizer and Moderna vaccines have shown in studies to be about 95% effective.
- The J&J effectiveness data was based on 28 days after the inoculation.

In case of Sputnik V, some of that concern was allayed when the phase III trial results (Logunov, 2021), published in February by the vaccine’s developers, suggested that it is 91.6% effective at preventing symptomatic COVID-19 infection and 100% effective at preventing severe infection.
Despite the absence of approval from the EMA or the WHO, several countries, including South Korea, Argentina and India, are already manufacturing Sputnik V. And India plans to pump out at least 850 million doses, to help speed up the vaccination of its embattled population. Many other countries, such as Hungary and Iran, are importing Sputnik V, and it has become a key plank of their vaccination campaigns (Logunov, 2020).
Data from seven vaccination trials help to identify a blood marker for protection against the disease. After people have been vaccinated against COVID-19, the levels of infection-blocking antibodies in their blood are a strong indicator of how much protection they’ve gained against the disease, according to a modelling study (Khoury et al., 2021). The research showed that the presence of even small quantities of these potent ‘neutralizing antibodies’ indicates that a vaccine is effective at protecting against COVID-19. The study is the best attempt yet to define features of the immune response that can act as a proxy for protection against COVID-19, known as a ‘correlate of protection’.

How long does the protection last?

- Because the vaccines are new, this is not yet known for sure.
- Based on other viruses that are similar to the coronavirion that causes COVID-19, the COVID-19 vaccines that are shown to be highly effective might protect people for a few years.

What about side effects?

People should expect to have some side effects, similar to what some people report after getting a flu vaccine. These experts said to expect temporary side effects such as soreness in your arm where you got the shot, fatigue, body aches, and perhaps a fever.
Sputnik’s side effects are also becoming clearer; studies so far suggest that they are similar to those of the other adenovirus vaccines, with the notable exception of rare blood-clotting conditions. Unlike for both the Oxford–AstraZeneca and Johnson & Johnson vaccines, there have been no reports of these disorders from Russian health authorities or from the other nations using Sputnik V.
A preprint (Pagotto, V. et al., 2021) from the Italian Hospital of Buenos Aires in Argentina reported no cases of clotting disorders or adverse events of special interest among 683 health-care workers vaccinated with Sputnik V. And an analysis of 2.8 million doses of Sputnik V administered in Brazil reported no deaths associated with vaccination, and mostly mild adverse events. Furthermore, a study posted as a pre-print in May, from the republic of San Marino, found no serious adverse events in 2,558 adults who received one dose of Sputnik V and 1,288 who received two doses (Montalti, M. et al., 2021).

HOW WELL DO THE VACCINES WORK IN THE REAL WORLD?

In Denmark, a clinical trial (Polack et al., 2020) in more than 40,000 people had already found the vaccine to be 95% effective in protecting recipients from symptomatic COVID-19. But Moustsen-Helms, who works at the Statens Serum Institut in Copenhagen, and her colleagues were among the first to test its effectiveness outside clinical trials, which can exclude some unhealthy individuals or those taking medicines that suppress immune responses.

The results (Moustsen-Helms et al., 2021) showed it was 64% effective in long-term-care residents with a median age of 84, and 90% effective in healthcare workers, given that immune responses in older people can be muted.

Among older adults who received the Pfizer–BioNTech vaccine, Israel has seen 94% protection from COVID-19 infection in people over 85 years old (Haas et al., 2021). This is remarkably high for that age group, and considerably higher than Moustsen-Helms's result of 64%, possibly in part because long-term-care residents are prone to be in poor health. Similarly, a UK study found that the Pfizer–BioNTech and Oxford–AstraZeneca vaccines were both 80% effective at preventing COVID-19 hospitalizations in people aged 70 or older (Bernal et al., 2021a).

Studies are under way to see whether vaccine effectiveness can be boosted even more by mixing and matching vaccines, and early results have been promising.

At the other end of the age spectrum, Pfizer–BioNTech and Moderna in Cambridge, Massachusetts, have recently completed clinical trials of their vaccines in adolescents, showing 100% and 93% protection in those aged 12–15 (Frenck et al., 2021) and 12–17, respectively.

Real-world data are not yet available. Initial laboratory tests suggested that antibodies raised by the Pfizer–BioNTech vaccine were less effective against the B.1.351 variant identified in South Africa, but it was unclear how that would affect protection against disease.

In May, researchers in Qatar published reassuring data showing that people who received two doses of the Pfizer–BioNTech vaccine were 75% less likely to develop COVID-19 from infection with B.1.351, and were almost completely protected from severe disease (Abu-Raddad et al., 2021).

The Oxford–AstraZeneca vaccine did not fare as well in another test: in South Africa, a small clinical trial suggested that the vaccine did little to fend off infections of the B.1.351 variant that, by that point, was causing most infections there (Madhi et al., 2021).

As a result, the South African government made the difficult decision to sell its doses and await a different vaccine. It is now rolling out the vaccine produced by Johnson & Johnson in New Brunswick, New Jersey, which in one clinical trial was 64% effective at blocking moderate to severe COVID-19 in South Africa at a time when B.1.351 constituted more than 94% of the infections in the trial (Madhi et al., 2021).

A vaccine made by Novavax in Gaithersburg, Maryland, which has not yet been authorized for emergency use, was 51% effective at preventing symptomatic COVID-19 among participants in South Africa who did not have HIV (Shinde et al., 2021).

There was still hope that it could protect against severe disease and death, a possibility that was not tested in the trial, which enrolled mostly young participants with a low risk of severe disease. Shabir notes that a later study in hamsters (Fischer et al., 2021) found that the vaccine prevented clinical disease caused by B.1.351. The coronavirion COVID-19 has proved to be much more prone to mutations than researchers first thought, and more variants are emerging all the time.

One variant of concern, called B.1.617.2, was first identified in India and is spreading rapidly in the UK, raising worries that it could be unusually transmissible. Public Health England (2021) has determined that two doses of either the Pfizer–BioNTech or the Oxford–AstraZeneca vaccines are 88% and 60% effective, respectively, at preventing symptomatic disease caused by this variant (Bernal et al., 2021b).

In the meantime, some researchers are looking to natural immunity as a guide. A study in more than 25,000 health-care workers in the United Kingdom found that a COVID-19 infection reduced the risk of catching the virus again by 84% for at least 7 months (Hall et al., 2021).

How soon that booster is needed could depend in part on the rate at which antibody levels decline — they could drop precipitously or plateau at a low level. One modelling study (Khoury et al., 2021) estimates that low levels of antibodies will be enough to offer significant protection against severe disease.

But Pfizer chief executive Albert Bourla has said that he expects a booster to be needed in about 8–12 months after the second dose of the Pfizer–BioNTech vaccine. Vaccine developers are now testing variant-specific boosters, too.

Moderna has released preliminary results showing that a booster vaccine using a spike-protein sequence from the B.1.351 variant increased the concentration of antibodies that neutralize SARS-CoV-2, and particularly the B.1.351 variant (Wu et al., 2021).

Researchers have been trying to fill this gap, and, so far, the data look promising. Results announced by Johnson & Johnson from clinical trials suggest that its vaccine is 74% effective against asymptomatic infections. Researchers studying deployment of the Pfizer–BioNTech vaccine in Israel have also reported that vaccination reduces the amount of virus found in infected individuals by up to 4.5-fold, suggesting that they could be less likely to shed that virus into the environment, where it might infect someone else (Levine-Tiefenbrun et al., 2021).

A study (Harris et al., 2021) by Public Health England has found that even a single dose of either the Pfizer–BioNTech or Oxford–AstraZeneca vaccine reduced the spread of disease from infected individuals to household members by up to 50%. It's likely that all the vaccines have some similar effect.

Shortly after inoculations with the Pfizer–BioNTech vaccine began, a few regions reported cases of a severe allergic reaction called anaphylaxis. But further study showed that the risk of this condition — which can be treated at the vaccination centre — is not much higher for the Moderna and Pfizer–BioNTech jabs than for other vaccines. For Pfizer–BioNTech, the risk is about 4.7 cases per 1 million doses (Shimabukuro et al., 2021); the risk of anaphylaxis from any vaccination is estimated at 1.3 in a million.

Sputnik V COVID-19 Vaccine Begins in Europe

The European Medicines Agency has begun a rolling review based on laboratory and clinical studies of the Sputnik V (Gam-COVID-Vac). The European Medicines Agency (EMA) announced on March 4, 2021 that its human medicines committee (CHMP) has begun a rolling review of the Sputnik V (Gam-COVID-Vac) COVID-19 vaccine. The review is based on results from laboratory and clinical trial studies that indicate the vaccine triggers the production of antibodies and immune cells. The rolling review will look at data as they become available and will continue until there is enough evidence to support a formal marketing authorization application. The usual standards for effectiveness, safety, and quality will be assessed by the agency for the vaccine, according to EMA.

Sputnik V (Gam-COVID-Vac) was developed by Russia's Gamaleya National Centre of Epidemiology and Microbiology.

EMA states that the vaccine is made up of Ad26 and Ad5 adenoviruses that "have been modified to contain the gene for making the COVID-19 spike protein; they cannot reproduce in the body and do not cause disease. The two adenoviruses are given separately: Ad26 is used in the first dose and Ad5 is used in the second to boost the vaccine's effect."

WHO approval of Chinese CoronaVac

CoronaVac is one of two Chinese vaccines already sustaining vaccination campaigns in more than 70 nations. Both should soon be much more widely available to low-income countries. The WHO has approved a second Chinese vaccine for emergency use.

CoronaVac was found to be 51% effective at preventing COVID-19 in late-stage trials, and researchers say it will be a key to curbing the pandemic. This overall protection is lower than that provided by the seven other vaccines already listed by the WHO.

But, importantly, trials suggest that CoronaVac is an inactivated-virion vaccine produced by Beijing-based Company Sinovac which is 100% effective at preventing severe disease and death. "CoronaVac will significantly contribute to the global fight against COVID-19 as a safe and moderately effective COVID-19 vaccine.

COMPARISON BETWEEN FOUR COVID VACCINES

Vaccine developer	Pfizer	Moderna	AstraZeneca	Johnson & Johnson
How it works	Messenger RNA	Messenger RNA	Inactivated cold virus	Modified cold virus
What percentage of people did it protect from getting infected in clinical studies?	95%	94.1%	70%	66.1% globally; 72% in the USs.; 86% effective against severe disease
How many shots do you need?	Two doses, 3 weeks apart	Two doses, 4 weeks apart	Two doses, a month apart	One dose
What are the side effects?	Fatigue, headache, chills, muscle pain, especially after the second dose.	Fever, muscle aches, headaches lasting a few days. Effects worse after second dose.	Injection site pain, fever, muscle aches, headache.	Injection site pain, headache, fatigue, muscle pain.
Who is it recommended for?	People 16 years and older	People 18 years and older.	Not yet available.	People 18 years and older.
Is there anyone who shouldn't get the vaccine?	People with a history of allergic reactions to vaccine ingredients including polyethylene glycol, and anyone with a history of allergic reactions to polysorbate	Not yet available.	Anyone who's had a severe allergic reaction to an ingredient in the vaccine.	Is there anyone who shouldn't get the vaccine?
What about people with lowered immune function?	There is limited safety data in this group	Not yet available.	What about people with lowered immune function?	There is limited safety data in this group.

SIDE EFFECTS OF THE SECOND DOSE OF THE VACCINE

It does appear that people have more effects after the second dose than the first. The reason is as the vaccine gets your body ready to fight the disease, some of the things in the human body does to fight the virus makes you feel a little under the weather. That's because the vaccine is like a training program for your immune system. A lot of what your body does immune system, the cells that are stimulated, the antibody production that's underway and all that stuff can make you feel, not so great for a day or 2 days.

Although these side effects can be unpleasant, they are temporary and an indication the vaccine is working. Older people tend to have fewer side effects than people in the 18 to 55 range, possibly because younger people have more active immune systems. Nature News on 06 July 2021 mentioned that **mounting evidence suggests Sputnik COVID vaccine is safe and effective:** Russia's vaccine is in use in more than 70 nations, but its adoption has been slowed by controversies and questions over rare side effects, and it has yet to garner WHO approval.

Russia's COVID-19 vaccine, Sputnik, has been the subject of fascination and controversy since the Russian government authorized its use last year, before early-stage trial results were published. Evidence from Russia and many other countries now suggests it is safe and effective — but questions remain about the quality of surveillance for possible rare side effects.
Sputnik V is also known as Gam-COVID-Vac and was the first COVID-19 vaccine to be registered for use in any nation, and it has since been approved in 67 countries, including Brazil, Hungary, India and the Philippines. But the vaccine (and its one-dose sibling Sputnik Light) has yet to receive approval for emergency use from the European Medicines Agency (EMA) or the WHO. Approval by the WHO is crucial for widespread distribution through the COVID-19 Vaccines Global Access (COVAX) initiative, which is providing doses for lower-income nations.
Some of that concern was allayed when the phase III trial results[1], published in February by the vaccine's developers, suggested that it is 91.6% effective at preventing symptomatic COVID-19 infection and 100% effective at preventing severe infection. However, some scientists criticized the authors for failing to provide access to the full raw data from the early stage trials, and also voiced concerns about changes in the vaccine's administration protocol and inconsistencies in the data.

VACCINES MADE FROM GENETIC MATERIAL CALLED mRNA?

- The Pfizer vaccine uses a snippet of genetic material called messenger RNA that doesn't contain the virus itself, but rather a genetic code associated with the coronavirion that triggers the body's immune response to fight it.
- The mRNA essentially teaches human cells to make something resembling the so-called spike protein found on the surface of the coronavirion.
- This prompts the body's natural defense to destroy it, if the vaccinated person is later exposed to the real virus.
- **The COVID-19 vaccine cannot give someone the virion and cannot affect or interact with our DNA or change our genes in any way.**

WILL COVID BECOME A DISEASE OF THE YOUNG?

A growing share of infections among unvaccinated youths in countries with high vaccination rates is putting the spotlight on the role of young people in the pandemic. The decision came in response to a trend that many countries with high rates of vaccination are experiencing: an ever-increasing proportion of new infections are in younger age groups. In the USs and the UK, COVID-19 has become a disease of the unvaccinated and who are predominantly young. This shift is occurring in many countries that vaccinated older people first, and are now reaching high levels of vaccination in the adult population.
It follows an earlier drop in age resulting from public-health measures to prevent the spread of COVID-19 among older people who are most at risk of severe disease, such as those in nursing homes, say researchers. Magnusson (2021) mentioned that the impact of COVID-19 in children on Norway's health-care system.
Also, she reported that although they didn't need specialist care, children often needed to see their doctor repeatedly for up to six months after contracting the virus. However, the overall risk of severe disease in children remains low, and in many countries that have observed the proportion of cases rising in younger age groups, the total number of cases has fallen, he points out.
And countries also need to consider the global context. Although the downward shift in the average age of infections in countries with high COVID-19 vaccination rates is an interesting phenomenon, it might be short-lived. A few scenarios could shift the balance back. Many countries could start to vaccinate younger people as Israel and the USs are already doing or new variants and waning immunity among older age groups could make them freshly sensitive. COVID-19 could still become a disease of the young. But how big a problem that is, is not a simple thing to respond to (Nature, 2021).

IMPACTS OF COVID-19 ON HEALTHCARE

Which Jobs Are in the Highest Demand in 2021?

The COVID-19 pandemic increased demand for healthcare workers, which was already growing due to the large aging population. The number of healthcare careers is expected to grow more than careers in many other industries over the next decade, adding approximately 2.4 million new jobs by 2029.

While healthcare is a safe field to pursue if you are looking for a stable career, the specific jobs that are in the highest demand will likely fluctuate based on the specific context and needs of the moment. As we begin 2021, here are what appear to be the healthcare jobs in the greatest demand for this year:

- Nurse Practitioner
- Medical Assistant
- Physical Therapist Assistant
- Occupational Therapy Assistant

As the COVID-19 pandemic evolves and more is revealed about gaps in our healthcare services, it will become clearer which healthcare careers may be the most fruitful to pursue in the coming years. Here are 6 healthcare jobs that have proven to be pandemic safe:

1. Medical Lab Technicians
2. Medical Transcriptionists
3. Claims Processors - Medical
4. Copywriters - Medical
5. Healthcare Data Analysts
6. Epidemiologists

So, even in a pandemic that decimates an economy such as COVID-19, there are some jobs that will still be in high demand and are essentially pandemic proof.

CONCLUSIONS AND RECOMMENDATIONS

The severe acute respiratory syndrome COVID-19 pandemic had a significant economic and social impact on the world. Since there is no effective medical treatment for COVID-19 at present, the international collaborative efforts are more focused on developing a safe and effective vaccine against COVID-19. It is recommended that HBM variables predicting a definite intention to take COVID-19 vaccination should be considered in the design of public health interventions to promote COVID-19 vaccination uptake at the community level. This explored various factors influencing the COVID-19 vaccination intention and the applicability of the health belief model to explore vaccination intention among the population of world. Increasing COVID-19 vaccine uptake among a larger section of the population is an important public health priority at the moment as many vaccine candidates approved for use have shown efficacy in preventing COVID-19 infection and associated complications. Vaccination is the most effective method for a long-term strategy for prevention and control of COVID-19 in the future.

Recent emergence and spread of variants have been reported with some concern regarding possible increased transmission and impact on immune responses. There is a real need for more studies regarding the public perception and acceptance of COVID-19 vaccines in the world. Further study should include the impact of COVID vaccine uptake on these rates.

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PP1.

INFLUENCE OF NITROGEN FERTILIZERS ON THE BIOLOGICAL ACTIVITY OF THE SOIL OF THE RAPESEED ROOT ZONE

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Abstract. *Soil microbiocenosis is a dynamic, well-balanced system with a complex spatial and trophic organization. The main biosphere function of soil microbiocenosis is the destruction of organic matter. The polyfunctionality of microorganisms in the rhizosphere gives them the opportunity to participate in opposite biochemical reactions of the soil, which underlie the preservation of metabolic equilibrium in nature. Agrotechnological methods of growing rapeseed require high doses of mineral fertilizers, especially nitrogen fertilizers. The use of high doses of nitrogen mineral fertilizers leads to changes in the balance of the chains in the ecosystem, including microbiological activity. It is microbiological activity that is the reactionary force that quickly reacts to external factors.* ***Method****: In our work, a study of the biological activity of the soil of the root zone of rapeseed for the action of nitrogen fertilizers is presented. The study was carried out in the soil of temporary field experiments on agrocenosis of winter rapeseed, variety Black Velikan. The conditions of the experiment assumed the introduction of nitrogen fertilizers – 120, 150 and 180 kg/ha. The control option did not include fertilization. Determination of the biological activity of the soil in the root zone of rapeseed plants included the determination of the microbial biomass of carbon, the intensity of carbon dioxide emission, antifungal and cellulolytic activity, and the phytotoxicity of the soil.* ***Results:*** *The study of the soil of the root zone of rapeseed plants showed a directly proportional relationship between the dose of nitrogen fertilizer applied and biological activity. It was revealed that the fungistatic status of the soil of the root zone of rapeseed plants under the condition of applying nitrogen fertilizers in comparison with the control grew by 2.3–5.5 times; cellulosic activity by 1.5, the intensity of carbon dioxide emission increased by 2.1–3.6 times.* ***Conclusions****: The content of microbial biomass was recorded at a high level; studies have shown that the content did not depend on the amount of fertilizers applied. Thus, the indicators of the biological activity of the soil of the root zone of rapeseed plants in the flowering phase indicate that the physiological and metabolic activity of microbial communities is at a high level and depends on exogenous sources of nitrogen.*

Keywords: *biomass, carbon dioxide emission, phytotoxicity, antifungal soil activity, cellulolytic activity, nitrogen fertilizers, rapeseed*

INTRODUCTION

Microbial communities of the soil are represented by a set of organisms interacting between themselves, which populate an ecological niche with homogeneous conditions and transform the energy and organomineral substances of this ecosystem. All connections in the community between members of associations of microorganisms are built on trophic interactions [1]. Most soil

microorganisms, with the exception of oligotrophs, remain inactive until an exogenous organic substrate is supplied [2].
The active role of the root system in most metabolic and synthetic processes, as a result of which complex substances are formed from the absorbed ions and assimilants of leaves, which are important not only for plants, but also for the soil microflora in contact with the root [3]. Plants cause not only changes in the structure of the microbial community of the rhizosphere, but also form special physicochemical conditions of the soil in the root zone. In turn, the properties of the rhizosphere environment can influence both directly and indirectly on the course of microbiological processes in it [4].
In agro-ecosystems, the cycle and nitrogen conversion cycle is characterized by a complex of interrelated biological, biochemical and chemical processes. A functional feature of nitrogen transformation in soil is their cyclicity. Therefore, the processes of mineralization are always accompanied by the processes of immobilization [5] and constantly occur in the soil. The most complete description of the interrelations of the processes of mineralization and immobilization was proposed by Janson [6] in the theory of a constantly operating intrasoil nitrogen cycle.
According to the theory of the intrasoil nitrogen cycle, in the process of immobilization, the ammonium form of nitrogen is more actively assimilated by the soil microbiocenosis in comparison with the nitrate form [5–7]. Each ecological-trophic and taxonomic group of microorganisms takes part in the processes of nitrogen immobilization-mineralization, the degree of which is determined by ecological-soil conditions [7, 9, 10].
The use of high doses of nitrogen mineral fertilizers in agricultural production leads to changes that disrupt the balance of chains in the ecosystem, formed over a long period. The peculiarities of the transformation of compounds of biogenic elements, including nitrogen, in agrocenoses depend on a number of factors, which include the climatic conditions of the region and a particular season, the geochemical influence of soils, the type of vegetation, anthropogenic impact and microbiological activity, is the least studied.
The structure of microbial populations (spatial, taxonomic, and functional) mainly depends on the totality of relationships that develop within communities. The article presents a study of the effect of nitrogen fertilizers on the biological activity of the soil of the root zone of rapeseed plants during flowering.

MATERIALS AND METHODS

The studies were carried out in the soil of the root zone of winter rapeseed plants, Black Giant (originator Vinnitsa State Agricultural Experimental Station, Ukraine). The predecessor is black steam. The accounting area of the plots is $25m^2$. The placement of variants in the experiment is systematic - sequential, repetition - fourfold. Experiment scheme: 1. Control (without fertilization) 2. $N_{120}P_{80}K_{140}$; 3. $N_{150}P_{80}K_{140}$; 4. $N_{180}P_{80}K_{140}$.
Soil – gray forest podzolic medium loam, humus content up to 2.06%, pH_{salt} 4.2, hydrological acidity 3.24 mg eq. per 100 g of soil, content of alkaline hydrolyzed nitrogen 74.2 mg/kg of soil (according to Cornfield), mobile phosphorus 174 mg/kg of soil and exchangeable potassium 115 mg/kg of soil (according to Kirsanov).
In autumn, the background of feeding rapeseed plants with nitrogen fertilizers was not carried out, since the excess of nitrogen ions in the soil solution reduces the frost resistance of plants [11]. The first feeding (for all experimental variants) of rapeseed plants with nitrogen fertilizers was carried out in the spring of next year, on frozen soil before vegetation. The second top dressing (for the 3rd and 4th variants of the experiment) was carried out two weeks after the resumption of the growing season before the beginning of the budding phase in the amount of 30 kg/ha. The third stage of feeding rapeseed plants was carried out only for the 4th variant, in the amount of 30 kg/ha, before flowering.
Soil samples of the rapeseed rhizosphere were taken in the 2nd decade of May (flowering phase), since it is believed that during the flowering of a plant, processes in the microbiocenosis are most active
The biomass of microorganisms in the soil of the root zone was determined by the rehydration method [12]. The intensity of carbon dioxide emission from the soil was determined by the Shtatnov absorption

method. The cellulose-destructive activity of the soil was determined by the Christensen method in the Zvyagentsev modification [13].
The phytotoxicity of the soil was determined by the Grodzinsky method in the Mochalov – Sherstoboev modification [14]. The antifungal activity of the soil was determined by decomposing soil samples of 0.5 g into wet disks of filter paper with a diameter of 2 cm, which were laid out in the center of a Petri dish for sowing on the lawn of test cultures of phytopathogenic fungus, which was grown on acidic Chapek medium for micromycetes, with the measurement of the transparent zone of no growth of the test culture around the paper after 5 days. [15]. Statistical processing of experimental results was performed with Statistica 6.0 program.

RESULTS AND DISCUSSION

Microbial groups as in the soil and also in the biocenosis cooperate with each other, creating extremely complicated complexes. Such groups use natural resources in their interactions, the functioning of which is ensured by a large number of trophic and energy connections between plants, animals, fungi and microorganisms. Biological activity of soil is a function of its living component, which demonstrated through the intensity of biological processes of the microbial group. In the process of vital activity, the soil microflora processes organic residues in the soil, and the microbial mass itself, the value of which is directly dependent on the content of organic matter, is an important source of nutrients available to plants.
In temporary field studies, it was found that the application of large amounts of mineral fertilizers, which are limiting factors in the formation of vegetative mass of rapeseed plants, high quality crops, caused some changes in the microbiocenosis of rapeseed rhizosphere. In agroecosystems studied the content of microbial biomass, carbon dioxide emissions, phytotoxicity, cellulose-destructive and antifungal activities under the application of nitrogen fertilizers (Table 1).
The obtained results indicate that an active increase in the content of microbial biomass was observed in the flowering phase (the most active phase of ontogeny of rapeseed plants) and its gradual decrease in accordance with the final phases of plant vegetation. As for the introduced nutrients, an increase in the microbial carbon content was observed for all three concentrations of nitrogen relative to control. But the difference between the options in determining this indicator was not significant. The intensity of carbon dioxide release is a very important integral indicator that determines the level of overall biological activity of the soil. The level of carbon dioxide emission intensity was directly proportional to the amount of nitrogen fertilizers applied. Thus, the difference in respiration intensity between variant $N_{180}R_{80}K_{140}$ and control variant was 30.4 mg of CO_2/kg of soil per day. Thus, the intensity of dioxide emissions was higher in the variants where nitrogen fertilizers were applied in the amount of 150 kg/ha and 180 kg/ha.

Table 1 Biological activity of the soil of the root zone of winter rapeseed plants (flowering phase)

Variant	Microbial biomass, μg C/g of soil	Intensity of CO_2 emission of soil, mg CO_2/kg of soil per day	Phytotoxicity of soil, %	Antifungal activity, mm	Cellulose-destructive activity, %
Control	174.4±11.2	11.5±1.6	6±1.1	1.88±0.08	27.6±1.1
$N_{120}P_{80}K_{140}$	201.7±14.8	24.3±1.1	10±1.1	4.32±0.15	26.4±1.5
$N_{150}P_{80}K_{140}$	209.6±20.1	35.7±2.4	12±1.2	6.28±0.31	24.7±1.8
$N_{180}P_{80}K_{140}$	198.6±14.3	41.9±3.7	13±1.2	10.42±0.42	39.3±2.6

The level of phytotoxicity of the soil of the root zone of rapeseed plants in the flowering phase was not high and ranged from 6 to 13%, which indicates a small number of phytopathogenic micromycetes in the root zone of plants at the beginning of the growing season.
The role in the formation of the fungistatic status of the soil belongs to the microbial group; in the conditions of the experiment the application of nitrogen fertilizers increased the antifungal activity of the soil. The fungistatic status of the rapeseed rhizosphere soil depended on the amount

of applied nitrogen, and the antifungal activity increased as the amount of applied fertilizers increased.
The antifungal activity of the rhizosphere soil of the variant where rapeseed plants were grown without adding additional food sources (control) in the physiologically most active phase of plant development was 2 times lower compared to the variant where nitrogen was applied in the amount of 120 kg/ha, and 5.5 times compared to the variant where nitrogen was applied in the amount of 180 kg/ha, the zone of suppression of the test culture was 2.1 mm for control and 11.4 mm – for the variant with the maximum amount of nitrogen applied.
According to the intensity of cellulose transformation, the soil of the root zone of rapeseed plants of the experimental agrocenoses was characterized by an average level of activity. In the flowering phase of rapeseed plants, the greatest development of cellulose-destructive microflora was noted in the variant $N_{180}P_{80}K_{140}$ – 36%.

CONCLUSIONS AND RECOMMENDATIONS

The results of the study of the biological activity of the soil of the root zone of rapeseed plants in agrocenoses show that there is a linear relationship between the studied indicators (biomass, carbon emissions, phytotoxicity, cellulose activity, antifungal activity) and the amount of nitrogen fertilizers.
It is shown that the fungistatic status of the soil of the rhizosphere of rapeseed plants with increasing the amount of applied nitrogen fertilizers increased by 2.3–5.5 times; cellulose-destructive activity of 1.5, the intensity of carbon dioxide emissions increased by 2.1–3.6 times.

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PP2.

ASSESMENT OF THE POTENTIAL RISK ELEMENTS AT METAL POST-MINING SPOIL HEAPS

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Abstract Motivation/Background: *Metallic minerals mining and smelting are connected with the environment degradation, including spoil heaps arising. Even after processing, spoil still contains high levels of the metals, metalloids as well as other potential risk elements and became a potential source of air, water and soil metalliferous contamination for dozens of years. Eastern Slovakia is well known for its mining history and presence of old mining excavation, yet the spoil heaps represent almost the third of them.*
Method: *We surveyed potential risk elements, including metals and metalloids in the substratum from overall nine spoil heaps (study sites) within the metal post-mining areas of Krompachy (KR) and Zlatá Baňa (ZB), located in Eastern Slovakia. The paper deals with characterization of substratum metalliferous contamination, with lay emphasis on the environmentally most relevant pollutants.*
Results: *At all study sites, iron and aluminium were in the highest content, at both localities, Fe exceeded permissible limits and background values almost 43-times. Overloading of permissible limits as well as background levels of arsenic, cadmium, lead and mercury was noticed too. Clustering indicates the identical origin of risk elements, since iron and aluminium are supposed to be purely lithogenic and the rest of them are in increased concentrations as a consequence of mining activities.*
Conclusions: *On the basis of results obtained, both of the areas could be characterized as highly polluted by environmentally relevant elements including arsenic, cadmium, copper, lead and especially mercury, i.e. heavy metals and metalloids found to be highly toxic even at low concentrations.*

Keywords: *heavy metals, mining activities, pollution*

INTRODUCTION

Areas of Krompachy (KR) and Zlatá Baňa (ZB), located in Eastern Slovakia, are both well-known for their mining history. Intensive gold, silver, cooper and also iron mining with consequential smelting activities started at the 13th century in Krompachy. At the 16th century, gold, antimony and colored metals mining started at Zlatá Baňa area, which is also well-known for the largest and the oldest opal deposits in the Europe. Although mining and smelting activities are significantly limited nowadays at both of localities, huge amounts of waste from the excavating related activities are presently deposed in a form of spoil-heaps. Spoil heaps are found to be environmental loads due to being potential source of water, air and soil contamination with potentially risk elements including heavy metals and metalloids. Negative consequences for local ecosystems persist for dozens of years, even after the cessation of mining operations. Yet, the spoil heaps represent almost the third from the 17.000 old mining excavations which could be found in Slovakia [1], [2], [3]. Metals are of the natural origin and several of them are found to be biologically essential components. But, if their levels increase after anthropogenic activities, wide spectrum of negative impacts are observed: heavy metals affect fauna behaviour, physiology or morphology; metalliferous pollution inhibits plants growth, cause chlorosis and plant death, decelerate photosynthesis, biomass production and nutrient assimilation; change quantity and species spectrum composition of affected flora and fauna; soil enzymatic production and activity are negatively correlated with heavy metal pollution too. Different flora and fauna groups are differently tolerable to metalliferous pollution. Humans intoxication is manifested by a wide range of carcinogenic, teratogenic or mutagenic diseases [4], [5], [6], [7], [8], [9], [10]. Just the communities within metal post-mining spoil heaps and in the surrounding environment are chronically exposed to potentially lethal dozens of environmental hazardous elements including heavy metals and metalloids. Thus, regular monitoring of risk elements sources is essential.

MATERIALS AND METHODS

Potential risk elements, including metals and metalloids were determined in the substratum from overall nine spoil heaps i.e., study sites: four spoil heaps were situated within the metal post-mining areas of Krompachy and five spoil heaps situated within Zlatá Baňa. Heaps are under different stages of vegetation succession, uncovered, with the dominance of bare-naked substratum and the occurrence of sparse trees as *Betula pendula* L. and mosses.

Figure (1): Spoil heaps at Krompachy and Zlatá Baňa study areas

A mixed substratum sample was taken once at every study site. The sample consisted of three sub-samples, randomly taken within the sampling plot of approximately 15 x 15 meters, located as near as possible to the centre of spoil heaps and from the depth of 5-15 cm [11]. Following elements were determined using Agilent ICP-OES spectrometer: aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), antimony (Sb) and zinc (Zn) in mg/kg. Prior to analysis, substratum was mineralized using microwave digestion Mars Xpress 5 in a mixture of 5 mL 67% HNO_3 and 5 mL deionized water. The mineralized solution was filtered and subsequently filled up with deionized water. Determined contents of potential risk elements were compared to permissible limits [12], [13] and background values [14], [15]. Contents of potential risk elements in substratum were used to determine:
(1.) Cd-degree of contamination indicating (a) low (Cd<8); b) moderate (8< Cd <16); c) considerable (16< Cd <32) and d) very high (Cd >32) degree of contamination; (2.) Cf-contamination factor indicating (a) low (Cf <1); b) moderate (1< Cf <3); c) considerable (3< Cf <6) and d) very high (Cf >6) contamination [34]); (3.) GeoI – Geo-accumulation index indicating (a) unpolluted area (GeoI ≤ 0); (b) unpolluted/moderately polluted area (0< GeoI ≤1); moderately polluted area (1< GeoI ≤2); moderately/heavily polluted area (2< GeoI ≤3); heavily/extremely polluted area (3< GeoI ≤4); extremely polluted area (4< GeoI ≤5) (4.) PLI - Pollution load index indicating (a) no pollution (PLI < 1), b) moderate pollution (1< PLI <2), c) heavy pollution (2< PLI <3) and d) extreme pollution (PLI >3) [16], [17], [18], [19], [20].
Mentioned factors were not proposed for the Ag and Mo because of missing background values and for Sb and Se because of the zero values at some of the sites. Mean values of the potential risk element´s contents of the entire study area of Krompachy and Zlatá Baňa were determined using univariate statistic, potential risk elements were clustering used Ward´s method and mutually correlated using Spearman's correlation analysis in the PAST [21].

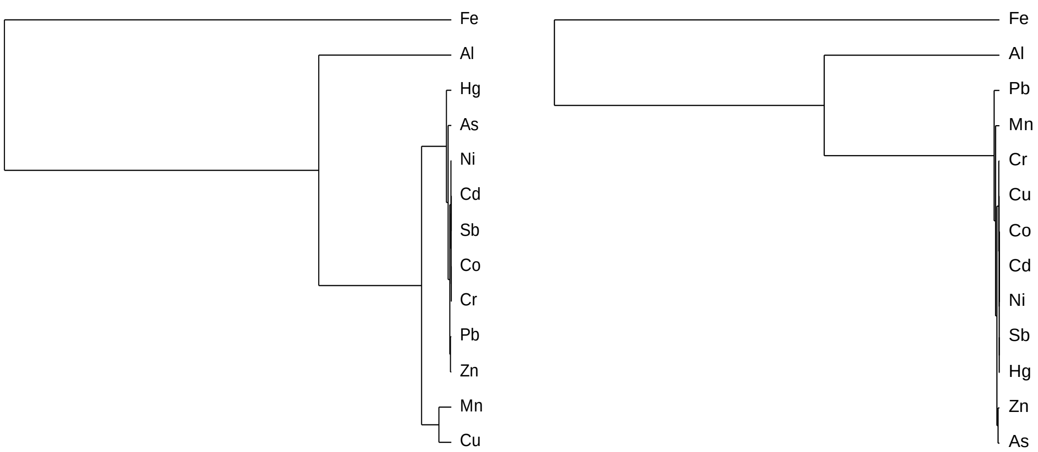
RESULTS AND DISCUSSION

At every from spoil heaps in Krompachy as well as in Zlatá Baňa included in the study, the highest content of iron was noticed from the elements determined in the substratum (Table 1, Figure 2).
Table 1: Permissible limit levels (PL) in mg/kg and background values (BV) in mg/kg of potential risk elements used within study and determined contents (mg/kg) of selected elements in the substratum from four spoil heaps were situated within the metal post-mining areas of Krompachy (KR 1-4) and five spoil heaps situated within Zlatá Baňa (ZB 1-5).

Table 1: Permissible limit levels (PL) in mg/kg and background values (BV) in mg/kg of potential risk elements

	PL	BV	KR-1	KR-2	KR-3	KR-4	ZB-1	ZB-2	ZB-3	ZB-4	ZB-5
Al	10	900	7 992	7 321	6 701	8 215	10 870	11 798	9 968	7 888	9 053
As	30	7	17.74	351.8	96.41	55.34	9.916	75.90	74.30	70.88	280.1
C	1	0.3	2.130	4.149	2.929	1.506	2.662	2.183	3.138	3.277	1.455
Co	15	25	9.029	12.39	13.12	5.227	3.694	1.775	2.162	6.102	0.371
Cr	60	10	12.78	9.418	17.81	11.58	47.36	64.54	11.08	4.541	5.864
C	50	25	15.30	3123	688.7	105.6	18.66	18.40	20.12	15.03	18.74
Fe	550	550	20 422	35 517	26 627	11 915	25 969	21 787	28 996	26 696	13 784
H	0.5	0.0	434.8	36.65	1.611	0.742	1.278	24.69	0.356	1.369	14.55
M	488	530	392.2	1268	915.0	329.1	79.32	41.99	38.69	383.7	48.07
Ni	50	25	19.14	39.64	15.19	10.85	5.017	1.645	2.074	3.216	0.353
Pb	70	25	1.726	11.96	21.61	51.25	11.51	10.04	9.586	70.66	513.5
Sb	1	0.6	0	3.769	0.947	0	2.568	1.653	1.942	0.628	2.047
Zn	150	70	26.66	110.3	92.13	106.3	26.21	18.54	20.39	107.3	70.41

In general, mean values of Fe for both entire localities exceeded permissible limits and background values almost 43-times. Aluminum was the second highest represented element at every from the spoil heaps studied. Provided elements clustering showed the individual position of Fe and Al in comparison to the rest of monitored potential risk elements. We suppose that detected potential risk elements became from identical source; however, iron and aluminum are purely lithogenic, till the increased concentrations of the rest of them are conditioned by mining activities.



(a) (b)

Figure 2: Dendrogram of monitored elements at Krompachy (a) and Zlatá Baňa (b) study areas based of their contents in substratum, provided using clustering, Ward´s method in the PAST [17].

Concerning As, Cd, Cu, Hg and Pb elements, which are found to be environmentally relevant, the most risk and highly toxic even at low concentrations [22], arsenic limit level was exceeded with the

exception of KR1 and ZB1 at every from the spoil heaps and, As background limit even every time. The most expressive it concerned KR2 and ZB5 spoil heaps when content of arsenic was higher than the permissible limit level more than 11 and 9 times consequently. Previous studies [1], [14], which reported arsenic environmental pollution of the Central Spiš area, relate it not only to the anthropogenic impact but also to the geochemical effects of the mineralized zones.
Cadmium content exceeded permissible as well as background at every sample from spoil heaps studied, the most expressive at KR2. At the same study site equally content of copper was more than 62 times as high as permissible limit. High content of Cu was also observed on the other from spoil heaps in Krompachy, KR3 and KR4, in spite of their mutual distance. Taken into account evaluating factors, entire post mining area of Krompachy could be found as considerable polluted by copper, or as hotspot of copper contamination [23], [24], (Table 2)..

Table 2: Values of Cd-degree of contamination, Cf-contamination factor, Geo-accumulation index and PLI - Pollution load index for the separate spoil heaps and entire study area of Krompachy.

	Cf				**GeoI**				**Cd**
	KR-1	**KR-2**	**KR-3**	**KR-4**	**KR-1**	**KR-2**	**KR-3**	**KR-4**	
Al	8.879	8.135	7.446	9.128	2.566	2.439	2.311	2.605	33.59
As	2.534	50.26	13.77	7.906	0.756	5.067	3.199	2.398	74.47
Cd	7.101	13.83	9.763	5.020	2.243	3.205	2.702	1.743	35.72
Co	0.362	0.496	0.525	0.209	-2.054	-1.598	-1.515	-2.843	1.591
Cr	1.278	0.942	1.781	1.158	-0.231	-0.672	0.248	-0.373	5.159
Cu	0.612	124.9	27.55	4.224	-1.293	6.379	4.199	1.494	157.3
Fe	37.13	64.58	48.42	21.67	4.630	5.428	5.012	3.852	171.8
Hg	5435	458.2	20.13	9.273	11.82	8.255	3.746	2.628	473.8
Mn	0.740	2.392	1.726	0.621	-1.019	0.673	0.203	-1.272	5.479
Ni	0.765	1.586	0.608	0.434	-0.970	0.081	-1.303	-1.789	3.393
Pb	0.069	0.478	0.864	2.052	-4.442	-1.649	-0.795	0.451	3.462
Sb	-	-	-	-	-	-	-	-	-
Zn	0.381	1.576	1.316	1.519	-1.978	0.071	-0.189	0.018	4.792
PLI	4.097	2.742	1.752	2.609					

Concerning lead, permissible and background limits overload were noticed only at ZB5 sites, with the more than 7-times higher value. We noticed exceptionally high mercury content: with the exception of ZB3 spoil heap, permissible limit was exceeded at every from the study sites. It could be supposed, that increased contents of Pb is connected with the solid waste from the copper production [25].
We also noticed exceeded permissible limits and background values by manganese, antimony, selenium and chromium (Table 3).

Table 3: Values of Cd-degree of contamination, Cf-contamination factor, Geo-accumulation index and PLI - Pollution load index for the separate spoil heaps and entire study area of Zlatá Baňa.

	Cf					***GeoI***					***Cd***
	ZB-1	**ZB-2**	**ZB-3**	**ZB-4**	**ZB-5**	**ZB-1**	**ZB-2**	**ZB-3**	**ZB-4**	**ZB-5**	
Al	12.08	13.12	11.08	8.765	10.06	3.010	3.128	-14.69	-9.901	2.746	55.09
As	1.417	10.84	10.62	10.13	40.01	-0.083	2.854	9.891	9.553	4.737	73.01
Cd	8.873	7.277	10.46	10.924	4.851	2.565	2.278	7.236	7.853	1.693	42.38
Co	0.148	0.071	0.086	0.244	0.015	-3.344	-4.401	-3.579	-3.516	-6.659	0.564
Cr	4.736	6.454	1.108	0.454	0.586	1.659	2.105	-2.795	-1.298	-1.355	13.34
Cu	0.747	0.736	0.805	0.601	0.750	-1.007	-1.027	-1.759	-3.046	-1.001	3.638
Fe	47.22	39.61	52.72	48.537	25.06	4.976	4.723	-5.358	-5.778	4.062	213.1
Hg	15.97	308.7	4.456	17.112	181.91	3.413	7.686	1.571	3.512	6.922	198.1

Mn	0.150	0.079	0.073	0.724	0.091	-3.325	-4.243	-7.374	-6.471	-4.048	1.117
Ni	0.201	0.066	0.083	0.129	0.014	-2.902	-4.511	-4.407	-4.849	-6.731	0.492
Pb	0.460	0.402	0.383	2.826	20.54	-1.704	-1.902	-4.177	-3.544	3.775	24.61
Sb	4.280	2.755	3.236	1.047	3.411	1.513	0.877	3.413	6.295	1.185	14.73
Zn	0.374	0.265	0.291	1.533	1.006	-2.002	-2.502	-2.455	-1.301	-0.577	3.47
PLI	1.389	1.457	1.014	1.679	1.386						

No significant correlations were observed between the elements at Krompachy area, at Zlatá Baňa, several correlations were observed as indicated in Table 4.

Table 4: Mutual correlations between elements in Zlatá Baňa detected using Spearman´s Rs correlation test in PAST.

	Al	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Sb**	**Zn**
Al	**0**												
As	0	**0**											
Cd	-0.4	-0.7	**0**										
Co	-0.3	-0.9*	0.9*	**0**									
Cr	1*	0	-0.4	-0.3	**0**								
Cu	0.1	0.3	-0.3	-0.5	0.1	**0**							
Fe	-0.2	-0.6	0.9*	0.7	-0.2	0.1	**0**						
Hg	0.2	0.6	-0.6	-0.5	0.2	-0.5	-0.8	**0**					
Mn	-0.5	-0.5	0.3	0.6	-0.5	-0.7	-0.1	0.1	**0**				
Ni	0	-1*	0.7	0.9*	0	-0.3	0.6	-0.6	0.5	**0**			
Pb	-0.6	0.2	-0.3	-0.1	-0.6	-0.3	-0.6	0.4	0.7	-0.2	**0**		
Sb	0.3	-0.1	-0.5	-0.3	0.3	0.6	-0.3	-0.3	-0.1	0.1	0.1	**0**	
Zn	-0.9*	-0.2	0.3	0.4	-0.9*	-0.3	0	-0.1	0.8	0.2	0.8	-0.1	**0**

CONCLUSIONS AND RECOMMENDATIONS

In spite of the significant limitation of smelting activities in the study areas of Krompachy and Zlatá Baňa post metal-mining spoil heaps remain a strong source of the environmental contamination with potential risk elements, including heavy metals and metalloids, which, as non-biodegradable, remain accumulated in the substratum for years [26], [27]. Especially it concerns environmentally the most risk, highly toxic As, Cd, Cu, Hg and Pb elements, which contents exceeded permissible limits several times. Spoil heaps as the contamination sources are very, *spatially*, variable – different content of the same element at different spoil heaps in spite of the localisation within the same study area and low mutual distance; as well as, *time*, variable, as the element´s contents are changing during the time [2]. As the risk elements could be spread to the surrounding environment [4] and recultivation and remediation are technically demanding, is seems important to avoid erosion of the spoil-heaps body by f.e. timber harvesting in the vicinity as much as possible.

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PP3.

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PP4.

ASSESSMENT OF CREEP ON LONG-TERM PERFORMANCE AND CALIBRATION FACTOR OF GEOSYNTHETICS IN GEOENVIOR-MENTAL APPLICATIONS

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Abstract: *Geosynthetics became one of the outstanding innovations with ever-growing significance for geotechnical and geo-environmental engineering industry. Earthwork construction over soft polluted sludge lagoons and tailing impoundments is mandatory to cap various waste materials. Low shear strength and high compressibility of such soil deposits expose short and long-term geotechnical challenges. Geosynthetics provide strengthening and allow accessibility over these deposits, which save money and reduce carbon footprint. Like all polymeric products, geosynthetics are susceptible to creep. However, creep data is not published by manufactures for community to disclose stiffness modulus degradation with elapsed time, which is necessary for finite element numerical modelling. This manuscript aims to assess creep effect and derive calibration factor between global to local strains. Field monitoring strain readings of high-elongation gauges glued to geosynthetics must be calibrated against "true" global strain. Geosynthetics polyester (PET) geogrid types Fortrac 35T, 55T, 80T, 110T and 150T were examined at the machine direction under constant strain load ranged from 20% to 0.05% strain/min using the multi-rib tensile method. Correlation charts were plotted to predict stiffness reduction versus logarithm of time. The long-term stiffness value decreases in non-linear manner with increasing logarithm of time but after 1000 hours, is considerably constant of 75% of the secant modulus at 2% strain (working loads). Calibration factor between global to local strains ranged from 2.08 to 1.99 was measured at 2% strain, in agreement with comparable factor of 2.2, which was introduced by Allen et al. (2002). Calibration Factor values slightly increased as strain rate decreased.*

Keywords: *creep effect, calibration factor, constant strain rate test, geosynthetics, stiffness degradation, global/local strain.*

INTRODUCTION

Geosynthetics became one of the outstanding innovations with ever-growing significance for geotechnical and geo-environmental engineering industry. Earthwork construction over soft polluted sludge lagoons and tailing impoundments is mandatory to cap various waste materials. Low shear strength and high compressibility of such soil deposits expose short and long-term geotechnical challenges. Geosynthetics provide strengthening and allow accessibility over these deposits, which save money and reduce carbon footprint. Like all polymeric products, geosynthetics are susceptible to creep. However, creep data is not published by manufactures for community to disclose stiffness modulus degradation with elapsed time, which is necessary for finite element numerical modelling. Advanced numerical analysis in conjunction with proper geosynthetics characterization through experimental tests are pragmatically deemed to perform accurate representative studies on associated response mechanisms of geosynthetics reinforced soil.

Creep phenomenon is a classically time-dependent elongation aspect for geosynthetics that affect the long-term performance and sustainable durability of the geosynthetic-reinforced structures. Creep impact is also function of the ambient temperature and service life after installation period. Globally, the creep behavior of geosynthetics is somewhat complicated phenomenon, index to the interaction and complexity of the subsequent components: (1) polymer nature and structure, (2) geosynthetics structure (nonwoven, woven, and integral structure), (3) rate of loading/ unloading conditions, (4) temperature conditions, and (5) soil environmental conditions at filed.

Filed monitoring strain readings of high-elongation strain gauges glued to geosynthetics materials must be calibrated against the "true" global strain as reported by Allen et al., (2002). Strain gauges bonded to woven geosynthetics (geogrids or geotextiles) typically generate a local "hard spot" causing under-registration of global actual tensile strains.

Based on series of Constant Strain Rate CSR tests, this manuscript aims to: (a) announce an overview about the geosynthetics behavior, non-linear tensile force– strain relationship (b) assess influence of creep on long-term performance and stiffness degradation, (c) derive calibration factor between global to local strains, and (d) promotes an appropriate geogrid simulation for Plaxis 3D FE modelling that can be used to predict geosynthetics behavior under service loadings rather than incipient collapse.

MATERIALS AND METHODS

a) OVERVIEW

The current research includes laboratory testing for 5 sets of designated specimens of geosynthetics geogrids Fortrac 35T, 55T, 80T, 110T, and 150T with varying strength and stiffness. **Table 1** listed the disclosed physical properties as per the technical data sheet introduced by the manufacturer HUSKER. The standard raw material is the high-modulus polyester (PET), which of high stiffness, low creep, and uniform product strength. The specimens were examined on a tensile test machine under Constant Strain Rate (CSR) in accordance with ASTM D6637, to investigate: (a) creep effect and secant modulus degradation with time and (b) calibration factor between global to local strains.

Table 1: Physical properties for the tested geogrid types as per HUSKER data sheets

PROPERTY	TEST	**35T**	**55T**	**80T**	**110T**	**150T**
Ultimate wide width (MD) Tensile Strength (kN/m)	ASTM D-6637	**35**	**55**	**80**	**110**	**150**
Elongation at ultimate tensile strength –MD	ASTM D-6637	≤10 %	≤10%	≤10 %	≤10 %	≤10%
Long term design strength-MD (kN/m)	GRI GG4	18.8	29.5	42.9	59	81

b) TEST APPARATUS AND SPECIMEN PREPARATION

TESTOMETRIC M500- 50CT Universal Testing machine was used to perform the geogrid load-extension under Constant Strain Rate CSR. The apparatus comprises main tensile machine, pressure jack and computer control unit. The tensile machine consists mainly of clamps, sensors for recording the tensile force, and other sensors to monitor the extent of grips separation. The clamps must be attached with suitable pressure to avoid slippage. Clamping pressure must be equal for all specimens under the same test to manage consistent judgment between the anticipated results. According to the Multi-Rib tensile method of ASTM D6637 "Standard Test Method for Determining Tensile Properties of Geogrids by the Single or Multi-Rib Tensile Method"; the specimen is schematically illustrated at Figure (1) to be a minimum of 200 mm wide and contains five ribs in the cross-test direction by at least three junctions or 300 mm long in the direction of the testing. The outermost ribs are cut prior to

testing to prevent slippage from occurring within the clamps as instructed by ASTM D6637, clause 8.2.4.
Thus, the width of intact ribs is 120 mm. The room ambient temperature was 20°c during test procedures to avoid adverse impact of the temperature in the test results. Figure (2) shows the particular test setup adopted for derivation of Calibration Factor CF between global to local strains. During the in-isolation tensile testing, strain gauge was mounted on one side of the geosynthetic specimen using soft glue adhesive. A KYOWA gauge type is used with model name KFG-5-120-C1-11L1M2R. Quarter bridge connection was used for 1-gauge 120Ω.

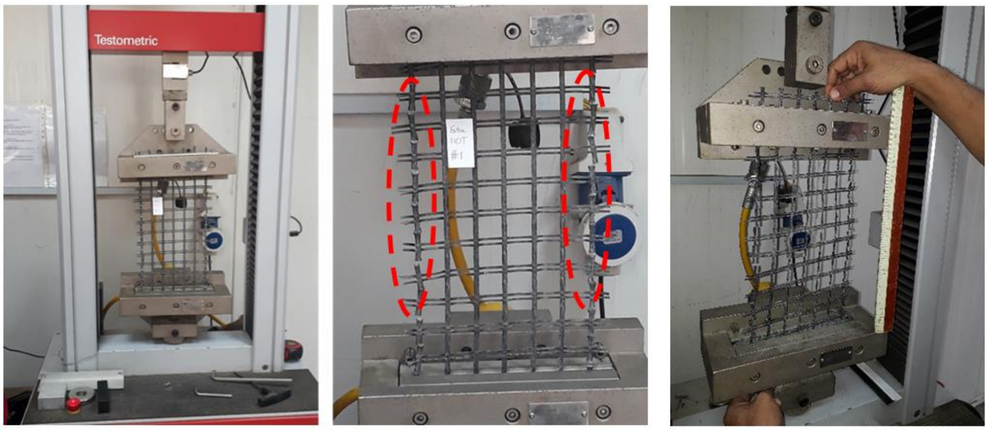

Figure (1): Specimen configuration; Multi-Rib tensile method -ASTM D6637

Figure (2): Test setup for Calibration Factor between local to global strain using CSR tensile test

STATISTICAL ANALYSIS AND GRAPHICAL PRESENTATION

Five single tests were performed on the Machine Direction (MD) for each specimen of Fortrac 35T, 55T, 80T, 110T, and 150T, in accordance with ASTM D6637 using the multi- rib tensile method. Constant Strain Rate CSR of 20%, 10%, 6%, 3%, 1% to slow rate loading 0.05% strain/min. were performed. The sample total width is 200 mm, while the intact width is limited to 120 mm. According to ASTM D6637, clause 8.2.4 “within test methods A, B and C the outermost ribs are cut prior to testing to prevent slippage from occurring within the clamps. For those cases where the outermost ribs are severed, the test results shall be based on the unit of width associated with the number of intact ribs”. Hence, calculation of the secant modulus ($J= EA_1$) at certain strain domain shall be the maximum tensile force divided by the total intact width per meter. For CSR of 0.05%/min, an interval of local strain readings (by automotive data logger) equals to 2 min is utilized to reveal 30 readings through strain domain of 3%. While, for CSR 10%/min, and due to the sensitivity of relatively rapid test loading; two sets were examined for local strain readings of intervals 2 sec and 1 sec, to result in approximately 30 and 60 readings, respectively to reach the failure.

RESULTS AND DISCUSSION

a. Assessment for variation of stiffness with time (creep)

Creep phenomenon is a classically time-dependent elongation aspect for geosynthetics that affect the long-term performance and sustainable durability of the geosynthetic-reinforced structures. Creep impact is also function of the ambient temperature and service life after installation period.

In general, creep phenomena can be divided into three main phases: (1) Primary creep phase, starts at a rapid rate and reduces with time, (2) Secondary creep phase, has a relatively uniform rate, and (3) Tertiary creep phase, has an accelerating sudden creep rate and ends up by failure of material at rupture time as reported by Han-Yong Jeon (2017).

As geosynthetics are generally manufactured from polymer materials, they exhibit a viscoelastic behavior, time, load, and temperature-dependent, under a sustained constant load. At time t, the total strain ε (t) can be expressed from the following equation: $\varepsilon (t) = \varepsilon_0 + \varepsilon_I + \varepsilon_{II} + \varepsilon_{III}$. Where: ε_0 is the instantaneous strain and ε_I, ε_{II}, and ε_{III} are the primary, secondary, and tertiary creep strains, respectively. Nevertheless, the final sudden progressive creep strain increase before rupture does not necessarily occur; it is normally revealed for Polyethylene (PE) and polypropylene (PP) not for Polyester (PET) at standard load levels and temperature.

Globally, the creep behavior of geosynthetics is somewhat complicated phenomenon, index to the interaction and complexity of the subsequent components: (1) polymer nature and structure, (2) geosynthetics structure (nonwoven, woven, and integral structure), (3) rate of loading/ unloading conditions, (4) temperature conditions, and (5) soil environmental conditions at filed.

Since the material is a polyester (PET) geogrid, time-dependent stiffness reductions are minimal (approximately 25-15% or less) as concluded by (Allen and Bathurst 2001); as introduced in **Table 2**. Also, temperature sensitivity of PET is not as great as that for HDPE or PP geosynthetics. For typical geosynthetics applications, the ratio of the modulus value at typical working conditions (i.e., strains on the order of 2% and loading times of approximately 1,000 hours) relative to the modulus obtained from typical CRS wide-width test (at strain 10 %/ min. ASTM D 4595) can be estimated for various Geosynthetics polymer types. Allen and Bathurst (2001) introduced a ratio stiffness modulus at 1000 hours $J_{1000hrs}$/ J_{D4549} for polyester (PET) = 0.75 to 0.85.

Table 2: Ratio stiffness modulus at 1000 hours (Allen and Bathurst 2001)

Geosynthetic Polymer	$J_{1000hrs}$/
PP	0.25 to 0.35
HDPE	0.25 to 0.35
PET	0.75 to 0.85

To convert the measured reinforcement strain to the most accurate estimate of the actual reinforcement load, each measured strain to be multiplied by the secant stiffness value that corresponds to the elapsed time. The stiffness value used was taken at a strain equal to the measured strain, but not more than the stiffness value at 1% strain. The time used to calculate the stiffness values was taken with respect to when the layer was installed in the field (Allen and Bathurst, 2014).

Consequently, series of CRS tests were conducted and stress strain curves were obtained for different strain rates of 20%/min, 10%/ min, 6% /min, 3%/min, 1%/min, and 0.05%/min. From the test results and measurement, the loads and time to reach 1% and 2% strains are calculated, and then secant stiffness as well. Long- term stiffness for strain equaled 2% at 1000 hour (creep data from Walters et al.,(2002)) was estimated as 0.75 multiplied by secant modulus at strain 2% measured from CSR of 10%/min (ASTM D 4595). Ratios between stiffness ($J_{2\%}$) to stiffness ($J_{1\%}$) at the same time are evaluated as presented in Table 3. Using average of these ratios, stiffness at strain 1% after 1000 hour can be estimated. The stiffness value decreases with increasing logarithm of time but is reasonably constant after 1000 hours, as reported by (Miyata and Bathurst, 2007).

Table 3: Summary of the derived Secant modulus at 1% & 2% strain for Fortrac 110T, 80T, 55T, 35T, and 150T with elapsed time

	CSR %/min.	time to 1% strain (hr)	time to 2% strain (hr)	Force at 1% strain (kN)	Secant modulus $J_{1\%}$ (kN)	Force at 2% strain (kN)	Secant modulus $J_{2\%}$ (kN)	Ratio $J_{2\%}/J_{1\%}$
110T	20	0.0008	0.00167	17.92	1792	25.34	1267	1.41
	10	0.0017	0.00333	15.83	1583	22.55	1128	1.40
	6	0.0028	0.00556	15.00	1500	21.84	1092	1.37
	3	0.0056	0.01111	13.96	1396	20.86	1043	1.34
	1	0.0167	0.03333	13.54	1354	20.66	1033	1.31
	0.05	0.3333	0.66667	12.08	1208	19.51	976	1.24
	Creep 0.75	1000	1000		1127		846	1.33
80T	21	0.0008	0.0016	11.87	1187	17.97	899	1.32
	10	0.0017	0.0033	11.04	1104	17.37	868	1.27
	6	0.0028	0.0056	10.63	1063	16.11	806	1.32
	3	0.0056	0.0111	10.13	1013	15.48	774	1.31
	1	0.0167	0.0333	9.88	988	15.10	755	1.31
	0.05	0.3333	0.6667	9.17	917	14.97	749	1.22
	Creep 0.75	1000	1000		838		651	1.29
55T	25	0.0007	0.0013	9.24	924	13.47	674	1.37
	10	0.0017	0.0033	7.29	729	10.72	536	1.36
	6	0.0028	0.0056	6.56	656	10.26	513	1.28
	3	0.0056	0.0111	6.25	625	9.90	495	1.26
	1	0.0167	0.0333	5.83	583	9.42	471	1.24
	0.05	0.3333	0.6667	5.21	521	9.12	456	1.14
	Creep 0.75	1000	1000		505		402	1.26
35T	20	0.0008	0.0017	5.80	580	8.84	442	1.31
	10	0.0017	0.0033	5.21	521	7.80	390	1.34
	6	0.0028	0.0056	4.79	479	7.18	359	1.33
	3	0.0056	0.0111	4.58	458	6.96	348	1.32
	1	0.0167	0.0333	3.96	396	6.30	315	1.26
	0.05	0.3333	0.6667	3.96	396	6.20	310	1.28
	Creep 0.75	1000	1000		381		292	1.30
150T	20	0.0008	0.0017	23.75	2375	32.56	1628	1.46
	10	0.0017	0.0033	19.58	1958	27.92	1396	1.40
	6	0.0028	0.0056	18.75	1875	27.56	1378	1.36
	3	0.0056	0.0111	16.67	1667	27.24	1362	1.22
	1	0.0167	0.0333	15.21	1521	25.02	1251	1.22
	Creep 0.75	1000	1000		1362		1047	1.30

For practical use, Figure (3) presents correlation design charts for the assessment of stiffness variation at $J_{1\%}$ and $J_{2\%}$ strains for the examined specimens versus logarithm of the elapsed time. These charts can be simply used to predict the secant modulus (after creep effect) corresponds to the elapsed time of each geosynthetics geogrid layer during staged construction at filed.

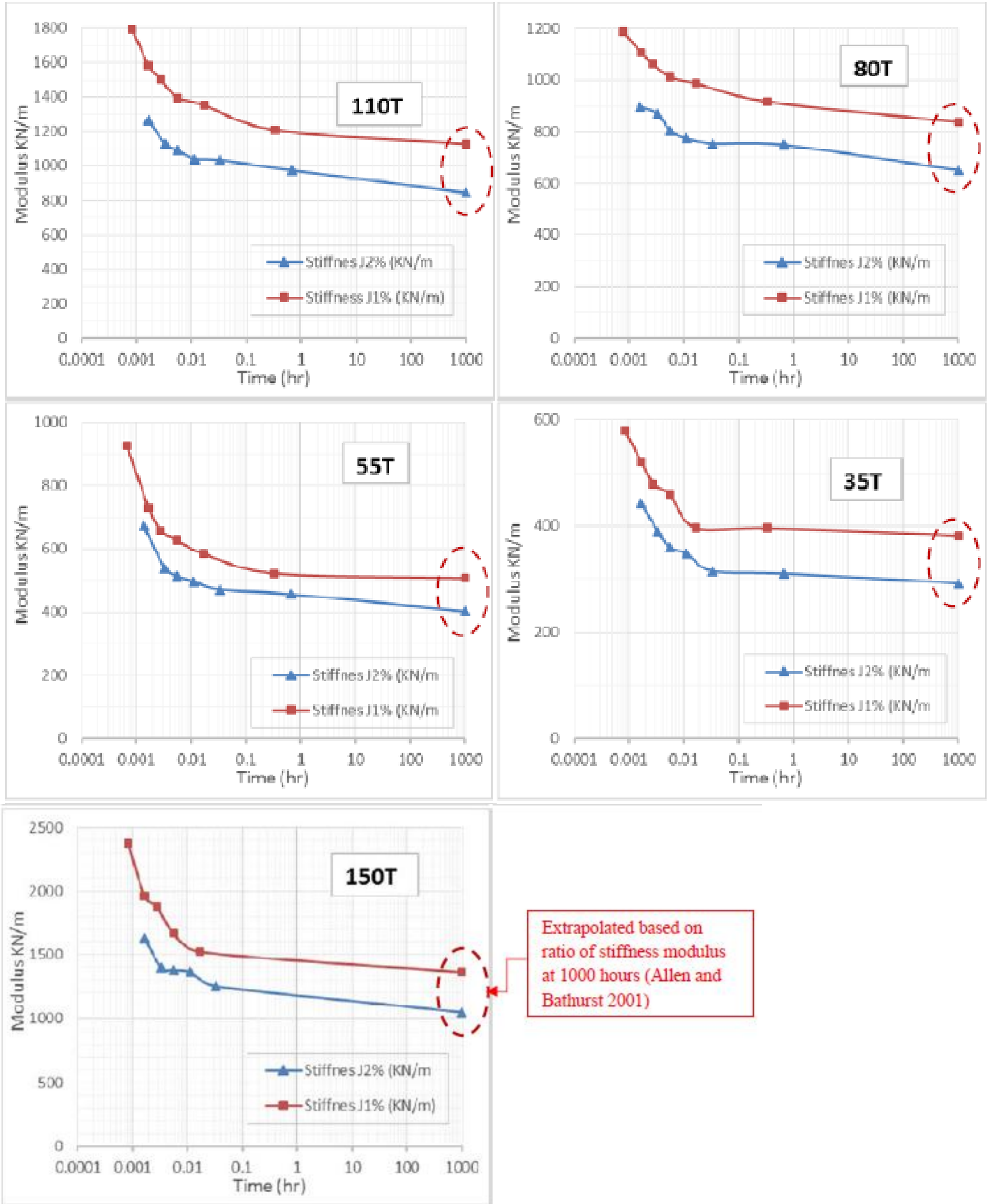

Figure (3): Correlation design charts for variation of secant modulus at 1% and 2% strain with elapsed time for Fortrac 110T, 80T, 55T, 35T, and 150T

Figure (4) demonstrates comparative trend for variation of stiffness with time between 1% and 2% strain for Fortrac 150T, 110T, 80T, 55T, and 35T. The vertical dashed line was plotted for reference to identify modulus J_{D4549} estimated at CSR 10%/min. Figure shows notable behavioural features: (a) non-linear degradation of secant modulus with elapsed time, (b) the derived modulus at strain1% is larger than corresponding at 2% regardless the strain rate of loading, and (c) the higher the stiffness, the higher the stiffness irrespective to strain rate of loadings.

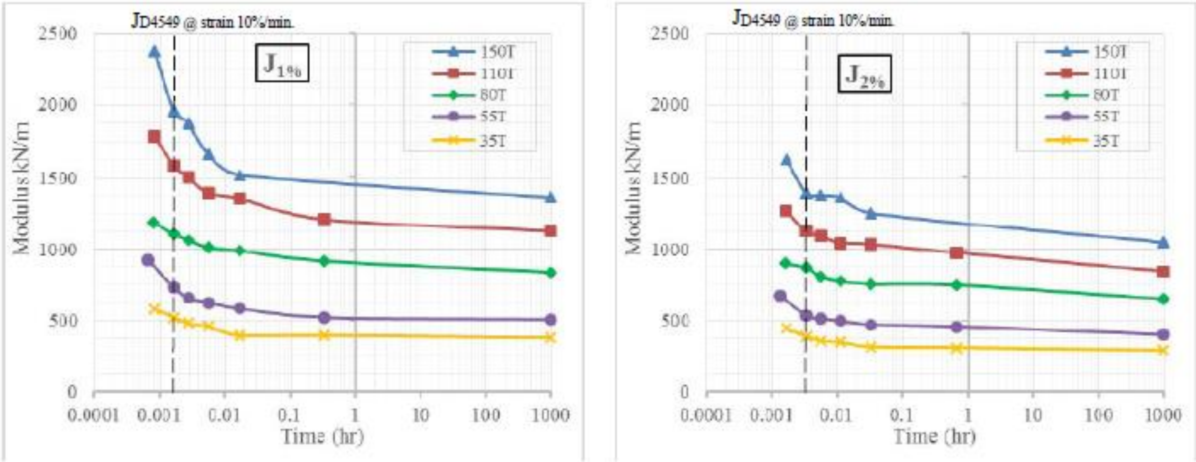

Figure (4): Comparative trend for variation of secant modulus with elapsed time between 1% and 2% strain for Fortrac 150T, 110T, 80T, 55T, and 35T

b. Calibration factor between local to global strain

Strain readings from high-elongation strain gauges glued to geosynthetics reinforced materials must be calibrated against the "true" global strain in the reinforcement as reported by Allen et al., (2002). In the current research, "local strain" or "gauge strain" values denotes strains recorded by a strain gauge at the point of attachment. "Global strain" refers to strain averaged over a length that is much larger than the strain gauge. Strain gauges bonded to woven geosynthetics typically generate a local "hard spot" causing under-registration of global actual tensile strains.

The Calibration Factor CF for a particular combination of gauge, bonding technique, geosynthetics polymer type, and location of gauge is typically established from constant strain rate in-isolation wide-width strip tensile testing in accordance with ASTM D4595 as reported by Allen et al., (2002). Hence, strain gauge readings in the field must be corrected to true global strains by factor derived from in-isolation tensile tests.

In order to better match the realistic (slower) loading conditions of a geosynthetics geogrid layer placed during staged construction, the data from the 0.05 %/min CRS test was used for consistent calibration. The geosynthetics PET geogrid types Fortrac 110T, 80T, 55T, and 35T were tested under CSR of 10%/min as nominal value introduced by ASTM 4595 and at a relatively low strain range of loading 0.05%/min in the domain of 0 to 3% strain, to match the maximum anticipated working conditions under service loadings (no need to reach the strain at break). Strain gauges were mounted on one side of the geosynthetic specimen during the in-isolation tensile testing using quarter- bridge.

For CSR testing of 0.05%/min, an interval of local strain readings equals to 2.0 min is utilized to reveal approximately 30 readings (by automotive data logger) through the strain domain of 3%. While, for CSR 10%/min, and due to the sensitivity of relatively rapid test loading and possible associated missing/ errors for recording the initial significant readings of local strain by data logger; two sets were examined for local strain readings of intervals 2 sec and 1sec, to result in approximately 30 and 60 readings, respectively to reach the failure. However, only limited number of readings (approximate to 10 or 20) at domain of strain 3% is not liable relative to CSR of 0.05%/min.

Figure (5) plotted response curves of the in-isolation local strain versus global strain for various tested specimens 35T, 55T, 80T, and 110T that used to derive the calibration factor. The response curves for constant-rate-of-strain tensile loading fall within a relatively narrow band. The significant under-registration of global strains is considered to be the result of the impregnation of the longitudinal polyester fibre bundles by the gauge epoxy glue, which creates a locally stiff region (hard spot).

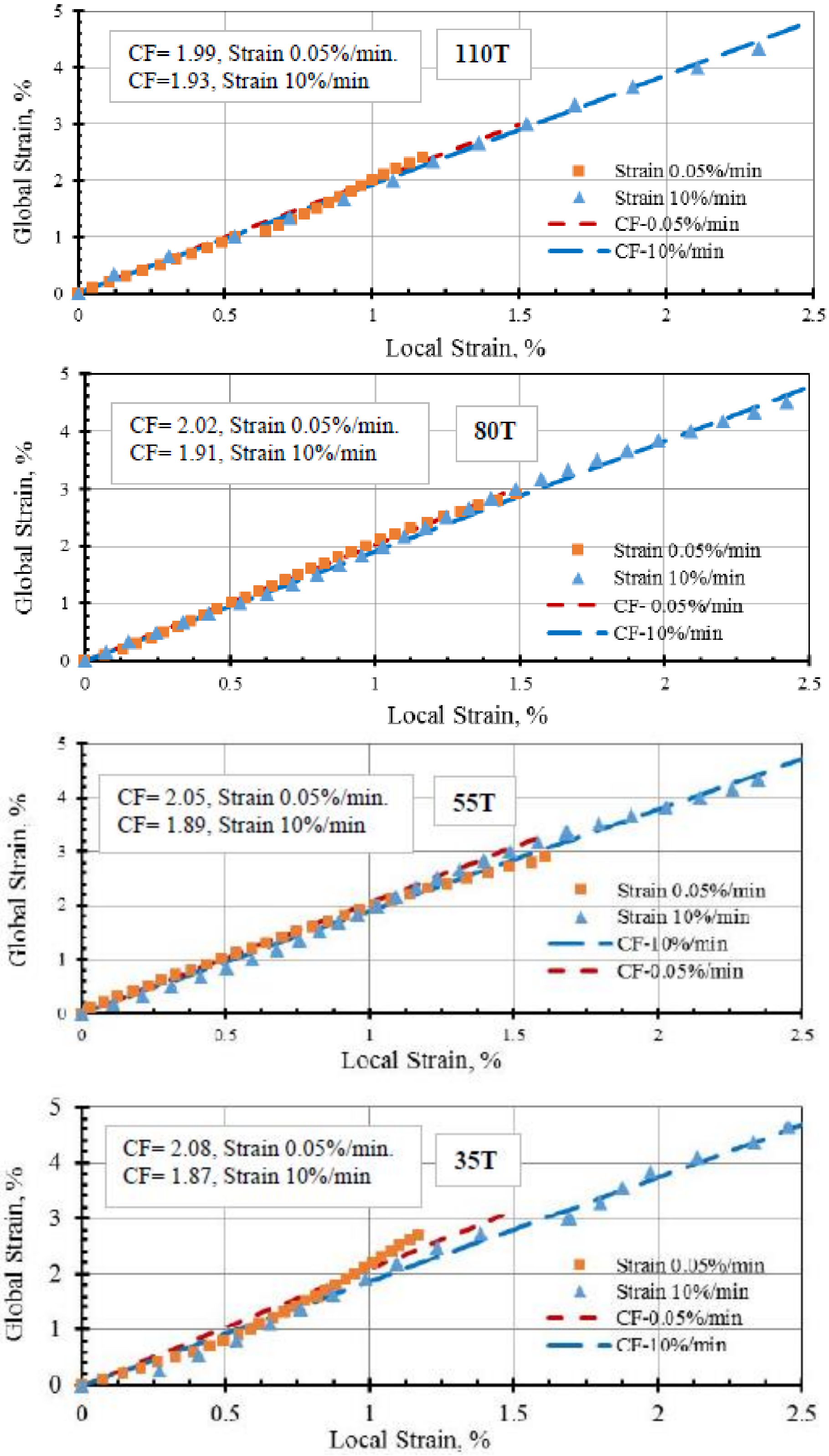

Figure (5): In-isolation local strain gauge response versus global strain for Fortrac 110T, 80T, 55T, and 35T

It should be pointed out that, bonded strain gauges during testing often gave a nonlinear response (or failed in the field) at strains ≥ 3%, hence, extensometers acknowledged the sole practical guaranteed devices of measuring geosynthetics strains at large strain domains. Calibration Factor (CF) values slightly increased as strain rate decreased. A nominal average best fit values for CF of 2.08, 2.05, 2.02

and 1.99 were derived appropriate for such foil strain gauges glued to Husker PET geogrid type 35T, 55T, 80T, and 110T, respectively. A single-value calibration factor from this data over a range of 0 to 2% strain is approximately 2. In other words, the strain gauge values must be doubled to represent "true" global strains. This CF estimation for strain levels ≤ 2% is in worthy agreement with comparable PET geogrid CF of 2.2, which was introduced by Allen et al., (2002). It can be argued that the actual loading history of a geosynthetics reinforcement layer during construction falls between the two idealized loading conditions performed in the laboratory.
It was identified that, to adapt the measured reinforcement strain to the most accurate estimate of the actual reinforcement load, each calibrated measured (global) strain to be multiplied by the secant stiffness value that corresponds to the elapsed time (after considering creep effect). The time used to calculate the stiffness values to be taken with respect to when the layer was installed in the field (Allen and Bathurst 2014).

c. Geosynthetics simulation at numerical plaxis 3d model

Principally, no successful mechanical model is yet introduced to properly identify the extensible polymeric reinforcement geogrid load as reported by Bathurst (2019). However, the linear approximation of secant modulus (after creep consideration) is considerable accepted in the strain domain up to 2% which covers a wide scatter of working conditions under service loadings as concluded by Hatami and Bathurst (2005). The geogrid characterization findings derived from the CSR testing are usually used to simulate the mechanical properties of the geogrid used at Plaxis 3D numerical FE modelling The linear "Elastoplastic- anisotropic" geogrid model introduced by Plaxis 3D seems appropriate to numerically simulate the geosynthetics behavior under service loadings. The derived parameters for the examined specimens are listed at Table 4.

Table 4: Elastoplastic- anisotropic geogrid parameters to be adopted at Plaxis 3D model

Type	35T	55T	80T	110T	150T
EA_1 (kN/m)	369	500	738	948	1186
EA_2 (kN/m)	3.69	5	7.38	9.48	11.86
GA (kN/m)	1.845	2.5	3.69	4.74	5.93
$N_{p,1}$ (kN/m)	18.8	29.5	42.9	59	80.6
$N_{p,2}$ (kN/m)	0.188	0.295	0.429	0.59	0.806

Where:
EA_1 and $N_{p,1}$ denote secant modulus and long term design strength at Machine-Direction MD, respectively derived based on the undertaken CSR test results. To simulate the uniaxial geogrid behavior, the secant modulus EA_2 and Long term design strength $N_{p,2}$ in cross machine direction CD to be introduced equal to 0.01 of that corresponding to MD to avoid numerical errors. Moreover, shear modulus GA is assumed equals to half of secant modulus EA_2 in cross-machine direction CD.

CONCLUSIONS AND RECOMMENDATIONS

The current paper focuses largely on, how to better identify the short and long –term response and material characterization of geosynthetics polyester (PET) geogrid under service loadings rather than incipient collapse. Like all polymeric products, geosynthetics are susceptible to creep. However, creep data is not published by manufactures for community to disclose stiffness modulus degradation with elapsed time, which is necessary for finite element numerical modelling. This manuscript aims to assess creep effect and derive calibration factor between global to local strains. Field monitoring strain "local" readings of high-elongation gauges glued to geosynthetics must be calibrated against "true" global strain. Hence, sets of geosynthetics polyester (PET) geogrid specimens Fortrac 35T, 55T, 80T, 110T, and 150T were examined at the Machine Direction MD under Constant Strain Rate CSR loading tests, in accordance with ASTM D6637 using the “multi-rib tensile” method. A series of CRS tests were conducted wide strain rates of 20%/min. (relatively high rate), 10%/ min, 6% /min, 3%/min, 1%/min, and 0.05%/min (relatively slow rate). Correlation design charts were plotted to predict stiffness modulus reduction versus logarithm of elapsed time. The main outcomes are as follows:

- The measured tensile force –strain relationship exposed a non-linear response of the examined geosynthetics PET geogrid specimens.
- The long-term stiffness value decreases in non-linear manner with increasing logarithm of time but after 1000 hours, is considerably constant of 75% of the secant modulus at 2% strain (working loads). Outcome is in good agreement with consensus that, for polyester (PET) geogrid, time-dependent stiffness reductions will be minimal (approximately 25-15% or less).
- The linear approximation of secant modulus after creep consideration is considerable accepted in the strain domain up to 2% which covers a wide scatter of geosynthetics reinforced structures conditions under service loadings.
- The derived stiffness at 1% is larger than corresponding for 2% strain, which reflects phenomenon of non-linearity and stiffness relaxation with progressive elongation.
- Strain gauge readings of geosynthetics measured in the field must be corrected to true global strains using in-isolation tensile response derived from the constant strain rate CSR loading tests of the instrumented specimens. Strain gauges bonded to woven geosynthetics typically generate a local “hard spot” causing under-registration of global actual tensile strains.
- Calibration Factor CF between global to local strains ranged from 2.08 to 1.99 was introduced appropriately for the tested polyester geogrid specimens at stain domain of 2%. In other words, the strain gauge measured values must be doubled to represent “true” global strains. This derived CF estimation for strain levels of 2% or less is in worthy agreement with comparable PET geogrid CF of 2.2, which was introduced by Allen et al. (2002).
- Calibration Factor values slightly increased as strain rate decreased. For example; specimen 35T; derived CFs are 2.08 and 1.87 for strain 0.05%/min. and strain 10%/min, respectively. Nevertheless, the CF derived based on the slow rate of strain loading 0.05%/min. seems appreciate to simulate the actual loading conditions and staged construction at site.
- Bonded strain gauges during testing often gave a nonlinear response (or failed in the field) at strains $\geq$ 3%, hence, extensometers acknowledged the sole practical guaranteed devices of measuring geosynthetics strains at large strain domains.
- Linear “Elastoplastic- anisotropic” geogrid model promotes by Plaxis 3D seems appropriate to numerically simulate the geosynthetics behavior under service loadings (strain domain $\leq$ 2%) after considering the stiffness degradation associated with creep effect. Sample set of admitted parameters were endorsed to be adopted at Plaxis 3D modelling of geosynthetics.

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PP5.

FAKOPP 3-DIMENSIONAL ACOUSTIC TOMOGRAPHY MEASUREMENTS, IN THE EASTERN ALPS, ALONG A DESIGNATED VERTICAL TRANSECT

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Abstract: *The global temperature increase in high mountain areas is higher than average, which can significantly affect the physiognomy of the vegetation, can change the dominant species of different alpine zones, or lead to the shift of vegetation belts. During our research, we focused on how the state of health of the dominant conifers in the montane belt and subalpine belt regions of Central Europe's mountainous areas changes along an elevation transect. However, the number of field measurements focusing on the health status of dominant trees in temperate mountains is limited. Our measurements were carried out in the Stuhleck Mountains along an elevation gradient from 850 to 1750 metres. Health status analysis of Picea abies and Pinus mugo have been completed by using FAKOPP 3D acoustic tomography, which is able to detect the size and location of decayed regions in the trunk non-destructively. For modeling, the relationship between the decay of trees and other factors simple linear regression models were used. The results showed that the individuals of Picea abies and Pinus mugo had the worst health status in the lowest and uppermost range of the taxa in the studied area. It could be a sign of the upward shift of their range. A positive significant correlation was found between the decay and the ratio of whole trunk/healthy wood both in the case of Picea abies and Pinus mugo. It seems that acoustic tomography measurements are adequate to indicate non-destructively the altitudinal optimum and upward shift of different taxa.*

Keywords: *climate change, conifers, vertical vegetation belts, ArborSonic FAKOPP 3D acoustic tomograph, health status.*

INTRODUCTION

Upper treeline ecotones are important life form boundaries and particularly sensitive to a warming climate. Changes in growth conditions at these ecotones have wide-ranging implications for the provision of ecosystem services in densely populated mountain regions like the European Alps [1].
The rate of increasing temperature in mountain systems is projected to be two to three times higher in this century than that recorded during the 20th century [2]. In addition, there is growing evidence that the rate of warming is amplified with elevation, such that high-mountain environments experience more rapid changes in temperature than environments at lower elevations. Elevation-dependent warming can accelerate the rate of change in mountain ecosystems [3].

Increasing temperatures are driving rapid upward range shifts of species in mountains. An altitudinal range retreat of 10 m is predicted to translate into a approximately 10-km latitudinal retreat based on the rate at which temperatures decline with increasing altitude and latitude, yet reports of latitudinal range retractions are sparse [4].
For example, the average warming within the last century was 1.6°C in south-eastern Switzerland, which is two times higher than the average increase for the Northern Hemisphere [5].
Climatic warming is expected to induce an upward shift in species and forest distribution in parallel with alpine tree line [6] [7] [8]. The latter is because regeneration and growth of trees there are limited by low temperature [9]. An instrumental analysis focusing on the health status of different-aged Quercus petraea stands in the Carpathian Basin showed that sessile oak stands located in a subatlantic area were the healthiest [10]. The most severely deteriorated stands occur in the continental region where the value in the 60 years old age group reached 4.24%. The aim of the searching with the Fakopp 3D acoustic tomography was the following; determine the state of health of Picea abies in the mountain, and Pinus mugo in the subalpine belt in a typical Eastern Alps mountain.

MATERIALS AND METHODS

The examinations were carried out in the Stuhleck Mountain, in the Eastern Alps along a vertical transect from 850 m to 1750 m above sea level. Among the dominant species of the given vegetation belt 3-3 tree individuals were measured in every 5 (between 1705-1750 m), 10 (between 850-1000 m) or 50 (1000-1700 m) meters. The measurements were completed in different layers from the soil respecting the various physiognomies of the species (0.4, 0.8, 1.2 m for P. abies and 0.2, 0.4 m for P. mugo respectively).
Measurements were made using the FAKOPP 3D acoustic tomography, which is able to detect the size and location of decayed or hollow regions in the trunk non-destructively [11] and calculate the ratio of the whole trunk and healthy wood. This non-destructive mobile instrument is suitable for determining the extent of rotting.
FAKOPP has been developed based on this considerable difference as well as on the fact that propagation speed of sound waves is in strong correlation with the mechanical characteristics of wood substance [12]. This advanced method of examination measures the propagation speed of sound within the tree.

GRAPHICAL PRESENTATION

Data input and processing was carried out in Microsoft Excel 365 online version.

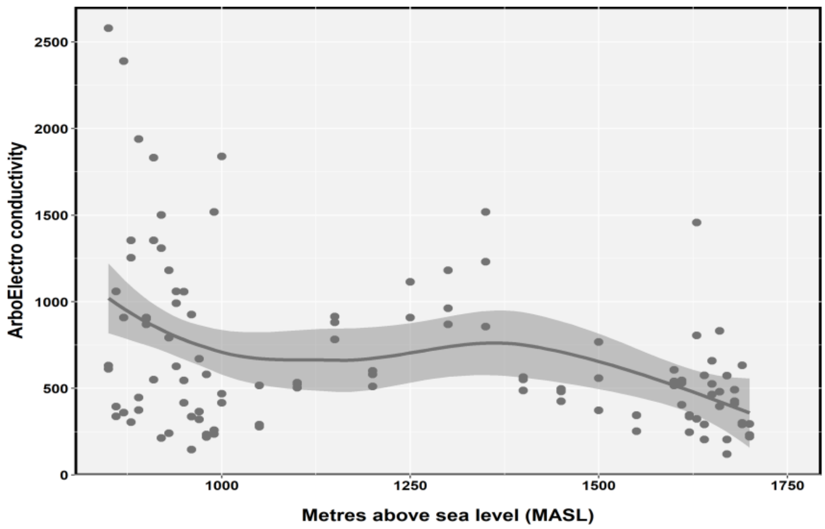

Figure (1): As the height increases, the rate of decay decreases.

Picea abies and *Pinus mugo* had the worst health status in the lowest range of the taxa in the studied area (Figure 1). It can be concluded, that there is a middle stronger relationship between the decay and the wall thickness ratio of whole trunk/healthy wood only in the case of *Pinus mugo* (Figure 2).

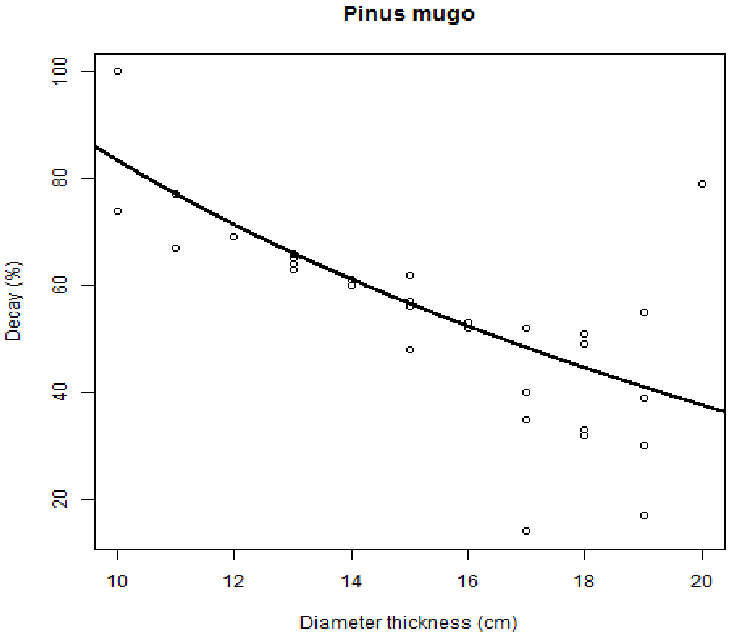

Figure (2): Figure 5. Correlation between the decay (expressed in percentage) and the wall thickness (ratio of whole trunk/ healthy wood) in case of *Pinus mugo*

RESULTS AND DISCUSSION

The results showed that the individuals of *Picea abies* and *Pinus mugo* had the worst health status in the lowest range of the taxa in the studied area. The higher deterioration rate observed in the lower limits of Norway spruce and shrubby pine stands could be a sign of the upward shift of their range. The latter is in harmony with [8] findings, that ongoing climate change shift tree species distribution. In addition, the higher rate of decay at the uppermost range of *Picea abies* and *Pinus mugo* may indicate that upward shift of trees coupled with increasing stress and declining fitness. The greater absolute and average decay of *Pinus mugo* compared to *Picea abies* could be explained by the most severe environmental conditions.

CONCLUSIONS AND RECOMMENDATIONS

The results showed that the individuals of Picea abies and Pinus mugo had the worst health status in the lowest range of the taxa in the studied area. It could be a sign of the upward shift of their range.
It seems that acoustic tomography measurements are adequate to indicate non-destructively the altitudinal optimum and upward shift of different taxa.

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PP6.

TOWARDS GREENER METAL-MEDIATED SYNTHETIC METHODS

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Abstract: *Metal-catalyzed cross-coupling reactions represent an important class of organic transformation used routinely in academic and industrial laboratories. A subset of those reaction, reductive cross-electrophile coupling of organic halides (R-X, X=Cl, Br, I), are traditionally carried out in the presence of stoichiometric amounts of metallic reducing reagents, and require amide solvents. In this short communication, a new methodology, based on Ni-catalyzed reductive coupling, for greener approach towards diarylmethanes is described. Improved reaction was achieved by applying some of the principles of green chemistry. While the reaction still requires metal catalyst, the protocol is more environmentally benign, and it allows for synthesis of this important class of compounds in an operationally simplified one-pot procedure.*

Keywords: metal-*catalyzed synthesis, cross-coupling reactions, green chemistry*

INTRODUCTION

Since the appearance of the concept of sustainability, the notion that every human activity should be accompanied with the assessment of its impact on the environment has become firmly entrenched in many areas of academic and industrial research and development [1]. In the pursuit of thriving society, it is inconceivable to achieve sustainability if fundamental chemical reactions and processes are not rendered less toxic, renewable, and restoring.

Hence, chemistry plays a crucial role not only in developing industrial production of necessary goods, but also achieving production in efficient, environmentally favorable and economically profitable manner. Therefore, as elegantly pointed out by P. T. Anastas, we refer to "Green Chemistry as the chemistry of sustainability" [2,3].

Although certain individual advancements towards cleaner chemical products and synthetic methods have been made in last several decades [4], area of green chemistry is still very actively researched and explored. Much of this research has been summarized in the form of 12 principles of green chemistry, published in 1998 in the seminal work by Anastas and Warner (Figure 1.) [5].

Consolidation of sustainability and principles of green chemistry are a leading priority in chemical industry, including fine chemicals and pharmaceuticals [6-10].

Currently, the main approach towards green chemical processes is at an industrial stage; however it would be advantageous to optimize synthetic methods at an earlier stage to improve the overall efficiency of the process.

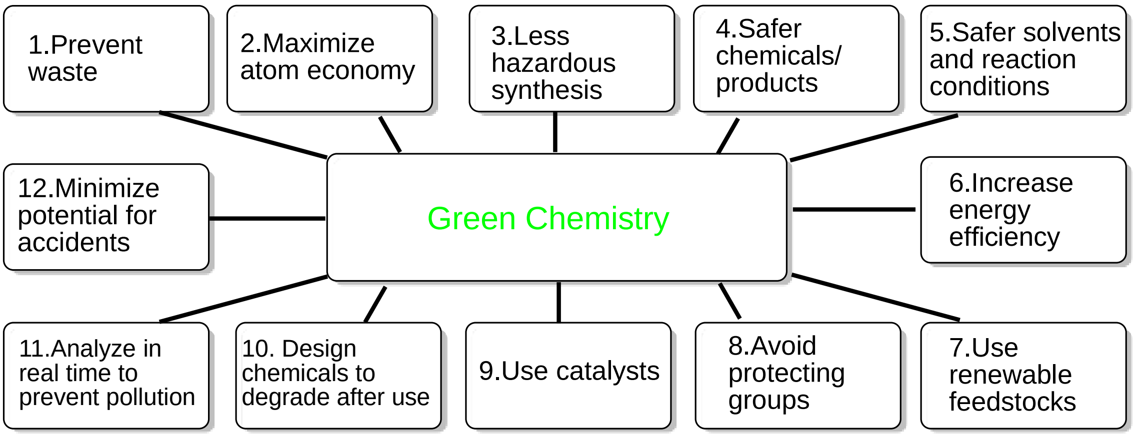


Figure (1): 12 Principles of Green Chemistry

Cross-Coupling Reactions

Since first reported in 1901 by Ullman [11], cross-coupling reactions have afforded a plethora of pathways to generate fine chemicals ranging from agrochemicals to biofuels to pharmaceuticals [12-15]. The pharmaceutical industry in particular has greatly benefited from cross-coupling reactions, allowing for rapid and effective drug discovery and application [13-15]. Moreover, the pharma industry has so embraced these synthetic pathways that a 2011 report in *Journal Of Medicinal Chemistry* found 7315 medicinal synthesis reports that utilize at least one cross-coupling step in the process [16].
Cross-coupling reactions are a class of organic transformations that generate new C-C bonds from pre-existing ones. They follow the general mechanistic pathway presented in the Figure 2. Traditional protocols require the use stoichiometric amounts of organometallic reagents (**R-M**, Figure 2.) which can be accessed from corresponding organic halides (**R-X,** Figure 2.) and metals. It is clear form the standpoint of green chemistry that these transformations will not be the first choice for application on an industrial scale. Aside form drawbacks that stem purely from the organometallic nature of the reaction, they are plagued with numerous other issues: necessity for toxic/expensive solvent, use of various additives, and use of catalyst that often promote undesirable side-reactions [17]. Greener alternative to these reactions are reductive cross-coupling reactions (XES, Figure 2.) which eliminate the use of pre-formed organometallic reagents and utilize organic electrophiles (**R-X,** Figure 2.) as coupling partners.

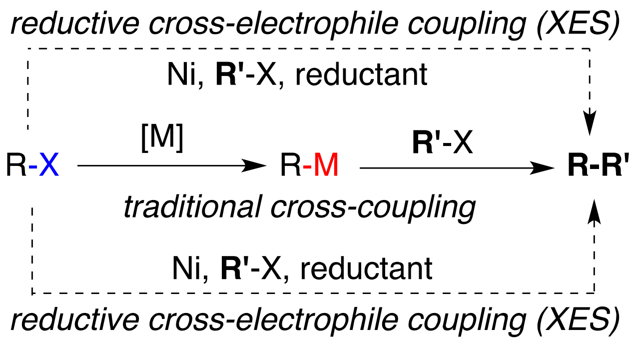


Figure (2): Cross-Coupling Reactions

Encouraged by the recent renewed interest in nickel catalysts due to their unique reactivity [18,19], we were interested in exploring greener alternatives (such as XES) to traditional cross-coupling reaction. In particular we were interested in devising a new method for synthesis of diarylmethanes (**3**, Figure 3.) as they can be found in many pharmaceutically active molecules [20], and since previous examples of their synthesis are still not general and broad in substrate scope [21].

MATERIALS AND METHODS

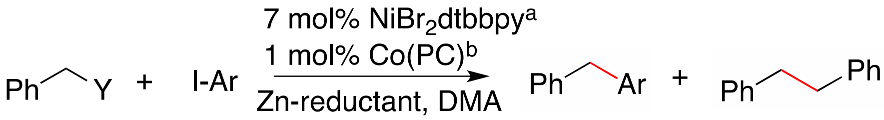
General Procedure for Synthesis of Diarylmethanes:

To a 1-dram vial equipped with a stir bar was placed reducing agent (1.0 mmol, 2.0 equiv), dtbbpy (6.70 mg, 0.025 mmol, 0.050 equiv), dodecane (10 µL, 0.044 mmol), benzyl alcohol (62µL, 0.60 mmol, 1.2 equiv), aryl halide (0.50 mmol, 1.0equiv), DIPEA (139µL, 0.800 mmol, 1.60 equiv), and DMA (2.0 mL). The mixture was stirred for 5 minutes at room temperature after which methanesulfonic anhydride (125 mg, 0.720 mmol, 1.40 equiv) was added. The contents were stirred for 60 min at room temperature, followed by the addition of $NiBr_2 \cdot 3H_2O$ (9.50 mg, 0.035 mmol, 0.070 equiv) and either 1 mol% (2.90 mg, 0.005 mmol, 0.010 equiv) or 3 mol% (8.60 mg, 0.015 mmol, 0.030 equiv) of Co(Pc). The reaction was then stirred either at room temperature or at 60 °C and the reaction progress was monitored by GC. Control reactions as well as optimization experiments also followed this general procedure, by either omitting or changing specified reagents.

RESULTS AND DISCUSSION

Choice of Feedstock

Starting from conditions originally developed for nickel-catalyzed cross-electrophile coupling, initial attempts to couple benzyl and aryl halides resulted in undesired dimerization (**4,** Figure 3) [22]. This is probably due to increased propensity of benzyl halides for radical/homo-coupling rather than cross-coupling reaction (entry 1, Figure 3). To address this issue and eliminate the undesired dimerization reaction a less reactive class of molecules was chosen. Fortunately, when benzyl mesylates (**1** (BnOMs), Figure 3.) were employed in the reaction a significant formation of the cross-coupled product was observed (entry 2, Figure 3). While successful at solving the problem at hand, the main shortcoming of these revised conditions was thermal instability of benzyl mesylates, and relatively short shelf-life. Fortunately, BnOMs can be conveniently generated *in situ* from corresponding benzyl alcohols (BnOH) (entry 3, Figure 3) [22]. This new reaction is very robust, unsusceptible to air or moisture, and it is general in substrate scope. Moreover, it relies on the use of abundant and green feedstock (benzyl alcohols). Data from *eMolecules* database (eMolecules, Inc) indicates that there are 6,152 commercially available benzyl alcohols (vs. 605 $ArCH_2Cl$). The feedstock of this new method eliminates the need for organometallic nucleophiles, and replaces them with more readily available and more stable electrophiles which decreases the costs and waste, and simplifies the system (one-pot procedure).



1 **2** **3** **4**

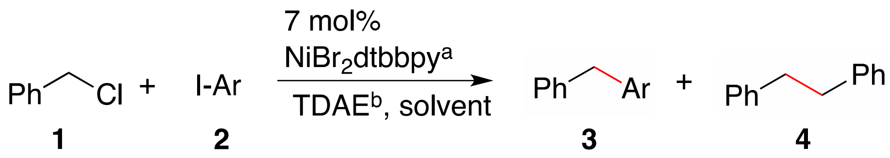
Entry	Y (1)	**3,** yield (%)
1	Cl	9
2	OMs	87
3	OH[c]	93

[a] 4,4'-di-tert-butyl-2,2'-dipyridine
[b] cobalt(II) phtalocyanine
[c] BnOMs was generated *in situ* using BnOH, Ms_2O and iPr_2NEt

Figure (3): Reductive Cross-Electrophile Coupling of Benzyl Mesulates With Arylhalides

Choice of Reducing Agent and Solvent

Although XES reactions circumvent the need for organometallic reagents, they still require metal powder which serves as a terminal reductant to complete the catalytic cycle. While zinc and manganese are abundant and readily available metals, generation of stoichiometric amounts of metallic waste can complicate waste management [23]. Additionally, there is a growing concern regarding the security of supplies in the long term [24]. There are reports where metallic powder has been successfully replaced with organic reductants in reductive cross-coupling reactions [25-30]. Although TDAE (tertakis(dimethylamino(ethylene)) has comparable reducing properties to zinc [31], our initial attempts to use this organic reductant led to the formation of the desired product, albeit the yields were lower comparing to metal reductants. To improve the reactivity of the system when TDAE is employed, we screened alternative solvents for this reductant (Figure 4). Fortunately, we were able to restore the reactivity of the catalyst and replace commonly used amide solvents (DMA, dimethylacetamide) with more favorable and greener solvents. The reactions proceeds exceedingly well in acetonitrile (MeCN) and propylene carbonate (90% yield in both cases) (entries 2 and 3, Figure 4), and less polar solvents as well (isopropyl acetate, 2-thyltetrahydrofuran, 79%, and 76%, respectively). (Amide and urea-based solvents have been under significant scrutiny in recent years, and since they have been subjected to increased regulation as they are more problematic [30].) In general, the reaction with TDAE affords the desired product in high yields for several solvents from solvent selection guide proposed by the ACS GCI Pharmaceutical Roundtable Solvent Selection Guide [33,34]. In summary, introduction of milder organic reducing reagent not only promotes the reaction in same efficient manner, but it also allows for the transformation to be carried out in greener solvents and therefore make it more appealing for application in both academic and industrial setting.



Entry	solvent	**3,** yield (%)
1	MeCN	90
2	propylene carbonate	90
3	isopropyl acetate	83

[a] 4,4'-di-tert-butyl-2,2'-dipyridine
[b] tetrakis(dimethylamino)ethylene

Figure (4): Organic Reductanat and Green Solvents for Diarylmethane Synthesis

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, a revised reaction conditions for reductive cross-coupling of benzyl mesylates with aryl halides provides a new and improved access to an important group of diarylmethane products. Applying the principles of green chemistry, the reaction was adapted to less toxic/hazardous reagents and additives. Furthermore, the entire procedure can be carried in a more simplified manner as a one-pot protocol. There are still challenges associated with these reactions and more research needs to be conducted to render them even more environmentally benign. With recent progress in the area of solid-supported catalysts, application of greener solvents (water, supercritical CO_2, etc.), and expansion of renewable feedstocks, it is realistic to expect that more improvements will be made. Finally, metal-catalyzed reaction may not ever be truly green, however, as nicely pointed out by P. G.

Jessop: “Green chemistry always involves comparisons. No solvent, product, or process can be inherently green; it is green in comparison to an alternative” [35].

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PP8.

LAWN REGENERATION RESULTS OF THE CONSERVATION MANAGEMENT ON THE HOMOKTÖVIS CONSERVATION AREA IN BUDAPEST

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Abstract*: In Budapest, despite the density of the population, precious plant communities still remain in a lot of mostly isolated fragments of habitats which – especially sandy lawns – are outstanding in the diversity of species and are rare inhabitants of endemic species. On the examined territory, since 2006 long term reconstructions of the habitats have been ongoing which strive for the decrease of the invasive woody species on the territory and the insurance of the habitat of the sandy lawn, as well as the long term conservation of the fragments of the lawn and the creation of the natural sandy lawn.*
Method*: The effects of the interventions on the vegetation were conducted on 7 sample areas, on 10-10 quadrats by examining the coenological entries; therefore we were able to provide the effects of the reconstruction of the habitats 14 years retrospectively.*
Results: *During the past years, due to the systematic planning on the fragments of the habitats, 9 hectares of new surface could be opened. Due to this, more than 40% of the entire protected area could become an area of lawn. Changes in the vegetation in the examined area were clearly observable during a 7-year-long period; species of the sandy grassland has become dominant, which can generally be regarded as positive from the nature conservation point of view.*
Conclusion*: As it has been confirmed by several authors the open sandy vegetation is more tolerant to degradation in well-formed grassland.*

Keywords: *Sandy grasslands, Nature conservation, habitat regeneration, open sandy vegetation*

INTRODUCTION

The area of Budapest came into existence on a surface of big variability regarding its natural geography, where the absolutely differing habitat types could exist within relatively small distances. In the following periods investigations appeared in growing numbers about the survey of the wildlife (Bajor 2009), which came to the same opinion: the old-world wildlife of the present capital due to its geographical conditions possesses a species richness and is singular in many respects. But to the

accurate realisation of the present interventions, it is of crucial importance the knowledge of the original wildlife and its change on the base of old surveys.
The professional lectures were published at first in Latin, but later also in Hungarian, or they were updated (Gönczy 1864). In this period, the initially monopolistic Linnaeus' approach dominated, but in the course of the research works in the Budapest area the common investigations of the species and their surrounding more and more prevailed. Next appeared a work, which accentuated the unique character of the landscape's flora (Borbás 1872). But since the early 1990s an increasing number draw attention to the constriction and degradation of the natural wildlife in the Budapest area (Pénzes 1942, Pénzes and Csízy 1956). At the end of the XX century the localized investigation of the remained fragments became characteristic, because the big, coherent areas practically ceased to exist (Rakonczay 1992, Simon 1994, Tardy 1996, Seregélyes 1996-1997, Pintér 2008).
From the beginning of the 2000s and beside the botanic fundamental researches started, also the research of the invasive plants. In Budapest, the invasive plants represent a serious problem in nearly every remained protected area and there they endanger directly the wildlife of the isolated habitats (Bajor 2011). Among these Robinia pseudoacacia, Ailantus altissima, Acer negundo, Eleagnus angustifolia, Solidago canadensis) and the milkweed (Asclepias syriaca) pose the biggest threat, their proliferation is of worsening level not only in the Budapest area, but already nationwide. According to some surveys, the surface of the area, which is already heavily infested by the invasive plants, exceeds 1 million hectares. Due to their not controlled proliferation, the costs of their suppressions are increasing from year to year (Mihály and Botta-Dukát 2004) therefore the importance of the habitat reconstruction activities is increasing.
The once rich grass vegetation at Budapest and in its surroundings dwindled drastically and survived only in small patches and fragments. The importance of the area, which constitutes the topic of this paper, is great, but above this they are crucial, because here it allows tracing back the influence of the environmental treatment on the vegetation.
One of the scenes of research was the nature reserve Sea Buckthorn Reserve area. However Raymond Rapaics carried out the first coenological records in the Újpest Habitat of the Sea Buckthorn Reserve, the concrete surveys became regular only after the first half of the 70s, thanks to the activity of Tibor Simon and his colleagues (Seregélyes 1996-1997). As early as that they recognize the outstanding importance of this area and this led finally to the first declaration as nature reserve area. But the first researches revealed that the most valuable parts stayed out and in 1999 the protection had been extended. But by this time – in spite of the closed character, according to its military presence – the degradation and fragmentation became significant, what was increased by the construction of the Újpest Settlement of the Waterworks Budapest at the expense of the sandy grass areas (Seregélyes 1996-1997). From this period onward the push of the non-native species – mainly the woodies – became noticeable.
Tamariska Hill, the last remained fragment of the one-time Király Forest, the other research area lies in the northern part of the Csepel Island. The investigation of the area's vegetation began early. Thereafter the first significant survey was carried out with the help of Ferenc Német, by Tibor Simon (Simon 1983) as well as Seregélyes et al.(2000). Using the results of the research it became mentioned in a descriptive booklet a lot of botanic value of the hill (Bognár 2005). Based on previous data, the Nature Conservation Society "Oak" in Gödöllő carried out a detailed botanic research and updated the list of the area's flora (Pintér 2006). About the area two years later an overall description of preservation character had been written (Bajor 2009).

MATERIALS AND METHODS

Their bedrock is decisively river sand, containing calcium, magnesium and bicarbonate. In their emergence played a big role the grain size of the sand, outspread by the Danube; the grain size is varying between 2-0.02 mm. The Újpest Sea Buckthorn nature reserve area received in 1974 the award of the nature reserve protection, what was proposed already in the 1950s (Seregélyes 1996-1997). The protection covered originally 5.7 ha, what was extended in 1999 to 24 ha and in 2013 to 40 ha. Presently an occurrence of 22 protected and increasingly protected plants is known from here. This legally protected habitat is named for the sea buckthorn (Hippophae rhamnoides), being its only

registered Hungarian wild occurrence; however some researchers contest it (Farkas 1998, Babulka et Turcsányi 1987). In order to preserve the grass fragments for a long time, conservation works are since 2006 going on in the buckthorn's Újpest habitat. We perform this activity by the help of the voluntaries of the Hungarian Ornithological and Nature Conservation Society's Budapest Group and of the students of the educational institutes, besides the procurement of the official permissions.
From 2006 to 2012 – strictly beyond the growing season – we carried out in the field all in all 28 times voluntary habitat management works. Up to this point, as a result of the appeals, 1200 voluntaries took part in our works. In the course of the interventions beyond the mechanical beating down of sprouts we employed also chemical parching. Thanks to the systematic planning, we could open an area of about 5 ha in the last seven years. Through this about 40% of the entire protected and treated area became again sand grassland. In the first period this value was only about the half of the present, i. e. only 20 % of the habitat was free of woody invasive plants.
In connection with the sites of the yearly treatments, we divided the area in seven sample areas,. The oldest treatment took place in 2006, next they were carried out continuously up to 2013. After the first treatment the maintenance takes place in every sample area yearly, if needed also eradication and mowing.
We investigated in the seven sample area sandy grasslands of different phases:

- I: no treatment, the area is for more than 20 years now natural grass,
- II: freed in 2006 from the alien species, a treated grass
- III: treated in 2007,
- IV: treated in 2008,
- V: treated in 2009,
- VI: treated in 2010.

RESULTS AND DISCUSSION

In the areas I and II species composition is nearly identical in every relevé, the common dominant species were *Festuca vaginata, Stipa borysthenica* and *Peucedanum arenarium. Fumana procumbens* was dominating in area I, but in area II its cover values decreased. In area II cover values of *Festuca pseudovaginata* increased significantly beside Festuca vaginata, what is characteristic and dominant species for sandy grasslands?
During the examined period the dominance relationships of the two Festuca species changed – the earlier the area was treated, Festuca pseudovaginata has become even less dominant, and however it reached a relatively high coverage in Area II. This species is occurred only once in the relevés from Area VI-VII. Similar to F. vaginata, Stipa borysthenica also occurs only in the area V and is missing in the quadratures of the study areas VI and VII, but its appearance is rather consistent in the records.
The distribution of the woody species is also important because this indicates the success of the conservation treatment. 6 invasive woody species occur in Area V - VII: Ailanthus altissima, Acer negundo, Populus canadensis, Robinia pseudoacacia, Fraxinus pennsylvanica and Celtis occidentalis. The native shrubs and trees occur in Area II – IV with less coverage; its amount became higher in Area V – VII.
Occurrence of weed species is a significant indicator of disturbance. The summary of the cover values of weeds in 2012. Their amount increases in Area III – VII with the highest values in the Area VI. In the pilot area VII and VIII is the weed cover ratio very high. On the area, where the shrub eradication took place for 5-4 years, the weed cover rate had been reduced only at some percentage.

CONCLUSIONS AND RECOMMENDATIONS

Nowadays the issue of habitat reconstructions is becoming more significant, an increasingly large growing scale of researches deals with the active restoration of valuable habitats (Török et al. 2009, Vida et al. 2008). However, the restoration of natural and semi-natural habitats in urban environment is almost unknown in Hungary (Kézdy and Tóth, 2013). In order to accelerate the process, over-

sowing with seed-mixture is being used in many cases (Török et al., 2009), since it is easier to achieve results, however it may not lead to success. With regard to the study site, over-seeding was not seemed appropriate because fragments of the original plant communities existed as a propagulum source (Pintér 2008).
Changes in the vegetation in the examined area were clearly observable during a 7-year- long period, species of the sandy grassland has become dominant, which can generally be regarded as positive from the nature conservation point of view (Borhidi 2003). As it has been confirmed by several authors (Kemény et al. 2001), the open sandy vegetation is more tolerant to degradation in well-formed grassland.

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PP9.

PUBLIC PERCEPTION, AWARENESS AND KNOWLEDGE ABOUT AIR QUALITY IN THE CITY OF ZRENJANIN

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Abstract: *Air pollution is one of the most important factors that can affect the quality of life in urban areas; given the fact that exposure to air pollution has harmful consequences for human health. The problem of air quality in the Republic of Serbia is currently attracting much more public attention than in previous years due to the appearance of many individually installed sensors for monitoring air quality, as well as the development of new applications easily accessible to the general population. To determine the perception, personal attitudes, and knowledge of citizens about air pollution, as well as ways of informing and reliability of data, a survey was conducted in the form of a questionnaire. The results showed that most citizens believe that the air they breathe is polluted or occasionally polluted; while as many as 80% of respondents do not feel sufficiently informed about air quality. In addition, the distrust of the citizens towards the official data on air quality is noticeable, as well as the reliance on the information obtained by measurements with low budget sensors of questionable reliability. Given these results, it is inevitable that more attention must be paid not only to improving the way of informing about air quality but also to educating the population.*

Keywords: air quality perception, air pollution, public concern

INTRODUCTION

The process of urbanization is characterized by the fact that more than three quarters of the European population is concentrated in urban areas, while air pollution is one of the most important factors that can affect the quality of life of citizens in urban areas [1-3]. Human exposure to air pollution leads to serious health problems. Every year, approximately 3.7 million people worldwide die prematurely due to ambient air pollution. In addition, air pollution contributes to respiratory and cardiovascular diseases, as well as lung cancer [4,5]. Sources of pollution can be different processes (e.g., industrial production and road transport) that are driven by different socio-economic phenomena of the population (consumer habits, choice of mode of transport and housing). In this context, the structure of urban areas can have a strong impact on pollution emissions [6].
The economic structure of the Republic of Serbia (RS) and the inadequate technical efficiency in terms of environmental protection explain the fact that the quantities of pollutants emitted into the air are large concerning the economic activity of the country. The increase in emissions of air pollutants in recent decades, mostly nitrogen and sulfur oxides, is a consequence of more intensive energy consumption based on the combustion of fossil fuels and the development of traffic [7].

Road traffic in the RS significantly contributes to poor air quality due to its increasing intensity, but primarily due to the obsolescence of vehicles and their dependence on fossil fuels [8].

Energy production in Serbia is based on the consumption of fossil fuels, with the largest number of energy plants being characterized by technological obsolescence, which causes high specific fuel consumption and low energy efficiency. In addition, the equipment for the purification of exhaust gases in the largest thermal power plants is inadequate. In addition to thermal power plants, significant emitters of pollutants into the air heating plants and industrial boilers, which make up 73% of the installed energy capacity.

At these plants, either there is no dedusting system or it is not maintained [8]. The most common way of heating apartments in Serbia is still solid fuels, mostly firewood and less coal. Heating with coal and wood causes significant emissions of sulfur and nitrogen oxides at the local level, as well as large emissions of soot [9]. Soot consists of very fine, small particles of different sizes (PM 2.5 from 0.1 to 2.5 μm and PM 10 from 2.5 to 10 μm). In terms of the impact of these particles on health, the biggest problem is their complexity regarding chemical composition and the presence of heavy metals that can adversely affect the respiratory system [7, 10]. Air pollution in urban areas largely depends on the meteorological conditions at the observed location [11, 12].

Although only a few studies have been done at the RS level that examines the relationship between air pollution and meteorological parameters, it can be concluded that the number of days with poor air quality increases during the cold part of the year. This is especially pronounced during anticyclones when temperature inversions are formed near the surface and wind speeds are low whereby the emitted pollutants are not adequately dispersed. During the winter period in the capital of RS, Belgrade, high pressure, light winds, temperature inversions up to a height of 700 or 1 000 m, fog and dew are common. These are the most unfavourable weather conditions from the point of view of local air quality and can last for days, issuing warnings about excessive air pollution [13].

The problem of air quality in RS is currently attracting much more public attention than in previous years, although it has been present for years. The development of the media has enabled the rapid and easy dissemination of information, which has contributed to placing this problem in the center of interest.

Air quality in Serbia is determined based on systematic monitoring conducted by the Environmental Protection Agency (SEPA). Systematic monitoring of air quality is performed by measuring the concentrations of basic pollutants in the network of automatic stations on the territory of the entire Republic. Reporting is done regularly daily, as well as on an annual basis in the form of annual reports, but also extraordinarily in the case of extremely high episodic pollution [9].

A special way of reporting, which is acceptable and understandable for the general population, is reporting through the Air Quality Index (AQI). However, there is no single methodology for calculating AQI that is internationally accepted so there are many different air quality indexes that represent the global urban air pollution situation [14, 15].

Recently, the number of air quality data obtained by measurements with low budget sensors has increased dramatically [16]. Due to the lack of consistent testing protocols that provide data on the performance and reliability of sensor operation, the quality of the data obtained in this way is highly debatable. Also, in this case, we cannot talk about their comparability with the data obtained by classical reference methods, as well as with the defined limit values.

Many associations of citizens interested in air quality install the mentioned sensors in the cities of RS, and the data from these devices can be monitored through various sites, whereby information is obtained in real-time. The problem that arises in this way is the great concern of citizens for air quality, which is not always justified because it is based on unreliable data.

In the city of Zrenjanin, which is located in the autonomous province of Vojvodina as the administrative center of the Central Banat District, air quality is officially measured at three measuring points, however, there are no automatic measuring stations and measurement results are not obtained in real time, but after processing.

The concern of the citizens of Zrenjanin for air quality initiated the implementation of citizen sensing for air quality monitoring. This paper aims to assess the knowledge, attitudes, and perceptions of the citizens of Zrenjanin about air pollution and ways of informing about this problem through a survey.

MATERIALS AND METHODS

In addition to theoretical research, a survey was conducted in the form of a questionnaire, to determine the perception, personal attitudes, and knowledge of citizens about air pollution as well as ways of informing and reliability of data on which they are based. The questionnaire was posted in "online" form, via the Google platform. It has been actively shared for 20 days. The questionnaire was formulated through two groups of questions: general and specific questions. The group of general questions included questions about gender, age, level of education, and place of residence within the municipality of Zrenjanin. The specific group of questions included questions of personal perception and knowledge about the ways of informing and reliability of data, as well as the question of personal actions in order to reduce air pollution. Table 1 shows a specific group of questions.

Table 1: Specific group of survey questions

Survey questions
Q1: How would you rate the air quality in Zrenjanin on a scale of 1-5?
Q2: Do you think that you are adequately informed about the air quality in Zrenjanin?
Q3: What should be improved in order to better inform citizens?
Q4: Which way are you informed about air quality?
Q5: What air quality data you check?
Q6: Did you know that there is air quality monitoring applications that generate data based on sensors set up by citizens?
Q7: How would you rate the reliability of the data obtained using these sensors?
Q8: Explain the previous answer
Q9: How would you rate the reliability of the Environmental Protection Agency's air quality data?
Q10: Explain the previous answer
Q11: In your opinion, what contributes the most to air pollution in Zrenjanin?
Q12: What measures do you propose to improve air quality in Zrenjanin?
Q13: How do you contribute to reducing air pollution?

RESULTS AND DISCUSSION

In the survey participated 154 respondents, 66 were male (42.85%) and 88 were female (57.15%). The age of the respondents is shown in Figure (1).

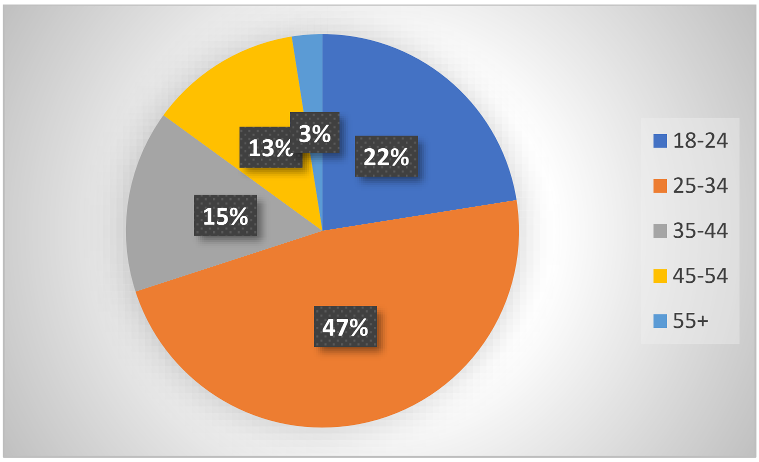

Figure 1: The age of the respondents

The age structure shows that the largest number of respondents is between 25 and 34 years old, 47% of the total number of survey participants. When it comes to the level of education, in Figure (2) it can be noticed that about a third of the respondents have a university degree (undergraduate studies), about a third of the respondents have a master's degree, while the rest are doctors, higher professional education students and high school students.

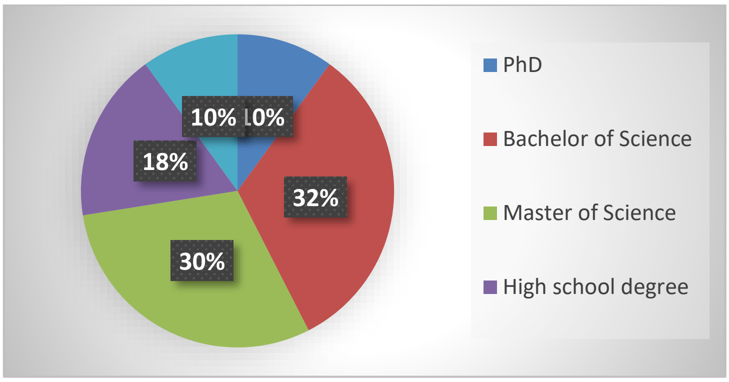

Figure 2: Level of education

Most respondents have a place of residence in the urban area of the city of Zrenjanin (87.5%), from the following parts of the city: Bagljaš, Centar, Duvanika, Gradnulica, IV Jul and Mužlja. 12.5% of the total number of respondents from rural areas within the municipality of Zrenjanin participated in the survey, with a place of residence in the settlements of Klek, Sutjeska, Botos and Melenci. The structure of the answers within the survey question Q1, which refers to the assessment of air quality in Zrenjanin, is shown in Figure (3).

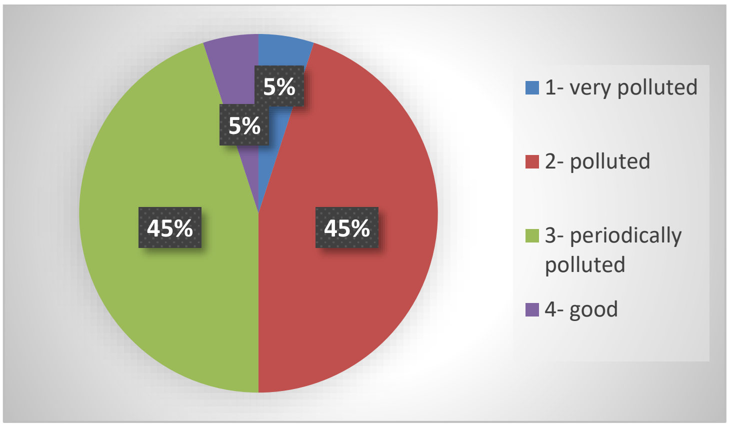

Figure 3: Q1answers

It is possible to notice that, when assessing the air quality in the city of Zrenjanin on a scale of 1-5, an equal number of respondents believe that the air is polluted (45%) and occasionally polluted (45%), while the remaining part of the respondents believe that the air is very polluted (5%) and good (5%).
Most respondents do not feel sufficiently informed when it comes to air quality, as many as 80%. Suggestions for improvement in this regard relate to raising public awareness, education on air pollution problems, improving internet media and applications that will contribute to better information flow.
Continuous monitoring and installation of new measuring stations would enable monitoring of several points of importance. The information generated in this way should be available in real-time, on the official websites of the institutions whose responsibilities are monitoring and reporting on air quality.
The most common improvement suggestion is the public and transparent propagation of real-time air quality information.
Respondents stated that they most often inform about air quality through local media (47.5%), followed by monitoring foreign sites and applications (35%), 12.5% stated "other" in response, while other respondents (5%) follow the website of the Environmental Protection Agency (SEPA). Other ways of informing citizens include public media, mobile phone applications, scientific sites, as well as obtaining information from experts involved in air quality research.
When it comes to the parameters that are monitored to determine air quality, these would be the Air Quality Index (AQI) and individual pollutant concentrations. The structure of the answer to question Q5 is shown in Figure (4).

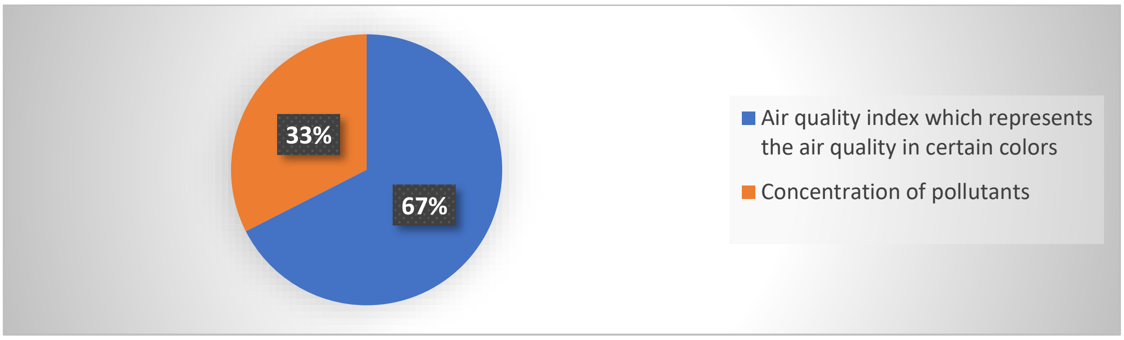

Figure 4: Q5 answers

The air quality index is a parameter whose values are monitored by 67.7% of respondents. AQI is used for hourly and daily reporting to the public and has the greatest benefit for the population to understand how air quality affects their health in the short term. A scale in different colours, denoting air quality classes, is used for reporting to the public via AQI and descriptive expression of air quality.
Concentrations of pollutants are most often monitored in official institutions such as the Environmental Protection Agency, to determine the exceeding of the maximum permissible level of pollutants in the air, which are prescribed by law. In general, for the population, knowledge about the concentrations of pollutants in the air is not significant if they do not know the legislation and the maximum permissible concentrations. Therefore, it is understandable that a larger number of respondents check the AQI parameter values.
As a large number of individually installed sensors for air quality monitoring have recently appeared, as well as the development of new applications easily accessible to the general population, the question arises as to how much citizens are aware of the existence of these sensors. The answers to question Q6 show that 60% of respondents have information about it, while the other 40% are not informed about their installation.
Almost half of the respondents rate the information generated in this way as reliable, while 42.5% believe that one should be careful when using it, i.e. that it is unreliable (10%). It is shown in Figure (5). A small number of officially installed measuring stations are one of the reasons why almost half of the respondents do not question the reliability of the data monitored via low-budget sensors. The unreliability is attributed to the inadequate installation of measuring sensors and the use of cheap and low-quality components for their manufacture.

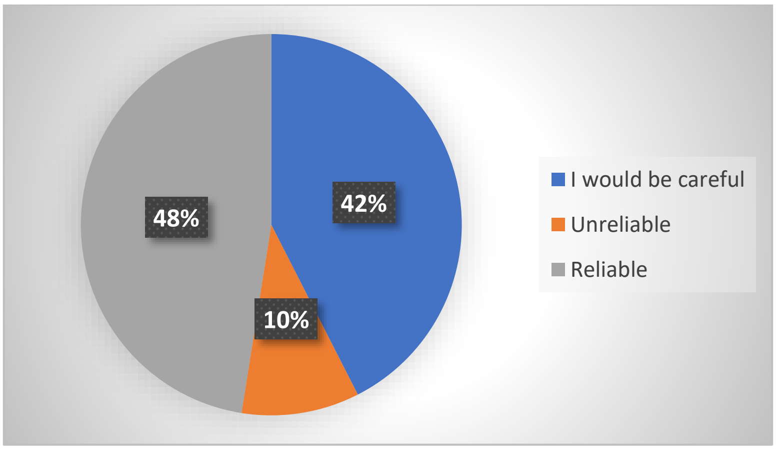

Figure 5: Low-budget sensors reliability

On the other hand, the data reported by the Environmental Protection Agency (SEPA) are assessed as unreliable (17.5% of respondents), or insufficiently reliable (55%). Respondents believe that it is possible to manipulate the given data in terms of mitigating the actual values of the parameters. Also, inadequate installation of measuring stations, in places that are not suitable for monitoring air quality parameters, results in data that are not representative. The impact of politics on reporting contributes to growing public distrust.

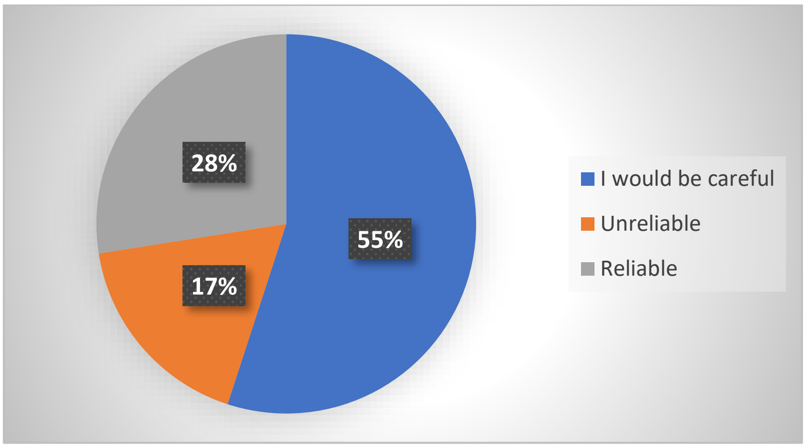

Figure 6: SEPA information reliability

According to the respondents, the biggest contributors to air pollution in Zrenjanin are rendering plant, road traffic, industry (rubber factory, chemical industry, etc.), individual fireboxes, landfills, as well as insufficient greenery in the city and its surroundings. Traffic affects to a large extent, primarily due to the structure of the vehicle fleet and the use of old imported cars. Industrial plants do not apply appropriate measures to reduce air emissions. A big burden in this regard are the individual fireplaces in the winter, when not only wood and pellets are used as fuel, but also other low-quality energy sources.
Measures that could be taken to improve air quality in Zrenjanin relate to the following:

- Construction of a bypass around the city and redirection of traffic, in order to reduce the load on the city center;
- Establishment of stricter regulations and penalties for non-compliance;
- Improvement of existing as well as the introduction of new preventive measures to reduce emissions from industrial plants within the municipality;
- Stimulating the purchase of new, more energy-efficient, and environmentally friendly stoves for individual households; limiting the use of inadequate fuels for these purposes;
- Setting up new measuring stations, adequate reporting and informing the population through public media;
- Improvement of green infrastructure, construction of new bicycle paths, and promotion of bicycle use in the urban environment;
- Education, raising public awareness on environmental protection and improvement of the air quality.

CONCLUSIONS AND RECOMMENDATIONS

The results of the survey conducted in this research show that the current provision of information and its dissemination does not meet the requirements of the public in the municipality of Zrenjanin. The vast majority of citizens believe that the air they breathe is polluted or occasionally polluted; while as many as 80% of respondents do not feel sufficiently informed about air quality. When it comes to the reliability of the data available to the general population, it turned out that citizens believe more in the reliability of the information they receive through air quality monitoring applications that generate data based on sensors set by citizens than the official data of the Environmental Protection Agency. Thus, it can be noticed that there is distrust among citizens towards official air quality data as well as reliance on information obtained by measuring low-budget sensors of questionable reliability. Given these results, the general conclusion is that air quality reporting should not be a mere presentation of data regardless of their usefulness or relevance to recipients, but that this problem must be approached from the aspect of educating the population to raise the level of knowledge, both about the problem of air pollution and about the ways of informing.

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PP10.

RELATIONSHIP BETWEEN EXPOSURE TO ENVIRONMENTAL TOXICANTS AND EPIGENETICS

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***Abstract*: *Worldwide, people are exposed to a huge number of environmental factors that have impacts on epigenetic mechanisms. Epigenetics is a field in modern biology that focuses on inherited changes in the gene expression caused by different mechanisms other than changes in the DNA sequence. Environmental epigenetics describes how environmental factors affect the cellular epigenetics and, in consequence, the human health. A foundational goal for the people is to improve their health, which typically includes positive changes in lifestyle and especially diet.* The aim of this review is to explain the *fundamentals of epigenetics, the* relationship between the environmental toxicants and the healthcare aspects of epigenetics with a focus on human systems, *environmental epigenetics and explains the influences of diet and environmental epigenetics on human health and disease. Epigenetic change is a regular and natural occurrence but can be influenced by several biotic and abiotic factors such as human age, parenting, the environment, diseases and disorders, drugs and addiction, lifestyle, diet and sport exercise. How the same genotype can give rise to different phenotypes under different environmental conditions. Identification of the fundamental epigenetic modifications and markers help to recognize the health disorders at earlier stages of the diseases, improving treatment outcomes and quality of life for many people.* Epigenetics could explain why *COVID-19 affects people differently. Extensive research in this field would do a great deal to protect public health, both now and in future generations.*

Keywords: *environmental toxicants, fundamentals of epigenetics, gene expression*, health care, human systems

INTRODUCTION

Epigenetic is defined as the heritable sequence-independent DNA changes and operates through histone modification (methylation, phosphorylation, and acetylation), DNA methylation, and micro-RNA-based mechanisms. Epigenetic change is a regular and natural occurrence but can be influenced by several biotic and abiotic factors such as human age, parenting, the environment, diseases and disorders, drugs and addiction, lifestyle, diet and sport exercise. How the same genotype can give rise to different phenotypes under different environmental conditions. The nature and pace of the environmental changes in climate, diet, predators, pathogens, pollutants or combinations of these pose intense and often novel selection pressures. The rate of extinctions in recent years reflects that, for many species, these pressures can overwhelm their ability to adapt and evolve appropriate responses.

Worldwide, people are exposed to a huge number of environmental factors that have impacts on epigenetic mechanisms. Epigenetics is the science of how environmental signals select, modify, and regulate your gene activity.
Epigenetics is a field in modern biology that focuses on inherited changes in the gene expression caused by different mechanisms other than changes in the DNA sequence. An Environmental epigenetics describes how environmental factors affect the cellular epigenetics and, in consequence, the human health. A foundational goal for the people is to improve their health, which typically includes positive changes in lifestyle and especially diet.
Pathogenesis, including the recent outbreak of coronavirus, can be effectively treated through the application of these bioactive molecules, vaccines, and nanomaterials. Microbiota coevolution with mammalian host results in the plethora of vital functions, such as metabolic signalling, energy metabolism, regulation of integrity, mobility of the gut barrier, and formation of the immune system. Various studies have demonstrated important roles of microorganisms in diverse fields, such as fermentation, biomolecules, bioremediation, antitoxicity, and diseases and health.
The environmental conditions named stresses are constantly shaping genomes of living organisms. Most of these external stimuli have a negative influence on growth, development, and reproduction (Arnholdt-Schmitt, 2004; Madlung & Comai, 2004). Avoidance represents the most common response to severe or long lasting environmental conditions. The organisms with a sedentary life style are unable to escape stress, and thus utilize mechanisms of tolerance and resistance.
Plants integrate into the environment through the efficient use of adaptation mechanisms that depends on the constant exchange of signalling molecules (Cronk, 2001). Plants are the organisms that continue their development throughout the entire life cycle. The germ line in plants is not predetermined but is established during the development. This allows plants to percept stress and integrate the memory of it through multiple feedback mechanisms. The only possible way of transmitting the memory of stress is via the epigenetic regulations involving DNA methylation, histone modifications and chromatin restructuring. These changes lead to the differential gene expression and allow establishing new epialleles, thus resulting in the destabilization of defined loci. The majority of these changes are neutral or deleterious, but in some rare cases they could be beneficial.
Constant exposure to certain stresses should lead to the selection of adaptive traits beneficial to these conditions. The retention and fixation of a necessary trait requires the selection from a number of neutral random changes in the genome. The fact that plants similarly respond to unrelated physical, chemical, or temporal environmental factors suggests the existence of complex inter-crossing perception and response mechanisms (Chinnusamy et al., 2004).

EPIGENETICS IN HUMAN LIFE

Epigenetics is a relatively new field that has transformed significantly over the years. Now more than ever, scientists from all over the world are investigating ways in which environmental factors and lifestyle choices can impact our health via epigenetic mechanisms. This includes the foods we eat, the quality of the air we breathe, and even when we go to sleep. What human eat is a collection of epigenetic studies that specifically detail the associations and implications various foods and nutrients may have on biological systems through epigenetic mechanisms. The studies range in complexity and some are conducted using animal models, while others focus on humans. Hundreds of epigenetic papers are being published as we eagerly form a more accurate understanding of the molecular underpinnings of the impact our lifestyle choices have on human epigenome and, ultimately, human health. Now it appears that our diets and lifestyles can change the expression of our genes. How? By influencing a network of chemical switches within our cells collectively known as the epigenome.
Perhaps the most intriguing principle of epigenetics is that meaningful changes to gene expression can occur without changing our underlying genetic “code of life.” Along with our genotype, our environment and experiences can influence our health via gene expression. Epigenetics is the study of potentially heritable changes in gene expression (active versus inactive genes) that does not involve changes to the underlying DNA sequence. It represents a change in phenotype without a change in genotype. This, in turn, affects how cells read the genes and can benefit or harm health and many

biological processes. Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, disease state, lifestyle, and environment.

EXPOSURE TO STRESS

Plants like other organisms have the ability to perceive stress and respond to it via differential changes in methylation pattern. Indeed, it has been shown that cold treatment promotes tissue-specific hypo-methylation of the particular genome regions, including those specific to retro-transposon sequences (Steward et al., 2002). Similarly, the exposure of tobacco plants to a tobacco mosaic virus induces demethylation of the *NtAlix1* stress-responsive gene, resulting in the continuous accumulation of the gene transcript (Wada et al., 2004). Many experiments have shown that such stresses as changes in growth conditions, exposure to salt, heavy metals, ultraviolet and ionizing radiation, herbicides and even pathogens influence the HR frequency in plants (Kovalchuk et al., 2004; Filkowski et al., 2003; Besplug et al., 2004; Molinier et al., 2005; Boyko et al., 2006). Some of these stresses are associated with the changes in a DNA methylation pattern in the progeny (Kovalchuk et al., 2003). The association of other stresses with the changes in methylation and genome instability remains to be established.

GENETIC & EPIGENETIC RESPONSES

The role of epigenetic control in the adaptation and acclimation process is hard to underestimate, as transgenerational changes in DNA methylation, histone modifications patterns and in the regulation of chromatin binding proteins are powerful tools of reversible changes in the gene expression. It has been revealed that the progeny of plants that are constantly exposed to ionizing radiation have hypermethylated genomes (Kovalchuk et al. 2003). Thus changes in DNA methylation patterns could be a part of the plant stress protection mechanism.

Traditionally, thoughts on the adaptation of populations to environmental pressures have focused on selection acting on phenotypes that ultimately reflect the genetic make-up of each individual, with consequent subsequent changes in the genetic profile of the population to better-adapted genotypes.

Numerous epidemiological studies in humans and experimental studies in laboratory and farm animals have now shown that inadequate or unbalanced nutrition in early life may ‘programme’ development and produce marked effects on the health status, physiology, metabolism and longevity of the adult. This phenomenon is often referred to as the developmental origins of adult disease (McMillen et al. 2008).

In humans, small body size at birth and during infancy is associated with increased rates of chronic diseases in adulthood, including hypertension, cardiovascular disease and insulin resistance. As well as diet-related developmental programming, there is long-standing evidence for ‘stress-related’ programming. In this phenomenon, experiences during pre-natal or early post-natal life have been shown to exert permanent effects on behavioural and physiological responses in the offspring (Weinstock 2008).

While the main drive to understanding ‘developmental programming’ as described above came from its implications for human health, application of the concept to wildlife raises the possibility that any environmental (including nutritional, stress, pollutant exposure and disease) impact experienced by parents may induce epigenetic responses in the offspring. These could then contribute to the different characteristics, lifestyle pathways, and fitness of animals born at different times of the year or phases of the population cycle. Gardner et al. (2009) gave the evidence and implications of programming effects in mammals, including specific effects on fecundity.

The essence of epigenetics is that environmental changes cause altered programmes of gene expression by modifying the chromatin platform on which the transcriptional machinery operates. These modifications may be long term and may even be transmitted from generation to generation.

The fact that epigenetic effects can be transferred through the germ line in mammals has been known for some years and is evidenced by the phenomenon of imprinted genes. Turner (2009) raises the important issue of whether environmentally induced epigenetic characters may be incorporated into permanent changes in DNA sequences. If it is true, this opens up a new dimension in terms of the trying to understand the effects of environmental pressures on population genetics and evolutionary responses. Guerrero-Bosagna & Jensen (2015) mentioned that Transgenerational epigenetic inheritance has gained increased attention due to the possibility that exposure to environmental contaminants induce diseases that propagate across generations through epigenomic alterations in gametes. In laboratory animals, exposure to environmental toxicants such as fungicides, pesticides, or plastic compounds has been shown to produce abnormal reproductive or metabolic phenotypes that are transgenerationally transmitted. Human exposures to environmental toxicants have increased due to industrialization and globalization, as well as the incidence of diseases shown to be transgenerationally transmitted in animal models. This new knowledge poses an urgent call to study transgenerational consequences of current human exposures to environmental toxicants.

HUMAN EXPOSURES TO ENVIRONMENTAL TOXICANTS

Human exposures to environmental toxicants have increased due to industrialization and globalization. The current state of globalization and climate change have helped the dispersion of toxicants in the environment by increasing the global transport of pollution, mobilization of legacy contaminants, and change in agricultural practices. As result, it is expected that the amount and time that humans are exposed to environmental contaminants will increase even further, with unpredictable health consequences (Stahl et al., 2013). Moreover, increasing production and environmental accumulation of new compounds, initially produced to substitute previous persistent contaminants, is also occurring. These emerging contaminants have been found in all environmental compartments across the globe (Burton & Metcalfe, 2014).

Epigenotoxicology is an emerging field of study that investigates the non-genotoxic epigenetic effects of environmental toxicants resulting in alteration of normal gene expression and disruption of cell function. Recent findings on the role of toxicant-induced epigenetic modifications in the development of degenerative diseases have opened up a promising research direction to explore epigenetic therapy approaches and related prognostic biomarkers. A comprehensive data on epigenetic alterations identified in various diseases, including cancer, autoimmune disorders, pulmonary conditions as well as cardiovascular, gastrointestinal and bone disease. Although data on abnormalities of DNA methylation and their role in the development of diseases are abundant, less is known about the impact of histone modifications and microRNA expressions. A number of different environmental toxicants have been identified for their role in aberrant DNA methylation, histone modifications, and microRNA expression. Such epigenetic effects were shown to be tissue-type specific and highly associated with the level and duration of exposure (Hodjat et al., 2017).

HUMAN EXPOSURES & DISEASES

The fundamental impact on the life quality of the entire human population is exposure to environmental toxicants. Such exposure is hidden by the fact that most times one cannot be aware of our contact with these compounds, which are numerous and of increasing production and availability. Worldwide trends show association between environmental exposures and the incidence of non-infectious diseases (Pimentel et al., 2007). Some of these diseases include the ones observed to be environmentally induced and transgenerationally transmitted in rodents, such as obesity, polycystic ovary syndrome (PCOS), or male fertility impairments (Guerrero-Bosagna & Skinner, 2014). For instance, in humans, obesity and overweight have experienced large increases from 1980 worldwide (Ng et al., 2014). Such increase is proposed to be induced by changes in the environment (Morgen & Sorensen, 2014). PCOS is one of the main endocrine abnormalities in women, affecting around 7% of them and associating with reduced pregnancy, diabetes, obesity, and metabolic and cardiovascular diseases (van Houten & Visser, 2014). Another example is male reproductive function. Trends in human populations consistently show decreasing male reproductive parameters in the last decades (Guerrero-Bosagna & Skinner, 2014). Interestingly, many male reproductive disorders share a common developmental origin and patho-physiological etiology. Therefore, the common factor among these diseases is that they are environmentally induced during early development.

Based on results in rodents, the current high incidences of these diseases in humans could be correlated to ancestral exposures to environmental contaminants such as DDT, BPA, phthalates, or hydrocarbon fumes (Skinner et al., 2013).

Food consumption is an important route to environmental exposures. Recent practices in the food industry derived from globalization and industrialization have also been correlated with negative consequences for human health. Vastly used agro-compounds include fungicides or pesticides known to produce transgenerational epigenetic effects that include developmental, reproductive, and metabolic abnormalities (Skinner et al., 2013). Natural estrogenic compounds present in grains, i.e., phytoestrogens, also gain relevance nowadays, due to the widespread emergence of soy-based food for both human and farm animal consumption. Consumption of phytoestrogens is known to have epigenetic and reproductive effects (Guerrero-Bosagna & Skinner, 2014).

On the side of animal-based food, the intensification of meat production in response to a growing world population, together with an increased demand for cheap food in large parts of the world, has caused large pressures on animal welfare on farms (Broom, 2010) and an increased exposure of humans to various pathogens, such as *Salmonella* and *Campylobacter* (Rostagno, 2009). In addition, increased exposure to drug residues from preventive and other treatments of farm animals is also an issue of concern (Rostagno, 2009). Disruption of the microbiome by inadvertent exposure to different chemicals emanating from the animal production industry may also affect health and behavior of humans in a range of as yet poorly investigated ways (Dinan & Cryan, 2012). Most emphasis on food based exposures has been on agricultural compounds; however, emphasis should also be placed on animal conditions and treatments when considering human environmental exposures and the emergence of non-infectious diseases.

POLICIES ON ENVIRONMENTAL EPIGENETICS

Today it is know that most non-infectious diseases are not explained by specific genetic variations but are rather related to environmental exposures during embryonic development or infancy (Madlung & Comai, 2004). Examples of these diseases include asthma, allergies, cancers, and obesity. Possibly, the widespread availability of environmental contamination and its future projections, together with the demonstrated biological possibility that these exposures can induce the onset of transgenerationally transmitted diseases, will have enormous effects in human health. Therefore, the new knowledge on environmentally induced transgenerational epigenetic inheritance should pose an urgent call for increased regulations on the production and use of environmental toxicants, as well as for research evaluating transgenerational effects derived from these exposures (in both humans and farm animals). It is becoming increasingly clear that the quality of life of our grandchildren depends on our current actions and exposures. In the same way, recent data strongly shows that many aspects of our health depend on what our grandmothers and grandfathers were exposed to in their lives.

An epigenetically-inherited trait can arise simultaneously in many individuals, as opposed to a single individual with a gene mutation. Moreover, a transient epigenetically-modified phenotype can be quickly "sunsetted", with individuals reverting to the original phenotype. Thus, epigenetic phenotype switching is dynamic and temporary and can help bridge periods of environmental stress. Epigenetic inheritance likely contributes to evolution both directly and indirectly (Burggren, 2016).

The earth is experiencing major changes in global and regional climates and changes are predicted to accelerate in the future. Rapid and extreme climate changes are predicted, raising questions as to the capacity of plants to adjust to and survive the new environments. Many species will be under considerable pressure to evolve, to migrate, or be faced with extinction. Clonal plants would appear to be at a particular disadvantage due to their limited mobility and limited capacity for adaptation. However, they have out lived previous environmental shift sand clonal species have persisted for millenia. Clonal spread offers unique ecological advantages, such as resource sharing, risk sharing, and economies of scale among ramets within genotypes. It was suggested that ecological attributes of clonal plants, in tandem with variation in gene regulation through epigenetic mechanisms that facilitate and optimize phenotype variation in response to environmental change may permit them to be well suited to projected conditions (Dodd & Douhovnikoff, 2016).

ENVIRONMENTAL EXPOSURES BECOME BIOLOGICALLY EMBEDDED

Epigenetic mechanisms influence the structure of chromatin, which is the complex formed of DNA and chromosomal histone proteins. Chromatin structure influences the accessibility of DNA to the gene transcription machinery, which drives differentiation of every cell type, all of which have the same DNA, by regulating the expression of different genes. A cell, organ, or person's phenotype is thus determined not only by genome but also by the epigenome. At present, there are three well-understood mechanisms by which epigenetic factors affect gene expression: 1) DNA methylation, 2) histone modification, and 3) non-coding RNA-mediated pathways.

DNA methylation usually results in gene silencing or reduced gene expression. Although there is abundant information about how these epigenetic mechanisms are deployed extensively in somatic tissues, their roles in the transgenerational transmission of chronic disease risks, via the germ line, are less well understood. One likely explanation of how epigenetic changes may be passed from one generation to another is that, during pregnancy, the foetal germ cells that will give rise to the mother's grandchildren are exposed to the same environmental factors as both the mother and the somatic tissues of the fetus. The impact of reduced rainfall and increased temperatures forecasted by climate change models on plant communities will depend on the capacity of plant species to acclimate and adapt to new environmental conditions. The acclimation process is mainly driven by epigenetic regulation, including structural and chemical modifications on the genome that do not affect the nucleotide sequence. In plants, one of the best-known epigenetic mechanisms is cytosine-methylation (Chano et al., 2020).

Amaral et al. (2020) mentioned that epigenetic marks and their machinery may change in response to environmental variations occurring throughout the life cycle of an organism, and in some cases, may be inherited in the progeny (Figure 1). Trees are known to recognize various abiotic and/or biotic stimuli occurring rhythmically (circadian or seasonal) or stochastically (Figure 1). These changes are perceived at different tissue levels, with most studies focusing on the leaves, roots, and meristems (SAM, shoot apical meristem; Cambium; or RAM, root apical meristem).

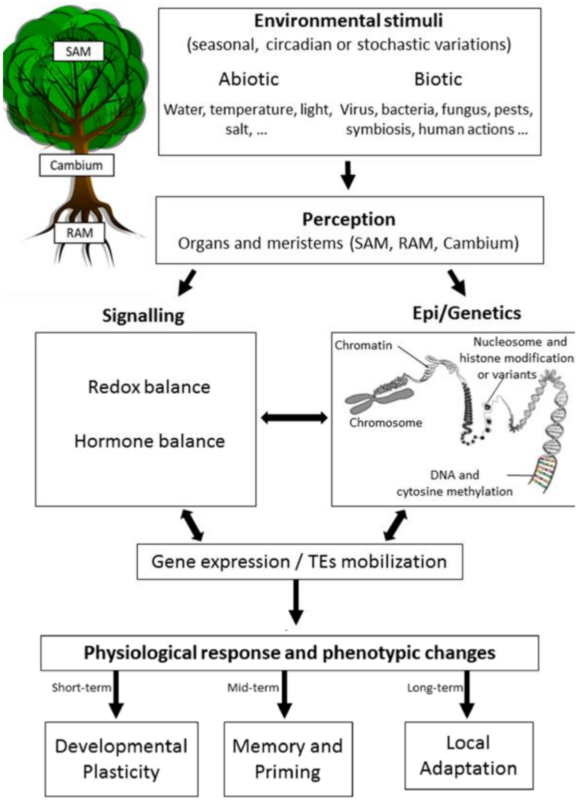

Figure (1) Epigenetic response to environmental changes in trees (Amaral et al., 2020)

Perception is then followed by signalling mechanisms that may include changes in the redox or hormonal balance, which have been shown to be related to epigenetic changes (chromatin remodelling, DNA methylation, non-coding RNA mechanisms, and histone modifications and variants). This complex crosstalk between signalling processes, epigenetics, and genetics results in an altered gene expression status and/or the mobilization of transposable elements (TEs). A physiological response is then observed together with phenotypic changes that allow trees to acclimate to the environmental changes initially sensed depending on the time-scale considered and the heritable transmission of epigenetic changes. The substantial differences in epigenetic diversity and the presence of specific epigenetic patterns found in response to each climatic treatment suggest that molecular responses underlying the reprogramming of gene expression and metabolic processes could be driving the functional stability of some dominant grass species (such as hare barley) under different environmental scenarios (Zhu 2016). The potential role of epigenetic regulation as a mechanism of adaptation to new environmental conditions is reinforced by the low genetic diversity that exhibited the study species when comparing the four climatic treatments.

ENVIRONMENTAL TEMPERATURE & HUMAN EPIGENETIC MODIFICATIONS

Environmental temperature is an important health determinant. As estimated by a multi-country study, 7.3% and 0.4% of mortality could be attributed to exposure to low and high temperatures, respectively (Gasparrini et al., 2015). As a major health determinant, environmental temperature is likely to be able to affect human epigenetic modifications which refer to changes of gene expression without alteration in the DNA sequence. Epigenetic modification includes DNA methylation, noncoding RNA (ncRNA),and chromatin regulation, while ncRNA mainly includes microRNA (miRNA) and long ncRNA, and chromatin regulation is mainly realized by histone modification (Alfano et al., 2018). It has been documented that epigenetic changes are associated with many human diseases, such as asthma, autism, diabetes, psoriasis and cardiovascular diseases (Petronis, 2010; Portela & Esteller, 2010). Meanwhile, the human epigenome can also be modified by environmental factors such as tobacco smoking (Li et al., 2018) and air pollution (Alfano et al., 2018). However, it is still unclear whether exposure to suboptimal environmental temperature could affect epigenetic modifications related to human health. By 2100, the global temperature is projected to increase by 2.7-4.8°C (International Energy Agency, 2015). In a warming world, whether and how people could adapt to heat exposure is attracting increasing concern. Meanwhile, epigenetic changes have been documented to play an important role in animals’ and plants’ adaptation to the temperature in their living environment (Liu et al., 2019). However, whether the evidence is also true for human beings remains unclear. The knowledge about the effects of environmental temperature on human epigenome is a potential key to understand the health impacts of temperature and to guide acclimation under climate change. It was performed that a systematic review on the epidemiological studies that have evaluated the association between environmental temperature and human epigenetic modifications (Rongbin et al., 2020).

Epigenetics represents a widely accepted set of mechanisms by which organisms respond to the environment by regulating phenotypic plasticity and life history transitions. Understanding the effects of environmental control on phenotypes and fitness, via epigenetic mechanisms, is essential for understanding the ability of organisms to rapidly adapt to environmental change. The review of McCaw et al. (2020) highlighted the significance of environmental temperature on epigenetic control of phenotypic variation, with the aim of furthering our understanding of how epigenetics might help or hinder species’ adaptation to climate change. It outlines how epigenetic modifications, including DNA methylation and histone/chromatin modification:

1. respond to temperature and regulate thermal stress responses in different kingdoms of life,
2. regulate temperature-dependent expression of key developmental processes, sex determination, and seasonal phenotypes,
3. facilitate transgenerational epigenetic inheritance of thermal adaptation,
4. adapt populations to local and global climate gradients, and finally
5. facilitate in biological invasions across climate regions.

Although the evidence points towards a conserved role of epigenetics in responding to temperature change, there appears to be an element of temperature- and species-specificity in the specific effects of temperature change on epigenetic modifications and resulting phenotypic responses. The review identifies areas of future research in epigenetic responses to environmental temperature change

PROBIOTICS & EPIGENETICS

Gut microbiota, known as the probiotics plays an important role in the physiological function of human body such as material metabolism, drug efficacy, immune homeostasis and immune response growth and development, and cognitive behavior. Gut microbiota plays a significant role in the manifestation of metabolic disorders and infections. Nowadays, gut microbiomes provide an ideal medium for the growth of beneficial bacteria.

Prebiotics and probiotics are found in natural foods. What might be the signs that our body needs a probiotic? One of these may be when various digestive disorders appear. The other is sugar, a high craving for sugary foods, but slowed metabolism and skin problems can also indicate that the balance of the gut flora is upset. Following the consumption of probiotics, a number of positive effects can be observed. We can have better digestion, have more energy, improve our mood and have clearer skin.

International Human Epigenome Consortium and the Human Epigenome Projects have been initiated to understand the overall epigenetic mechanisms involved in human diseases and health. The changes in non-coding RNA were believed to cause obesity, diabetes, and neurodegenerative diseases and those affecting lung and liver (Figure 2).

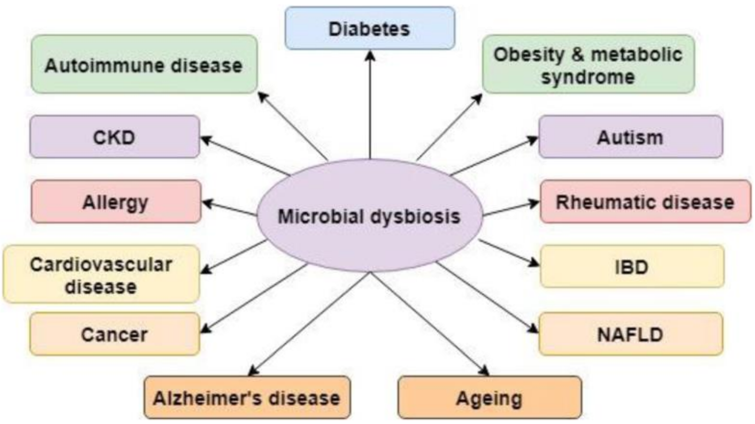

Figure (2) Microbial dysbiosis and diseases. CKD: Chronic kidney diseases, IBD: Inflammatory bowel diseases, NAFLD: Non-alcoholic fatty liver diseases

The epigenetic DNA imprinting is usually the most active during the early 1,000 days period from conception. During this journey, early nutrition plays a major role in regulating developmental programming. This phase of development possibly decides the individual susceptibility to diseases like obesity, diabetes, cardiovascular diseases, and other chronic non-communicable conditions that may occur later in life.

EPIGENETIC ALTERATIONS & MICROBIOME

DNA methylation is one of the most ubiquitous and fundamental mechanisms of epigenetics alteration that occurs on CpG Island (50-C-p-G-30) by DNA methyltransferases which are highly susceptible to nutrient availability and are influenced by gut microbial metabolism.

Probiotics are healthy, living bacteria that are essential for a healthy gut to function. It is very important for the gut to function healthily so that our immune system is also in excellent condition.

This is because the functioning of the intestinal system and the immune system are closely related. Regular consumption of probiotics helps prevent intestinal diseases, thereby maintaining the health of our immune system. Maintaining the health of the intestinal tract is essential, as if this stage of our digestive system is not in the best condition, the absorption of nutrients may also be insufficient. Good intestinal bacteria are needed for a healthy digestive system. It is important to strive to have the right amount of beneficial gut bacteria, as they support the functioning of the immune system, reduce allergies, improve cholesterol levels, and can inhibit the growth of harmful bacteria such as *Salmonella* or *E. coli*. Probiotics refer to beneficial bacteria, and prebiotics are plant fibers that provide food for the growth of good gut microbiomes.

MICROBIOTA BASED HEALTH INTERVENTIONS

Protein sources modulate energy efficiency and obesity progression by affecting gut microbiota. The terrestrial animal-based proteins have been found to be more obesogenic than those of seafood or vegetables. Human and animal-based studies have suggested that the level of *Lactobacillus* and *Bifidobacterium* can be increased after consuming soy foods and is beneficial for reducing the risk of diseases. Metabolites (such as betaine, folate, choline, and vitamin B12, etc.) are potentially implicated in the synthesis of 6-methyltetrahydrofolate (methyl donor) to generate S-adenosylmethionine, which participates in DNA methylation. The gut metabolites like folate, acetate, and butyrate are responsible for epigenetic modifications through the regulation of various enzyme activities. Probiotics when are given out in sufficient amounts confer health benefits on the host.
Lactobacilli containing fermented milk products have been reported for the longevity of Bulgarians. The mechanism of probiosis comprises stimulation of epithelial cell proliferation, immunomodulation, manipulation of intestinal microbial communities, differentiation, and fortification of the intestinal barrier, and the suppression of pathogens.
Faecal microbiota transplantation carries therapeutic potential against functional gastrointestinal disorders, obesity, metabolic syndromes, and inflammatory bowel disease through the administration of faecal microbiota into the recipient’s intestinal tract from a healthy donor. This transplantation changes the microbial composition of the gut of the recipient and makes them healthier.
Many drugs are playing the bidirectional communication with the microbiome. Regular physical exercise associates strongly with cardiovascular health benefits during ageing through the mechanism of epigenetic modifications. Identification of the fundamental epigenetic modifications and markers help to recognize the health disorders at earlier stages of the diseases, improving treatment outcomes and quality of life for many people.

DISEASES & DISORDERS

Lot of articles that explore the connection between epigenetics and diseases and disorders, including cancer, cardiovascular disease, diabetes, autoimmune disease, and more are published (Figure 2).
Type-2 diabetes comes with a slew of health issues, but one complication that patients often endure is muscle weakness. Poor muscle function makes everyday tasks difficult and reduces activity, which in turn, makes the condition even worse. While exercise and diet can help maintain a patient’s mobility, more information is needed to address the underlying causes that lead to diabetic muscle damage. Scientists at Lund University in Sweden have looked into this matter and have found evidence that one particular gene may be epigenetically impaired, contributing to muscle deterioration. The gene, simply known as VPS39, plays a role in skeletal muscle regeneration and maintenance. According to the study, it was found to be down regulated due to altered epigenetic mechanisms in individuals with type-2 diabetes (T2D). Their findings were published in the journal *Nature Communications* (Davegårdh, 2021).
Insulin resistance and decreased muscle quality are hallmarks of T2D. Understanding how these two are correlated could no doubt help those living with the disease live healthier lives. With T2D, insulin production is impaired, leaving excess sugar in the blood. Because muscle tissue is poor at absorbing the glucose for fuel, it starts to deteriorate and loses function.
Muscle tissue, much like other tissues in the body, has regenerative properties. Specific stem cells (myoblasts) are activated in the muscle in response to injury and certain exercises. The process of

stimulating these stem cells to form into new muscle fibers (myotubes) is called myogenesis, and it occurs through a series of epigenetically regulated events. Determining whether epigenetic modifications effect myogenesis intrigued the Lund researchers (Davegårdh, 2021).
Epigenetic factors are critical during development and help regulate cell-type specificity, chromatin stability, and gene expression. Environmental influences like exercise, diet, age, and diseases can alter epigenetic mechanisms like DNA methylation in muscle tissue as well as other tissues. It was discovered that irregular epigenetic and transcriptional changes during myoblast differentiation in individuals with obesity, a major risk factor for T2D. For this study, the investigated genome-wide expression and DNA methylation in the primary myoblasts and myotubes of people with and without T2D. The participants in this experiment were matched by age, gender, and body mass index, and all cell handling conditions were kept equivalent. The results revealed 42 unique genes that were differentially expressed in the myotubes (fibers), while 20 of these genes, including the VPS30, were identified as being differentially expressed in the myoblasts (stem cells) as well. The researchers also analyzed mature muscle cells, and they compared epigenetic patterns before vs. after cell differentiation in the two groups (Davegårdh, 2021).

EPIGENETICS & CORONAVIRION INFECTION

Epigenetics could explain why COVID-19 affects people differently. Extensive research in this field would do a great deal to protect public health, both now and in future generations. Emerging viral infections pose a major threat to global public health. In the last two decades, the world has dealt with several fatal outbreaks from the Swine Flu to Ebola to Zika infections and more.
The latest to appear is COVID-19, which emerged in December, 2019 in Wuhan, China and spread quickly around the globe. Although this disease is new, the virus itself is not entirely unknown. It's actually a type of coronavirion (CoV) that is similar to SARS-CoV and MERS-CoV, all which attack the respiratory system and derive from animal origin. Information about COVID-19 is currently still evolving, and health officials don't know for certain the true severity of the disease. However, researchers have investigated comparable pathogens in the past and have found similar mechanisms may be involved in the development of these types of infections. Perhaps the data from these studies could be useful in shedding light on how new viruses, like COVID-19, are able to spread and mutate so quickly in humans. Because viruses are constantly changing, they are usually difficult to treat, and ultimately, it's up to the host's immune system to clear the infection. Vaccinations and antiviral drugs have aided in lowering the mortality rates for these types of diseases, but they are not always available and can have harmful side effects.

Viral Infections & Epigenetics

In viral-host interactions, DNA/RNA methylation, chromatin remodelling, and histone modifications are known to regulate and remodel host expression patterns.
In viral-host interactions, DNA/RNA methylation, chromatin remodelling, and histone modifications are known to regulate and remodel host expression patterns. Epigenetics is described as the study of both genetic and non-genetic factors that control phenotypic variation. Primarily brought about via external and environmental influence, these modifications change the activity and performance of a gene without changing the underlying DNA code. RNA virion of COVID-19 shows strong associations with RNA modifications. For instance, m6A, m6Am, and 2′-O-me have been found to play important roles in the viral life cycle.
In particular, they can affect the structure of the virion, replication, innate immune response, and innate sensing pathways. Generally, virions like those from the family of coronavirions and influenza are not able to change genetic sequence. However, they can alter the epigenome, allowing them to defeat a host's immune system and successfully spread infection. Exactly how this occurs is not fully understood. But recent advancements in high throughput technology have allowed researchers to evaluate the epigenetic landscape at a genome-wide scale, as well as on a sequence-specific level.

Newer studies are using these technologies and finding that diverse viral groups utilize common and unique strategies to antagonize the immune system.

Epigenetics & the infection with COVID-19

The COVID-19 pandemic spread around the globe in a matter of months, leaving devastating human health and economic consequences in its wake. From a viral point of view, this incredible success is the result of a careful balance between its deadliness and contagiousness. While the number of deaths is high mark as of December 2020, many who are infected show only mild symptoms or no symptoms at all, carrying out their usual activities and spreading the virion in an exponential manner. What is the reason behind the striking differences in disease progression?

Old age, underlying health conditions, and being male have all been linked to more severe disease outcomes. Yet, many young and seemingly healthy people have died of COVID-19, leaving experts puzzled. While the explanation is likely a complex interaction of several contributing factors, epigenetics might play an important role (Corley & Ndhlovu, 2020).

The so-called cytokine storm is the culprit behind the death of many COVID-19 victims. Cytokines are small protein molecules that are released in the body during an infection by components of the innate immune system, the first line of defense against the virion. While they are critical in the response against the infection, for some reason certain people produce them in excessive amounts. This overdrive of the immune system causes cytokines to mistakenly attack the body's own cells, leading to cell death and organ failure. In a study published in *Clinical Immunology,* researchers studied the epigenetic regulation of cytokines in people suffering from Systemic lupus erythematosus, a chronic autoimmune disease. They found that key cytokine genes were hypomethylated in lupus patients, meaning that they were set to produce cytokines in larger quantities.

The different epigenetic regulation of cytokines thus could explain why many lupus patients suffer from severe forms of COVID-19. Another possible explanation lies in the epigenetic regulation of the *ACE2* gene, the human receptor for SARS-CoV-2, the virion that causes COVID-19.

Upon infection, virions use the replication machinery of the host's cells to multiply themselves. The *ACE2* gene plays an instrumental part in the infection process, as SARS-CoV-2 has to bind to the *ACE2* receptor on the cell's surface to be able to enter the cells.

DNA methylation patterns differed by gender in lung tissues and by age in epithelial cells. In the previous study of lupus patients, *ACE2* also showed different methylation patterns when compared to healthy individuals, with lupus patients showing a lack of DNA methylation.

As COVID-19 disease outcomes were found to increase in severity with age, underlying health conditions, and in males, *ACE2* DNA methylation patterns could play a role in COVID-19 disease progression. While further studies will have to connect the DNA methylation patterns of key cytokine genes and *ACE2* with COVID-19 severity in patients, a deeper look into the epigenetics of the COVID-19 infection could open up avenues towards accurate risk detection as well as personalized treatment options for the disease (Sawalha et al., 2020).

Epigenetic drugs against cancer

In recent years, in-depth studies of epigenetic machinery and its deregulation have led to the development and use of a wide range of modulatory molecules directed not only at chromatin enzymes (histone acetyltransferases, histone deacetylases, histone methyltransferases, histone demethylases and DNA methyltransferases) but also toward the emerging class of chromatin associated proteins, so-called "histone readers." Chromatin modifiers are attractive therapeutic targets for the development of new cancer therapies.

EPIGENETICS & PRIMARY CARE

Epigenetics is the study of how changes to chromosome structure record and/ or transmit changes in the expression of genes. Epigenetic mechanisms act during development to control mechanisms such as cell proliferation and differentiation, tissue formation, organogenesis, and the emergence of physiological function. They also act throughout life to regulate gene expression over the long term. Epigenetic mechanisms respond to a wide range of biological signals, including stimuli from the external and social environments. It seems likely that processes are involved that regulate the production of inflammatory cytokines and stress hormones such as noradrenaline and cortisol. Together the accumulated effect of these stress-related biological signals is known as allostatic load. It refers to allostasis, the process of restoring physiological set-points after exposure to stressors (which may be environmental or social).

EPIGENETICS & PREVENT BRAIN AGING

One unfortunate side effect of aging is loss of brain function. For most people, it starts off here and there with some "forgetfulness" or a modest decline in other thinking skills. But brain loss can be progressive as we age and lead to more problematic conditions such as dementia or Alzheimer's. One possible key to maintaining brain function and staying sharp-minded could be as simple as living a varied and active life. An accumulation of errors in the epigenetic machinery is one of the primary hallmarks of aging or key factors causing cellular damage.

Prior research has shown that genome-wide DNA methylation changes are highly associated with aging and age-related diseases, such as those involving brain deterioration and neurodegeneration. Certain lifestyle factors, including physical exercise, cognitive stimulation, and socialization, have been shown to offset age-related brain decreases in humans, allowing for what is called "reserve" or "maintenance" of brain function. Normally, methyl marks that safeguard the genes relevant for growing new neurons and cellular connections diminish in aging. The good news is epigenetic factors are not necessarily permanent — meaning it may be able to slow down, possibly even reverse, age-related brain dysfunction either through lifestyle changes or potential therapies (Zocher, S. et al., 2021). An Epigenetic Clock is a sophisticated way of tracking our "real" age by measuring methylation or demethylation at particular DNA sites (Kanherkar, 2014). The uses for epigenetic clocks are manifold. The most obvious use comes as a diagnostic tool, one that is already being offered by some companies as a direct-to-consumer test.

The second is as an in vitro screening method, something to inspect the effects of pharmaceuticals on cells in a petri dish. This lets researchers analyze subtle molecular signs of aging, paving the way for the rapid discovery of potential anti-aging therapeutics (Lujan, 2019).

Accurately estimating biological age has tremendous value. This is because aging has a negative effect on every aspect of our health. It's not a substitute for more specific tests, like fasting glucose levels, but paints a big picture.

Like any other biomarker, it does not stand alone, but it is complementary to the growing ensemble of tests modern medicine now has at its disposal. Epigenetic clocks made headlines with the publication of a paper that found all-cause mortality could be predicted based on methylation patterns in the blood (Marioni et. al, 2015). In other words, the team found they could guess when someone was going to die from any number of natural causes. Some epigenetic modifications are well-entrenched, but not all are set in stone (Kanherkar, 2014). The sands of time flow downwards for us all, but the pace varies. In other words, what is not completely reversible can still be influenced by our choices, if not through lifestyle changes, then through future therapeutics meant to produce specific changes in the epigenome. While the usefulness of epigenetic clocks is not questioned, it is not clear why they work. It's not obvious as to whether changes in DNA methylation are the cause or result of aging (Eckler, 2019).

THE FUTURE OF EPIGENETICS

Personalized medicine is becoming more popular as science identifies the varying effects of different substances on an individual's health. Not only is there a need for further research on how our environment and lifestyle choices (e.g., like diet) impacts epigenetic marks on human DNA, but how the outcomes will differ depending on a person's body. Epigenetics can be utilized for diagnostic tests, or markers that indicate.

CONCLUSIONS

The velocity of future climate change is commonly viewed as necessitating rapid plant movements as natural selection cannot operate fast enough to generate novel beneficial gene combinations. The epigenetic juggernaut is gaining momentum and is likely to touch most if not all aspects of biology in the coming years. The importance of epigenetics in human disease is being intensively studied, the role that epigenetics plays in inheritance and beyond to evolution is receiving much less attention, even though the implications may be far reaching.

DNA methylation is a dynamic epigenetic mark. DNA methylation at specific loci is induced by heterochromatic histone modifications and small interfering RNAs (siRNAs). It can be concluded that various genetic and epigenetic mechanisms of living organisms response to stress and other environmental and psychological factors as well as on pathogen stress. The current status of wildlife populations will undoubtedly change in response to the increasing pressures from the abiotic and biotic environments. The public and political concerns about the impact of climate and environmental change in humans tend to centre on such factors as food production and availability, water sources, the impact of extreme climatic events, sea levels, vegetation patterns etc. and the economic, political and social consequences of all these factors, including immigration and emigration. Abiotic stresses induce methylation or demethylation at specific loci. Small RNAs probably has direct methylation or demethylation enzymes to these loci.

Systematic analysis of siRNAs, DNA methylation, and stress response of mutants defective in siRNA biogenesis, DNA methylation, and demethylation will help unravel the role of RNA-directed DNA methylation (RdDM) in development and stress responses.

It was found that the existence of a specific, epigenetically controlled mechanism that promotes rearrangements in gene loci in the progeny of organisms infected with a compatible pathogen. Effects of environmental impacts on human reproduction do not normally feature at all, except for the particular case of pollutants and their potential for adverse effects on human reproductive and general health.

There is a danger that the seeming success of reproduction of the human species provides a false sense of security for the public, media and politicians with respect to wildlife survival, the maintenance of viable ecosystems and the capacity for recovery of damaged ecosystems and endangered species. It is now clear that anthropogenic environmental changes may affect both the reproductive success and the survival of many wildlife species by multiple routes and in often unpredictable ways. Since man does not exist in isolation, these wider impacts of anthropogenic macro-environmental changes need to be understood by society at all levels.

Future studies are needed

Future studies are needed to understand the specificity of the regulation including signal production, maintenance and “inheritance”. More studies are needed to broaden our understanding of temperature related epigenetic changes, especially under a changing climate.

The effect of long-term exposure to local temperature, potential non-linear effect, the effect of hot temperature, the interaction between temperature and other environmental factors are also yet to be explored. Right now, we are in the midst of fighting one of the most prolific viral outbreaks of the 21st century. Because this one has been so efficient at spreading, it is a reminder of not only how important it is to be prepared, but also more knowledgeable about emerging epidemic-prone diseases.

Future epigenetic studies should expand upon what we already know about these types of viruses so that more specific aspects of immunity, such as inflammatory response and apoptosis, can be explored. It is important to note that any information gained from these types of studies should be made globally and publicly available across political and international boundaries.

Having this information in a rigorous peer-reviewed manner will increase the utility and leverage of already existing data systems, which may be vital in developing both vaccines and therapeutic treatments. Furthermore, it could also allow newly emerging agents to be more promptly identified, and thus control measures put into place sooner.

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TL1.

PEOPLES' PERCEPTION IN PLASTICS WASTE MANAGEMENT TO PROTECT MARINE LITTERING, ENVIRONMENTAL QUALITY AND PUBLIC HEALTH AND ACTIONS THEREOF

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***Abstract**: Plastic waste, takes longtime to decompose, is dumped into landfills, water bodies and a portion leads to marine environment creating threat to environment and human health. Plastics turn into micro and nano-plastics which are consumed by animals & birds and, mammals & fishes in marine environment. Micro plastics find its way to the human body through eco-systems following different routes. Before plastic wastes gradually fills up our oceans and landfill sites, there is an immediate need to address this cause in order to reduce generation of plastic wastes. This study assesses the awareness of people regarding plastic waste management and its impact on environmental and health through survey. The survey is carried out both online questionnaire and in situ visits and interviews in several sectors, namely, educational institutes, health care units, industrial units and residents. Studies are carried out for efficient usage of non recyclable plastics wastes as a fuel for effective waste management in our society by the method of co-processing in cement plants, leading to implementation of circular economy to reduce marine littering. This study finds that the awareness of the citizens are still have to improve to enhance the efficacy of waste management and treatment efficiency which will help in determining the future course of actions for co-processing in cement plants and other potential use, assessing the amount that leads to marine environment. The study has also mapping the behavioral pattern of citizen on use and disposal of non-recyclable plastics.*

***Keywords:** Co-processing, Environmental pollution. Marine littering, Nonrecyclable plastics, Plastic waste, recycling*

INTRODUCTION

Plastics have become an unavoidable aspect in our daily life. From toothbrush to cars, almost everything we use contains plastics. They are relatively inexpensive to manufacture and can be used in

a wide range of varieties due to its ability to be shaped and molded into anything with quite ease compared to other materials. Plastics are also durable, resistant to corrosion, lightweight and resilient; it can be fabricated easily, and has great protective and preservation value. It also provides has thermal, electric and acoustic insulation value, reducing costs through greater efficiency. Its characteristics significantly reduce energy consumption and greenhouse gas generation, both in production and in use. Plastic production has been rising significantly since the 1950s. Since 1950s, more than 8.3 billion metric tons of the plastic has been produced worldwide. India produces plastic of 3.3 million tons of plastic per year out of which only 9% were recycled and 12% were incinerated. Plastic production is set to double in the next 20 years and quadruple by 2050 Despites such versatility and efficient usage, it has become an evident threat to our mankind due to its indestructibility. It takes 400-1000 years to decompose. Only 9% other the total plastics are recycled and the rest are practically non-recyclable products. These plastic wastes are generally dumped into landfill sites and with time they are led to the oceans, migrating into our ground water, hence contaminating our environment. These plastic wastes are known as Non-Recyclable Plastic Wastes (NRPW). Of the 8.3 billion, 6.3 billion tons have become waste, clogging our oceans and landfills, also killing marine life by getting mixed with their food.
The MacArthur report says there will be more NRPWs than fish by 2050 if a solution is not found to fight against this marine littering (McArthur Foundation, 2016). Figure (1) and Figure (2) show the pictures of accumulation of Non-Recyclable plastic waste in the landfills, slowly finding its way to the oceans causing marine littering and killing marine lives.

Figure (1): NRPWs in landfills at Baidyabati, WB

Figure (2): some examples of NRPWs

POLLUTION AND ITS ADVERSE IMPACT ON ENVIRONMENT

The accumulation of plastic and products made of plastic in the environment lead to plastic pollution which imposes a hazardous effect on wildlife and human food chain. The plastics have a chemical configuration by which they are resistant to environmental degradation rusting to high incidences of environmental pollution due to slow degradation. Plastic pollution occurs by plastic goods which vary according to its chemical configuration. It depends on the method of its polymerization and the method of natural degradation (Subha Ganguly, 2019).

EFFECT ON ENVIRONMENT SOIL: Harmful chemicals are released by seepage in the groundwater and in the ecosystem especially in the soil from the plastics. Polymer and nylon degrading bacteria like *Pseudomonas*, nylon-eating bacteria and *Flavobacteria* contribute to the release of methane gas from the breakdown of nylon which contributes towards greenhouse gas and global warming.

Figure (3): plastics wastes littered and scattered on the ground

***EFFECT ON WATER*: ** Plastic contaminates the water bodies and oceans by storm-water runoff, flowing into watercourses or directly discharged into coastal waters. This pollution enters the food chain thereby causing hazardous long term carcinogenic effect to fishes, animals and human beings due to the release of diethylhexyl phthalate, lead, mercury and cadmium. Oceans are generally contaminated from micro-plastic debris which floats on the sea surface. Every year, about 8 million tons of plastic waste escapes into the oceans from coastal nations. That's the equivalent of setting five garbage bags full of trash on every foot of coastline around the world.

Figure (4): Plastics waste floating in water bodies

***MARINE LITTERING*:** Plastic bags and abandoned nets are a risk to turtles, dolphins and seals. Turtles for instance may eat plastic bags since they mistake them for jellyfish. Plastic ingestion can guide to ulceration gastrointestinal blockages and internal perforation and death (IEEP, 2016). About 15 % of the species affected through entanglement and ingestion are on the IUCN Red List. Of particular concern are the critically endangered Hawaiian monk seal *Monachus schauinslandi*, endangered loggerhead turtle *Caretta caretta*, and vulnerable northern fur seal *Callorhinus ursinus* and white chinned petrel *Procellaria aequinoctialis*. Population level effects are evident in some species such as the northern fulmar *Fulmarus glacialis* (van Franeker et al., 2011), and the commercially important lobster *Nephrops norvegicus* (Murray & Cowie, 2011).

***EFFECT ON ANIMAL*:** Ingestion in animals can occur either due to mistaking plastic as food or prey or consuming accidentally during feeding or normal behaviour. Ingestion can cause serious harm by blocking digestive track of the animal or causing internal injuries which may lead to death. The impact of ingesting larger plastics (>5mm) is well documented. In the present situation, 60% of seabirds have plastic in their gut, with this figure expected to rise to 99% by 2050 (CSIRO, 2015). Nearly 700 species, including endangered ones, are known to have been affected by plastics. Nearly every species of seabird eats plastics.

Figure (5): Effects of plastic waste on aquatic life and birds

IMPACT ON TOURISM**:** The visible presence of marine litter has an in-depth impact on the aesthetic value and attractiveness of beaches and shorelines. Plastic debris along coastal beaches reduces the aesthetic value of the beaches ultimately affecting recreational experience of the visitors often resulting in sharp decline in number of visitors at the beaches. This results in substantial loss to coastal tourism industry. Entanglement in marine litter such as derelict nets and rope can also be serious hazards for swimmers and divers (NOWPAP MERRAC; 2013). Moreover, there is also a strong relationship between the visible presence of marine litter and the attractiveness of marine waters for recreational purposes (Fanshawe and Everard 2002). In addition, due to inconvenience, marine litter can also pose health risks and safety hazards to divers, recreational boaters, fishers and other coastal visitors. Industrial items (e.g., discarded chemical drums, batteries and appliances) leach toxic compounds, while medical/personal hygiene items (e.g., disposable diapers and sanitary products) contaminate the water, posing a health risk to swimmers and other users (UNEP; 2017) as shown in Figure (6).

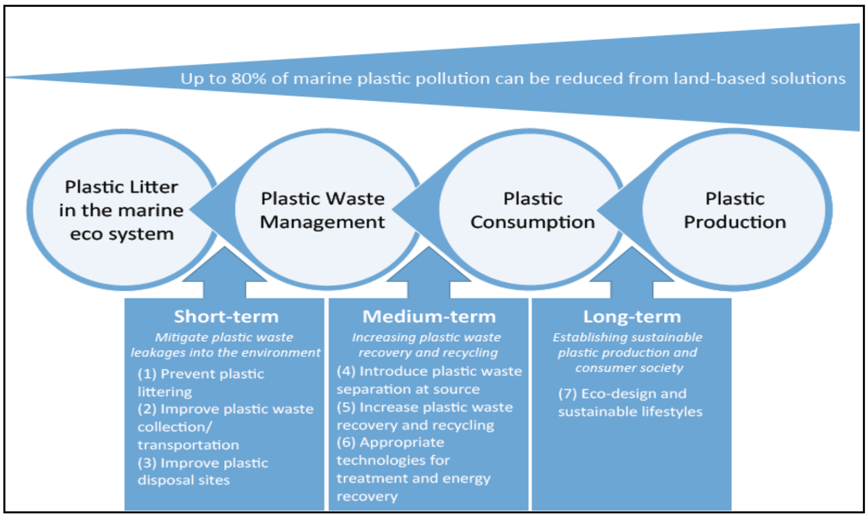

Figure (6): Proposed Strategies regarding reduction of Marine Littering Problem (UNEP; 2019)

PUBLIC HEALTH EFFECTS OF PLASTIC WASTES

It is generally believed that plastic polymers are lethargic and of little concern to public health, however, different types of additives and the residual monomers possibly retained from these polymers are responsible for the suspected health risks.

Most of the additives present in plastics are potential carcinogens and endocrine disruptors. Ingestion, skin contact and inhalation are the main routes of exposure of humans to these additives. Dermatitis has been reported from skin contact with some of the additives present in plastics. Micro-plastics are major contaminants that can bio-accumulate in the food chain after ingestion by a wide range of freshwater and marine lives leading to a public health risk. Human consumption of animals exposed to micro-plastics and plastic additives can be detrimental. Bio-monitoring studies on human tissues have shown that plastic constituents persist in human population through the measurement of environmental contaminants. It has been observed that there are lots of adverse effect of plastics waste littering and dumping on the environment and human health. Possible effects on environment, human health and animal have been computed in Table 1.

Table 1: Effects of plastic pollution on different aspects

Activities	Effects on Environment	Effect on Human Health	Effect on Animal
Plastic dumping in sewage	Sewage sludge is used as fertilizer and hence causes contamination of soil with plastics, also in tap water.	Consuming of food containing microplastics cause various respiratory, skin diseases.	Animal dies consuming plastics
Plastic dumping in open	Soil gets contaminated, Leaching of plastics to ground water	Consuming of food containing microplastics cause various respiratory, skin diseases.	Birds and animal accidently eat plastics and harm themselves.
Marine littering	Ocean water fills with microplastics which are consumed by marine lives.	Harmful too, as we eat fishes which has consumed plastics.	Consumed by marine lives, causing death
Open burning	Emission of harmful gases to the environment.	Those gases are harmful to human being.	Those gases are harmful to animal.
Landfilling	Leaching of plastics into soil, ground water contaminating it	Lack of hygiene, harmful for human being in plastic contaminated areas.	Many animals survive on food waste found on landfill sites, dies by eating the plastics present in them
Incorrect disposal of plastics	If not segregated, plastic mixes with other waste and causes contamination of both land and water bodies.		
Plastic additives	Leaching of harmful chemical present in plastics into soil and water		

LEGISLATION REGARDING REDUCTION OF MARINE LITTERING

There are several EU legal instruments already in operation that could have a role in tackling marine litter, addressing litter sources from a diversity of sectors. But they have limitations also. Some notable strategies and legislation are mentioned here regarding preventing marine littering issue. They are shown in Table (2).

Table 2: Few EU policy instruments with a potential to prevent marine litter (Newman, S, et al, 2013)

Policy instrument	Aims	Impact on marine littering monitoring	Limitations
Marine Strategy Framework Directive (2008/56/EC)	To achieve 'Good Environmental Status' in European seas by	This legislation has great impact on socially and economically. Descriptor 10 on marine litter requires that 'properties and	– Problems regarding implementation – Difficulty in proving and costing the level

	2020.	quantities of marine litter do not cause harm to the coastal and marine environment' (Annex I (10)).	and extent of biological 'harm'
Landfill Directive (1999/31/EC)	To establish technical requirements for the operation of landfills with the aim of reducing their impact on the environment, including the pollution of surface water	This legislation states Landfill location must consider the proximity of water bodies and coastal waters, and landfill design must aim to avoid pollution of soils and waters from the landfill (including from wind-blown waste).	– Inadequate implementation by Member States – Cost exemptions for small landfills serving islands or remote settlements can lead to Leakages or wind-blown waste reaching the marine environment.
Urban Waste Water Treatment Directive (91/271/EEC)	To reduce the pollution of freshwater, estuarial and coastal waters by domestic sewage, industrial waste water and rainwater	Urban waste water is a source of marine litter eg sanitary towels, tampons, plastic cotton wool bud , sticks etc Improved treatment can only remove a proportion of waste (and larger pieces, not microplastics). Product design has a much greater potential for improvement, particularly for microplastics.	– Poor compliance record – Implementation problems – Current requirements are not strict enough to prevent marine litter items entering the sea.
Common Fisheries Policy (European Maritime and Fisheries Fund) (Regulation (EC) No 2371/2002)	To ensure exploitation of living aquatic resources that provides sustainable economic, environmental and social conditions.	– The current European Fisheries Fund supports measures to remove lost fishing gears from the seabed. – The proposal for a new European Maritime and Fisheries Fund includes a measure to collect marine litter from the sea	– Extension of fishing-for-litter measure still in proposal form. – Proposed measure requires safeguards to ensure it doesn't lead to overfishing.
Port Reception Facilities Directive Øhlenschlæger, J P, Newman, S and Farmer, A (2013)	To reduce the amount of pollution in seas and on coastlines of Member States caused by ship-generated waste	Ship generated waste is an important source of litter and tightening of the Directive could have a big impact on this source. For that, Ports must have adequate facilities for the prompt reception of waste and cargo residues from ships.	All ship generated waste is to be delivered to port reception facilities , which can cause inadequate implementation

US National Marine Debris Monitoring Program and Legislations regarding Marine Littering

The National Marine Debris Monitoring Program (NMDMP) was developed to standardize marine debris data collection in the US by using a scientifically valid protocol to determine marine debris status and trends. This program was conducted over a five-year period between 2001 and 2006. The results indicate that land-based sources of marine debris account for 49 % of the debris surveyed nationally (Sheavly, 2010). Other legislation relevance to marine litter could have a significant impact on the amount of waste in the ocean. It includes Shore Protection Act aims to minimize trash, medical debris, and other harmful material from being deposited into coastal; The Beaches Environmental Assessment and Coastal Health Act aims to reduce the risk of diseases to users of the coastal recreation waters.

South Korea Initiatives on Marine Litter

As for legal and institutional restructuring, the “National Basic Plan for the Marine Debris Management” was institutionalized in 2008 by most of the concerned central Government agencies This plan is referring the Marine Environment Management Law as its legal base and sets two quantitative goals: reduce the amount of marine debris annually entering the ocean from 159,800 t (2007) to 127,840 t (2013) and increase the collection rate from 34 % (2007) to 45 % (2013). Nevertheless, studies showed that this marine debris policy is not successful in dealing with the marine debris issue since the policy focuses on collecting debris already at sea rather than preventing it from entering the ocean initially.

Taiwan Legislation relevance to Marine Littering

The Marine Pollution Control Act is the national legislation of MARPOL 73/78 and London Protocol. The act regulates that waste shall remain on board or be discharged into reception facilities, unless specific conditions apply for legal discharge. However, thus far, specific conditions have yet to be promulgated.

Scotland Marine Litter Strategy and National Litter Strategy

The Scottish Government and Marine Scotland recently initiated a process to advance the Marine Litter Strategy and the National Litter Strategy to jointly manage litter in Scotland’s terrestrial (including inland waters), coastal and marine environments.
Main aim of this legislation is to prevent the incidence of litter through a combination of approaches: to create education and awareness, infrastructure and tools, and enforcement and deterrence (The Scottish Government 2013).

MATERIALS AND METHODS

We are working under the project called OPTOCE which is funded by Norwegian company SINTEF and is in collaboration with Jadavpur University, West Bengal. In this study, we have conducted surveys to assess the quantity of plastic usage in various sectors and to study the life chain cycle of plastic products and how the plastics are being turned into non-recyclable plastic waste leading to the oceans as marine litter.
The objective of our study is to gain some knowledge about people’s perception regarding the harmful effects of plastic in our environment. The study was mainly based on Kolkata, West Bengal and its surrounding areas. The methodology we adopted was consisted of several components. We designed a survey which was based on a series of questions which were regarding usage amounts and disposal methods availed by people.
A total of five questionnaires was designed which is designed for different sectors like- Household, Industries, Educational sectors, Health units and commercial sectors like shops, restaurants, etc. The questionnaire was turned into a google form and was circulated throughout Kolkata and its surrounding areas, India. Hand to hand printouts of the google questionnaire form was also circulated manually. Industries and health units were contacted separately and were visited for the survey. The study samples were selected using random sampling technique (Marshal 1996). Among the visited households, at least one member of the family was picked randomly for the study regardless of his/her age, educational status, sex and occupation as long as he/she was willing. The number of male respondents was higher than female respondents due to the fact that males showed more readiness to be interviewed and fill the questionnaires provided. To collect the data, semi-structured questionnaires were prepared in English. Prior to the administration of the questionnaires, conversations were held

with the selected respondents to explain the objective of the study. Those respondents who were willing but not able to attend the questionnaires by themselves were helped by data collectors. Based on the collected data, the respondents were grouped into different categories. Analysis of the collected data was carried out using MS Excel and Minitab. After the collection of the data's from the survey, they were filled out in spreadsheet using MS Excel and then they were categorized according to their educational qualifications, age groups, number of members in their families, etc. The period of the household survey was 30 days (December, 2020). Then the data's were to be used for statistical analysis for us to get the missing data's by extrapolating them. We have used MS Excel and Minitab for the purpose of making diagrams and graphical analysis. Finally a quantitative model about people's perception would be proposed. The model will be further checked for its adequacy in case. Limitations in this study include Non-respondents to the survey, Intentions not to share correct data, limited data availability, and reaching some target groups.

ANALYSIS & RESULTS

People were randomly selected for the purpose whose age varied from a range of 15 years to 80 years. Their educational qualification was found to be diverse. No specific selections were made in case of their education, income or any status of living. In other words the survey was completely random. Educational qualifications of the surveyed people ranged from below secondary to PhD levels. Location of the surveyed people ranged from various parts of Kolkata and its surrounding. Maximum surveys were received from West Bengal till date. People from both urban and rural areas were confirmed. People belonging to various classes were observed in the surveyed list. The numbers of members in a family ranged from 1 to 11 at max. This confirms the survey was done on diverse range of family backgrounds which included nuclear homes, apartments, complex, joint families. Schools and colleges were also selected based on random sampling technique (Marshal 1996).
Industries and health units were visited personally for the questionnaire filling. Sectors, others than household were pretty hard to reach due to the current COVID 19 pandemic situation. The following figures shows the percentages of people reacting to different types of question listed with the diagram as well. So the results indicated that most of the people were more or less aware of the adverse effects of plastic pollution since very few of them were heavy plastic users.
It was also seen that people mostly used utensils made of plastics. Only 10.4% said that they never recycled their plastic products and dumped them directly. 68% of surveyed person confirmed they disposed of their plastic waste daily. While 27% said it was weekly when they disposed of their plastic waste and only 5% said they got rid of their plastic waste once in a month.
Generally in India, people dispose their waste before segregating. So the periodicity of disposing solid waste and plastic waste can be assumed to be the same.

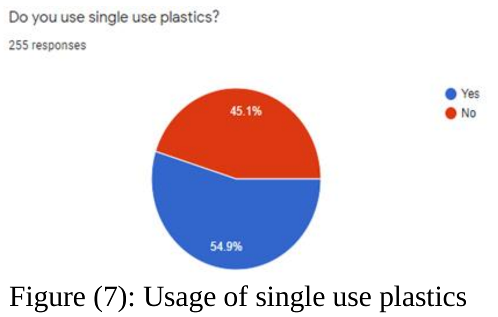

Figure (7): Usage of single use plastics

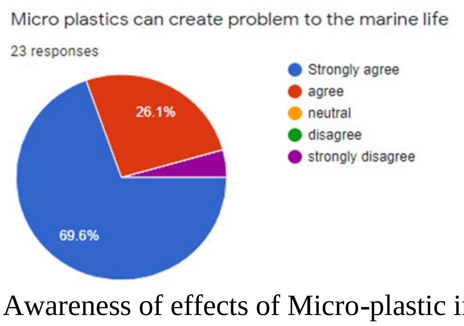

Figure (8): Awareness of effects of Micro-plastic in ocean

A total of 25 educational institutes were also surveyed by random sampling technique. Number of students and employees ranged from 50 to 5000. Some of the pie diagram from the responses is shown below (Figures 9 and 10).

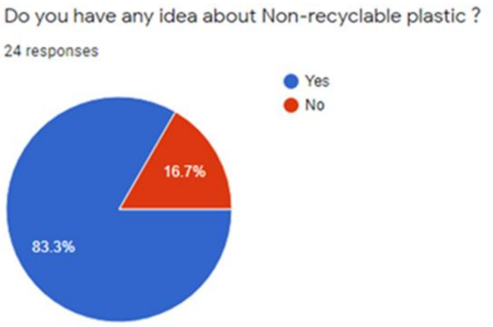

Figure (9): Awareness of NRPW

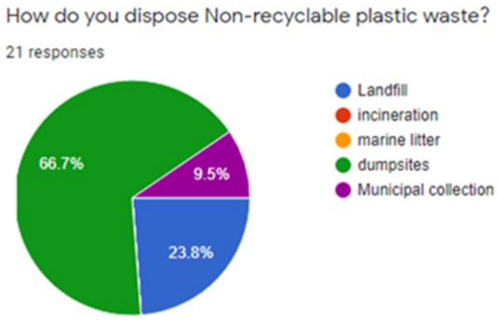

Figure (10): Disposal of NRPW

82% of the surveyed institutes said they disposed of their wastes daily by handing them over to the municipal workers. 54% of them were organizing awareness program regarding plastic pollution on a timely manner. When asked about how the usage of plastics can be reduced, 29.2% suggested recycling more while 16.7% supported a ban on the plastic products. Most common plastic product used in these institutions was plastic furniture and stationeries which are basically long term plastic products. 69.6% said they were aware how micro-plastics can be harmful to marine life. A lot of marine life is wasted daily due to consuming of micro-plastics. 91.7% disagreed with dumping of waste in watersides as they were aware of plastic causing harm to aquatic life. Roadside dumping was also opposed by 70.8% of surveyed people.

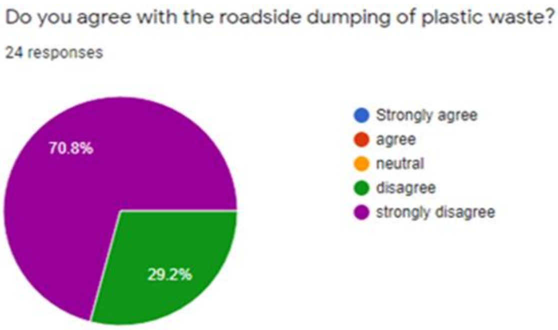

Figure (11): Roadside dumping opinion

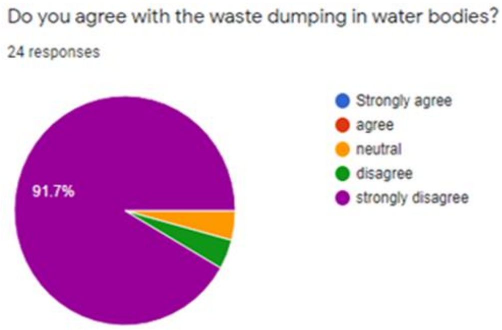

Figure (12): Water side dumping opinion

DISCUSSIONS

From the survey conducted in households, it can be concluded that people were fairly aware of the adverse and harmful effects of plastic wastes in our environment. People were starting to avoid plastic bags and encouraging usage of biodegradable products more than before. Rate of self burning and roadside littering of plastic wastes were found to zero. Very few people had no idea about marine littering and non recyclable plastics but the majority of the people said they were aware about it. Still there is a need to spread more awareness among people specially youths in order to educate them. These can be done with entertaining lectures, workshops and programs. Many of them had also started using alternative items to plastics. But they were very rare in number.

Effect of COVID 19 on plastic pollution

COVID 19 effect: It was found out while surveying in the hospitals that, COVID 19 had a significant effect on the plastic waste management system. The quantity of wastes had increased manifold across countries keeping in account the social distancing measure of staying at home. The intensified usage of single-use products and panic buying has increased production and consumption, hence jeopardizing efforts towards reducing plastic pollution. However, several countries have thus far instituted policies to ensure sustainable management of waste while protecting the safety of waste handlers. Owing to the global adoption of personal protective equipment such as face masks, future research should aim at developing biodegradable and environmentally friendly protective gears including face masks, gloves, overalls, among others, to accelerate the agenda towards achieving sustainable production and consumption while reducing environmental costs.

ROLE OF PUBLIC IN REDUCTION OF PLASTIC WASTE

The general public has a leading role in the process of reducing plastic pollution: only our recourse can make a change, from the moment one decides to purchase a certain plastic product until the time one disposes it and it is further taken up for recycling or conversion into a new product or at least for energy recovery by incineration or co-processing. With the right information and awareness, the general public can be successful in fighting this catastrophe.

The evaluations have shown that students changed quantitatively their perception of beach litter causes and derived problems, and they improved their knowledge about the main marine litter sources and the role of the sea in waste transport and deposition along the coast.

NGOs can contribute to this process significantly by connecting different categories of stakeholders to provide adequate information, by starting to take actions and mobilizing volunteers, by launching supportive projects, dissemination events, and awareness raising programs, and by showcasing success stories and best practices to provide models to other communities, they can be the engine triggering a society response to decrease plastic pollution. Several NGOs are taking up responsibility to educate people about the effects of plastic waste and how it can be reduced to create a plastic free environment.

Creating educational programs and field events for pupils and teens to raise their support for pollution reduction measures and nature conservation activities is another important measure to be taken, provided that young persons are more receptive to environmental problems. This can contribute to induce a behavioral change for the young generations, as they will be aware of the risks posed by plastic pollution to human and wildlife health and can positively influence their networks.

CONCLUSIONS AND RECOMMENDATIONS

1) It can be concluded that people were fairly aware of the adverse and harmful effects of plastic wastes in our environment though huge amount of plastic waste are produced still which is also due to unawareness of people. There is a need to spread more awareness among people specially youths in order to educate them.
2) If the plastic waste is managed properly, is effect on environment and living live can be improved significantly. They will no longer pile up our landfill sites and marine littering can also be blocked, thereby curing health of marine lives.

   Circular economy principles can be applied to global plastic packaging flows which could transform the plastics economy and drastically reduce negative externalities such as leakage into oceans.
3) Every stakeholders and SMEs should be made aware and brought under strict policies and rules regarding plastic waste management. Also they should research and design business models so that plastic waste can be recycled to form new products or even used more efficiently for energy recovery by incineration or co-processing. Such models will encourage people more and also provide job opportunities for the local people.
4) Co-processing of plastic wastes in cement kiln will be of tremendous change of shift in waste management scenario in the country. The Indian cement industry can also lead India towards Alternate fuel utilization thereby using circular economy methodologies. This co-processing of wastes shall also solve the problems regarding effective waste management, carbon emissions, Marine littering, saving lands from being used as landfills.

CO-PROCESSING OF PLASTIC WASTE IN CEMENT KILN

Co-processing is defined as the process where waste are used as raw material, or as a source of energy, or both to reduce usage of natural mineral resources (material recycling) and fossil fuels such as coal, petroleum and gas (energy recovery) in industrial processes, mainly in Energy Intensive Industries (EII) such as cement, lime steel, glass, and power generation. Waste materials used for Co-processing are referred to as Alternative Fuels and Raw materials (AFR).

Worldwide, wastes suitable for Co-processing have an energy potential equivalent to nearly 20% of the fossil fuel energy used by the EII and coal-fired power plants. By 2030, the thermal substitution rate of waste could rise to nearly 30%. In the EU-25 countries of Europe, the available energy potential in waste currently represents nearly 40% of this demand, and this is expected to rise to almost 50% by 2030.

The EU cement industry already uses more than 40% fuels derived from waste and biomass in supplying the thermal energy to the grey clinker making process. Although the choice for this so-called alternative fuel (AF) is typically cost driven, other factors are becoming more important. Use of AF provides benefits for both society and the company: CO_2-emissions are lower than with fossil fuels, waste can be co-processed in an efficient and sustainable manner and the demand for certain virgin materials can be reduced. Clearly, the societal benefits can be enlarged if more member states increase their AF share. Ecofys found that local factors constrain the market potential to a much larger extent than the technical and economic feasibility of the cement industry itself.

Roughly 60% of the waste that could be used for Co-processing is biomass and therefore carbon neutral. In this way Co-processing offers a significant potential for the reduction of greenhouse gas emissions from fossil fuels. Furthermore, diverting industrial waste streams from landfills and incinerators without energy recovery contributes to reducing overall CO_2 emissions when used to substitute fossil fuels through Co-processing.

It is therefore important and necessary to dispose the waste contains in an environmentally sound manner and cement kiln co-processing can be explored for the effective management of this hazardous waste. Plastics are being used in cement manufacturing as fuel run furnaces. The cement industry can reduce its fuel costs by as much as 20% or more by using single-use plastics as an alternative fuel source. India is one of the largest producers of cement, producing about 300 million tons of cement per year. Hence it has a huge opportunity to co-process plastic wastes in cement factories, both as a raw material and a source of fuel.Figure (13) shows how the waste can be effectively used and emissions of harmful gases can be reduced.

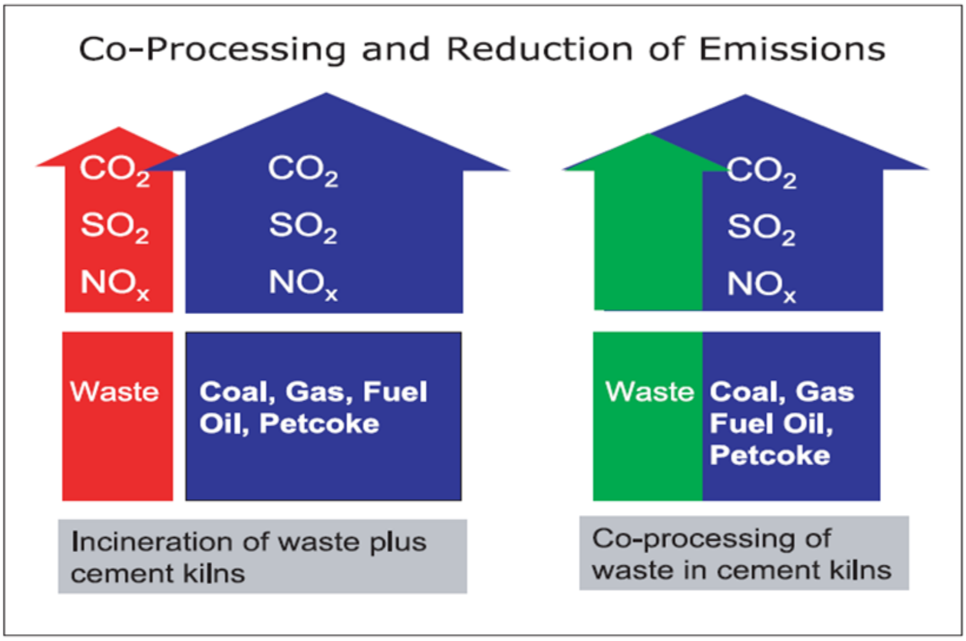

Figure (13): Co-Processing & Emission Reduction; (Source: Indian center for plastics in the environment (ICPE)

Here is how emissions were reduced by co-processing them in cement kilns, instead of directly incinerating them. Co-processing can be a key player in reduction of accumulation of plastic waste which is finally leading to the ocean and causing our environment harm. Co-processing has been found to be very effective in reducing plastic pollution from various studies. A lot of countries has been practicing co-processing of AFR in cement kilns and they have even reached 50% TSR. India produces 3.3 million tons of plastic waste each year and India is also the second largest producer of cement in the world. So India has the perfect chance to uitilize its plastic waste in cement kilns, provided quality on various aspects like health, environment, production is maintained.

Table 3: Some actions that can be taken for reduction of plastic pollution are also suggested:

Sl No.	Action that can be taken	Impact
1	Choose Reusable items (stopping of usage of single use plastics, disposable bottled water or drinks, etc)	Significant reduction on single usage of plastic products.
2	Boycott micro-beads (toothbrush, facial scrub, small plastic products)	Reduction of plastic migration into ground water due to their small sizes
3	Cooking more and eating homemade food	Reduction in usage of plastic containers provided by restaurants
4	Buying secondhand items more	Reduction of plastic packaging
5	Put pressure on manufacturer	Introduction of alternative items
6	Taking own bag to shopping, buying things in bulk	Reduction of plastic carry bag
7	Educate more	General awareness about harmful effects of plastic pollution

SOME RECOMMENDATIONS AND REGULATORY ACTIONS TAKEN REGARDING MONITORING OF MARINE LITTER

Regulatory and market-based incentives

A recent analysis highlighted the effectiveness of small incentives in reducing waste mismanagement in Australia and the United States. An incentive of as little as 5–10 cents through container deposit legislation (CDL) or cash for containers was effective in reducing beverage container waste. The proportion of beverage containers found in coastal surveys from states with incentives was approximately 40% less than in states without incentives—and was consistent between the two countries (Hermawan, S.; 2019)

Transparency and labelling: Improvement on transparency on the chemicals contained in plastics can be suggested – to help with decisions on remanufacture and recycling. In addition, the transparency on where the personal care and cosmetic products (PCCPs) do and/or do not contain plastics.

Waste management measures: Investing in waste collection infrastructure (at ports), waste management infrastructure and wastewater treatment facilities to avoid scattering of litter into the marine environment - particularly in coastal areas or near rivers (IEEP; 2019).

Banning of unnecessary and damaging products or activities: Unnecessary plastic products can be banned and also damaging recycle products can be dealt with fine (Brink, P. T. et al; 2019).

Enhancing the Circular Economy: The circular economy is an economic system that wants to retain as much value as possible of products, parts, and materials by approaches such as optimal reuse, refurbishment, remanufacturing, and recycling. But still it will be a difficult task to achieve 100 percent success rate. However it can be solved by investing more into education and R&D department of the cement industry, which offers various opportunities for fundamental and applied R&D work. Only with expertise and sufficient research the cement industry will be able to further reduce the environmental footprint of its operations and its products in their final application.

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TL3.

IMPACT ASSESSMENT OF ANTHROPOGENIC ACTIVITIES ON AIR QUALITY OF ULAANBAATAR, MONGOLIA USING LICHEN AS THE BIOINDICATOR

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Abstract: *Lichen is a composite organism that arises from green algae or cyanobacterium (phycobiont) and fungal partner belongs to the Ascomycota and Basidiomycota (mycobiont), living together in symbiotic relationship. The interaction between lichens and air pollution has been used as a means of monitoring air quality since 1859. The most important pollutant was sulphur dioxide produced by coal burning industry and power stations. City of Ulaanbaatar, the capital of Mongolia was initially designed for a half million residents. Due to intense rural-to-urban migration after transition to market driven economy, the population of the capital is nearly tripled, which resulted in huge area of informal settlements and elevated number of vehicles. Tents and small buildings in above settlements heated by conventional stoves by burning coal and wood, while the most vehicles on the road are imported second-hand cars. During last two decades, air quality of capital city Ulaanbaatar is considered as an emerging issue and above two are primary sources of outdoor air pollution. The most abundant air pollutants are nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and carbon monoxide (CO) which have disastrous effects on health when inhaled over prolonged periods of time. Due to the quantities of these pollutants would be far more abundant and thus cause the catastrophic effects to the air quality that is seen in Ulaanbaatar. Various techniques have been developed to monitor air quality, including biomonitoring using lichens. In this study, we monitored types of epiphytic lichens covering sensitive to air pollution fruticose type and relatively tolerant foliose type of lichens. Unlike air quality index, lichens clearly show negative impact of poor air quality on surrounding ecosystems. We examined lichens on Larix sibirica, the most abundant coniferous tree of the area. Both foliose and fruticose types of lichens are abundant in Larix sibirica dominated forests located to the northern areas of Ulaanbaatar. It indicates the area is free of pollution. In contrary, fruticose type lichens, especially representatives from the genera Usnea, Cladonia and Vulpicidia are absent in coniferous forest to the southern areas of Ulaanbaatar. These results clearly show downwind ecosystems have been badly affected by poor quality air of the capital. Additionally, there is none of those bark dwelling lichens are observed on the three along the crowded roads, parks and residential areas which indicates the extreme pollution of the air. Finally, biomonitoring using lichen as the bioindicator is discussed, and future recommendation is provided in the end of the study.*

Keywords: A*nthropogenic activities, air quality of Ulaanbaatar, Mongolia, lichens, bioindicator*

INTRODUCTION

Lichens are mutualistic associations of a fungus and an algae or cyanobacterium. In nature, they occur on rocks, bare grounds, and trees, the latter known as epiphytic lichens. Due to their dependence on airborne nutrients, lichens are very sensitive to air pollution especially to sulfur dioxide. They do not bear defensive tissue, therefore quickly absorb, and accumulate sulfur dioxide in their tissue, which subsequently leads to a damage of chlorophyll in phycobiont algae so that photosynthesis is inhibited. Because of this sensitive characteristic, lichens are widely used as environmental indicators or bio-indicators. Depending on their degree of tolerance, some species may present with certain concentration of pollutants, while all species may vanish with extremely poor air quality. On this ground, lichens provide a relevant, sensitive, and measurable indicator for long-term monitoring (Das *et al*., 2013; Giordani, 2007).

One of the places where lichens could be used as bio-indicator is city of Ulaanbaatar, the capital of Mongolia. The city is characterized by its high elevation (1350 meters above sea level), low precipitation (200-300 mm), and its location in a basin (Hauck, 2008). The city was initially designed for a half million residents. However, due to 1990s intense rural-to-urban migration, population of the capital was far more exceeded than its infrastructure capacity and ended up with informal settlements or huge ger areas (Figure 1).

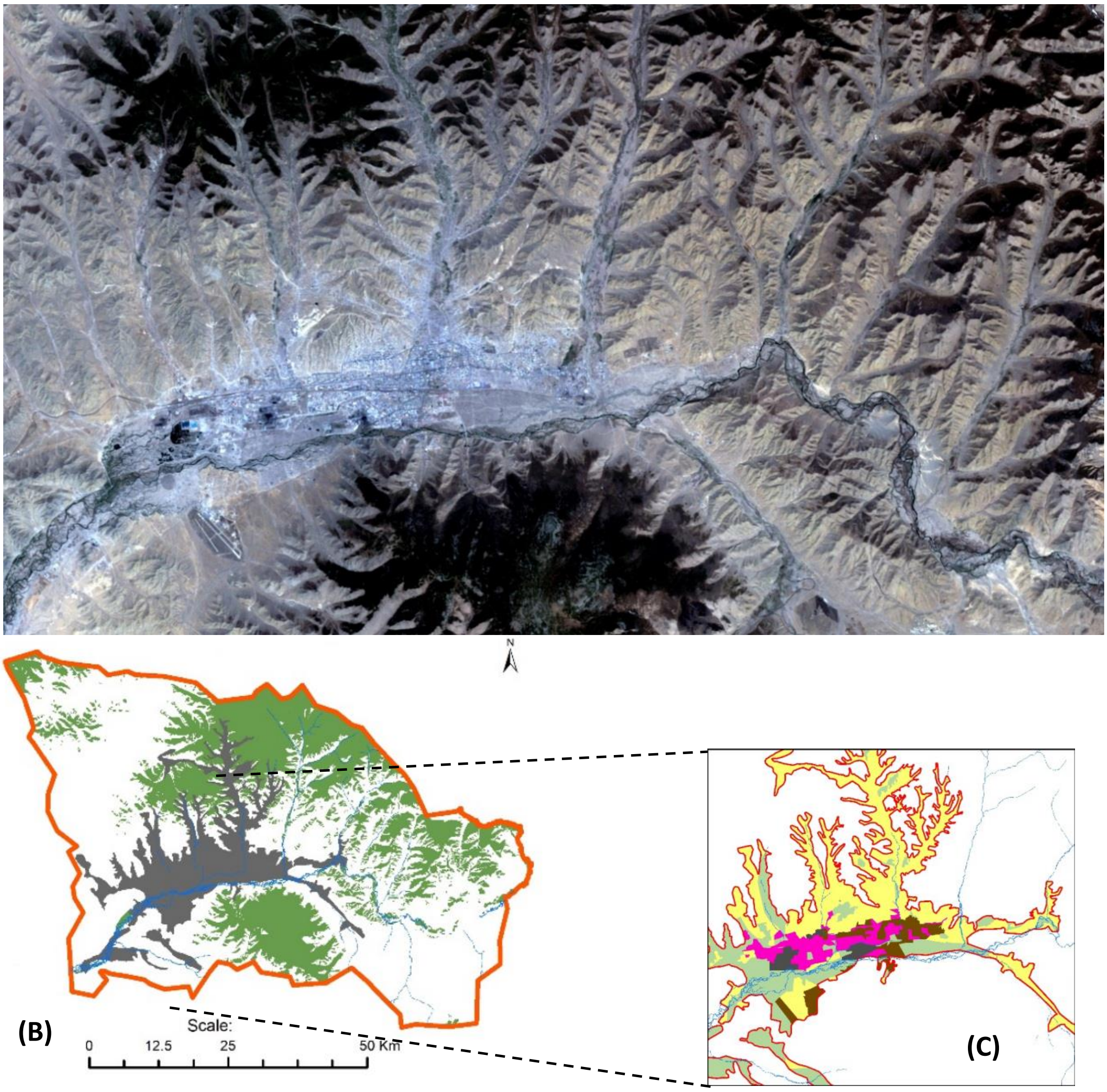

Figure (1): Map of the city of Ulaanbaatar

(A) Landsat image (https://landsat.visibleearth.nasa.gov); **(B)** Settlement areas of the city, where grey - settlement areas, green –forest areas, and blue – rivers; **(C)** Land use map as of 2010, where yellow – ger areas (Myagmartseren et al., 2018)

Since Ulaanbaatar is the coldest capital in the world with annual mean temperature of minus 3.7ºC (Hilbig et al., 2004), the major cause (c.a. 80%) of air pollution comes from coal burning stoves which are the sole way to heat ger homes under sub-zero temperatures. There are 130.5 thousand families in ger areas burn roughly 525 thousand metric tonnes of coals, 290 thousand cubic metres of firewood, and other combustible materials each year (http://agaar.mn/static/stove-distribution). As a result, concentrations of the major pollutants far outreached the guideline values provided by World Health Organization (WHO). For example, on the coldest days of the year, daily average of $PM_{2.5}$ level reaches 687μg/m^3, which is 27 times higher than the level WHO recommends as safe (https://www.unicef.org/mongolia/environment-air-pollution). Similarly, sulphur dioxide, the major pollutant from coal burning reaches 170 μg/m^3, which is eight times higher than a national air quality standard. The second major source (10%) of air pollution is 340 thousand vehicles on the road and most of which are (79%) aged more than ten years and with substandard quality. Total of three thermal power plants are responsible for 6% of Ulaanbaatar air pollution, and the rest 4% is windblown dust (Davy et al., 2011).

Supporting reason for dense air pollution of Ulaanbaatar is its geographical location in a basin. The city is located along the Valley of Tuul River. Surrounding mountains reduce vertical and horizontal dispersion of air pollutants, especially during wintertime (Figure 1). Polluted air accumulates in the city area and shows number of negative impacts on human and ecosystem health. Air quality of capital city Ulaanbaatar is considered as an emerging issue and dozens of measures taken during the last two decades; however, no significant improvements have been achieved so far.

Study on lichens in relation with air quality started in mid 90s (Galsan et al., 1995; Enkhtuya, 1999) and further studies examined Ulaanbaatar air pollution effect on lichenous flora of surrounding ecosystems, biochemical and chemical analysis of lichens, bioindicator roles of local lichens and biodiversity of lichens (Ganbold et al., 2007; Hauck, 2008; Baljinnyam et al., 2009; Delgermaa et al., 2016).

In this study, we examined epiphytic lichens on *Larix sibirica*, the most abundant tree of the local forest at four sites close to Ulaanbaatar city. Two sites were chosen from Bogd Khan Mountain to the south and downwind of the city and two were from Chinggeltei Mountain to the north and upwind of the city. Both foliose and fruticose types of lichens were abundant at the sites to the northern areas of Ulaanbaatar. The result indicated the area was free of air pollution and wind flow prevents the area from pollution. In contrary, fruticose type lichens, especially representatives from the genera *Usnea*, *Cladonia* and *Vulpicidia* are absent in coniferous forest to the southern areas of Ulaanbaatar. Furthermore, relatively tolerant foliose type lichens show morphological changes such as size reduction, shape deformation and colour change or fading. This result clearly shows downwind ecosystems have been badly affected by poor quality air of the capital. Additionally, there were none of those bark dwelling lichens observed on the threes along the crowded roads, parks and residential areas which indicated the extreme pollution of the air within the city. Our result show that lichens could act as reliable and cost-effective bio-indicator which clearly differentiates mild or heavy pollution of the air. On the other hand, from the lichen community and their current biodiversity loss, we could foresee how anthropogenic false activities may effect on other forms of life if pollution exposure lasts further.

MATERIALS AND METHODS

Study sites. Total four sites were selected in this study. Site A and B located in Bogd Khan Mountain area which is to the south and downwind of the city. The Site A located at 1762 meters above sea level, where wind flows mostly from the west, rather not from the city. Site B was just 1.62 kilometers apart from Site A, however 298 meters below and located downwind the emission from Thermal Power Plant III. With this reason, the Site B was suspected as the most polluted site along with other three sites (Figure 2). Site C and D were from Chinggeltei Mountain to the northern side and mostly upwind of the city. The Site C was chosen because its location along the Selbe River. During winter, when air pollution reaches its highest peak, the polluted air fills up the valley and reaches Site C if wind flow is weak. That is why we suspect this site to expose to mild pollution.

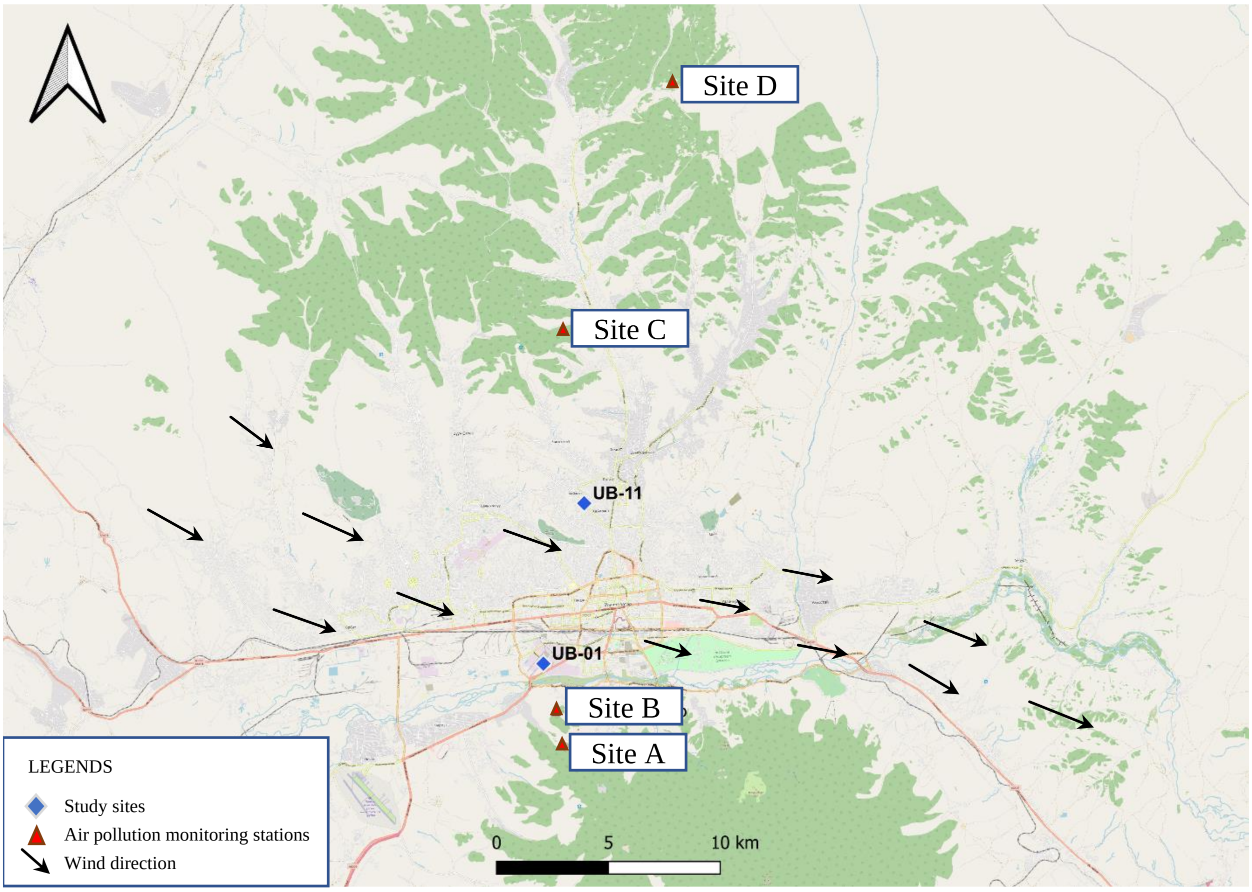

Figure (2): Location of the selected sites

The last Site D was chosen because its distance from the source of pollution. All of the above four sites belonged to the same type of forest or coniferous forest where larch tree dominates (Figure 2). Details of the selected sites were given in Table (1).

Table (1): Details of the selected sites

Site of study	Bogd Khan Mountain (downwind the city)		Chinggeltei Mountain (upwind the city)	
	Zaisan	Khiimoriin ovoo	Yargait	Khandgait
Latitude	47º86′26.2″N	47º87′61.5″N	48º02′93.9″N	48º12′71.2″N
Longitude	106º88′28.6″E	106º87′91.7″E	106º88′90.8″E	106º95′85.8″E
Altitude	1782 m	1464 m	1545 m	1731 m
Distance from city center by road or path	8.6 km	7.0 km	14.7 km	26.9 km
Forest type	Coniferous	Coniferous	Coniferous	Coniferous

Lichen sampling. Siberian larch *Larix sibirica* was used in this study because of its abundance and morphological characteristics of bark that is rough and suitable for lichen settling. Lichen colonies were collected with the help of forceps, placed in sample bags and transferred to a laboratory.

Identification of lichens
Lichen specimen was collected in polyethylene bags and transferred to a laboratory. The samples were air dried and identified using light microscopy with the help of chemical tests (Orange et al., 2010).

Total lichen coverage. Total fifteen individual larch trees, whose diameters ≥20 cm were selected at each site. Quantification of lichens was done by placing a lichen grid (20X20 cm) on the tree at 0-20 cm and 130-150 cm heights from ground level (Enkhtuya, 2007).

RESULTS AND DISCUSSION

Lichen diversity. Currently there are 225 species of lichen recorded in Bogd Khan Mountain (Enkhtuya, 2007). However, in this study, we recorded collectively 12 lichen species at the A and B sites selected from Bogd Khan Mountain area (Table 2); 10 and 8 species from the Site A and B, respectively. Most lichens observed from Bogd Khan Mountain belonged to foliose and crustose growth types which are relatively tolerant to air pollution. For example, according to Hawksworth, 1970, foliose type lichen *Hypogymnia physodes* could tolerate 70µg/m^3 mean winter SO_2 concentration and appear around the basis of the trees, but do not extend up the trunks. In our study, mostly all sensitive species from fruticose type lichens were vanished and foliose type species - *Hypogymnia physodes, Flavopunctelia soredica and Parmelia sulcata* were observed as dominant lichens mostly around the basis of the trees. Especially, two tolerant species *Flavopunctelia soredica* and *Parmelia sulcata* comprised more than 90% of the total lichen coverage at the Site B.
From fruticose growth type, only one species - *Evernia mesomorpha* was observed in this area. To note, only one colony of *Evernia mesomorpha* was recorded among fifteen larch trees used at the Site B.

Table (2): Lichen species recorded at each study site

Species	Morphology	Bogd Khan Mountain		Chinggeltei Mountain	
		Site A	Site B	Site C	Site D
Cladonia coniocraea	Fruticose			+	+
Cladonia fimbriata	Fruticose			+	+
Evernia mesomorpha	Fruticose	+	+	+	+
Usnea fulvoreagens	Fruticose			+	+
Usnea hirta	Fruticose			+	+
Usnea lapponica	Fruticose				+
***Usnea* sp.**	Fruticose				+
Flavopunctelia soredica	Foliose	+	+	+	+
Hypogymnia bitterii	Foliose	+		+	+
Hypogymnia physodes	Foliose	+	+	+	+
Melanelia exasperatula	Foliose	+			+
Melanelia olivacea	Foliose			+	+
Parmelia soredica	Foliose		+	+	+
Parmelia sulcata	Foliose	+	+	+	+
Vulpicidia pinastri	Foliose				+
***Buellia* sp.**	Crustose			+	+
Lecanora piniperda	Crustose	+			+
Lecanora symmicta	Crustose			+	+
Lepraria sp	Crustose	+			+
***Rinodina* sp.**	Crustose			+	+
Trapeliopsis flexuosa	Crustose	+	+	+	+
Trapeliopsis granulosa	Crustose		+		+
Hypocenomyce scalaris	Squamulose	+	+		
Total		**10**	**8**	**15**	**22**

+ present

For the two sites C and D from Chinggeltei Mountain area, upwind the city, the most sensitive fruticose type lichen species were observed as abundant lichens (Table 2). Well known bio-indicator species from a genus *Usnea*, which exist when mean winter sulfur dioxide concentration is below $40 \mu g/m^3$ only were observed at both Sites C and D. Furthermore, golden-coloured foliose type lichen *Vulpicidia pinastri*, which is extremely sensitive to any type of air pollution, was recorded at Site D. This result indicated an excellent air quality at this site. We collected and identified total 22 species of lichens from Chinggeltei area; 15 and 22 species from the Site D and C, respectively. Some of the lichens were photographed and shown in Figure (3).

Hypogymnia physodes

Parmelia sulcata

Flavopunctelia soredica

Hypogymnia bitterii

Vulpicidia pinastri

Evernia mesomorpha


Usnea fulvoreagens


Usnea hirta


Trapeliopsis granulosa


Lecanora symmicta


Cladonia fimbriata


Cladonia conicraea

Figure (3): Lichen species observed near Ulaanbaatar city

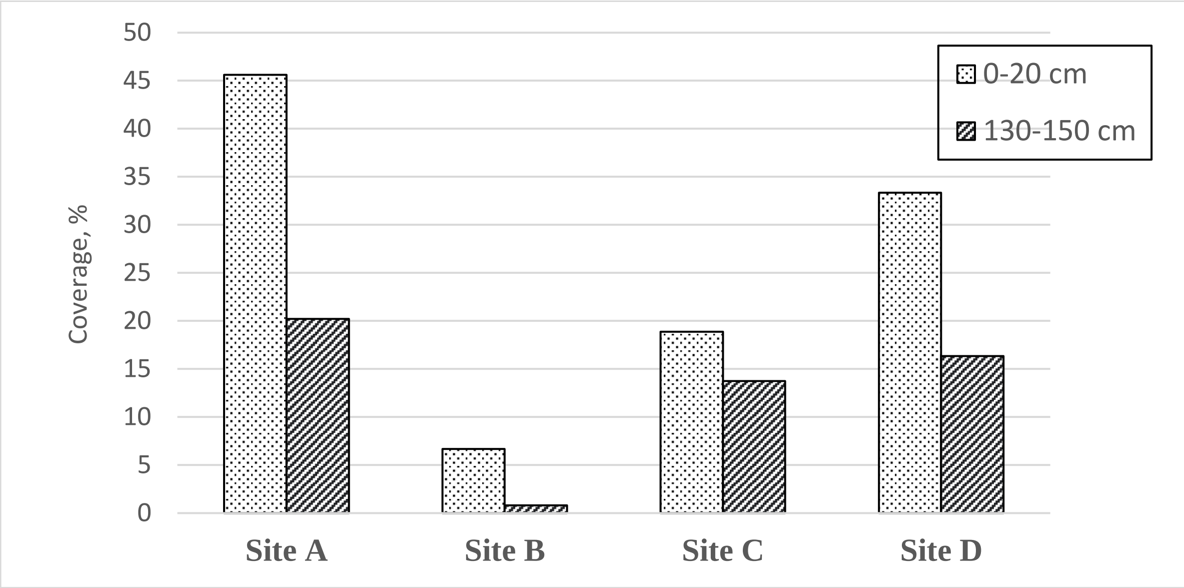

Figure (4): Total lichen coverage at the selected study sites

Lichens as bioindicator of air quality

Sulfur dioxide level in the air can be estimated qualitatively by studying the epiphytic lichens growing on trees (Hawksworth, 1970). In contrary, higher vascular plants and trees respond to air pollution at much slower rate (Muir et al., 1988). We observed lichen diversity within and outside of the city of Ulaanbaatar. All species of the trees and bushes near roads, parks and residential areas of Ulaanbaatar city do not bear any of the species of lichens. This result simply suggests a disastrous amount of air pollution that is mean winter sulfur dioxide concentration is around or exceeded 170μg/m^3. This finding also meets measurement results from the air quality monitoring stations.

Bogd Khan Mountain area, downwind of the city bear only lichen species tolerant to mild pollution, but size, colour and morphology are already changed, and this result indicate plant stress under polluted conditions.

CONCLUSION

In this study we observed clear change in lichen diversity downwind and upwind of the capital city Ulaanbaatar. The results suggest that polluted air generated from conventional stoves from ger areas transferred to the southern part of the city and show its negative impact to Bogd Khan Mountain ecosystems. Lichens are the first organisms respond to environmental pollution and warn us; but if such anthropogenic false activity still remains for the future, many other important life forms may degrade in term of prolonged exposure time.

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CYTOTOXICITY EFFECTS OF THREE PESTICIDES ON MITOTIC CHROMOSOME ON ROOT CELLS OF *ALLIUM CEPA.*

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Abstract: Motivation/Background: *Pesticides constitute a heterogeneous category of chemical specifically designed for the control of plant diseases. The wide production and use of chemical pesticides cause a serious pollution to the surrounding environment. These chemicals, a part from affecting the target pest, also affect plants, animals and human.* ***Method:*** *After growing roots of Allium cepa till they reached 2–3 cm in length, they were treated with three concentrations (0.001, 0.0025, and 0.005 mg/ml) of mancozeb (fungicide), dimethoate 40 and lanate 90 (insecticides), and distilled water was used as control, for 24hour exposure.* ***Results:*** *The tested concentrations decreased the mitotic index compared to the control; the mitotic index values were significantly decreased with an increase in the concentration of the pesticides. In addition, the different treatments caused diverse types of chromosomal abnormalities during cell division. The proportion of chromosomal aberrations was significantly increased from a control value. The chromosomal abnormalities that were observed during the study are stickiness, early chromatin condensation at prophase, chromosome multipolarity, fragmentation of chromosomes, c-metaphase and micronuclei appearing in interphase cells were.* ***Conclusions:*** *The result indicates that three pesticides had cytotoxic activities on mitotic index and chromosomal aberration. Hence, the use of these pesticides should be under control in agricultural fields.*

Keywords: *Allium cepa, Chromosomal aberration, Environment, Mitotic index, Pesticides and Pollution.*

INTRODUCTION

Pesticides are useful for controlling pests, increase yield and improve the shelf life of agricultural products. Their use in disease prevention has skyrocketed, without considering their harmful side effects on plants, animals and human beings [1]. Although the use of these chemicals has become essential, but their ingredients have induced acute toxic effects [2,3]. Pesticide toxicity is not necessarily a result of direct application; certain pesticides accumulate into the food to a toxic level and have an effect on public health [4]. Dryanowska [5] showed that the frequency of cancer increases among people who have been exposed directly or indirectly to pesticides or fungicides.

Pesticide residues are a common cause of soil and water pollution, especially in developing countries. They remain available into the environment, posing a local and global threat to habitats and human populations. [6]. Cytological investigations have been carried out to detect the harmful effects of agricultural chemicals on various crop plants [7,8, 9,10]. Constant use of these chemicals may result in changing the hereditary constitution of an organism [11,12]. Genetic damage on plant [13,14], alters mitotic activity, chromosomal structure and changes stability of DNA template and expression of proteins, increased the genetically abnormalities and decreased the mitotic index (MI) [15]. Generally, Pesticide application leads to overproduction of reactive oxygen species (ROS) resulting lipid peroxidation, cell membrane damage, protein oxidation, enzyme inactivation, DNA and RNA damage [16].

The *Allium cepa* assay is an efficient test for chemical screening for toxic effects of environmental contaminants and has been widely used to study, because its high sensitivity, low cost, availability throughout the year, easy and rapid performance along with small number and large chromosomes [17]. In addition, the mitotic index (MI), could be used as a reliable parameter for evaluating the cytotoxicity of various agents [18]. Therefore, the aim of the present study was to examine the cytotoxic effects of these chemicals on mitotic activity of *Allium cepa*.

MATERIALS AND METHODS

TEST CHEMICALS

Three pesticides were used in the current study, which are commonly available on the market and widely used: Dimethoate 40 and Lannate 90 are insecticides, and Mancozeb is a fungicide (Table1). Every pesticide was prepared in three concentrations (0.001, 0.0025, and 0.005 mg/ml), with distilled water as diluents and as a control.

Table 1: Some features of pesticides

Chemical name	IUPAC name	Molecular formula	Molecular weight
Mancozeb	zinc; manganese (2+); N-[2-(sulfidocarbothioylamino)ethyl] carbamodithioate	$C_8H_{12}MnN_4S_8Zn$	541.1g/mol
Dimethoate 40	lego; O, O-dimethyl-S- (N-methylcarbamoylmethyl) phosphorodithioate	C5H12NO3PS2	229.12.1g/mol
Lannate 90	S-methyl -N-(methylcarbamoyloxy) ethanimidothioate	$C_5H_{10}N_2O_2S$	162.21g/mol

EXPERIMENTAL PROCEDURES

Onion bulbs, free from agricultural pesticides and growth inhibitors were grown in the dark at a constant temperature of 25±1°C. The bulbs were placed onto cylindrical glass, and receptacles were filled with distilled water, which was renewed every 24 hours for the purpose of aerate. The experiments began when the newly growing roots had reached 2–3 cm in length. After which each bulb was planted on a 50 ml capacity beaker filled separately with the pesticide concentration, except the control which was treated with distilled water only, and used as a comparative sample for the effects of tested pesticides. For each concentration of the pesticides three replications were made. The *Allium cepa* bulbs were left into the filled beakers for 24 hours.

MICROSCOPIC PARAMETERS

At the end of the exposure period, roots were cut and immediately treated in a chilled Carnoy's fixative (ethanol: acetic acid = 3:1) for 24 h before being transferred to 70% ethanol. The root tips were then hydrolyzed in 1 N HCl at 65°C for 3 min. Two root tips of each bulb were squashed on slides, and then stained with acetocarmine for 15 min [19].

One slide was prepared per bulb for each replication, a total of 2,000 cells, were scored per concentration to study the different types of chromosomal aberrations in the dividing cells. The mitotic index, mitotic inhibition and chromosomal aberration were determined from the scores obtained for the dividing cells based on these formulae:

$$\text{Mitotic Index (MI)} = \frac{\text{Total number of dividing cells in the treatment}}{\text{Total number of cells}} \times 100$$

$$\text{Mitotic inhibition} = \frac{\text{Mitotic Index of control - Mitotic Index of treatment}}{\text{Mitotic Index of control}} \times 100$$

$$\text{Chromosomal Aberration} = \frac{\text{Total number of abnormal cells}}{\text{Total number of cells}} \times 100$$

STATISTICAL ANALYSIS

One-Way Analysis of Variance (ANOVA) with Duncan multiple range tests (P <0.05) were used to compare the differences among the treatments using the SPSS version 13.

RESULTS AND DISCUSSION

EFFECT OF PESTICIDES ON MITOTIC INDEX

Plants are used for determination of environmental pollutants such as pesticides because they are indirect recipients of a cytotoxic. Therefore, they are important materials for cytotoxic test and environmental monitoring of places affected by pollutants. Higher plants such as *A. cepa*, *V. faba* and *T. paludosa* have large monocentric chromosomes in reduced numbers and are accepted as suitable test material for the study of environmental mutagenesis [20, 21, 22, 23].

The cytological effects of the pesticides (mancozeb, dimethoate 40 and lannate 90) on the *Allium cepa* root tip cells are shown in Table (2). The various treatments with pesticides have a marked reducing effect on mitotic index values as compared with the control. The mitotic index values were significantly decreased with an increase in the concentration of the three pesticides. The mitotic index reached a minimum value of 0.86%, 1.34% and 1.43% after treatments with the concentrations 0.005mg/ml of mancozeb, dimethoate and lannate respectively, compared with the control (9.00%). The highest percentage of mitotic inhibition was observed at 0.005mg/ml of mancozeb (90.42%), which reflect their cytotoxicity. Our results are in accord with the previous reports, where mitotic inhibition and cytotoxicity of pesticides were demonstrated [24, 25, 26, 27, 28]. Reduction in mitotic activity may be due to the blocking of mitotic cell cycle during interphase [29], inhibition of DNA synthesis [30,31,32], a block in the G2-phase of the cell cycle [33], inhibition of nuclear-proteins synthesis essential for normal mitotic sequence [34], damage to deoxyribonucleic acid (DNA) at any stage of the cell cycle [35], change in the relative duration of the mitotic stages [36], thus preventing the cell from entering mitosis [37].

Table 2: Effect of pesticides on mitotic index

Treatments	concentration mg/ml	Total number of cells observed	Total number of dividing cells	Normal cells%	Chromosomal aberration%	Mitotic index%	Mitotic inhibition %
Control		2214	180.6	82.67	17.33	9.00	
mancozeb	0.001	2846.3	38.3	17.46	82.54*	1.29*	85.66
	0.0025	2635.3	31	11.81	88.19*	1.194*	86.73
	0.005	2959.3	24.6	8.99	91.01*	0.862*	90.42
dimethoate 40	0.001	2630.6	37	17.04	82.96*	1.40*	84.44
	0.0025	2032.6	28	10.75	89.25*	1.37*	84.77
	0.005	2360.3	31	7.57	92.43*	1.34*	85.11
lannate 90	0.001	2210	67.3	29.55	70.45*	3.04*	66.22
	0.0025	2100	47.6	17.31	82.69*	2.15*	76.11
	0.005	2788.6	40	15.39	84.61*	1.43*	84.11

(*) The mean difference is significant at the 0.05 level.

CHROMOSOMAL ABERRATION

Different concentrations of pesticides (0.001, 0.0025 and 0.005mg/ml) induced various types of chromosomal aberrations during cell cycle. The proportion of chromosomal aberrations was significantly increased from a control value of 17.33% to 91.01%, 92.43% and 84.61% in root treated with the highest concentration (0.005mg/ml) of mancozeb, dimethoate and lannate respectively, Table (2). Chromosomal aberrations are changes in chromosome structure that results from a break or exchange of chromosomal material [38].

Chromosomal abnormalities such as chromatid breaks, bridges and stickiness of chromosomes during cell division, were observed after treatment with different concentrations of mancozeb. Chromosome stickiness, C metaphase, chromosome breaks and micronuclei cells at interphase stage were the most common abnormalities observed after being treated with different concentrations of dimethoate.

In addition, observed stickiness, ring chromosome and c-metaphase at metaphase, early chromatin condensation at prophase, chromosome multipolarity, fragmentation of chromosomes at anaphase and late chromosome at telophase were observed after treatment with different concentrations of lannate (Figure 1).

Stickiness was the most frequent chromosomal aberration observed in the root tips of *Allium cepa* treated with three types of pesticides. Other researchers suggest that sticky chromosomes reflect highly toxic effects and probably lead to cell death [39].

Genotoxicity is one of the serious side effects of pesticide exposure [40] stickiness may be due to breakage and exchange of the basic folded units of chromatids and the stripling of the protein covering of DNA in chromosomes [41]. This stickiness is presumably due to intermingling of chromatin fibers which lead to subchromatid connection between chromosomes [42]. Stickiness can also be explained as physical adhesion of the proteins of the chromosome [43]. While the bridges cause structural chromosome mutations.

Anaphase bridges could happen during the translocation of the unequal chromatid exchange, due to dicentric chromosome presence, or due to the breakage and fusion of chromosomes and chromatids [44, 45]. In addition, bridges may arise due to chromosome breaks, stickiness and breakage and reunion of the broken ends [46].

On the other hand, C-mitosis (colchicine metaphase) is the result of damaged mitotic apparatus due to genotoxic substances in the cells, followed by a random scattering of the chromosomes over the cell [47, 48]. The abnormal spindle formation and the production of this type of abnormalities may be due to the effect of this pesticide on the cyclin-dependant kinases activity [49].

Disturbed spindle resulted in inability of chromosomes to move to the poles, and it is stimulated by many chemicals [50, 51]. Micronuclei often result from the acentric fragments or lagging chromosomes that fail to incorporate into daughter nuclei during telophase of the mitotic cells and can cause cellular death due to the deletion of primary genes [52, 53, 54]. Chromatid breaks indicate a clastogenic effect of the tested pesticides [55].

Chemicals that induce chromosome breakage are known as clastogens and their action on chromosome is generally regarded to involve an action on DNA [56]. Laggards were observed which are due to the failure of the chromosome to move to either of the poles [57].

The result of our study indicated that three pesticides can induce cytotoxic and genotoxic effects on the meristematic cells of *Allium*. It is similar to what have been reported earlier by different studies [58,59, 60, 61].

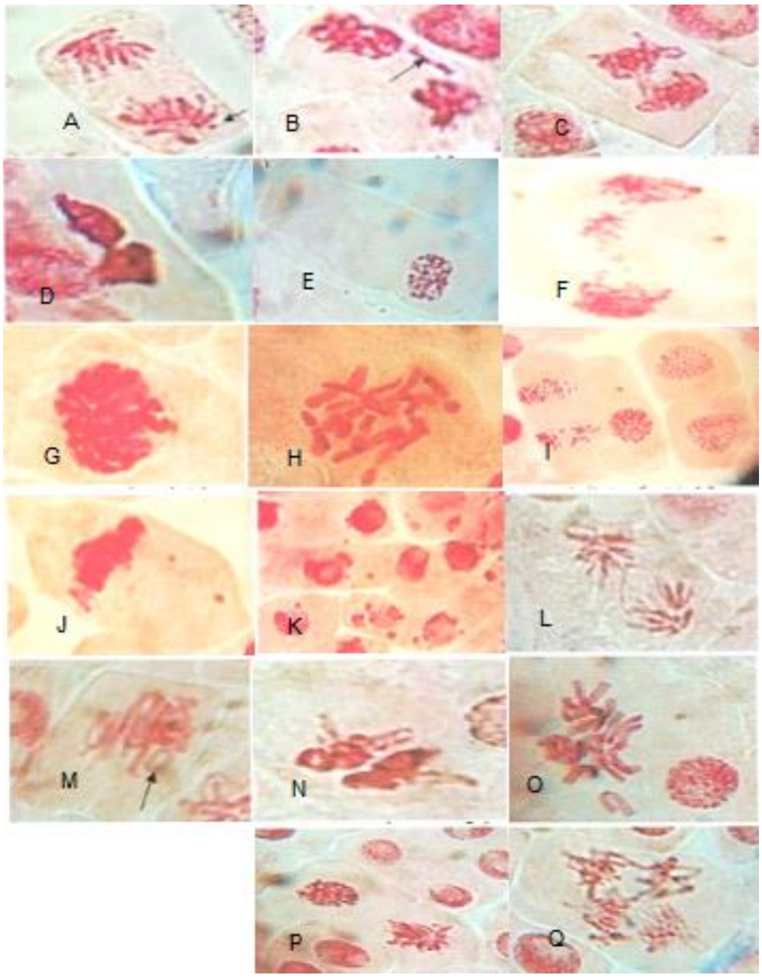

Figure (1): Chromosomal abnormalities caused by three pesticides in root meristem cells of *A. cepa:*
A-E mancozeb; (A) multipolarity and fragment (0.001 mg/ml); (B) fragment(0.0025 mg/ml); (C) bridge(mancozeb 0.005 mg/ml); (D) chromosomes stickiness(0.005 mg/ml); (E) fragmentation(0.005 mg/ml); F-K dimethoate; (F) fragmentation(0.005mg/ml); (G) early chromatin condensation(0.001 mg/ml); (H) C metaphase (0.001 mg/ml); (I) fragmentation(0.005 mg/ml); (J) sticky and fragment(0.0025 mg/ml); (K) micronuclei cells (0.005 mg/ml); L-Q lannate; (L) multipolarity(0.0025 mg/ml) ;(M) ring chromosome(0.005 mg/ml); (N) sticky and chromosome Laggard(0.0025 mg/ml) ; (O) C metaphase(0.005 mg/ml); (P) sticky and binucleate cells (0.005 mg/ml); (Q) multipolarity(0.005 mg/ml).

CONCLUSIONS

The result of this study indicates that the treatment of these three pesticides (mancozeb, dimethoate 40 and lannate 90) decreased the mitotic index and was statistically significant. Also the applications of these pesticides induced chromosomal abnormalities in mitotic cell division, such as stickiness, chromosome bridges on anaphase stage, C metaphase, lagging, fragments and micronuclei. Decrease in the mitotic index and increase in the chromosomal aberration frequency indicates that they had a cytotoxic effect on cells division; chromosomes abnormalities indicate that the three pesticides have a clastogenic property that leads to cytotoxic effects. Hence, the use of these pesticides should be under control in agricultural fields.

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TL6.

GEOCHEMICAL EVALUATION OF GROUNDWATER: A CASE STUDY OF THE SIDI FARAG FARMS, BENGHAZI CITY, NE LIBYA

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Abstract: *In this work, we conducted a geochemical assessment of groundwater in the Sidi Farag farms to determine the possibility of use in drinking and irrigation. The results showed that the water contains high TDS contents (brackish water). The water is characterized by the dominance of Na and Cl (NaCl type) as a result of evaporation, seawater intrusion and weathering of rocks. The water is supersaturated with halite, gypsum, calcite and dolomite. Moreover, the water is seriously affected by Na, Cl, Pb and Hg and therefore is not drinkable. In addition, the water is not suitable for irrigation.*

Keywords: *Drinking Water, Hydrochemistry, Irrigation, Libya, Sidi Farag, Water Quality.*

INTRODUCTION

Assessment of water sources is very necessary due to the increase in the world's population (e.g., Sudhakar and Narsimha, 2013; Flem *et al.*, 2018). Water used for drinking and irrigation purposes may contain contaminants, which may harm human health (e.g., Shaltami *et al.*, 2017). The Al Talhiah reservoir, Sidi Mansour wells and household wells are the main water sources of the Benghazi city. Shaltami *et al.*, (2019) conducted a geochemical evaluation of these sources and the results of their study are as follows:

1) The Al Talhiah reservoir, Sidi Mansour wells and household wells contain fresh water ($CaHCO_3$ type), brackish water (mixed type) and saline water (CaMgCl type), respectively.
2) The water is of hard type.
3) There is a clear supersaturation with calcite, dolomite, gypsum and halite.
4) Contaminants should be removed from the water of the Al Talhiah reservoir and Sidi Mansour wells before use for domestic purposes, while the water of the household wells is not recommended, because it is highly polluted and the treatment process will be expensive.
5) The water can be used for irrigation purposes.

The Sidi Farag village is located in northeastern Libya (Figure 1); it is a suburb of the Benghazi city. This village is basically an agricultural area. Groundwater in the village farms is used for both

drinking and irrigation. The goal of this work is to assess the groundwater quality in the Sidi Farag farms and its suitability for human uses.
In order to achieve this objective, we determined the concentrations of major ions and heavy metals in addition to total dissolved solids (TDS), electrical conductivity (EC) and potential of hydrogen (pH). The main anthropogenic source is sewage water intrusion in the groundwater in some places in the farms. Four wells were selected from the study area. The coordinates of the studied wells are as follows: 32° 1' 61" N 20° 12' 72" E, 32° 1' 71" N 20° 12' 69" E, 32° 1' 17" N 20° 12' 09" E, and 32° 1' 46" N 20° 12' 64" E.

Figure (1): Satellite image showing the location of the Sidi Farag village.

METHODOLOGY

We selected 8 water samples from the studied wells. TDS, EC and pH was identified using Denver Instrument Model 50. Major ions and heavy metals were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES), and inductively coupled plasma-mass spectrometry (ICP-MS). The analytical work was carried out in the National Water Research Center, Ministry of Water Resources and Irrigation of Egypt.

RESULTS AND DISCUSSION

Hydrochemistry

The chemical analysis results of the studied samples are shown in Table 1. The TDS values indicate that the water is of brackish type.
The average of the Cl/Na ratio in the water (1.72, in average) is slightly higher than that in seawater (1.17, Shaltami *et al.*, 2018). Moreover, the plot of alkalinity (Alk) versus pH (Figure 2) shows that the studied samples are hard water.

Table 1: Chemical analysis data (concentrations in mg/l, except for EC in μs/m) of the studied water

Parameters	Sample No.							
	S1	S2	S3	S4	S5	S6	S7	S8
pH	8.12	8.08	8.37	8.44	8.05	8.19	8.22	8.10
EC	1108	1090	2008	1071	2210	1872	1673	1881
TDS	3250	3250	6825	6825	6510	6510	4355	4355
K	199.98	222.00	217.17	310.23	357.16	343.45	157.54	134.17
Ca	633.41	815.32	793.91	991.00	977.87	744.55	622.91	278.72
Na	6009.19	9223.00	17877.14	20337.92	16110.42	13653.00	1822.23	1744.41
Mg	205.09	291.11	228.28	456.33	415.18	390.32	167.44	174.00
Cl	9000.31	14007.00	29090.22	41119.52	28748.18	30191.31	2944.51	2618.93
HCO_3	871.45	727.26	655.09	690.00	689.88	809.54	650.91	648.25
SO_4	2189.71	2087.82	684.21	787.55	1217.00	766.61	800.00	667.08
Fe	0.66	0.50	0.50	0.71	0.52	0.50	0.67	0.72
F	1.10	1.15	0.78	0.89	0.95	1.00	1.00	1.22
B	1.59	1.88	1.71	1.60	1.57	1.29	1.10	1.87
Pb	0.09	0.09	0.10	0.08	0.07	0.06	0.11	0.09
Hg	0.009	0.006	0.005	0.010	0.008	0.005	0.009	0.006
As	0.27	0.31	0.31	0.33	0.20	0.20	0.29	0.20
Cd	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cu	1.76	1.70	0.98	1.09	0.90	1.66	1.51	1.82
Zn	1.93	2.00	2.00	2.55	2.69	1.38	1.72	1.19
Cr	0.01	0.01	0.02	0.04	0.01	0.02	0.03	0.03
Ni	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Br	0.69	0.81	0.77	0.93	0.68	0.62	0.65	0.93

The total hardness (TH) ranges from 1410.2 to 4348.45 mg/l. The TH and Alk were calculated using the following equations:

$$TH\ (mg/l\ CaCO_3) = 2.5\ Ca\ (mg/l) + 4.1\ Mg\ (mg/l)$$
$$Alk = [HCO_3^-] + [OH^-] + 2[CO_3^{2-}]$$

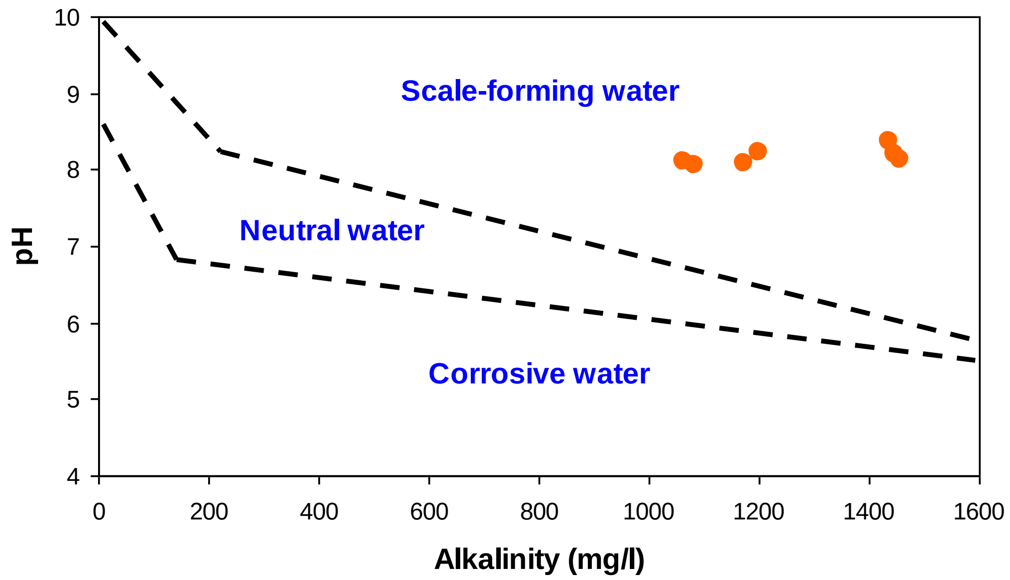

Figure (2): Plot of alkalinity vs. pH showing the hardness of the studied water (Fields after Singh and Hussian, 2016).

In addition, the dominance of evaporation, seawater intrusion and weathering of rocks in the samples are evident in the plots of $Cl/Cl+HCO_3$ versus TDS (Figure 3) and $Na/Na+Cl$ versus $Ca/Ca+SO_4$ (Figure 4).

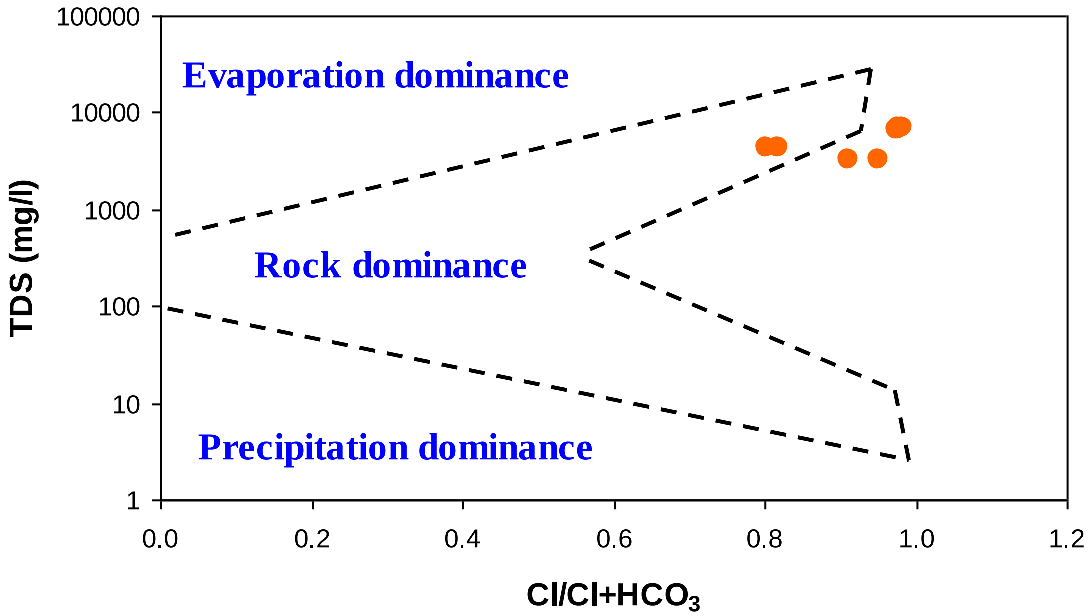

Figure (3): Dominance of rock and evaporation on $Cl/Cl+HCO_3$ vs. TDS of the studied water (Fields after Gibbs, 1970).

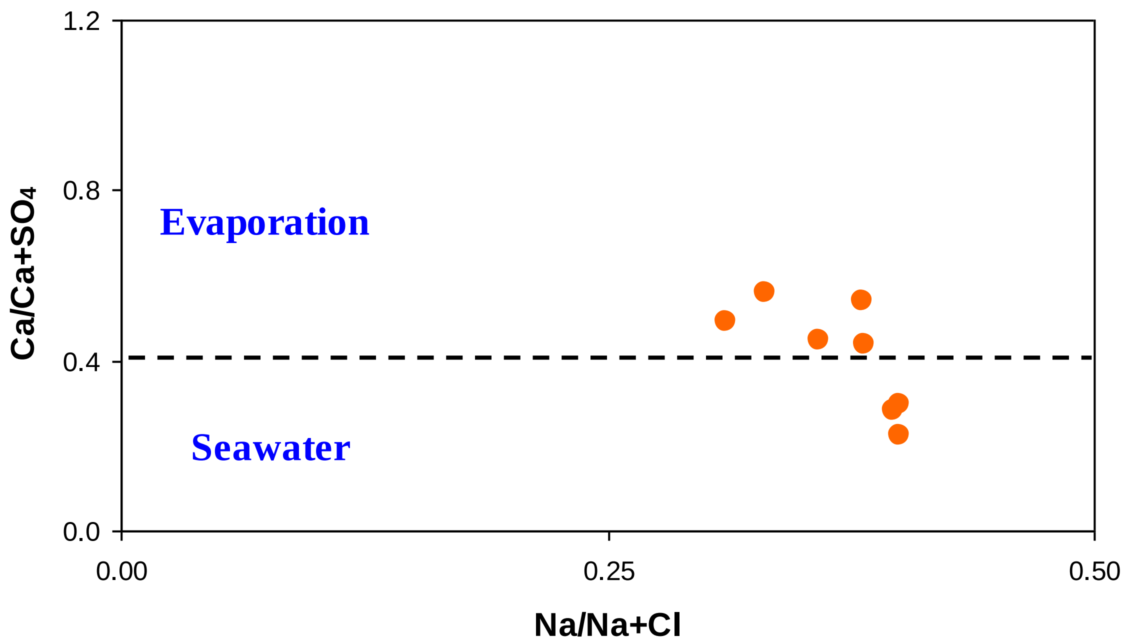

Figure (4): Plot of $Na/Na+Cl$ and $Ca/Ca+SO_4$ showing different origins of the studied water (Fields after Hounslow, 1995).

The hydrofacies of water was determined using the Piper diagram (Figure 5). Clearly, the water samples are of NaCl type. The Schoeller and Stiff diagrams (Figure 6) support this assumption. According Shaltami *et al.*, (2019) the saturation index (SI) can be calculated as:

$$\text{Log SI halite} = \log a_{Na} + \log a_{Cl} + \log \text{Ks halite}$$
$$\text{Log SI gypsum} = \log a_{Ca} + \log a_{SO4} + \log \text{Ks gypsum}$$
$$\text{Log SI calcite} = \log a_{Ca} + \log a_{HCO3} + \log \text{Ks calcite}$$
$$\text{Log SI dolomite} = \log a_{Ca} + \log a_{Mg} + \log a_{HCO3} + \log \text{Ks dolomite}$$

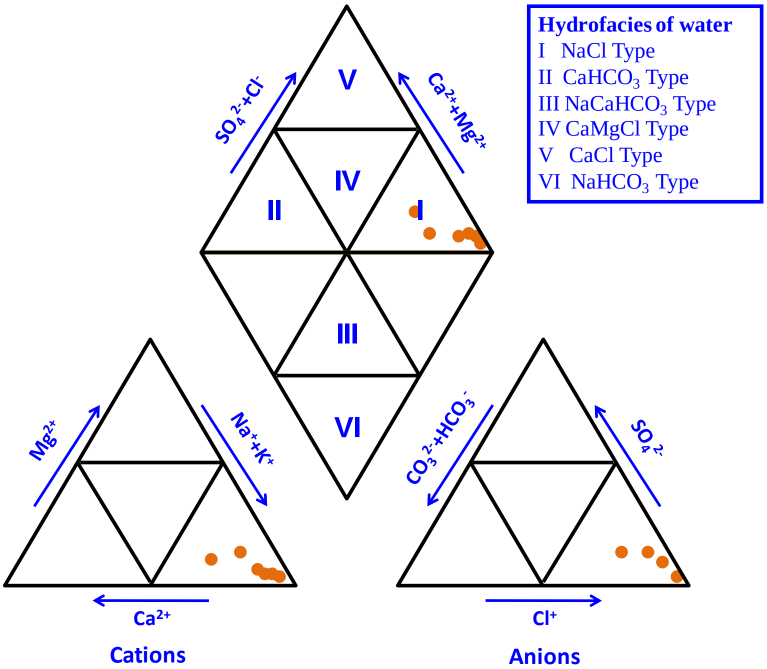

Figure (5): Piper diagram showing the hydrochemical facies of the studied water (Fields after Tweed *et al.*, 2005).

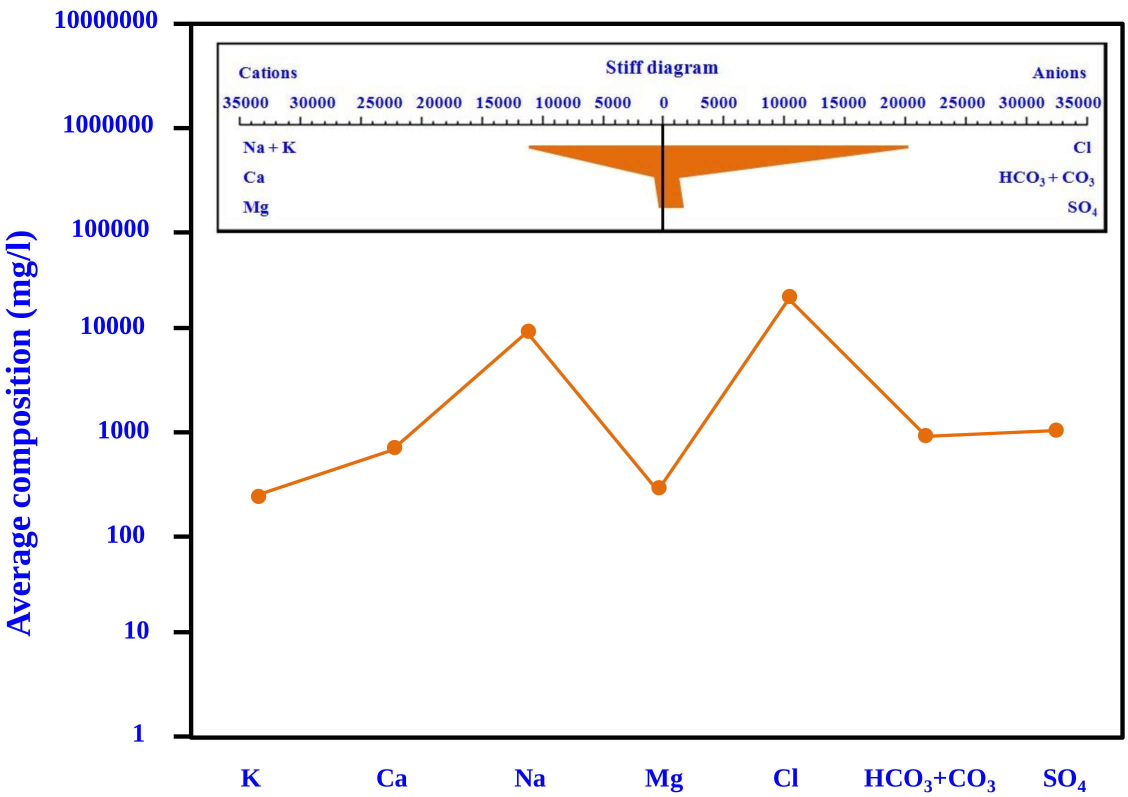

Figure (6): Schoeller and Stiff diagrams showing average composition of the studied water.

The water samples display high Log SI values (Figure 7), suggesting supersaturation with halite, gypsum, calcite and dolomite.

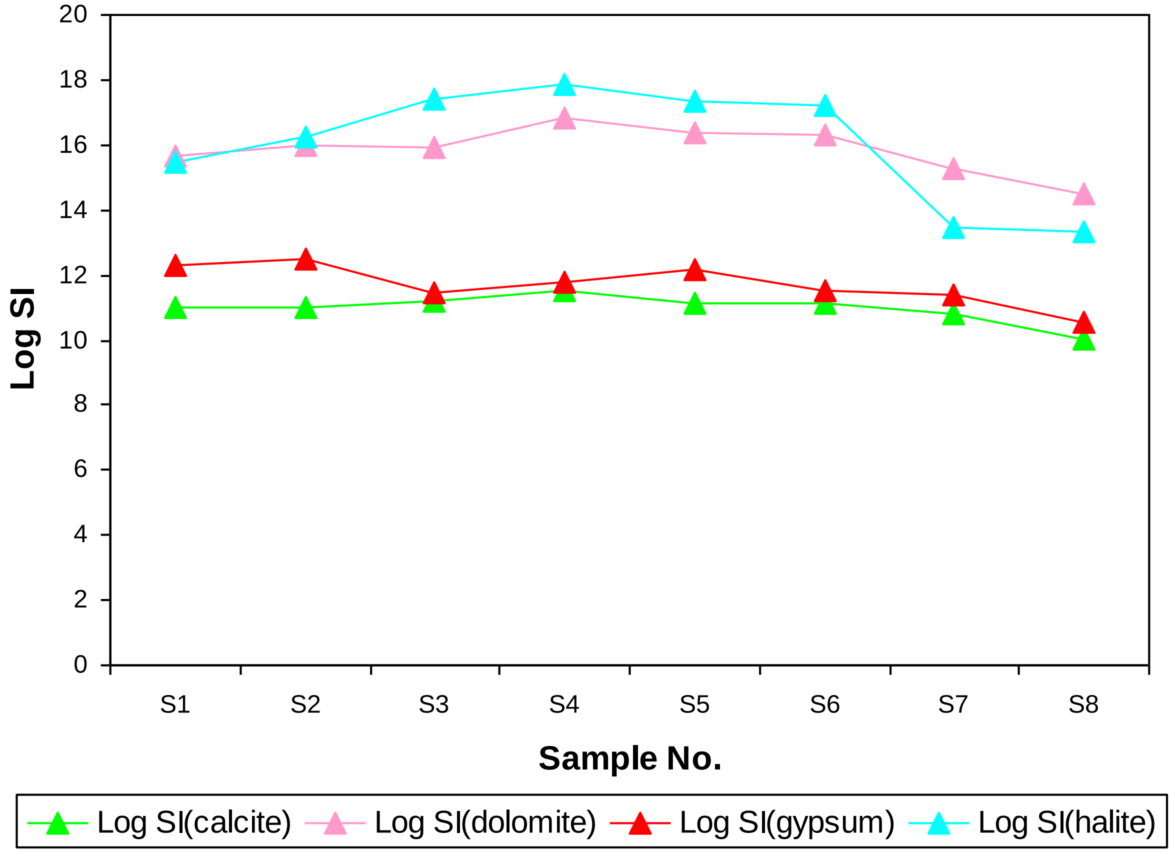

Figure (7): The mineral saturation indices of the studied water.

Drinking Water Quality

The values of major ions and heavy metals (except for F, B, Cu, Zn, Cr and Ni) in the studied water are above the permissible limit of WHO (2018, Table 2). According to Caerio *et al.*, (2005) the metal index (MI) is calculated as: MI = C/MAC. Where, C is the metal concentration (mg/l) and MAC (mg/l) is the permissible limit of WHO (2018).

There are six classes of MI: very pure <0.3 (class I), pure 0.3-1 (class II), slightly affected 1-2 (class III), moderately affected 2-4 (class IV), strongly affected 4-6 (class V) and seriously affected >6 (class VI).

Generally, the water samples are pure with F, B, Cd, Cu, Zn, Cr and Ni, slightly to moderately affected by K, Ca, Mg, HCO_3, SO_4, Fe, As and Br, and strongly to seriously affected by Na, Cl, Pb and Hg.

Table 2: The permissible limits of WHO (2018) for drinking water (concentrations in mg/l)

Contaminant	WHO
pH	8
K	100
Ca	200
Na	200
Mg	150
Cl	250
HCO_3	600
SO_4	600
TDS	500
Alk	200
TH	500
Fe	0.3
F	1.5
B	2
Pb	0.01
Hg	0.001
As	0.1
Cd	0.002
Cu	2
Zn	3
Cr	0.05
Ni	0.02
Br	0.5

Irrigation Water Quality

The plot of EC versus sodium percent (Na %) suggests that the water samples are not suitable for irrigation (Figure 8).

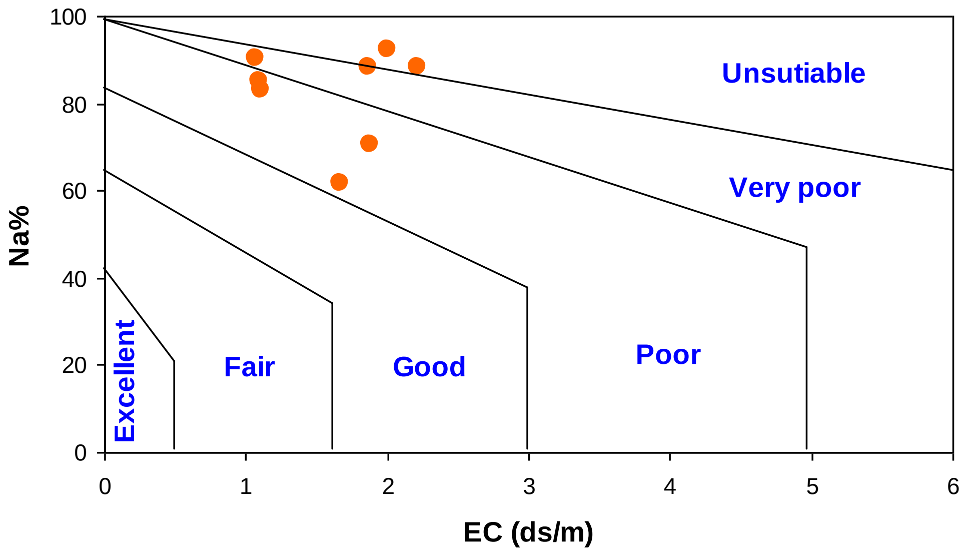

Figure (8): Plot of EC vs. Na% showing the classification of irrigation water (Fields after Johnson and Zhang, 1990).

This assumption is also supported by the irrigation parameters such as pH (8.2, in average), sodium adsorption ratio (SAR = 80.76, in average), magnesium adsorption ratio (MAR = 39.77, in average), lime deposition potential (LDP = 21.71, in average) and Kelley's ratio (KR = 7, in average). Furthermore, the samples display high corrosivity ratios (CR = 36.35, in average) indicating corrosive nature. In addition, the heavy metal concentrations are below the recommended limits of Rowe and Abdel-Magid (1995, Table 3). The irrigation parameters are calculated as:

$$Na\% = (Na*100)/(Ca+Mg+Na+K)$$
$$SAR = Na /\sqrt{(Ca+Mg)/2}$$
$$MAR = [Mg/(Mg+Ca)]100$$
$$KR = Na/(Ca+Mg)$$
$$LDP = (HCO_3^{-} + CO_3^{2-})$$
$$CR = [Cl/35.5 + 2(SO_4/96)] / 2[(HCO_3+CO_3)/100]$$

(All concentrations are expressed in meq/l)

Table 3: The recommended limits of Rowe and Abdel-Magid (1995) for irrigation waters (heavy metals in mg/l)

Contaminant	Long-term use	Short-term use
Fe	5	20
F	1	15
B	1	2
Pb	5	10
As	0.1	2
Cd	0.01	0.05
Cu	0.2	5
Zn	2	10
Cr	0.1	1
Ni	0.2	2

CONCLUSIONS

The conclusions of this work can be summarized in the following points:

- Brackish water is dominant in the Sidi Farag farms.
- The hydrofacies of the water is NaCl type.
- Weathering of rocks, evaporation and seawater intrusion are prevalent in the water.
- There is a clear supersaturation with halite, gypsum, calcite and dolomite.
- There is a significant pollution by Na, Cl, Pb and Hg.
- The water is not appropriate for irrigation.

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TL7.

EFFECTS OF SUGAR BEET FIBER AND INULIN ON PHYSICOCHEMICAL AND SENSORY PROPERTIES AND VIABILITY OF BIFIDOBACTERIUM BIFIDUM IN RED GRAPE JUICE

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Abstract: Background: *Dietary fiber is a prebiotic compound that can increase the viability of probiotic microorganisms in food products during storage and exert healthful effects on the consumers. The purpose of this study was to investigate the effect of different concentrations of inulin and sugar beet fiber on physicochemical and sensory properties and viability of Bifidobacterium bifidum in red grape juice.* ***Methods:*** *0.7, 1, 4 and 2.1% sugar been fiber and 1, 2 and 3% inulin were added to red grape juice inoculated with 10^8 cfu/mL of B. bifidum. pH, Brix, viability of B. bifidum, color parameters (L^*, a^*, b^*) and sensory attributes (taste, flavor, color, mouthfeel, texture, consistency and total acceptance) were measured on days 0, 5, 10, 15, 20 and 25.* ***Results:*** *The results showed that with increasing the amount of inulin and sugar beet fiber, Brix of red grape juice increased significantly and pH value showed a significant decrease. Viability of B .bifidum in all treatments reduced significantly during 25 days of storage, however the number of living bacteria in all treatments containing sugar beet fiber and inulin was above 10^6. Addition of inulin and sugar beet fiber had a significant effect on the changes in the color of red grape juice samples. As the amounts of inulin and sugar beet fiber increased from 1% and 1.4%, respectively, sensory properties tended to decrease significantly. Given the fact that there was no significant difference between the treatment containing 1.4% sugar beet fiber+1% inulin and control sample, this treatment was selected as the superior treatment for health and nutritional advantages.* ***Conclusion:*** *It could be concluded that a synbiotic product with acceptable sensory properties could be produced using inulin up to 1.4%.*

Keywords: *Bifidobacterium bifidum; Fiber; grape juice; Inulin*

INTRODUCTION

Today in many societies the role of food in human health and nutrition is very important. Given the primary role of food as a source of energy and growth and its effect on human health, the tendency of

food product and consumer market towards functional foods has been increased. Functional foods are products which promote health status in addition to providing basic nutrition. Among the functional foods, foods containing probiotic microorganisms are of particular importance.
Since probiotics exert beneficial effects on the intestinal flora balance and general body health, product and consumer market for functional foods are developing. Probiotics are living microorganisms that exert beneficial effect on the health of host when consumed in sufficient amounts. Foods and products containing probiotics are introduced as processed products containing living probiotic microorganisms in sufficient quantities (Bozkurt et al., 2006). The use of compounds which enhance the growth of probiotics stimulates the growth and activity of these bacteria in GI system especially in the intestines. These compounds are commonly carbohydrates and are known as prebiotics. Among these compounds are dietary fibers which increase the viability of probiotics and are used as functional products in food industries. One source of fiber is sugar beet fiber which is obtained from the sugar beet pulp. Sugar beet pulp is a byproduct from the sugar extraction process. Processing 100 tons of sugar beet is resulted in 6 - 10 tons of dry pulp with different sugar contents (sendra et al., 2008). Among the foods that may be a good carrier for the probiotic , the demand for non-dairy probiotic products has increased due to milk lactose intolerance in some people and high cholesterol content of dairy products. Since fruits and vegetables contain beneficial compounds such as minerals, antioxidants, dietary fiber and vitamins and are free of milk allergens they can be a good choice for manufacturing non-dairy probiotic drinks (Mohammadi, 2012). The important difference between probiotic products and other functional foods is that their active or functional compounds are living organisms, i.e. bacteria not chemical compounds (Saarela et al., 2011). The most important potential advantages of probiotic foods include improving and balancing GI microflora, stimulating immune system, anticancer activity, treating lactose intolerance and irritable bowel syndrome (IBS), preventing and treating diarrhea and lowering blood cholesterol. Resistance of probiotic bacteria in foods is a very important challenge faced by researchers because many factors affect cell viability during processing and storage (Roberfroid et al., 2000). Non-dairy probiotic products can be used in cases such as lactose intolerance, milk protein allergy hypercholesterolemia and people's reluctance to include dairy products in their diet (Champagne et al., 2008). Additionally, juices are rich in nutrients and contain no starters which prevent probiotics from competing with starters to obtain the nutrients. Dairy products containing *B. bifidum* or *Lactobacillus* species and sugars fortified with fructooligosaccharides or inulin have been on the market over 10 years. The viability of *Bifidobacterium* and *Lactobacillus* species in orange, apple, pineapple, green apple, blueberry, lemon, peach, strawberry, mango, pear, grape, kiwifruit, pomegranate, etc. juice has been studied (Cruz et al., 2006). Grains and fruits wastes is an important source of dietary fiber which can be used in manufacturing food products as energy-efficient and cost-effective fillers with technological applications in functional food industry. Interactive effects of fibers and food components during production and storage can change the amount of the nutrients bioavailability as well as ultimate flavor and texture.
According to the American association of cereal chemistry the edible part of plant or similar carbohydrates which are resistant to digestion and absorption in the small intestine and are fully or partially fermented in the large intestine are called dietary fiber. Daily intake of 38 g and 25 g of dietary fiber is recommended for men and women, respectively (Davidson, 1998). A direct link between consumption of high-fiber diets and reduced risk of some chronic diseases such as colon cancer, constipation, obesity, diabetes and cardiovascular diseases has been demonstrated.
Today there are various methods for enhancing the viability of probiotics in foods and gastrointestinal system however the most realistic method seems to be the study of fermentation characteristics and stimulation of probiotics in the intestine via increasing their metabolic capacity which is in the fact the notion of probiotics (Gibson and Roberfroid., 1995). Dietary fibers can be selectively metabolized by the intestinal flora and alter the microbial population to increase the beneficial bacterial.
Sendra et al. claimed that the addition of citrus fiber to fermented milk enriched with probiotics increased their viability and growth likely due to rapid conversion of lactose into lactic acid (Sendra et al., 2008). In the present study, sugar beet fiber and inulin were used to produce functional grape juice and enhance the viability of *Bifidobacterium bifidum* and the physicochemical and sensory properties as well as the viability of *B. bifidum* over 25-day storage were investigated.

MATERIALS AND METHODS

In order to produce probiotic grape juice containing sugar beet fiber, grape juice concentrate was purchased from Sunich Company, Iran. *Bifidobacterium bifidum* (ATCC 29521) in the form of lyophilized ampules was purchased from the collection of Iranian research organization for science and technology (IROST) and sugar beet fiber was purchased from Hegmatan sugar company, Iran. Short-chain inulin with 8 degree of sterilization and 99.5% purity was purchased from Roosendaal Company, Netherlands. All chemicals were obtained from Merck, Germany.

Preparation of samples

Preparation of sugar beet fiber

Sugar beet fiber was prepared from sugar beet pulp (residual sugar beet after sugar extraction). The pulps were prepared and the frozen for later use. After defrosting, the pulp was cleaned manually. Then the cleaned fibers were homogenized in a mixer with 69% ethyl alcohol to thoroughly bleach the filtered extract. They were placed in oven at 50℃ for 12 h until their moisture reached 9-11%. The dried pulp was ground and sieved with mesh 250-300 mm (Özboy and Köksel, 2000).

Preparation of *B.bifidum* suspension

To activate the bacteria, the vial containing the bacteria was transferred to Broth MRS medium. Then it was incubated at 37℃ for 24 h under anaerobic conditions. Next the bacterial suspension was centrifuged and the resulting precipitate was dissolved in 0.1% peptone water. The bacterial density (1.5×10^8 CFU/mL) was determined using MCFarland method by spectrophotometer (in fact, the light absorbance in the bacterial suspension at 625 nm should be 0.08-0.13) (Moussavi et al., 2011).

Preparation of synbiotic drink

To prepare the functional red grape juice, 1, 2 and 3% inulin and 0.7, 1.4 and 2.1% sugar beet fiber were added to red grape juice (Table 1) and they were homogenized by a laboratory homogenizer (Fan Azma, Iran) at 20 Mpa. The drinks then were inoculated with 108CFU/mL pure lyophilized culture of *B.bifidum* and kept at 4℃. Physicochemical and sensory properties, colorimetry parameters and viability of *B.bifidum* were measured on days 0, 5, 10, 15, 20 and 25.

Table 1: Coding of research treatments

Row	Treatment's code	Inulin (%w/w)	*Bifidobacterium bifidum (cfu/m)*	Sugar Beet Fibre (%)
1	T1	1	10^8	0.7
2	T2	1	10^8	1.4
3	T3	1	10^8	2.1
4	T4	2	10^8	0.7
5	T5	2	10^8	1.4
6	T6	2	10^8	2.1
7	T7	3	10^8	0.7
8	T8	3	10^8	1.4
9	T9	3	10^8	2.1
10	T10	-	10^8	-

Tests

Brix and pH

Brix and pH of the functional grape juice were measured according to Iranian national standard No. 3414 (2019).

Viability of *B. bifidum*

To prepare the first dilution, 1 mL of the homogenized grape juice sample was added to 9 mL of sterile 1.0% peptone water. Serial dilutions were prepared by adding 1 mL of each dilution to 9 mL of sterile 1.0% peptone water. The samples then were cultured on RCA agar by pour plate method in anaerobic jar with gas pack A at 37°C for 72 h. The colony count then was performed (Krasaekoopt et al., 2003).

Color evaluation

Color was evaluated by colorimeter (Model D25-DP9000) with standard black and white plates for reference and the white sample of height 1.5 m was set to standard plates, X=81.2m Y=83.32 and Z=98.03. CIE system was used: L^*(lightness) blackness (0)/whiteness (100); a^*(greenness/redness); b^*(blueness/yellowness) (120–+120) (Vmesha et al., 2013).

Sensory evaluation

Sensory properties of juice sample including aroma, flavor, color mouthfeel, texture, consistency and total acceptance were measured by 25 semi-trained panelists in Karaj agricultural engineering research institute on 5-point hedonic scale (Krasaekoopt et al., 2010).

RESULTS AND DISCUSSION

Brix measurement

The results of measuring Brix of probiotic grape juice containing different concentrations of inulin and sugar beet fiber during 25 days of storage are given in Figure (1). The results showed that with increasing inulin and sugar beet fiber content, Brix of samples increased significantly. The highest and the lowest brix values were found for the samples containing 3% inulin + 2.1% sugar beet fiber (T9) and control sample (T10), respectively on day 25. The increase could be attributed to increased starch, pectin and fiber compounds (Roller et al., 2004). Brix of all treatments decreased significantly ($P<0.05$) on day 25 with the decrease being more pronounced for control and samples containing lower amounts of inulin and sugar beet fiber. Due to the presence of inulin and fiber in the samples, sugars are metabolized by probiotic bacteria at a low rate so Brix decreases moderately (Gibson et al., 1995). Mesbahi et al. (2009) studied the addition of tomato skin and seeds to ketchup to improve its nutritional value and sensory properties and found that the addition of tomato pomace is significantly increased Brix of ketchup which is in agreement with the results of the present study. Bahmani et al. (2021) reported that growth and activity of *L. acidophilus* during storage lead to the consumption of bacterial substrates and reduce brix. They inoculated pineapple juice with *L. casei* and reported that Brix of all samples reduced with time which is consistent with our results.

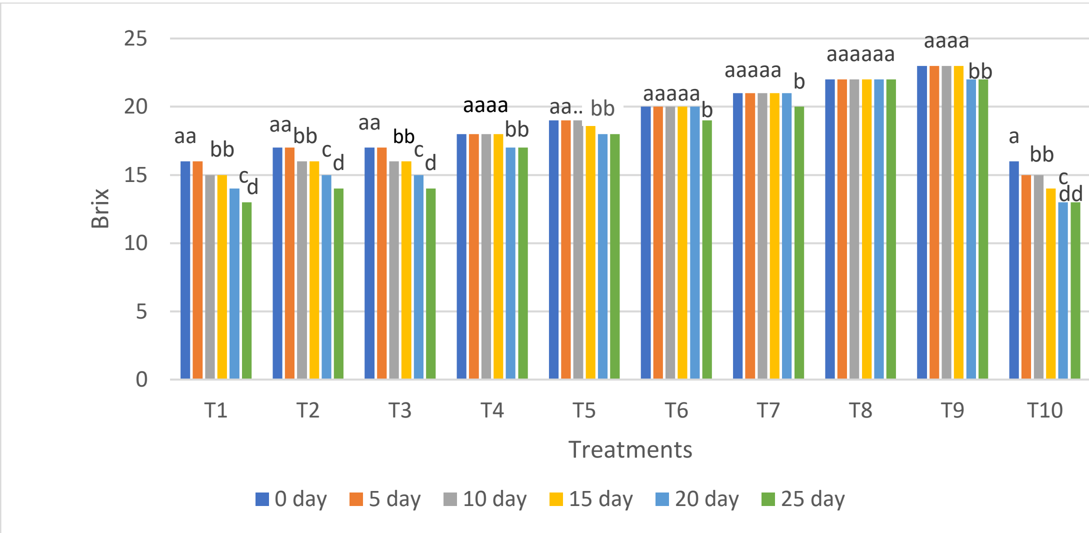

Figure (1): Brix of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25-day storage

pH measurement

The results of measuring pH value of probiotic grape juice containing different concentrations of inulin and sugar beet fiber during 25 days of storage are given in Figure (2). The results showed that with increasing inulin and sugar beet fiber content, pH value of the samples decreased significantly. The highest (2.38) and the lowest (1.72) pH values were observed for control sample (T10) and the samples containing 3% inulin + 2.1% sugar beet fiber, respectively on day 25. pH value of all treatments decreased significantly ($p<0.05$) over time with the decrease being more extreme for the samples containing inulin and sugar beet fiber. Inulin provides a sugar substrate for probiotic bacteria and fermentation and organic acids production result in acidification of the environment and decrease in pH of juice (Yoon et al 2004). Nadali et al (2014) reported that pH value of probiotic set yogurts containing red grape concentrate decreased over storage (Nadali et al., 2004).

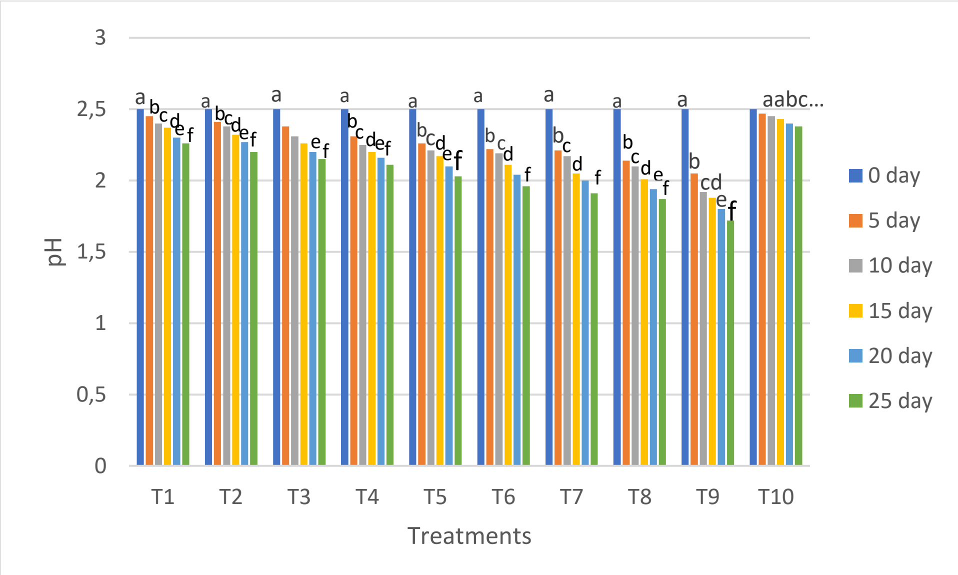

Figure (2): pH value of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25-day storage

Viability of *B. bifidum*

One of the main indicators of synbiotic juices production is the viability of probiotic bacteria. The results of measuring the viability of *B. bifidum* in probiotic grape juice samples containing inulin and sugar been fiber during 25 days of storage are presented in Table 2. The results showed that the number of *B. bifidum* in all treatments decreased significantly. This decrease in the samples containing higher concentrations of inulin and sugar beet fiber occurred moderately as the highest (7.05^{10} g cfu/ ML) and the lowest (5.03^{10} g/cfu/ ML) viability was found for the treatment containing 3% inulin+2.1% sugar beet fiber and control sample, respectively on days 25. It is worthy to note that the number of probiotic bacteria in all treatments containing inulin and sugar beet fiber was higher than 10^6 cfu/ML while it decreased to less than 10^6 cfu/ML in control sample likely due to the decrease in nutrients content.

Inulin and sugar beet fiber provide energy and organic acids thereby enhancing growth and viability of probiotic bacteria over storage (Bahmani et al., 2021). Given the fact that minimum number of bacteria for a probiotic product is 106 cfu/ML, the conditions needed for bacteria to survive in food products must be provided (Akin et al., 2007). Gazavi et al. (2018) reported that the number of probiotic *L. acidophilus* in pomegranate juice dropped to zero in less than two weeks. They stated that the ability of bacteria to grow and survive largely depends on their capability of adapting to the environment and the probiotic content in the food.

Ghorbani et al. (2019) showed that the viability of all probiotic bacteria in pomegranate (P>0.05) juice samples decreased significantly over storage which is in agreement with the results of the present study.
Tirpathi and Giri (2014) reported that the factors effecting the survival and activity of bacteria in probiotic juices included food parameters such as pH value, acidity, oxygen, water activity, salt and sugar, chemical and synthetic flavorings and colorings and processing parameters such as pasteurization, rate of cooling, packaging, storage methods, level and volume of oxygen and microbiological factors such as bacterial strains and their incubation.
Chen et al. (2005) studied prebiotics such as fructooligosaccharides and iso-maltooligosaccharides, a peptide growth enhancer and sodium alginate as coatings for microencapsulation of prebiotics such as *L. acidophilus* and *B. bifidum* and found that these compounds significantly increased the bacteria viability. In consistence with their results, Mahdian et al. (2013) investigated the effect of sugar been fiber on the viability of *L. acidophilus* in probiotic yogurt and reported that the viability increased significantly as the sugar beet content increased.

Table 2: Evaluation of viability of Bifidobacterium bifidum (logcfu/ml) in grape juice samples containing different concentrations of inulin and sugar beet fiber and control sample during 25 days of storage

Treatments	Storage time (days)					
	0	5	10	15	20	25
T1	8.00±0.00^{aA}	7.51±0.05 ^{dB}	6.89±0.01 ^{dC}	6.49±0.15 ^{gD}	6.19±0.09 ^{dE}	6.06±0.08 ^{gF}
T2	8.00±0.00 ^{aA}	7.63±0.08 ^{cB}	7.08±0.09 ^{cC}	6.58±0.17 ^{fD}	6.32±0.14 ^{cE}	6.14±0.10 ^{gF}
T3	8.00±0.00 ^{aA}	7.68±0.11 ^{cB}	7.18±0.08 ^{cC}	6.71±0.15 ^{eD}	6.47±0.05 ^{cE}	6.27±0.04 ^{fF}
T4	8.00±0.00 ^{aA}	7.64±0.06 ^{cB}	7.26±0.03 ^{cC}	6.89±0.16 ^{dD}	6.56±0.21^{bE}	6.33±0.10 ^{eF}
T5	8.00±0.00 ^{aA}	7.70±0.17 ^{abB}	7.31±0.09 ^{bC}	6.95±0.12 ^{dD}	6.65±0.15 ^{bE}	6.45±0.07 ^{dF}
T6	8.00±0.00 ^{aA}	7.75±0.13 ^{abB}	7. 36±0.18 ^{bC}	7.01±0.06^{dD}	6.73±0.20 ^{bE}	6.62±0.03 ^{cF}
T7	8.00±0.00 ^{aA}	7.77±0.09 ^{abB}	7.39±0.17 ^{bC}	7.17±0.09 ^{cD}	6.81±0.15 ^{bE}	6.70±0.11 ^{bF}
T8	8.00±0.00 ^{aA}	7.89±0.16 ^{aB}	7.47±0.12 ^{bC}	7.31±0.06 ^{bD}	6.94±0.14 ^{bE}	6.81±0.10 ^{bF}
T9	8.00±0.00 ^{aA}	7.91±0.14 ^{aB}	7.77±0.08 ^{aC}	7.55±0.04 ^{aD}	7.15±0.13 ^{aE}	7.05±0.04 ^{aF}
T10	8.00±0.00 ^{aA}	7.18±0.21 ^{eB}	6.56±0.06 ^{eC}	6.16±0.12 ^{hD}	5.54±0.16 ^{eE}	5.03±0.05 ^{hF}

* The difference small letters show a significant difference (P≤0.05) in each column.
* The difference capital letters show a significant difference (P≤0.05) in each row.

Color measurement
Lightness (L^*) measurement

The results of measuring lighthess (L^*) of probiotic grape juice containing different concentrations of inulin and sugar beet fiber during 25 days of storage are presented in Diagram 3. The results showed that L^* index for all treatments decreased significantly over time. As the amount of inulin and sugar beet fiber increased, L^* value decreased significantly (P≤0.05). The highest L^* value was found for control sample (T 10) followed by T1 containing 1% inulin and 0.7% sugar beet fiber. The lightness of treatments containing inulin and sugar beet fiber decreased likely due to the accumulation and density of fiber particles in the product which prevented light from passing resulting in a decrease in lightness. Majzoobi et al. (2010) studied the effect of using sugar beet pulp on the quality properties of Barbari bread and reported that bread containing sugar beet pulp showed a significant decreased in lighthess. Shoorideh et al. (2009) used inulin as a substitute for sucrose in the formulation of dark chocolate and reported that the treatments containing higher inulin content showed lower lightness value. They stated that increasing inulin resulted in an increase in water absorption and a decrease in light scattering thereby decreasing the lightness. Rodrigues et al (2000) replaced fat by inulin in the formulation of ground meat and reported that using inulin at concentrations of above 2% reduced the lightness significantly being in agreement with the results obtained in the present study. Mantzouridou et al. (2012) used inulin-based dessert as a carrier of probiotic bacteria and reported a significant decrease in lightness caused by inulin.

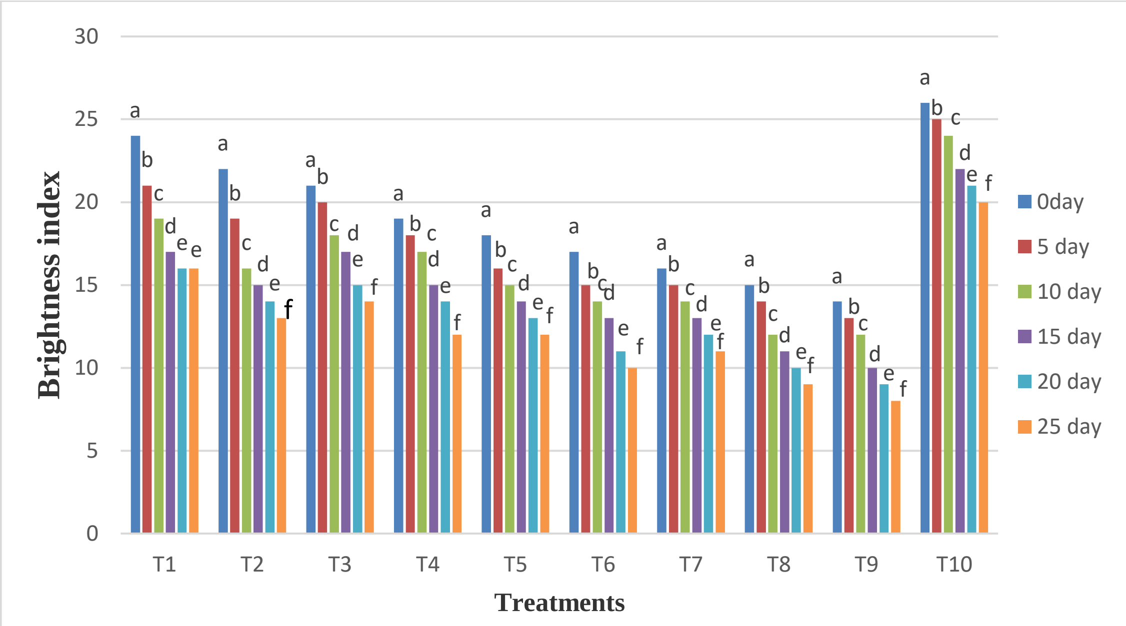

Figure (3): Measurement of lightness (L^*) of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage

Redness (a^*) measurement

The results of redness (a^*) index of probiotic grape juice containing different concentrations of inulin and sugar beet fiber during 25 days of storage are given in Figure (4). The results showed that storage time had no significant effect on changes in redness (a^*) of the treatments. As the amount of sugar beet fiber increased redness (a^*) value increased significantly ($p \leq 0.05$) likely due to the presence of red pigments in the fiber. Majzoobi et al. (2010) investigated the effect of sugar beet pulp on the quality properties of Barbari bread and reported that redness (a^*) of the bread increased as the sugar beet fiber content increased which is consistent with the result of the present study. Saadatmandi et al. (2011) enriched tortilla chips with sugar beet fiber and reported that using the fiber increased redness (a^*) of tortilla chips significantly. Increased inulin content in grape juice samples did not have a significant effect the changes in redness (a^*) index. The lowest and the highest redness (a^*) values were observed for control sample and the treatment containing 2.1% sugar beet fiber (T3, T6, T9).

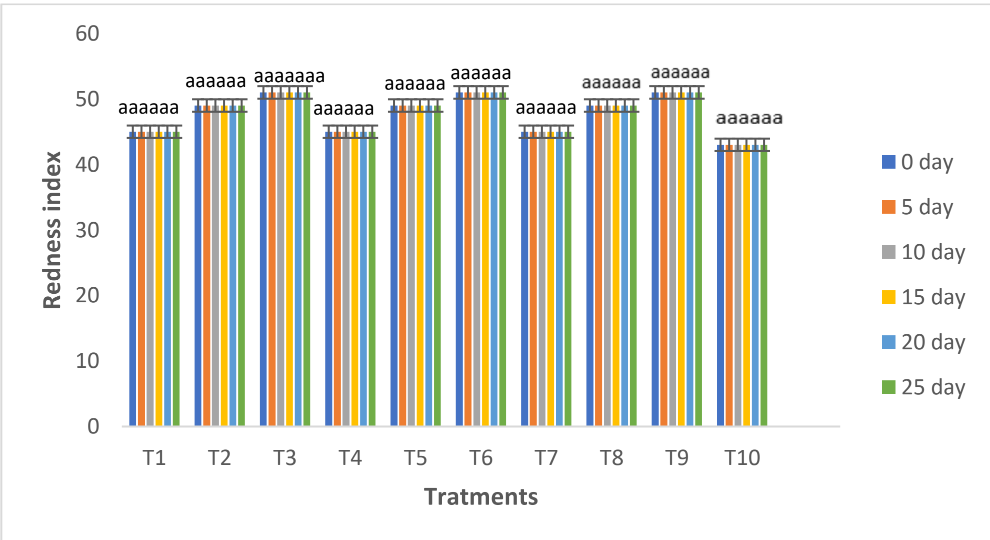

Figure (4): Measurement of redness (a*) of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage

Yellowness (b*) measurement

The results of measuring yellowness (a*) of probiotic grape juice containing different concentrations of inulin and sugar beet fiber during 25 days of storage are presented in Diagram 5. The results showed that storage time did not have a significant effect on the changes in yellowness (b*) of the treatments. Yellowness (b*) value showed a significant ($p \leq 0.05$) increase as the inulin content in the grape juice samples increased. The reason could be the presence of starch granules and long chains of inulin which affect the light transmission. Afshinpajooh et al. (2011/0 studied the effect of inulin on colour parameters of pasta dough and reported that the use of high concentration of inulin resulted in a significant increase in yellowness (b*) of the samples which is consistent with the results obtained in this study. Addition of sugar beet fiber or increasing its concentration in grape juice samples had no significant effect on the changes in yellowness (b*). The lowest and the highest yellowness (b*) values were found for control sample and the treatments containing 3% inulin (T7, T8, T9).

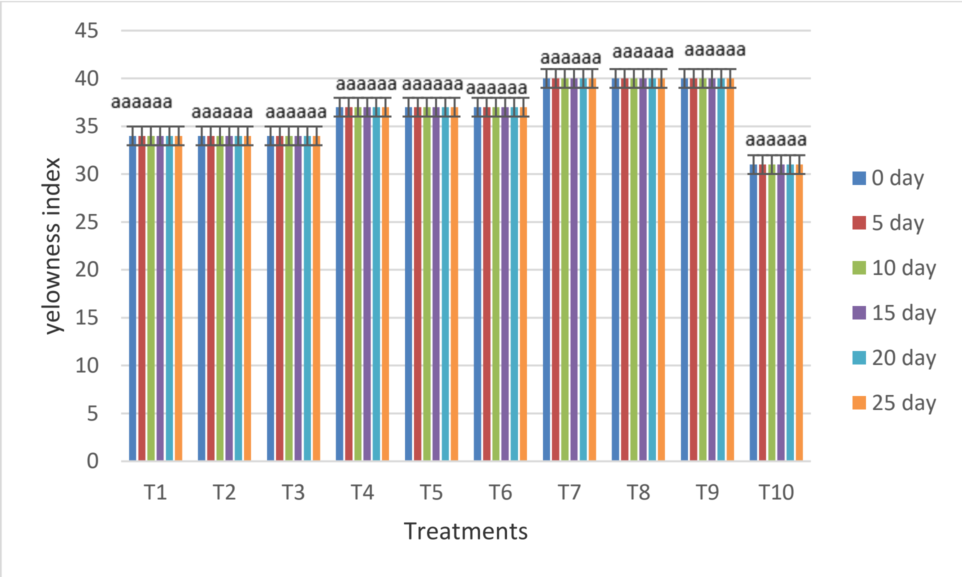

Figure (5):-Measurement of yellowness (b*) of grape juice samples containing different concentrations if inulin + sugar beet fiber and control sample during 25 days of storage

Sensory evaluation
Flavor measurement

Scores given to the flavor and taste of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage are shown in Figure (6). The results revealed that with increasing storage time, the flavor and taste scores decreased significantly. As the inulin content increased above 1%, the flavor and taste scores decreased significantly. The highest flavor and taste scores were found for control treatment and the treatments containing 1% inulin showing no significant difference from each other. Increasing the inulin content above 1% reduced the flavor and taste scores significantly. One reason could be the development of oily feeling which is desirable only in dairy products and ice cream.
Shoorideh et al (2010) investigated the effect of inulin as a substitute for scores in the formulation of dark chocolate and reported that the use of high concentrations of inulin decreased the sensory scores significantly due to the greasy feeling developed in the samples. They reported that inulin had lower solubility than simpler sugars such as sucrose so it developed an undesirable stickiness in the mouth.
Damanafshan et al. (2012) evaluated the sensory properties of low-fat cake containing inulin and reported lower flavor and taste scores for the sample containing inulin as compared to control sample. The reason was the greasy mouthfeel which was developed by inulin.

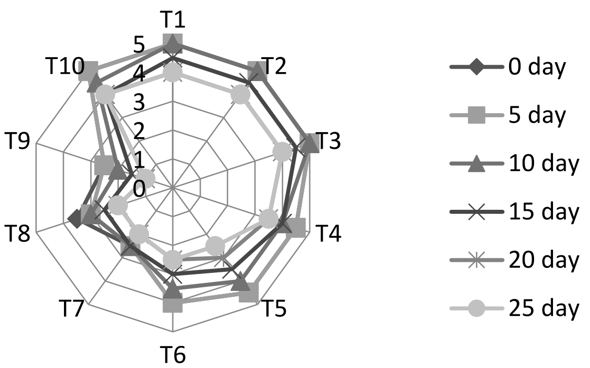

Figure (6):-Measurement of flavor and taste of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage

Aroma measurement

Scores given to the aroma of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage are given in Diagram 7. The results showed that with increasing storage, the aroma score decreased significantly. As the concentration of inulin increased above 1% and of sugar beet fiber increased above 0.7% the aroma score decreased significantly. The reason could be the accumulation of pectins, fermentations and production of undesirable gases by *B. bifidum* in the grape juice samples over storage. Majozoobi et al. (2009) also reported that the use of high concentration of sugar beet pulp fiber in Barbari bread had an adverse effect on the aroma of bread and the bakery yeast activity. Damanafshan et al. (2011) incorporated inulin into the formulation of cake and reported that the addition of inulin to cake significantly decreased the aroma of the samples.

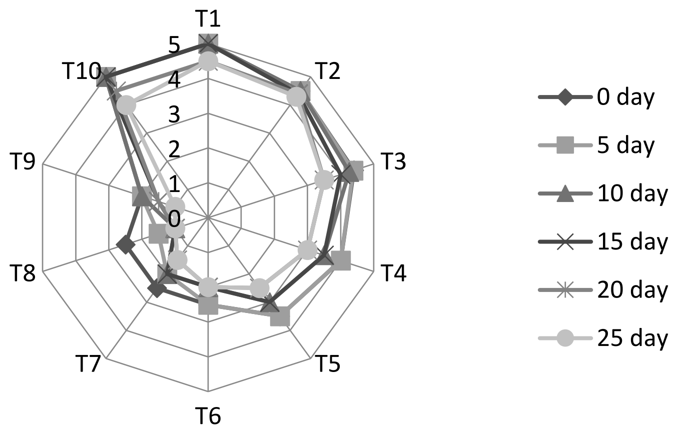

Figure (7):-Measurement of aroma of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage

Apparent colour measurement

Scores given to the apparent colour of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage are displayed in Figure (8). The results showed that with increasing storage time the apparent colour score for all treatments decreased significantly. With increasing the concentration of inulin above 1% and sugar beet fiber above 1.4%, the apparent colour score showed a significant decrease.

The highest apparent colour score was observed for control sample and the sample containing 1% inulin + 0.7% sugar beet fiber showing no significant difference from each other. Increasing the inulin content above 1% and sugar beet fiber content above 1.4% are resulted in significantly lower apparent colour score likely due to the presence of colour components of inulin and sugar beet fiber. Majzoobi et al. (2010) also reported that the use of high amounts of sugar beet fiber pulp fiber in Barbari bread negatively affected the Millard reaction and the colour of bread.

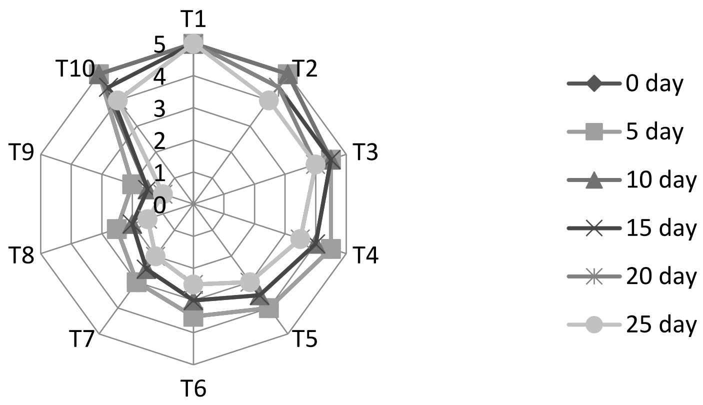

Figure (8): Measurement of apparent colour of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage

Mouthfeel measurement

Scores given to the mouthfeel of grape juice samples containing different concentrations of inulin + sugar beet fiber are shown in Figure (9). The results revealed that with increasing the inulin content above 1% and sugar beet fiber above 104%, mouthfeel score decreased significantly. As the storage time increased, the mouthfeel score for all treatments reduced significantly with this decreasing trend being more moderate for the samples containing 1% inulin+0.7% sugar beet fiber. The reason for the decrease in the mouthfeel score could be the metabolization of sugar and starch by *B. bifidum* which produced undesirable compounds. The highest mouthfeel score was found for control sample and the sample containing 1% inulin + sugar beet fiber showing no significant difference from each other. Increasing the concentration of inulin above 1% and of sugar beet fiber above 1.4% significantly decreased the mouthfeel score likely due to the greasy mouthfeel score caused by high concentration of inulin. Homayounirad et al. (2012) investigated the effect of inulin and stevia on the physical properties of milk chocolate and found that inulin increased mouthfeel score significantly. Saadatmandi et al. (2011) used sugar beet fiber in the formulation of tortilla chips and reported that using sugar beet fiber at concentration of above 1% significantly decreased mouthfeel score.

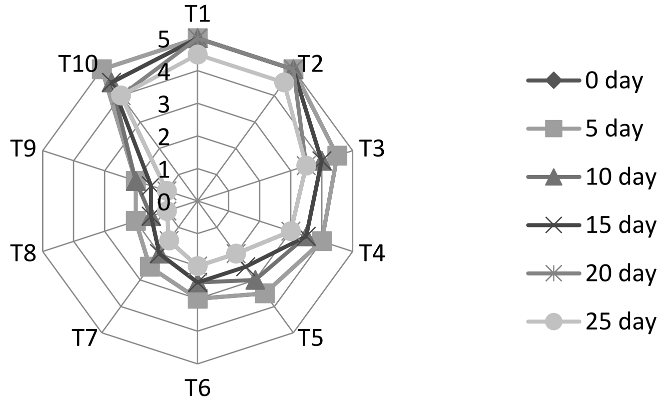

Figure (9): Measurements of mouthfeel of grape juice samples containing different concentration of inulin + sugar beet fiber and control sample during 25 days of storage

Texture and consistency measurement

Scores given to the texture and consistency of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage are depicted in Diagram 10. The results showed that with increasing inulin content above 10.4 %, the texture and consistency score for all treatments decreased significantly however the decreasing trend was more moderate for the treatments containing 1% inulin and 0.7 and 1.4% sugar beet fiber. The highest texture and consistency score was observed for control treatment and the treatment containing 1% inulin + 0.7 and 1.4% sugar beet fiber showing no significant difference from each other. Increasing the concentration of inulin above 1% and of sugar beet fiber above 1.4% significantly decreased the texture and consistency score. Texture and consistency are greatly affected by the food components.

Since inulin increased the viscosity and developed an oily feeling, its increased concentration had an undesirable effect on the consistency of grape juice. Homayounirad and hojat Ansari (2014) also reported that using 1.5% and 2% inulin in the formulation of apple juice decreased the consistency score significantly being in agreement with the results obtained in the present study.

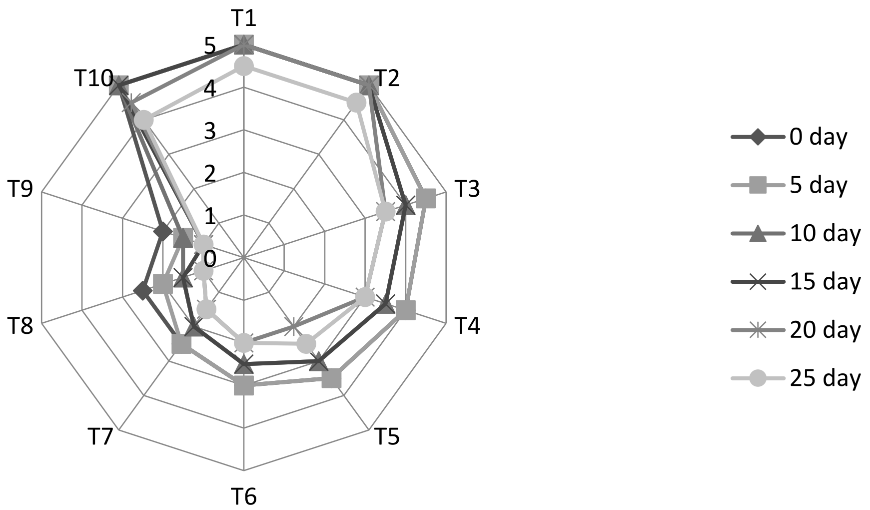

Figure1(0): Measurements of texture and consistency of grape juice samples containing different concentration of inulin sugar beet fiber and control sample during 25 days of storage

Total acceptance measurement

Total acceptance or ultimate desirability includes a set of all evaluation criteria. Scores given to the total acceptance of grape juice samples containing different concentrations of inulin + sugar beet fiber and control sample during 25 days of storage are presented in Figure (11). The results showed that with increasing the concentration of inulin above 1% and sugar beet fiber above 1.4% the total acceptance score for all treatments decreased significantly however this decreasing trend was more moderate for the treatment containing 1% inulin and 0.7 and 1.4% sugar beet fiber.

The highest total acceptance score on day 1 was found for control sample and the treatment containing 1% inulin and 0.7% and 1.4% sugar beet fiber showing no significant difference from each other. Increasing the inulin content above 1% and sugar beet fiber above 1.4% resulted in a significant increase in the total acceptance score.

Homayounirad and Hojat Ansari (2014) studied the sensory properties of clear apple juice containing inulin, fructooligosaccharides and polydextrose during 1-mounth storage at ambient temperature and reported that the samples containing inulin had higher acceptability and the acceptability of samples containing polydextrose was lower.

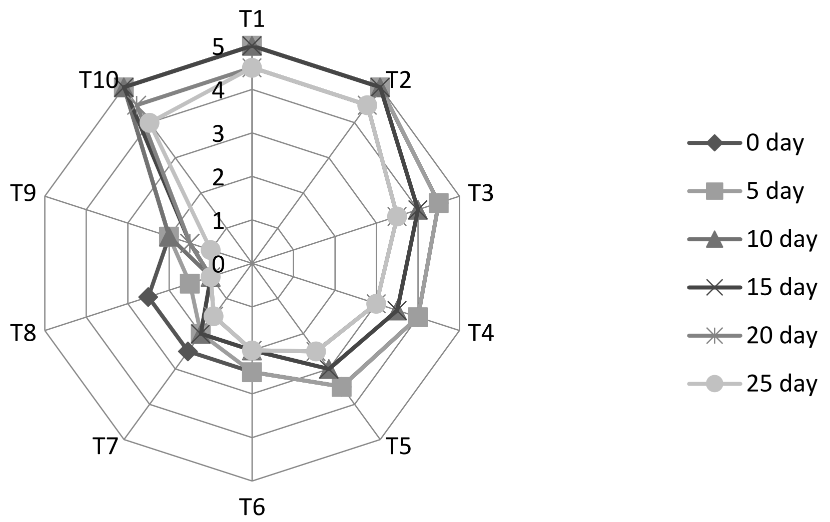

Figure (11): Measurements of total acceptance of grape juice samples containing different concentrations of inulin sugar+ sugar beet fiber and control sample during 25 days of storage

CONCLUSION

This study was aimed at producing a functional red grape juice containing inulin and sugar beet fiber. To do so, inulin at concentrations of 1, 2 and 3% and sugar beet fiber in amounts of 0.7, 1.4 and 2.1% were added to res grape juice inoculated with *Bifidobacterium bifidum*. Physicochemical and sensory properties, colour, and viability of *B. bifidum* on days 0.5, 10, 15, 20 and 25 were evaluated. The results showed that as the amounts of inulin and sugar beet fiber increased the Brix and pH values of the red grape juice samples increased and decreased, respectively. The incorporation of inulin and sugar beet fiber into the formulation of the red grape drink had a significant effect on the viability of probiotic bacteria during storage as the number of living bacteria in all treatments containing inulin and sugar beet fiber at the end of 25-day storage was above 10^6. Using concentrations of inulin above 1% and of sugar beet fiber above 1.4% decreased the sensory properties significantly. There fore, the addition of inulin up to 1% and sugar beet fiber up 1.4% to red grape juice is suggested to benefit from healthful properties of probiotic bacteria.

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TL8.

VANADIUM PENTOXIDE AND THEIR NANOCOMPOSITES FOR WATER TREATMENT

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Abstract: *The access to fresh water for world population is limited which is further debilitated by water pollution. To improve water purification, nanomaterials have been successfully utilized for efficient and complete removal of variety of pollutants from water. Nanomaterials in the form of nanoparticles, nanowires, nanorods, nanosheets and nanocomposites can easily be prepared in desired size and morphology with specific required physical and chemical properties. The hybrid methods such as combination of nano adsorbent with metal oxide nanoparticles have been the first hand choice for researchers as adsorbent material for water purification applications. The unique characteristics exhibited by nanocomposites include strong solution solubility, high reactivity, greater mechanical strength, dispersibility, hydrophobicity, hydrophilicity and high working efficiency. This review is meant to evaluate multiple ways to synthesize numerous vanadium pentoxides (V_2O_5) nanocomposites and their applications in removing water pollutants. Simple nanocomposites and bimetallic nanocomposites have been the top choice for researchers for removal of water pollutants such as oil, hydrocarbons, dyes, pesticides, antibiotics etc. The advanced adsorption techniques coupled with nanocomposites have proved to be exhibited efficient, affordable and rapid for water purification. Similarly, the surface modification of nanocomposites is instrumental in achieving characteristic features including high adsorption capacity, low bulk density, and significant reusability.*

Keywords: *Nanocomposite, Pollutant, Vanadium Pentaoxide, Water treatment, Dye removal*

INTRODUCTION

Prevalently, water can be described as a natural source found on earth or universal solvent due to the properties such as power solubility. Water in a pure state is essential for human beings and as well for other living organisms. As a matter of fact, without water concept of life is unimaginable. However, the primary threat that is currently being experienced worldwide is water tainted [1]. Basically, water pollution involves unwanted materials that become in contact with water bodies and thus making them not suitable for drinking or survival for aquatic animals. The prime contributors to water pollution include industrial wastes (organic substances), agricultural perspectives, radioactive waste material, marine dumping issues, inadequate sewage treatment, to mention a few. The pollution of water has been observed to harm both humans and the environment. According to the world health organization,

about 1.7 million people die due to the pollution water and 4 billion health cases related to water pollution [2]. Consequently, water pollution carries adverse impacts on social perspectives and as well economic growth.
The common pollutants found in water bodies from industrials firms are organic substances. Dyes and pigments are the widely organic substances used in production industries such as food and fabrics.
When accidents are released into the environment, they lead to severe health and environmental problems [1, 49]. To overcome this tragedy, mechanical, physical, and chemical methods have been utilized. However, such a technique has been costly in terms of installation and maintenance. More so, some of them show low performance during water treatment. This has made the researcher switch their gears on technology, and it has seen to improve water purification. Nanotechnology is among the technologies that have been applied in water treatment [3, 48]. The nanomaterials and nanocomposites have prioritized since they have high working efficiency, removing pollutants, low expenses, and readily available. Other unique characteristics that make nanomaterials suitable in removing organic substances include strong solution solubility, highly reactive, nano size, solid mechanical property, dispersibility, hydrophobicity, hydrophilicity, porosity characters, among others [4, 51]. Thereby, this study will evaluate vanadium pentoxides and their nanocomposites in removing organic substances in water.

SYNTHESIS OF VANADIUM PENTOXIDE NANOCOMPOSITES

Vanadium pentoxide has been widely applied in various fields, particularly in industrial process plants [5]. For instance, V_2O_5 is used as a catalyst during the contact process and oxidation processes. Recently, studies revealed that the composites of Vanadium oxides exhibit excellent characteristics for removing organic substances in the waste water [6]. Vanadium pentoxides prepared using hydrothermal method and annealed in different temperatures range up to 750°C [50]. Zhu et al., reported V_2O_5 nanorods prepared through electro-spinning method in which cheap starting materials such as NH_4VO_3 and $H_2C_2O_4$ were used as starting materials as described in Figure (1). The resulting nanorods were utilized as potential cathode material [7].

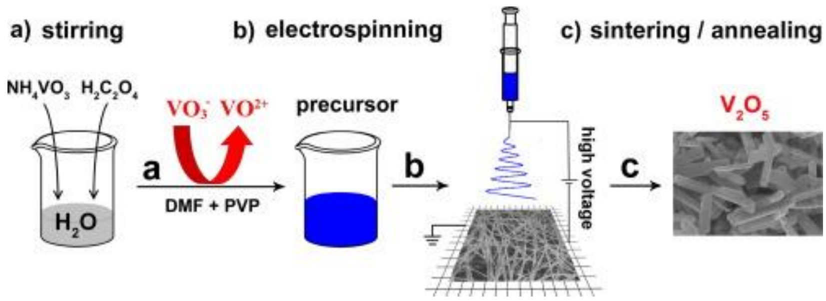

Figure (1): Electro-spinning reaction process for preparation of V_2O_5 nanorods [7]

Similarly, V_2O_5 nanosheets supported on CNTs were prepared by Yu et al., using facile hydrothermal method. The scheme of preparation is presented in Figure (2), the nanosheets were further utilized for preparation of microflowers [8].

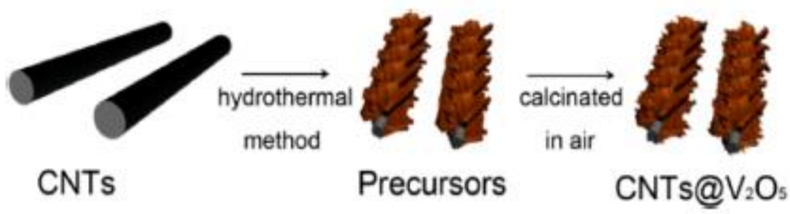

Figure (2): Hydrothermal method for CNTs supported V2O5 nanosheets [8]

This review will outline numerous reported methods using vanadium pentoxide nanomaterials along with metal oxides in the form or nanosheets, nanorods, nanofibers or nanocomposites and their specific applications for water treatment.

APPLICATIONS OF VANADIUM PENTOXIDE NANOCOMPOSITES

NiO: V_2O_5 Composites

Vanadium oxide (V_2O_5): Nickel Oxide (NiO) is prepared with vanadium nitrate (NH_4VO_3), absolute ethanol, vanadium nitrate (Ni $(CH_3CO_2)_2$, and acetic acid [14]. The first sol-gel method is used to synthesis the V_2O_5 nanomaterial where the solvent is suspended to produce the solution and then converted into the gel. For instance, 0.6g vanadium nitrate is mixed with 100ml of ethanol and methanol. Drops of acetic acid are added to adjust the P^H of 1.8. The mixtures are then stirred and heated at a temperature of 50^0C [14]. The solution is then evaporated at ambient temperature and calcined for two at least two hours at a temperature of 500^0C [10]. After preparation of V_2O_5 nanomaterial, V_2O_5 photocatalyst of NiO: V_2O_5 Composites was prepared. 0.4g nickel acetate was mixed with 80ml organic solution and stirred for about 2hrs. Nanomaterial of V_2O_5 was added to the mixture and stirred with magnetic stirring, and then heated till the solution becomes thick [14]. The composite is dried at ambient temperature and calcined at 250^0C. The synthesized NiO: V_2O_5 composites were then characterized via TEM and SEM microscope [14]. The nanocomposite material was then used to remove oil and methyl orange from waste by harnessing the photocatalytic activity. NiO: V_2O_5 composites' efficiency were 88%, and 93% when exposed to light and heating, respectively [14].

V_2O_5 -CeO_2 nanocomposite catalysts

The materials used to prepare the V_2O_5-CeO_2 nanocomposites include cerium nitrate hexahydrate, deionized water, and hydrazine monohydrate. CeO_2 is prepared via simple precipitation [2]. For example, 4M $Ce(NO_3)_3.6H_2O$ is dissolved into 200ml distilled water. The mixture is stirred and heated at 90^0C, and 0.5ml hydrazine is added and further heated for about 2hrs [2]. CeO_2 nanoparticles are washed with distilled water and ethanol and dried at 70^0C for at least a night.
V_2O_5-CeO_2 nanocomposites are synthesized by aligning the CeO_2 nanoparticles with V_2O_5 via simple thermal decomposition and the addition of NH_4VO_3 [34]. The analysis of nanocomposites is performed via FE-SEM, TEM, XRD, and EDS [34]. The degradation of methylene blue by V_2O_5-CeO_2 nanocomposites showed more than 98% efficiency was under 25 min irradiation [34].

Sn-doped V_2O_5 Nanoparticles

The reagents used to synthesis Sn-doped V_2O_5 nanocatalysts are Tin (IV) chloride pentahydrate, ammonium hydroxide, potassium sulfate, distilled water, and hydrogen peroxide [30]. Sn-doped V_2O_5 nanocatalysts are prepared with 0.5-6wt%Sn doping via the sol-gel process. The precursors of these nanoparticles in and vanadium (v)[30]. To illustrate this, about 2.7g $V_2O_{5\ is}$ added to 50ml of distilled water and hydrogen peroxide solution and then stirred. Through ultrasonic agitation, the solution is diluted, where the solutions turn to brick-red. The solution is further diluted with 128ml distilled water [30]. The prepared gel is then diluted with 357ml water while stirring. The V_2O_5 solution is vigorously stirred and then evaporated at 95^0C. The prepared particles are sieved to the required particle size [30].
The photoluminescence properties, crystalline microstructures, and optical are characterized via X-ray diffractor, TEM, and UV-vis spectrophotometer. Sn-doped V_2O_5 nanocatalysts are used to degrade ammonia via a microbial fuel cell and photocatalysis [9]. After the degradation of ammonia, it was confirmed that Sn-doped V_2O_5 nanocatalysts had the optimal degradation of 96.4% [30].

V_2O_5 -ZnO coated carbon nanofibers

The reagent utilized in the synthesis of V_2O_5-ZnO coated carbon nanofibers are ethylene glycol, zinc acetate, methanol, acetic acid, sodium hydroxide, enoxacin, cinoxacin, acetonitrile, and ammonium metavanadate [4]. V_2O_5 -ZnO nanofibers are prepared by adding 3g ammonium metavanadate and 3g of zinc acetate into 30ml ethylene glycol [4]. About 1g of carbon nanofiber is added to the mixture, and the solution is constantly stirred [4]. The carbon nanofiber is dispersed through the mixture via sonication for about 20 mins. After 24hrs the precipitates were cooled, centrifuged, washed, and then dried at 120^0C for about 12h. The prepared V_2O_5 -ZnO coated nanofibers are then annealed at 320^0C for 3h. The properties of synthesized V_2O_5-ZnO nanofiber are analyzed via XRD, FTIR, SEM, and TEM. After the analysis, the results revealed that V_2O_5 -ZnO nanoparticles are incorporated on the surface of carbon nanofibers [4]. The parameter such as initial P^H, adsorbent amounts, and contact time is enhanced by the Box-Behnken method. The condition optimum is found to be 6.5, 0.18 g for P^H and adsorbents. This articulates the adsorption capacity of 71.4 and 87.7mg/g for cinoxacin and ciprofloxacin, respectively.

V_2O_5 -montmorillonite nanocomposites

V_2O_5-Mt nanocomposites are prepared by adding montmorillonite into ethylene glycol while stirring. 2M, NH_4VO_3 is added to the solution, and then the solution is then mixed till yellow precipitate is obtained [40]. The residues are then washed with distilled water and ethanol and then calcinated at 600^0C [40]. The removal of hydrogen peroxide by the nanocomposites was found to be 30 to 400µM. This shows the potential of removing the organic compounds from wastewater.

Au metal nanoparticles (NPs) decorated V_2O_5 (Au-V2O5)

The materials used to prepare Au-V_2O_5 are Rhodamine-6G chloride (Rh-6G), Ammonium metavanadate, sulfuric acid, and chloroauric acid. Au-V_2O_5 nanocomposites are prepared by template-free one-step hydrothermal method. 0.1M Ammonium metavanadate is added into 80ml water, and the solution is stirred [37]. Sulfuric acid is then added to the solution to achieve a P^H of 3. The surface decoration of Au-V_2O_5 is enhanced by the addition of Chloroauric acid. The solution is then into the hydrothermal reactor, where then is sealed and heated at 120^0C [37]. After 24hrs, the reactor is cooled to room temperature and thus forming the precipitation. The precipitates are washed with ethanol and water and then dried at 70^0C.
XRD, FE-SEM, UV-V, and TEM are used to characterize optical, structure, and microstructure properties of Au-V_2O_5 nanocomposites [33]. The photodegradation of rhodamine-6G dye was induced via catalytic activity. The maximum removal was confirmed to be 96% [33]. Additionally, the photocatalysts exhibited increased reaction rate constant, reduced charge combination, and finally increased lifetime.

Uniform V_2O_5 Nanocuboid Nanocomposites

V_2O_5 Nanocuboid was prepared by adding V_2AlC powders into a Teflon container followed by 40ml of 49% concentrated hydrofluoric acid [12]. The mixture is kept at room temperature while stirring. The Mxene is placed uniformly in a silica boat and put into a horizontal tube furnace. Dry air is kept furnace under 550^0C [12]. The airflow rate of 0.5L/min kept throughout while calcinating the V_2O_5 Nanocuboid. Microstructures, crystal structure, and morphologies are characterized via XDF, SEM, and TEM. The adsorption of the capacity of V_2O_5 Nanocuboid nanoparticles is reported to be 55mg [12]. Consequently, the nanocomposites utilized low energy during the desalination of water.

Aerogels of V_2O_5 -Nanowire Dispersion

The required reagents include ammonium metavanadate, Hydrochloric, distilled water, and Aniline.
V_2O_5 -Nanowire is synthesized by adding few drops of water into ammonium metavanadate. The paste is then dried over the oven at 110^0C [16]. 1g of the paste is mixed with 10m of 1M HCl, and the

mixture is vigorously stirred. 10ml distilled water is then added, and the solution is sedimented, where red precipitate is formed. The red residue is dispersed in hot at 90^{0}C, and the solution is stirred thoroughly where the top layer solution is removed [16]. This procedure is repeated severally till uniform V_2O_5 -nanowire dispersions are generated.

About 0.42 ml aniline monomer is dispersed into 40ml distilled water. The prepared V_2O_5 -Nanowire dispersion is molded into rectangular shapes [16]. Finally, the hydrogel of V_2O_5 -PANI is formulated by immersing the molded distribution into an aniline monomer. Gelation is achieved by keeping the composite at room temperature for at least a few hours [16]. The Aerogels- V_2O_5 composite is then freeze-dried at -60^{0}C for at least a day. The analysis of Aerogels- V_2O_5 composite was examined via FTIR, SEM, and TEM. After characterization, the composites are found to have low density (0.02 g/cc), maximum EMI shielding efficiency of 34 Db, and specific shielding efficiency of 1662.2 dBcm^3/g [16].

Pd-V_2O_5 Heterostructure Nanorods

The materials required to prepare the Pd-V_2O_5 nanocomposites are palladium chloride, Rhodamine-6G, sulfuric acid, and ammonium metavanadate. The Pd-V_2O_5 nanorods are formulated via the low-temperature hydrothermal method. 0.1m ammonium metavanadate is dissolved into distilled water and then stirred to attain homogeneity. The sulfuric acid solution is then added to the mixture to achieve a P^H of 3 [5]. The required amount of palladium chloride is added to the solution and then stirred [7]. The mixture is then transferred into a Teflon-lined stainless-steel autoclave at a temperature of 120^{0}C for one day [5]. Afterward, the precipitates are cooled to room temperature, centrifugated, washed with water and ethanol, and finally dried at 70^{0}C for at least 8h [5]. The synthesized sample is then heat-treated to a temperature of 500^{0}C. The structural analysis of synthesized Pd-V_2O_5 heterostructure nanorods is examined via FE-SEM, TEM, and XRD. The photodegradation of rhodamine-6G by Pd-V_2O_5 nanorods is investigated via photocatalytic activity [5]. The degradation performance of the nanorods results to be 98% [5]. This performance was enhanced by the highest reaction rate constant (1.8544/h) and the largest BET surface area (19m^2/g).

V_2O_5-TiO_2 with S and N nanocomposites

The sol-gel method is used to prepare the TiO_2 modified with S and N. 0.064 M tetrabutyl titanate is mixed with 0.064M tertrabutyl titanate and 0.128 mol of acetylacetone [23]. While stirring, the solution is diluted with 50ml ethanol with urea and thiocarbamide. The solution is heated at 60^{0}C and then dried at 120^{0}C [23]. The prepared sample is calcined at 500^{0}C for at least 3 h. The V_2O_5 is then incorporated into S-N-TiO_2 via the impregnation method. The properties of V_2O_5-TiO_2 nanocomposites are characterized via XRD, TEM, TPD, and SEM. The properties analyzed are BET surface area and temperature-programmed desorption [23]. The surface area, active sites, Lewis's acidity, and reducibility are enhanced by S and N [23]. The strong interaction between TiO_2 and Vanadia shows more active oxygen species on the surface of the catalyst [23]. Thus, eliminating nitrogen oxides was done through reacting with oxygen.

Fluorescent Polyimide Ag and V_2O_5 Nanoparticles

Fluorescent Polyimide Ag and V_2O_5 Nanoparticles are prepared by reacting perylene tetracarboxylic dianhydride and *p*-phenylenediamine [24]. Then Ag and V_2O_5 are added to the mixture at 160^{0}C under a nitrogen atmosphere. Thermal properties and chemical functionality, and morphology were determined via DSC, FTIR, and FE-SEM. The nanoparticles are then used to degrade rhodamine-6G, Cr (VI), and *p*-nitrophenol [24]. The Nanocomposites show high degradation performance.

Magnetic Fe_3O_4@V_2O_5/rGO nanocomposite

The material used in the formulation of the stated nanocomposites includes sulfuric acid, hydrochloric acid, anhydrous $Fecl_3$, graphite powder, potassium permanganate, silver nitrate, NH_4VO_3, $FeCl_2.4H_2O$, Na_2SO_4, NH4Cl, $NaNO_3$, ascorbic acid, and $NaBH_4$ [8].

Graphene oxide is first prepared by adopting graphite powder via adopting Offerman. $Fe_3O_4@V_2O_5$/rGO nanocomposite is prepared via ex-situ where, Fe_3O_4 is deposited on the surface of V_2O_5/rGO via a chemical co-precipitation method. $Fe_3O_4@V_2O_5$/rGO nanocomposite characterizations are examined via SEM, XRD, TEM, XPS, FTIR, and VSM techniques. Afterward, the $Fe_3O_4@V_2O_5$/rGO nanocomposite exhibited outstanding photocatalytic activity on the degradation of dyes under sunlight irradiation [8].
Table 1 presents comprehensive comparison of numerous V_2O_5 nanomaterials applied for removal of wide range of target pollutants from water. The literature suggested that V_2O_5 nanocomposites have been highly efficient for water purification and can yield removal efficiency up to 98% in some cases.

Table 1: Comparison of V_2O_5 nanomaterials for removal of pollutants from water

Metal Oxide	Type of nanomaterials	Method of Preparation	Target Pollutant	% Removal Efficiency	Ref.
$ZnO-V_2O_5$	Carbon Nanofibers	Precipitation	Antibiotics	83.7	[4]
$Au-V_2O_5$	Nanorods	Precipitation	Rhodamine-6G Dye	96	[5]
$NiO:V_2O_5$	Nanocomposite	Sol-gel	Oil methyl orange	88/93	[14]
Sn-doped V_2O_5	Nanocatalyst	Sol-gel	Ammonia	96.4	[30]
$CeO_2:V_2O_5$	Nanocomposite	Precipitation	Methylene blue Dye	98	[34]
V_2O_5-montmorillonite	Nanocomposite	Simple	Hydrogen Peroxide	87	[40]
$ZnO-V_2O_5$	Heteronanorods	Gas-phase	Methylene blue Dye	87.5	[45]

V_2O_5 and different metal oxides nanosize were prepared by our research group in Sustainability Solutions Research Lab, Faculty of Engineering, university of Pannonia, and modified with MWCNTs using hydrothermal method. This metal oxides nanosize / MWCNTs nanocomposite were used for water treatment applications for removal of dyes and hydrocarbons from water [46]. The modification and nanocomposites preparation could be explaining by Figure 3. Modification of MWCNTs with metal oxides nanosize will provide more opportunity for increasing the possibility of bonding and removal of pollutants from water for example oil and dyes. Figures 4 and 5 explain the mechanism of kerosene and dyes removal over metal oxides nanosize modified MWCNTs [47].

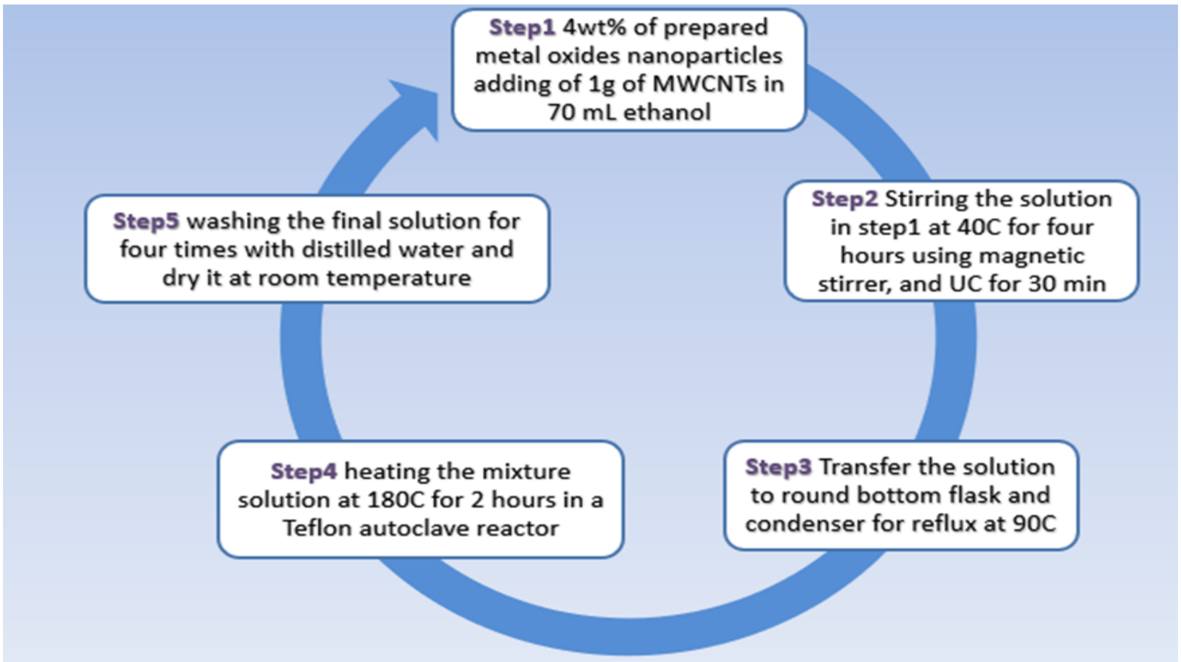

Figure 3: Explanation steps for MWCNTs modification with vanadia nanoparticles and different metal oxides nanosize.

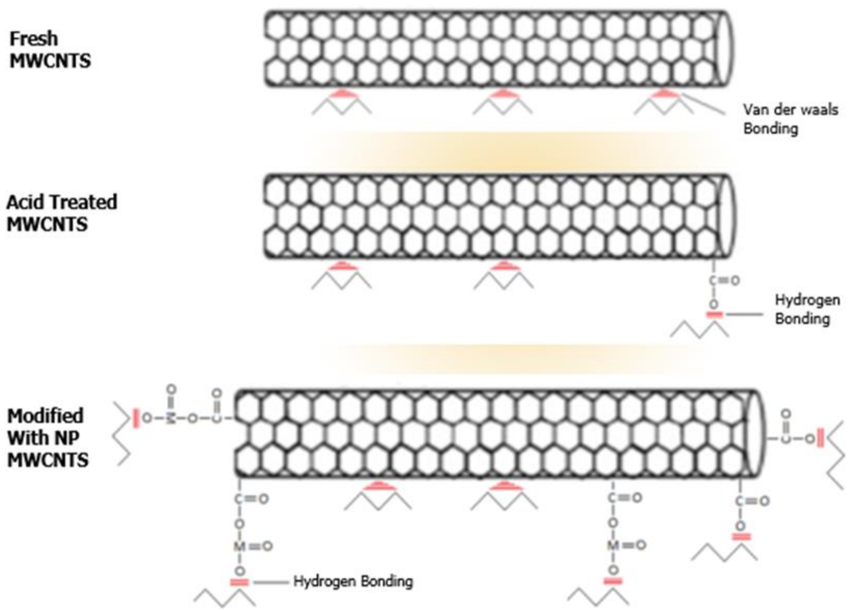

Figure 4: Mechanism of kerosene removal from water using fresh, acid treated and modified MWCNTs with metal oxides nanoparticles [47].

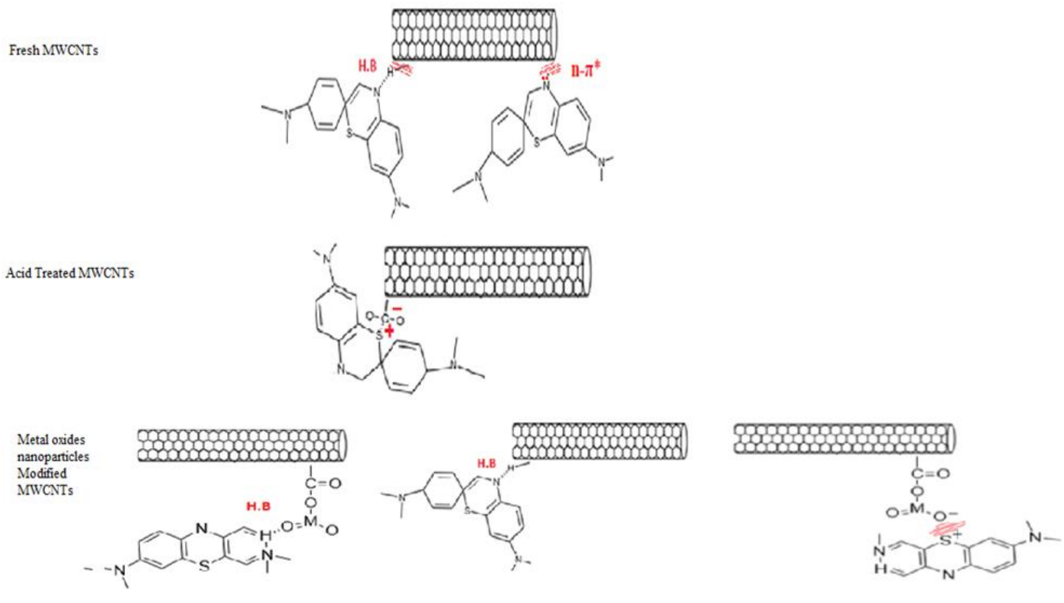

Figure 5: Mechanism of methylene blue from water using fresh, acid treated and modified MWCNTs with metal oxides nanoparticles [47].

CONCLUSIONS

The availability of the fresh water is the major focus of the environmentalist and the policy makers around the globe. The water pollution has equally disturbed human and marine life. The invent of nanotechnology has been quite beneficial for water purification. The ability of nanomaterials to exist in wide variety of size and structure has paved ways for scientists to synthesize nanocomposites with specific physical and chemical properties.

The review of numerous applications of vanadium pentoxides (V_2O_5) nanocomposites and their surface modification with MWCNTs and other materials have resulted in manifold increase of their sorption capacity for removal of oil and organic pollutants.

These nanocomposites have been proved to be environment-friendly because these do not require large amounts of chemical on the one hand while does not generate waste on the other hand. These nanocomposites have exhibited the sorption capacity and removal efficiency of nearly to 100%. V_2O_5 nanocomposites are considered as efficient and cost-effective sorbent material and it is likely that these materials will have bright future for numerous applications in water decontamination.

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TL9.

THE ROLE OF EDUCATIONAL PATHS IN THE ENVIRONMENTAL EDUCATION

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Abstract: *The Óbuda University decided to make an educational path in the year of 2019. The financial background was provided by the III. district of Budapest from a European Union source. In the planning phase, the educational path originally imagined as a mixed type observational trail both physical and online stations to present the natural and cultural sights of the Kiscelli-plateau. The primary aim of the trail was to use as an option of the environmental education of the 10-18 years old age group, however the applicability of the BSc environmental engineering education was also examined. The environmental engineering training is also educated in English in the University. For this reason, every station's website has its own English version. To draw the aim age group's attention, we designed websites for half of the stations, but the scalability requires making online background surface in every station of the pathway. This is also important for the future developmental possibilities.*
It is important to emotionally affect for the visitors in the environmental education. The educational path could fulfil its role, because in every possible station, we determine the age of the cultural sights and search maps from the same era. We use an actual satellite image for the opportunity to compare the actual condition with an older state. Visitors could also see the slow geological events affect and the relatively fast anthropogenic transformation of the area. This illustrative display could form the mind of the audience.

Keywords: *Environmental education, educational path, online observational trail, interactivity*

INTRODUCTION

In 2019, Kovács Csaba presented my work "Developing observation trail at the example of Kiscelli-Doberdó educational path" at the Faculty of Light Industry and Environmental Engineering of the Óbuda University. In the same semester, a thesis was prepared from the presentation. The result is a complex nature and environmental trail, which primarily benefits the younger age group as well as the university students. The project was funded by the KEHOP 1.2.1. During the design phase, we tried to build the route in a way that it could be used by BSc Environmental Engineering students of Óbuda University. It is important to note that the information about the attractions is accessible to the target audience and to anyone, in addition to the environmental engineering students. The knowledge transfer is achieved through thought-provoking background material and a mixed physical and online format that provides an experience for students on other courses at the university and for all visitors.

The aim was to create an educational trail with a real educational value, which would make the visitor aware of the extent of man's ability to form nature. Here I had in mind a visually accessible visual aid, based on maps of military surveys of Hungary and modern satellite images. The stations follow each other spatially, while representing an era in chronological order. One of these timelines runs through geological landmarks, which at certain stations allows us to follow changes over millions of years. The other timeline covers a narrower range, focusing on changes of anthropogenic origin, covering a period of roughly three centuries, as illustrated by military surveys. This shows the changes in the proportion of forests, fields and inhabited areas, which changed rapidly in relation to geological processes. The observation of the influence of human activity can lead to action, or at least make the visitor reflect on the extent to which man is shaping its environment.

Keeping in mind the expectations of the 21st century, the trail will not only provide knowledge transfer in the traditional way by displaying information panels but will also provide an online backdrop. This will allow for simple, low-cost, continuous improvement. In this way, certain data can be update and the interactivity of the trail can be improved. And with the ubiquitous availability of smart devices, it makes sense to use them for this purpose.

In 2020, the trail reached a milestone in terms of usability, when it was decided that it should be more prominently used in environmental engineering training. The publication "The possibilities of project-based education using the example of the Kiscelli-Doberdó nature trail", does not only examine the potential for educational use, but also looks specifically at the range of subjects in which the nature trail could be used for field work and measurements, in the light of the criteria for project-based education. Practical aspects and project-based teaching are becoming increasingly prominent in the educational strategy of Óbuda University.

The trail was originally planned to be completed in early 2020. However, the pandemic delayed the work and changed the design aspects, as an online interface was created for all stations, even those that originally existed in physical form.

MATERIALS AND METHODS

Public health role

Health is a state of complete physical, mental and social well-being, not merely the absence of disease or disability. [1.] The establishment and operation of a public health system is the responsibility of the state, but it relies on the active involvement of individuals and society. Public health is the science which studies the conditions for preserving, protecting and improving human health: it studies the effects of the environment on man and the requirements of a healthy lifestyle. [2.] In considering the impact of man on the environment, it is also worth educating people to be aware of the impact of the environment on them. This can be achieved if they are aware of their environment and understand the anthropogenic processes whose impact on the environment is ultimately harmful to humans. In my opinion, a nature trail can play a useful role in this. At a local level, it can influence the thinking of individuals who know a place well by highlighting connections that they may not realise. The state of the environment and environmental protection are therefore important elements of a prevention-based approach to public health, and a trail can be used to explore the links.

Opportunities for project education

The attractions offered by the Kiscelli-Doberdó observation trail can provide a good basis for environmental education to use at the university. In the education system of Óbuda University, project pedagogy and project-based education are becoming increasingly important. The students of the Faculty of Light Industry and Environmental Engineering are the BSc Environmental Engineering students who can benefit from this. The model curriculum for the course includes several subjects (e.g. Water Quality Protection, Nature and Landscape Protection) where a project approach can be applied. [3.] This implies that a longer-term project task is expected to be carried out independently. Where this would not be appropriate for the value of certain stations on the trail, classroom tasks can be carried out on site, aided by the proximity of the university. [4.] In the project approach, it is important that teachers and docile are equal partners, the difference being in their different competences.

Detailed definition of trail type

The type of the Kiscelli-Doberdó educational path must be known to fulfil its role. In detail it is mixed, based on the value presented or the theme. It aims to present geological and natural values, but botany is also present at the fifth station. An essential element is the historical thread, linked to the time of origin of the socio-historical values of each station.

In terms of the way in which the knowledge is conveyed, the trail is mixed type. The difference with the traditional stake-track, as defined, is that the numbered stakes are accompanied by QR codes, so that the knowledge associated with the stations can be viewed on linked websites rather than in a programme booklet.

The way the trail is presented is demonstrative, both for the target age group and for other visitors.

The trail is a complex environmental trail, which makes it particularly suitable for the primary objective of environmental education. Its thematic nature cannot be completely ruled out either, since there are three main aspects that are present at almost all the stations: natural, geological and historical.

In relation to the site, the Kiscelli-Doberdó trail is local. Each station focuses on themes specific to the area, and if it does describe a particular discipline (e.g. water quality protection), it does so in relation to the features that can be observed here.

The length of the trail makes it a walking trail, because its length is less than 2 km. The difference in altitude over this distance is 44 m. Good accessibility is an important criterion for walking trails. Fortunately, no attention has been paid to accessibility, as the Kiscelli plateau is a popular hiking area with well-maintained dirt roads and asphalt pavements. The exception is the Schmidt spring, which is in a slightly more inaccessible location deep in the forest. [5., 6.]

Implementation basics, tabletop stations, online stations

The design of the virtual trail had to take into account the requirements of the mobile devices. The scaling of the web pages is dynamic, adapting to the orientation of the devices. The font size is large to make it easier to read and links are provided on the web pages to access further information.

An important step in the design was the creation of a history thread, for which I positioned two images in the same position using GPS coordinates based on a historical map and a current satellite image. On a desktop or laptop computer, it is an advantage to have the two images side by side for comparison, but on a mobile phone screen they will be too small to compare effectively. I have also made their layout vertical. Rotating the mobile phones would of course solve the problem, but convenience and ease of use were important considerations in the design.

It is also the reason for the clean design of the pages and the contrasted appearance. When creating the web pages for the stations, I did not design any distracting elements on the site, simple black background, white letters and images make up the whole site, sometimes with tables and external links. Another undeniable advantage of simplicity is the quick loading speed and lower data traffic requirements. As free wireless internet access is not available in the trail area, this is not a negligible factor.

The linking of the pages, the clarity and the creation of an English version of the site has created a new need, resulting in the tabular header that is a common feature of all the websites. In the table, a larger font size and a yellow colour indicate the number of the current station. This makes it easier to see how many stations the visitor has visited and how many are still to come. In addition, the numbers are also links, so that the visitor can view the other stations without scanning a QR code, if they wish. Another important element of the table header is the ability to change the language of the website. In the top left-hand corner of the page, you can click on the English or Hungarian flag to display the page in the language of your choice. The default version is Hungarian for stations with a QR code on the pile, while the default version is English for stations with a code on the board. If the page has already been opened in a particular language, you no longer need to bother with setting the language when navigating by numbers. (Figure 1)

Figure (1): A common table header for websites

The creation of an English version of the trail came up during the design work. The trail is located in Budapest, in a busy part of the city, so serving English-speaking visitors to the city might be a reason worth mentioning, but it was not the main motivation. At the Rejtő Sándor Faculty of Light Industry and Environmental Engineering also offers a BSc programme in Environmental Engineering in English, among several other subjects. A part from group age between 10 and 18, the second most important target audience is the university students. In view of this, it was appropriate to create an English version of each station. Project-based learning, which also plays an important role among them, was also an important motivating factor. In the sites marked with a stake, which were originally intended to be online stations, the QR code leads to the Hungarian website, from where the English version can be accessed by clicking on the British flag in the header. Once implemented, the stations originally designed as a signboard will each have a QR code to access the English website.

The importance of environmental education, pedagogical methods

In the 21st century, the importance of environmental education has increased in the face of climate change due to anthropogenic influences. Environmental education is understood to mean the shaping of consciousness through pedagogical methods that lead to the development of a responsible, environmentally aware attitude and provide the necessary emotional motivation. Environmental education is a form of consciousness-raising which, like general education, has an influence on the whole personality [7.], and plays an important role in ensuring that individuals do not act out of compulsion for their environment, but also feel a genuine sense of responsibility for environmental protection. As with all educational processes, the younger age group can be targeted for more effective results. This study explores this potential for awareness-raising through the use of nature trails.

Today, environmental education is compulsory in schools in Hungary. According to a survey by the National Institute of Public Education, environmental education should be compulsory in primary schools. Unfortunately, this does not represent a comprehensive approach, because we can only talk about environmental education in connection with nature science subjects. Furthermore, the limited available financial resource for environmental education is also a problem. It is important to emphasise that the educational work carried out in the school setting is most rewarding when children experience similar educational patterns in close family circle. It is primarily the behaviour of parents that determines personality. [8.] For this reason, it is worth considering the creation of demonstration sites and strategies suitable for environmental education, which highlights the vulnerability of our environment to anthropogenic influences not only in schools but also in other leisure settings.

School-based environmental education can be integrated into the curriculum for each age group, subject by subject. Unlike current practice, however, it can be integrated into the curriculum not only in the nature science subjects but also in the human subjects. Children generally see examples of nature conservation, but it is also important to be aware of the social and economic impacts. According to information from the National Institute of Public Education survey, 85% of schools integrate environmental education into compulsory subjects. 4% of institutions report that they devote a specific lesson to this. 11% of the institutions surveyed in the representative survey mentioned that environmental education is integrated into the curriculum as well as being taught as a separate subject. [7.]

There are many opportunities for schools to mainstream environmental education beyond the classroom. The institutions surveyed reported varying rates of use of workshops, camps, field trips, projects and environmental activities, ranging from 20% to 56% across the categories. A particularly important role was given to field trips. The schools that completed the questionnaire mentioned this type of knowledge transfer method with a rate of more than 84% as a tool that could be used in environmental education outside the classroom. In the light of these figures, trails can therefore play a useful role in promoting environmental education [8.]

The role of trails in environmental education

As seen above, nature trails could play a significant role in developing environmental awareness, yet in a pedagogical questionnaire on nature trails, only 0.5% of respondents associated the word nature trail with environmental education. [6.]
Of course, the fact that environmental education is not the first thing that comes to mind for the majority of visitors does not mean that nature trails are the most effective way of promoting an environmentally oriented approach. This means that the requirements for nature trails should meet the following requirements: promoting direct harmony between man and nature, enriching general knowledge of species, acquiring knowledge of the immediate environment, understanding ecological relationships and raising awareness of environmental responsibility. [9.]

RESULTS AND DISCUSSION

The type of stations and the way in which the information is transmitted will be described below. The original plans for 2020 were to have 4 stations with boards and 4 with QR-code poles, but due to the pandemic, the 3rd district of Budapest asked the constructor, the Óbuda University, to provide an online version for each station.

Launch station

The starting station of the trail differs in theme from the other locations. It does not mention a specific attraction but gives general information about the trail and the most important facts about it. The starting point should be indicated along the Doberdó Street, and in any case on a signboard, as the attractions in the area should be prominently marked at the beginning of the road. The sign should include a map showing the exact location of the stations and the route to each point. (Figure 2) In addition, other information of interest to visitors may be mentioned, such as the altitude, the elevation of the route taken, or the length of the route.

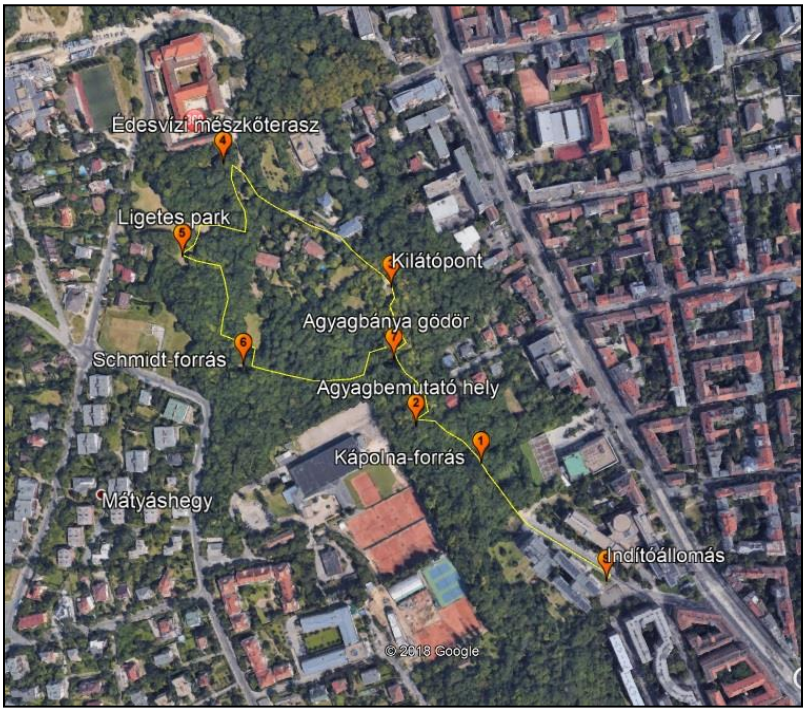

Figure (2): The route and stations on the map

The Chapel Spring

The first actual station on the Kiscelli-Doberdó educational path is the Chapel Spring. There are two major features on the site, one of which is the spring that gives the station's name and the hydrological interest. The other is the Chapel of the Holy Blood, which is noteworthy from a cultural and historical point of view.

The karstic landforms of the region are characterised by the presence of karstic water beneath the surface. This means that the deposits in the lower layers of the soil easily reach the surface where the mountain slopes are. However, the Chapel Spring itself is not karstic but groundwater, and its water is cold. Unfortunately, its water output is decreasing in the long term. In 1998, it produced 20 l/min. In contrast, in September 2006 the spring's water output was 1.5 l/min, and is therefore not protected by the law, because it is under 5l/min. Its water is not drinking water, its chemical oxygen demand, nitrite and sulphate content exceeds the limit values. [10, 11, 12.]

Another important aspect of the station is its cultural significance. The Kiscelli plateau was the property of the Zichy family in the 18th century. The Chapel of the Holy Blood is also associated with the family. It was built in 1702 for a statue brought from the village of Mariazell in Austria. [11.]

The historical line begins with this period. The closest map of the country to the year of the Chapel's construction is the first military survey, carried out from 1763 to 1787 by order of Maria Theresa. 21 partial surveys were made, producing maps at a scale of 1:28800, covering the whole country, with a clearly visible representation of settlements, gardens, fields, forests and topography. [11.] To achieve environmental education, a contemporary map can be juxtaposed with a modern one to show the changes that have occurred over the centuries as a result of human activity (Figure 3).

Figure (3): The area of the educational path in the 1700's and the present

Kiscelli clay demonstration site

The second station of the Kiscelli-Doberdó trail, the clay demonstration site, in addition to the geological features of the area, pays special attention to the Kiscelli clay. The clay itself is common sediment, usually containing fine-grained components. It is formed by the weathering of feldspathic rocks, where atmospheric carbon dioxide causes potassium and sodium silicate to dissolve from the feldspar, and the non- crambled minerals form the clay itself. Clay is defined as sediments with a grain size of less than 0.039 mm. [13.]

In addition to the calcareous content of the clayey soil, it is important to examine the clay, which is also the first Kiscelli Clay Formation observed in the area and described here. Here it is worthwhile to deal specifically with the characterization of this type. The Kiscelli Clay is a marine sedimentary rock deposited during the Palaeogene period, which began 65.5 million years ago and ended 23 million years ago. It was deposited in the Tethys Sea, the predecessor of the Mediterranean Sea, and is a common rock layer in the local mountains. It is typically found near the surface, with thicknesses generally varying between 50 and 500 m. [14.] At the station, it is important to be aware of the fact that the wall is largely composed of limestone, with the clay layer itself beneath. The colour of the

Kiscelli clay is originally bluish grey, but the parts of the clay that have been exposed by the forces that formed the surface, or by human activity, have acquired a yellowish tinge as a result of an oxidation process that takes place during the transformation of pyrite into limonite. This calcium carbonate coating gives the formation its unique texture. [14., 15.] The information on the website of the second station provides information on all this in both English and Hungarian. The so-called acid test, used at the university to test the clay, is presented in tabular form for the use of the excursionists and students. The values of the empirical scale, arranged in tabular form, can be used to test the lime content of the clay. A recognition image is also included on the site, showing the bluish-grey tint mentioned above. (Figure 4)

Figure (4): Crab fossil in the Oligocene layer of the Kiscelli clay [16.]

Lookout spot

The location of the station is on the eastern edge of the Kiscelli plateau, so the panorama covers the sights of Óbuda and Pest to the east. The problem that makes the site unsuitable for a lookout point is the dense vegetation that can be observed at present. It would be shames to disturb natural assets that are important even in their present state, especially in view the fact that the main purpose of the nature trail is environmental education. It should be added, however, that the view is definitely better in late autumn to early spring, after the foliage has fallen.

As this is the closest station and, unlike the 2007 version of the trail, the calvary is not a separate station, it is worth mentioning here. The events of the Way of the Cross can be observed through the images of Jesus' Passion, which can also be found in Roman Catholic churches. The phenomenon became common in the Baroque period, and similar works were created in Hungary from the 17th century onwards. The twenty stages can be observed along the Doberdó Street, from the Calvary to the chapel. They were built between 1821 and 1822. [11].

This period coincides with the Second Military Survey of Hungary, which was carried out between 1806 and 1869. The survey was begun by order of Emperor Franz I, but was not completed until the reign of Emperor Franz Joseph I. The scale of the maps is 1:144,000, which makes them more detailed than the First Military Survey used at the first station, but there is a drawback: they used a different method of depicting topography, so they are not as illustrative. [17.] See Figure (5).

Figure (5): The area of the educational path in the 1800's and the present

Freshwater limestone terrace

The fourth station on the Kiscelli-Doberdó educational path -unlike the previous one- provides a deeper insight into the natural formations of the area, including the geology. The area is observed to have subsided along the slopes from the Three Border Mountain to the Kiscelli-plateau with dolomite bedrock. The water's work in shaping the surface has eroded a large part of the hills, but the hard source limestone on the Danube bank has protected the underlying clay layer, forming the Kiscelli escarpment. [18.] See Figure (6)

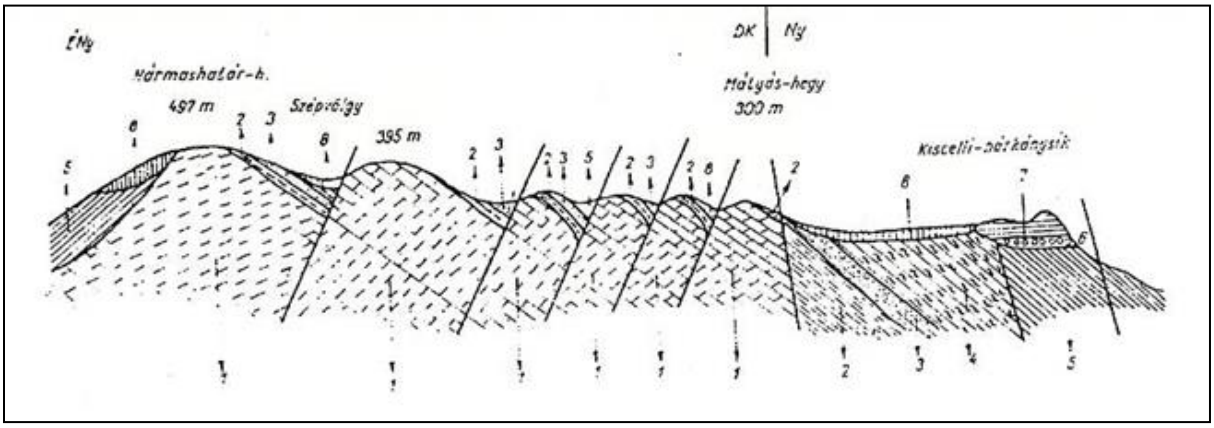

Figure (6): Geological section of the Three Border Mountain (after K. Hoffmann (1883))

The freshwater limestone terrace is sufficiently unique to allow the station to detail its formation. The type of limestone is noteworthy, as it tends to form in the wake of saltwater areas worldwide. It is a chemical sedimentary rock, formed between 1 and 2 million years ago during the Pleistocene. The rock material could have been derived in two ways, from spring water by purely chemical means and by plant mediation. [19.] The limestone terrace observed here is not a natural formation, but the result of the activity of a late limestone quarry. The vertical wall is fully stratified and shows traces of crustal movements in the post-Pleistocene periods. It should be added that naturally exposed limestone can be found in the area. [10, 11] In accordance with the rules laid down during the construction work, the information provided by the fourth station is of general application, shedding light on the geological background. This is also a board station, as the knowledge on the panels does not change.

Glade Park

The fifth stop on the trail, the grove, provides an opportunity to study the plants found here, as this was not previously the case. Most of the trees in the area are non-native. The hornbeam-oak

population has been significantly reduced by centuries of anthropogenic influences. The area around the station was the first to fall victim to tree clearance following the expansion of the town and subsequently lost to the expansion of clay and limestone mining. [10, 15]
I considered a panel format to be the most appropriate for the design of the botanical station, as the current land use is unlikely to change the vegetation composition in the long term. The focus should be on the presentation of protected grasses, which are the group of vegetation most threatened by the forested nature of the area. The layout of the website is similar to the one shown in the picture below. Species are classified according to the same four criteria, with the addition of a bioindication procedure that does not require any instruments. This station is also used for the presentation of leaf necrosis, which indicates the presence of pollutants in the atmosphere. Anthropogenic sulphur dioxide in the air causes brown discolouration of the leaves, a process that leads to the death of plant cells involved in photosynthesis. The phenomenon is first observed at the edges of the leaves and then gradually progresses from there towards the inside. At the station, a schematic illustration is used rather than a photograph to help visitors to become more familiar with the species.

The Schmidt Spring

Like the Chapel Spring mentioned above, this is also a cold-water groundwater spring that springs from the boundary between the slope sediment and the clay. Its source is located at an elevation of 150 m above sea level, at the base of a tree. In the late 1990s, its water output exceeded that of the Chapel Spring, but today it is unfortunately not measurable. Its condition has not improved to date, but rather deteriorated. The area around the spring is contaminated with inert waste, and a lot of earth, concrete and asphalt has been dumped in the vicinity, which, if not the water quality, certainly degrades its appearance. At present, the spring's point of origin cannot be determined, but the wetter surface and the veins running from it are a reminder that groundwater rises to the surface here. [15]

Clay mine pit

The seventh and final stop on the Kiscelli-Doberdó nature trail is the clay pit, which both commemorates the mining activity that once took place in the area and gives us a chance to see the Kiscelli Plateau in the 1900s. Mining activity was present in the area in the 1850s to serve the brick factories that were established here. By the first quarter of the 1900s, however, brickworks production in Óbuda was disappearing. Some of the mining areas were filled in, while others were not. [11.] An example of the latter is the pit marked as a stop on the nature trail. It continues the thread of history already seen at the previous stations, related to brick making. The website “egykor.hu”, where photographs illustrating the historical trajectory are available from the 1870s, is a great help in visualising the station. For a comparison with the present day, the Third Military Survey of Hungary would be useful, but instead I used a series of aerial photographs of Budapest in 1944 provides the basis for comparison. The photographs cover a sufficient area for an overview of the trail and its surroundings, and the quality is also good. And it certainly provides a more visual basis for comparison than the maps (Figure 7)

Figure (7): The area of the educational path in the 1900's and the present

CONCLUSIONS AND RECOMMENDATIONS

The Kiscelli-Doberdó observational trail was originally conceived as a way of raising environmental awareness among 10-18 years old age group. In addition, not only the students of Óbuda University, but also the teachers can benefit from the trail, as the educational strategy of the faculty can be developed in a variety of ways in accordance with the university's policy, which is determined to focus on practical education.
An important aspect in the design of the trail was the diversity of the stations, both physical and online, and the variety of values presented. The historical thread that is presented brings visitors closer to the changes that have shaped the environment over the centuries and illustrates the intense human impact on such a relatively small, sparsely populated area.
Another noteworthy achievement is that the English version has also been produced, which means that the trail can play a role in raising awareness not only among students of the English-language course at our university, but also among non-Hungarian-speaking visitors. It is hoped that the knowledge and experience gained here will be incorporated into the daily lives of visitors, thus helping to protect the environment and promote a healthier lifestyle.

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TL10.

THE USE OF MICROALGAE COMPOUNDS IN THE IMPROVEMENT OF AGRICULTURAL PRACTICES

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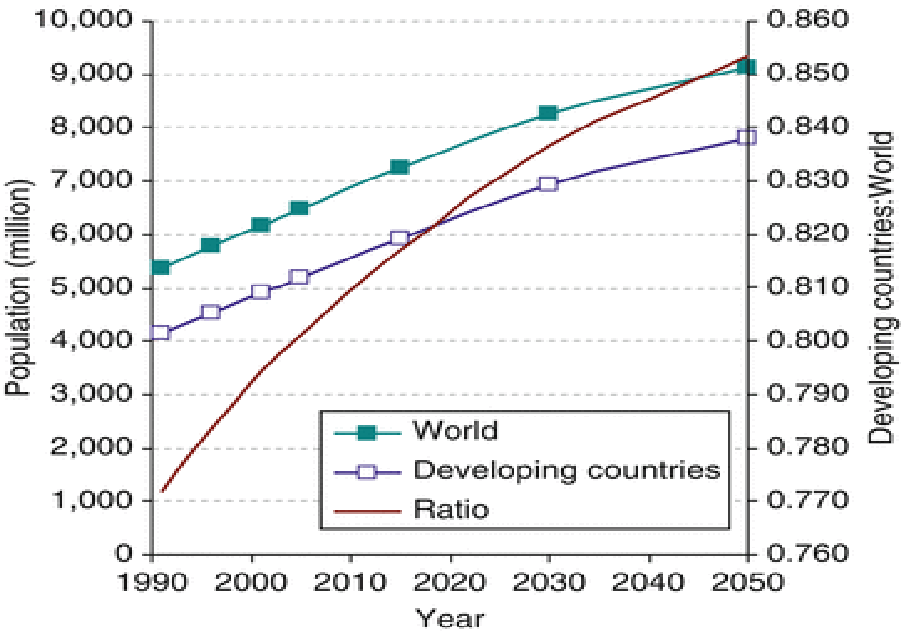
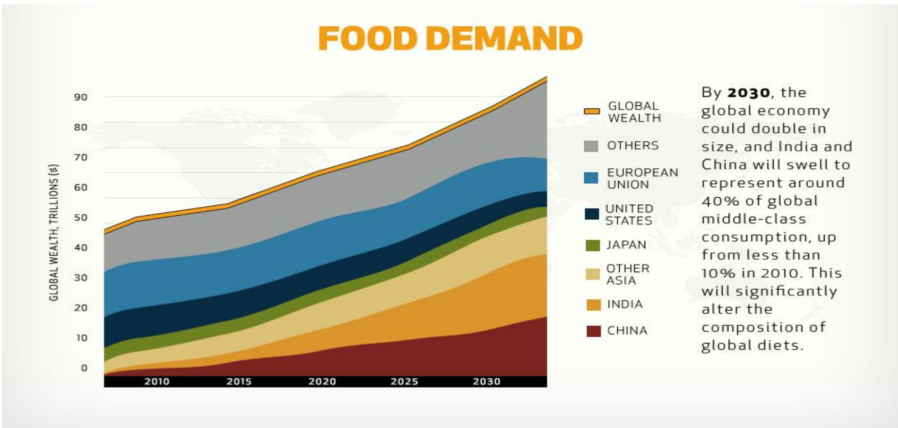
Abstract: *The increase in worldwide population observed within the last decades has contributed to an accrued demand for food supplies, which might solely be attained through an improvement in agricultural productivities. Moreover, agricultural practices should become more sustainable, because the overuse of chemically-based fertilisers, pesticides and growth stimulants will cause serious environmental problems (Gonçalves, 2021).*
One new approach for the development and extremely effective agriculture is the use of biological compounds like the extract of microalgae biomass.
Currently, microalgae are attracting the interest of agrochemical industries and farmers, because of their biostimulant (MBS) and biofertiliser (MBF) properties (Ronga et al., 2019). Until recently, microalgae were mostly investigated as a practical approach for the production of lipids and for environmental purposes, like the mitigation of greenhouse gas (CO_2) emitted by industrial processes, and for effluent treatments (Oancea et al.,2013; Gonçalves,2021).
On the opposite hand, investigations of microalgal product appropriate for crop productions stay largely unexploited.
In the light of this point, this study highlights a number of the present researches and future development priorities and examining the factors supporting the employment of MBS and MBF for managing crop productions and abiotic stresses.

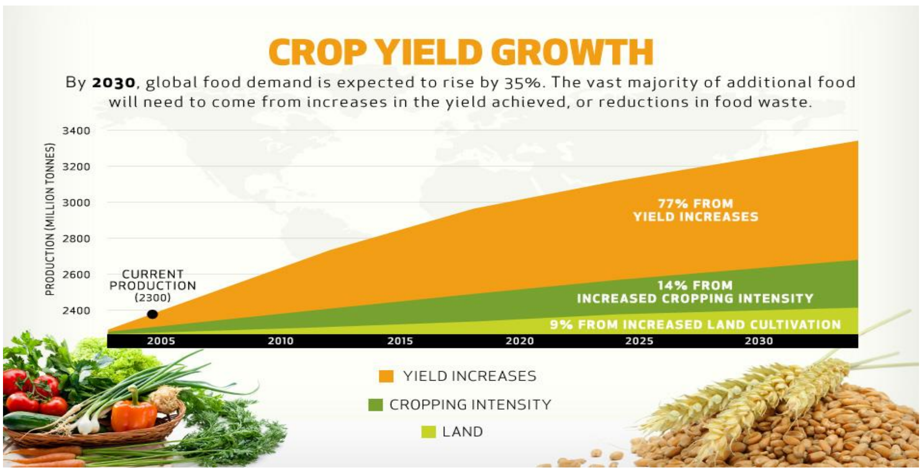
Keywords: *Abiotic stresses, Biofertiliser, Biostimulant, Microalgae, Crop productions.*

INTRODUCTION

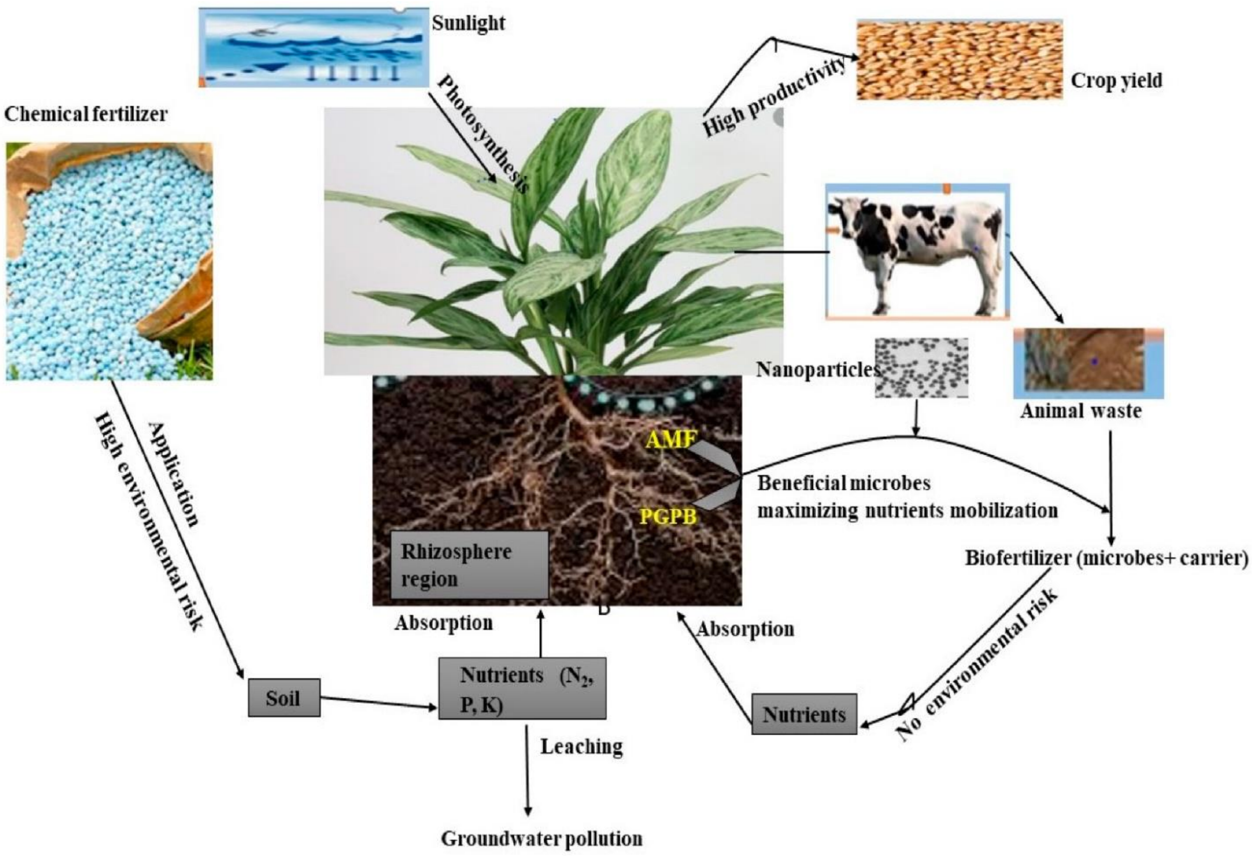
The increase in worldwide population observed within the last decades has contributed to an accrued demand for food supplies, which might solely be attained through **an improvement in agricultural productivities.**



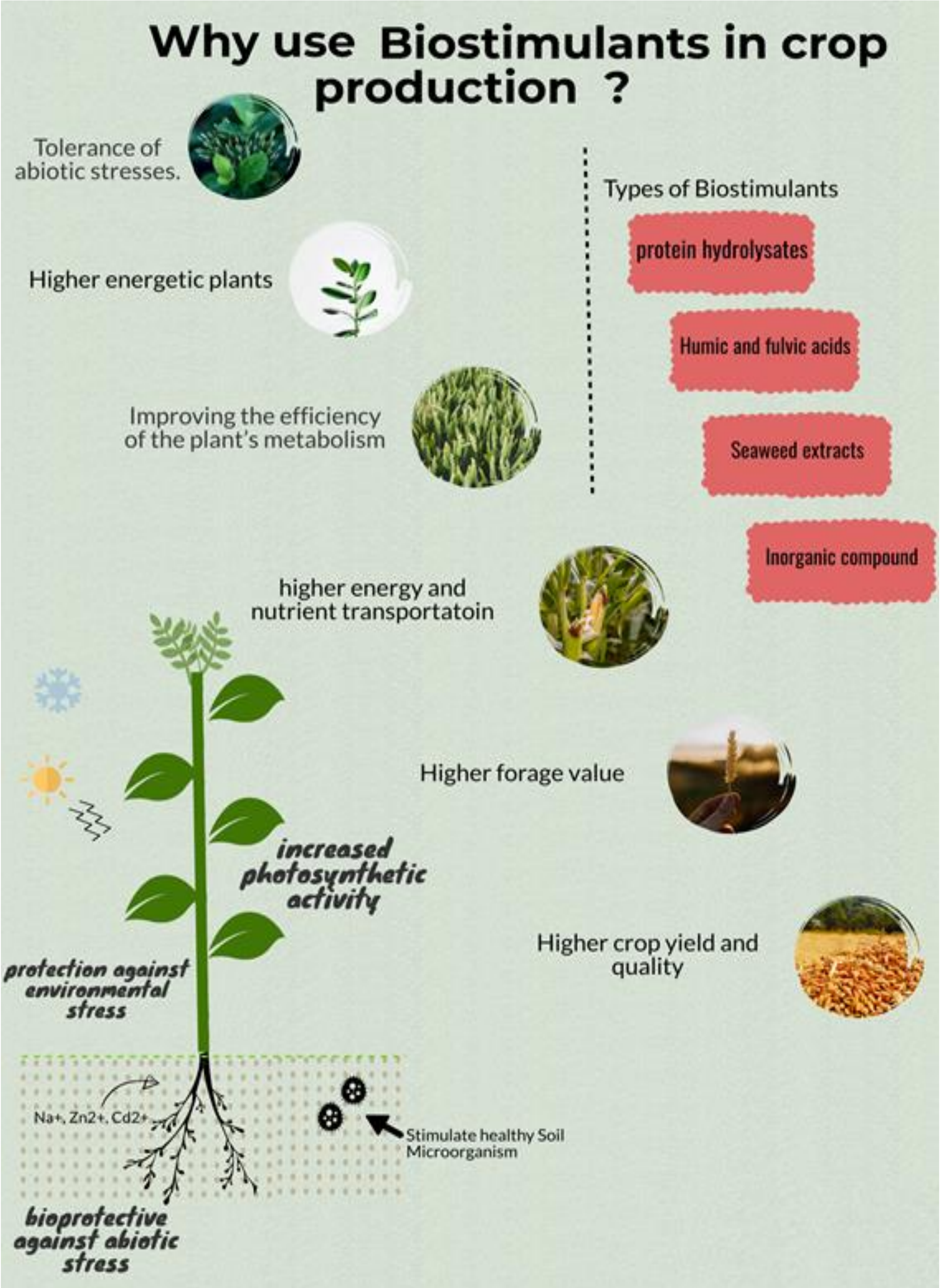




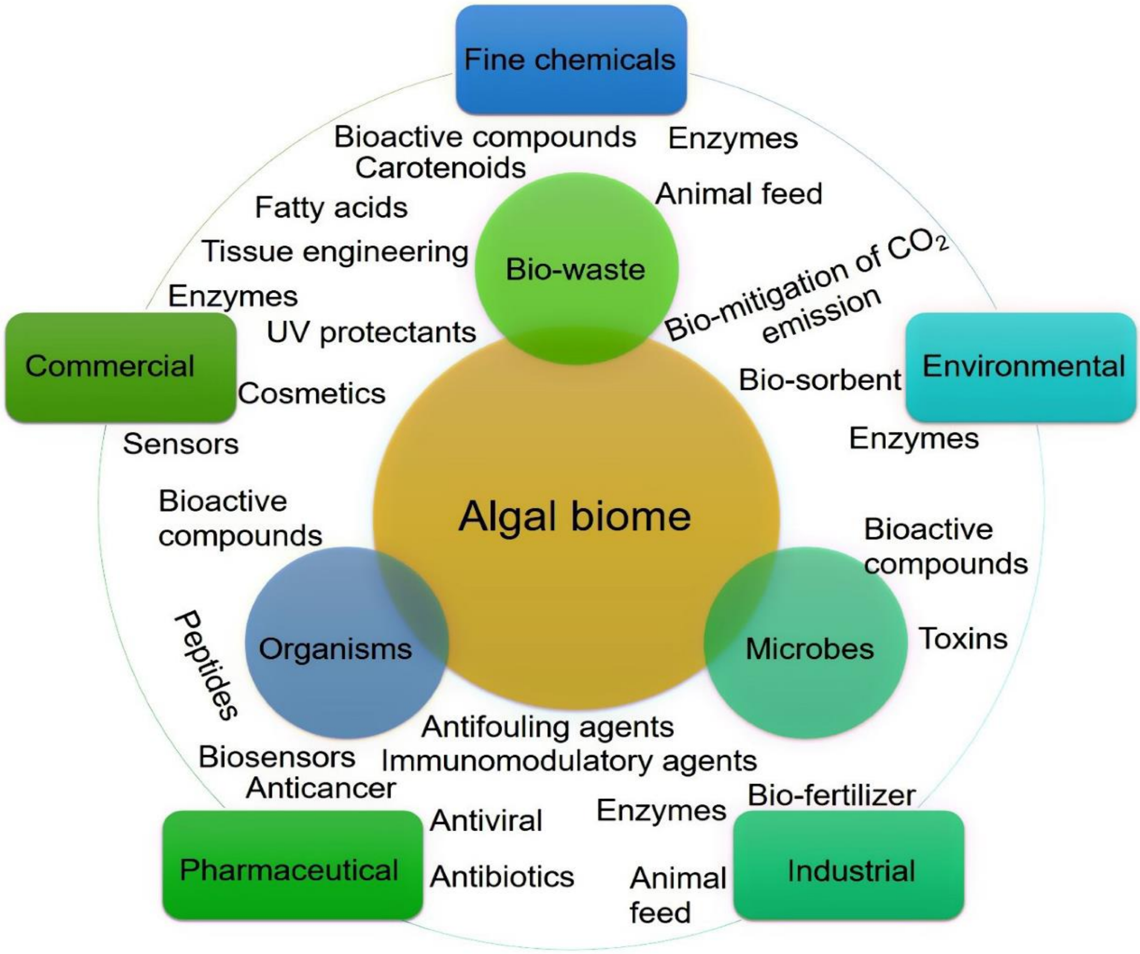
Moreover, agricultural practices should become more sustainable, because the **overuse of chemically-based fertilisers**, pesticides and growth stimulants will cause **serious environmental problems** (Gonçalves, 2021).

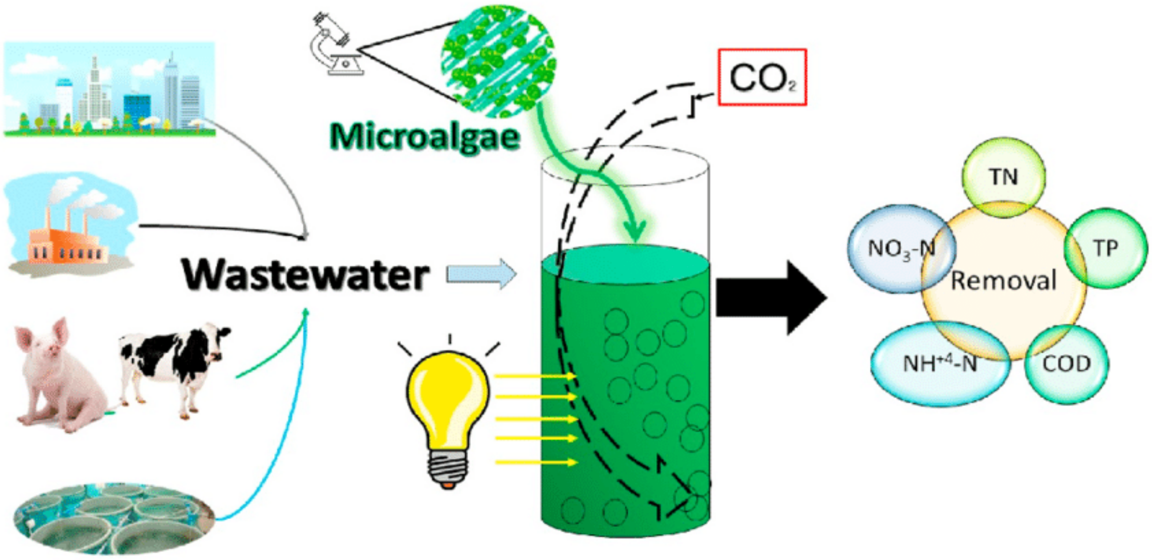

Sunlight
Photosynthesis
High productivity
Crop yield
Chemical fertilizer
Nanoparticles
Animal waste
AMF
PGPB
Beneficial microbes
maximizing nutrients mobilization
Rhizosphere
region
Biofertilizer (microbes+ carrier)
Application
High environmental risk
No environmental risk
Absorption
Absorption
Soil
Nutrients (N2, P, K)
Nutrients
Leaching
Groundwater pollution

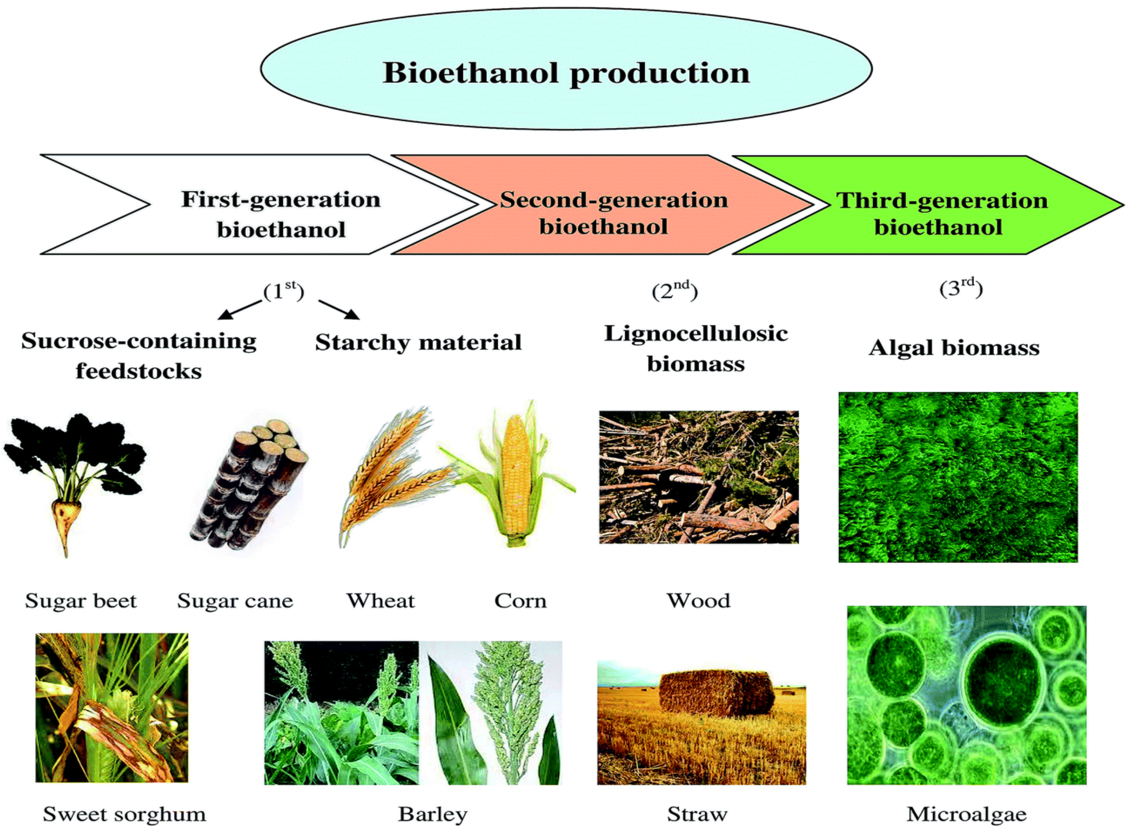
One new approach for the development and extremely effective agriculture is the use of **biological compounds** like **the extract of microalgae biomass**. Currently, microalgae are attracting the interest of agrochemical industries and farmers, because of their biostimulant (MBS) and biofertiliser (MBF) properties (Ronga et al., 2019).



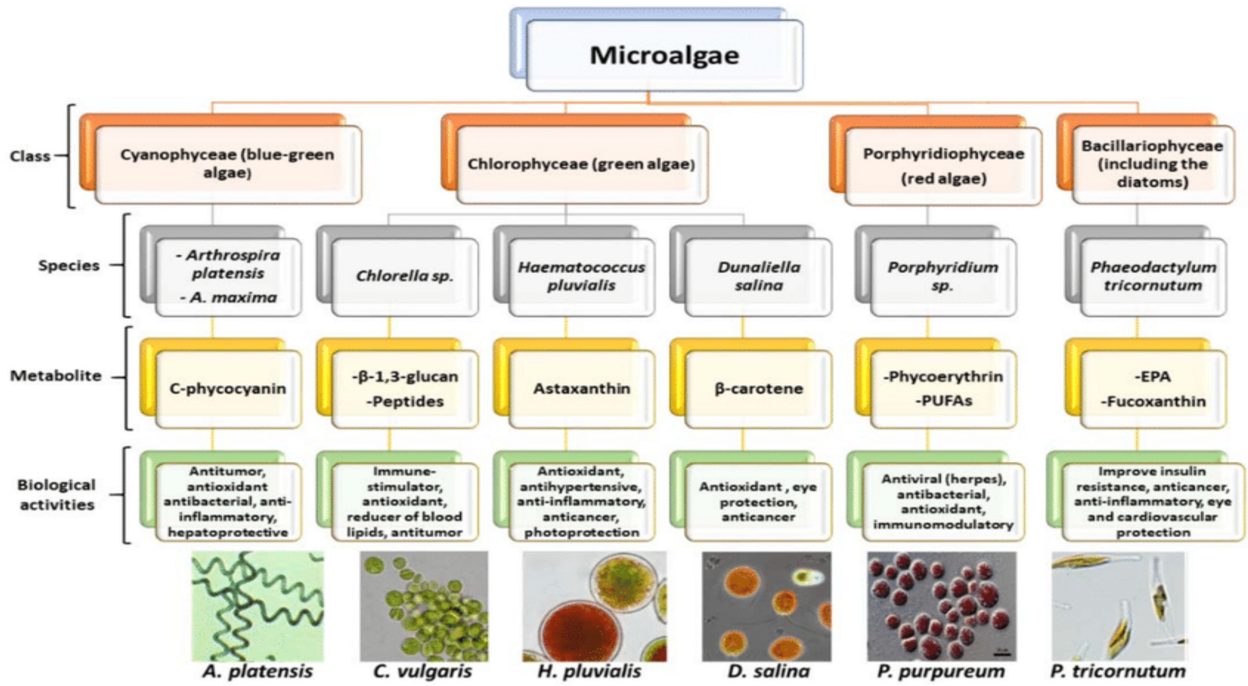
Until recently, **microalgae** were mostly investigated as a practical approach for the **production of lipids** and for environmental purposes, like the **mitigation of greenhouse gas (CO_2)** emitted by industrial processes, and for **effluent treatments** (Oancea et al., 2013; Gonçalves, 2021). On the opposite hand, investigations of microalgal product appropriate for **crop productions** stay largely unexploited.


Fine chemicals
Bioactive compounds
Enzymes
Carotenoids
Animal feed
Fatty acids
Tissue engineering
Bio-waste
Bio-mitigation of CO2 emission
Enzymes
UV protectants
Commercial
Cosmetics
Bio-sorbent
Environmental
Sensors
Enzymes
Bioactive compounds
Algal biome
Bioactive compounds
Peptides
Organisms
Microbes
Toxins
Antifouling agents
Biosensors
Immunomodulatory agents
Anticancer
Bio-fertilizer
Antiviral
Enzymes
Pharmaceutical
Industrial
Antibiotics
Animal feed


Microalgae
CO2
Wastewater
TN
NO3-N
TP
Removal
NH+4-N
COD

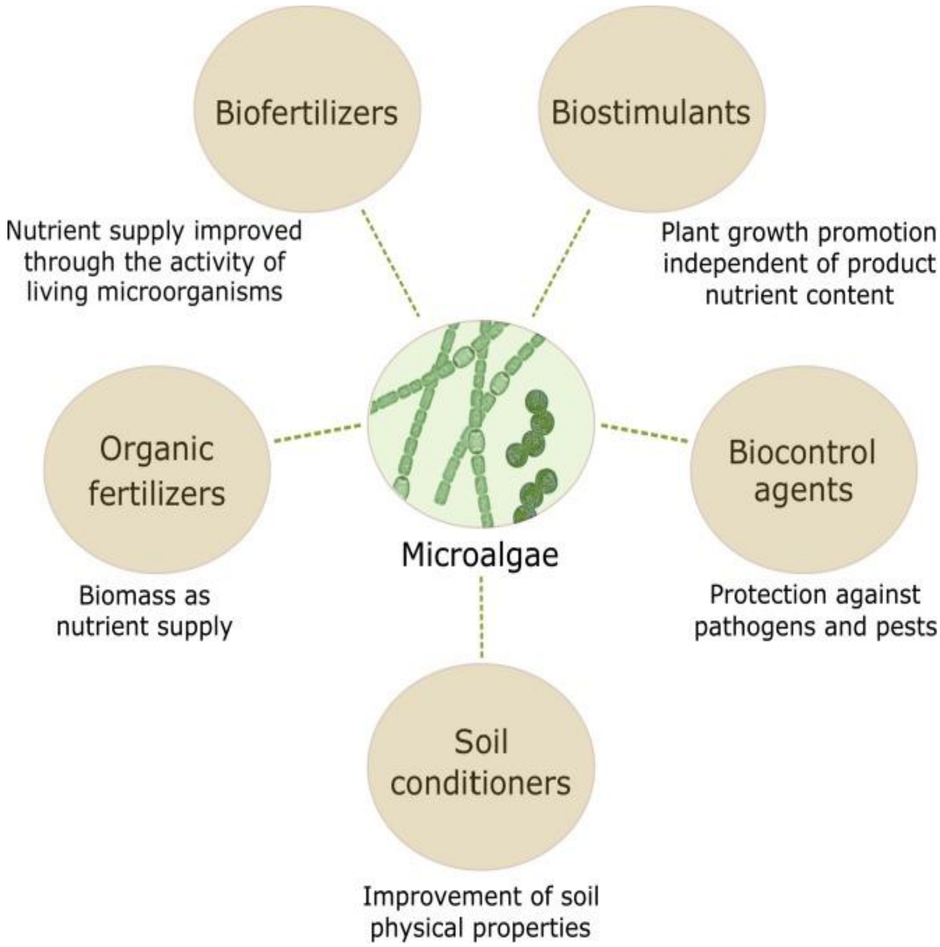


Many studies demonstrated the remarkable potential of compounds extracted from algae and cyanobacteria such as **phenols, terpenoids, free fatty acids, polysaccharides, and carotenoids** in crops production.



Main microalgae classes, their most important species, and associated biological activity "microalgae images were obtained from the "Microalgae strain catalogue", second edition, published in the Enhance Microalgae Project".

Furthermore, **microalgal metabolites** can play an important role in **soil decontamination and fertilization**; **plant protection** against biotic and abiotic stress factors; and plant development. Moreover, microalgae and cyanobacteria also present **phytohormones**, which are known for their activity as **plant-growth promoters.**



As **algal biomass represents an important resource for developing sustainable and environmentally friendly approaches to agriculture** besides forming the base of food webs, a definite need exists to create greater awareness and facilitate in-depth research to diversify and intensify their applications in the years to come.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TL11.

THE EVALUATION OF PESTICIDES ON OPPORTUNISTIC *PSEUDOMONAS AERUGINOSA* AND ITS ANTIBIOTIC RESISTANCE

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Abstract: Background*: Nowadays, researchers have pay attention to the various effects of micro-pollutants in the environment. In the present thesis, I focused on the effect of pesticides on the growth and antibiotic resistance profile of* Pseudomonas aeruginosa*, an emerging opportunistic pathogen microorganism.* ***Method****: 5 ppm concentrations of three pesticides (glyphosate, S-metolachlor, and terbuthylazine) were used for the treatment of 5 clinical and environmental strains of* P. aeruginosa*. Optical density of the treated bacterial cultures was detected after 1, 3, 24, 48, and 120 h to determine growth curves. Antibiotic resistance against 10 chosen agents was tested by MIC test strips (Liofilchem) containing a quantitative gradient of antibacterial drugs. Pesticide degradation rate of the examined strains was determined by gas chromatography.* ***Results****: Glyphosate and S-metolachlor both could be utilized as nutrients by the examined strains; therefore in the examined concentration they did not inhibit the growth of* P. aeruginosa *irrespectively to the origin of the strain (clinical or non-clinical). By contrast, terbuthylazine might have a negative effect on P. aeruginosa and limits its growth, but the differences were not significant.* P. aeruginosa *strains could degrade the S-metolachlor around 34-54% and terbuthylazine around 21-54% in 5 days. In addition, glyphosate caused a significant increase of MIC values on cefepime and ticarcillin and decreased the MIC values on tigecycline. S-metholachlor and terbutylazine have slightly increased the resistance of P. aeruginosa to ticarcillin, too but no other changes were observed.*

Keywords: *Antibiotic resistance, micro-pollutants, pesticides, Pseudomonas aeruginosa*

INTRODUCTION

With the rapid growth of population and the gradual improvement of human living standards all over the world, the requirements for agriculture have been correspondingly increased, thus, a lot of chemical products have been used to increase the amount of agricultural production. One group of the widely used chemical products is pesticides. Pesticides are used to control factors that may have a negative effect on crops or plants. But in recent years, it was increasingly reported that pesticides can cause serious pollution in the environment. As it was recently verified, pesticides may have various sublethal effects on natural microbiota as well.

Antibiotic resistance, namely the ability of some bacteria to evolve resistance to several classes of antibiotics, is an emerging issue all around the world mainly caused by the improper use the antibiotics. WHO has classified antimicrobial resistance as a widespread and serious threat to humans of any age, in any country (WHO, 2014). One of the most dangerous emerging bacterial species with the ability to cause disease and develop antibiotic resistance is *P. aeruginosa*, an opportunistic microorganism that can be detected in various environments.

Our knowledge about the effects of low concentration agrochemicals on emerging opportunistic pathogens is still limited, therefore our research aimed to determine the effects of some chosen pesticides on the growth rate, antibiotic resistance, and the degradation rate of the chosen bacterial species *P. aeruginosa*. Based on our results, we could analyse the effects of pesticides on the environment from a new aspect. The major steps of our investigation were as follows:

- Based on the scientific literature, we have chosen three pesticides, that are (considering their annual volume and health effects) the most important emerging micro-pollutants in Hungary and Europe: glyphosate, S-metolachlor, and terbuthylazine (Hvězdová et al., 2018).
- We have chosen one of the most important environmentally relevant opportunistic microorganisms, *P. aeruginosa*, and obtained five reference strains of this species for the planned investigations
- We have set a 5-day long experiment and checked the initial and final antibiogram, the growth rate, and the degradation capacity of the examined strains

MATERIALS AND METHODS

The main phases of the experiment are summarized in Figure (1).

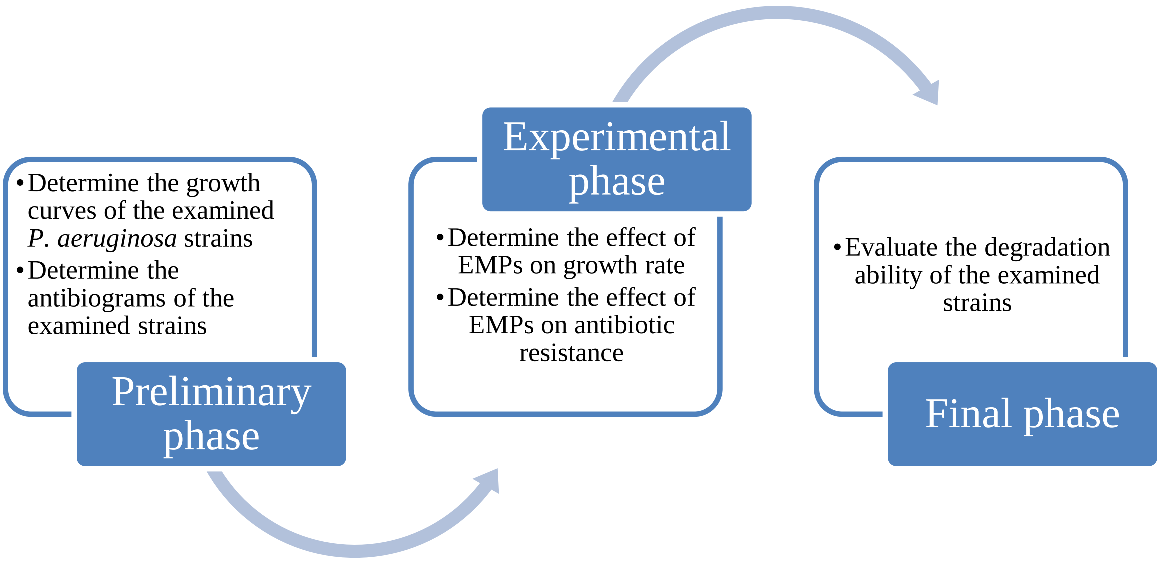

Figure (1): The summary of experimental processes

Bacterial strains and their origin

Five different reference strains of opportunistic pathogen *P. aeruginosa* were obtained from the National Collection of Agricultural and Industrial Microorganisms (NCAIM, MATE, Hungary) and the strain collection of the Department of Environmental Safety (MATE). The origin and the known properties of the examined strains are summarized in Table 1 and Figure 1.

Table (1): The origin and main properties of the examined P. aeruginosa strains

Marks	Culture collection no.	Other collection no.	Origin	Main features
1	ATCC 27853	B.01876, CECT 108, etc.	Clinical (isolated from blood)	Quality control strain for antibiotics susceptibility tests
2	ATCC 15442	B.02060, B.02358, etc.	Animal drinking water	Disinfectant and preservation testing (European standard EN 1040)
3	ATCC 10145	B.02040, DSM 50071	Non-clinical	Quality control strain for API and Sensitise products
4	PAO 11	B.02360, DSM5168	n.d.	Plasmid pMO821
5	P66	-	Zalaegerszeg, Hungary, Hydrocarbon contaminated groundwater	Environmental strain from the Department collection

Figure (2): Liquid cultures of the examined *P. aeruginosa* strains (credit: D. Jiang)

Experimental settings

Each experiment was set in Luria-Bertani (LB) medium (10.0 g tryptone, 5.0 g yeast extract, and 9.0 g NaCl in 1000 ml distilled water). The experiment was performed in triplicates with all five bacterial strains therefore, 15 flasks with 45 ml LB were used for each herbicide experiment with an abiotic control of herbicides. LB medium in conical flasks was supplemented with 5 ml of overnight (<24h) bacterial suspension with an optical density of OD_{600}: 0.6 checked on GenesysTM 10S UV-Vis Spectrophotometer (Thermo Scientific). See Figure (3).
Glyphosate (Pestanal®, CAS 1071-83-6), S-metolachlor (Pestanal®, CAS 87392-12-9), and terbuthylazine (Pestanal®, CAS 5915-41-3) were obtained from Sigma Aldrich Ltd. Stock solutions were prepared in dimethyl sulfoxide (DMSO, CAS 67-68-5, purity ≥99.99%, Fisher Scientific) with a final concentration of 2500ppm (Tóth et al., 2019). Herbicide concentrations in conical flasks were set to 5 ppm. Flasks were inoculated in Certomat BS-1 rotary shakers (Sartorius) at 28°C, with 170 rpm (round per minute) for 120h.

Determination of growth curves

This experiment was set to determine the growth curves of the selected strains of *P. aeruginosa* which were treated by glyphosate, S-metolachlor, and terbuthylazine separately. The growth rate was determined using the optical density of bacterial cultures determined by GENESYS™ 10S UV-Vis Spectrophotometer at 600 nm wavelength (Figure 3 and Figure 4).

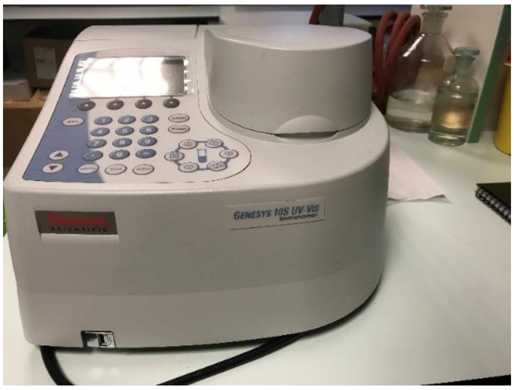

Figure (3): The Genesys 10S UV-Vis spectrophotometer which was used during the lab work (credit: D. Jiang)

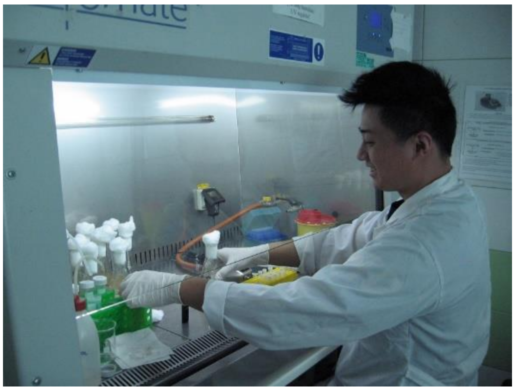

Figure (4): The operational process of measuring the optical density (credit: E. Kaszab)

During the 120 hour long experimental period, OD values were determined in the 1st, 2nd, 4th, 8th, 16th hours and then once in every 24 hours. Data on the detected OD values were visualized and analysed by GraphPad Prism 7. Software.

Antibiotic resistance

The antibiotic resistance assay was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006) using MIC Test Strips (MTS) (Liofilchem) to determine Minimal Inhibitory Concentrations. The principle of the antibiotic resistance investigation is to prepare a bacterial suspension of the examined strain in physiological saline with 0.5 McFarland density and spread onto the surface of Mueller Hinton agar (Sigma Aldrich). MTS is positioned by sterile forceps on the surface of the medium (see Figure 5.) and plates are incubated at 35°C for 24h. According to the visible breakpoints, we can determine and compare the minimal inhibitory concentrations (MIC values) to the available database of EUCAST to characterize the bacterial strain as resistant (R), intermediate (I), or sensitive (S).
The list applied antibiotics and their evaluation based on their MIC values are summarized in Table (2). (source: http://www.eucast.org/clinical_breakpoints/)
As a baseline, we have determined the antibiotic resistance profiles (antibiograms) of each strain without pesticide treatment. Treated bacterial strains were re-isolated after 120h incubation. Triplicate samples were mixed, and their mixture was used for antibiotic resistance assay.

Table (2). The examined antibiotics and their evaluation based on their MIC values (EUCAST)

Name of antibiotic	MIC breakpoints	
	S ≤	R >
CEFTAZIDIME	0.001	8
CEFEPIME	0.001	8
IMIPENEM	0.001	4
MEROPENEM	2	8
TICARCILLIN	0.001	16
GENTAMICIN	4	4
TOBRAMYCIN	4	4
CIPROFLOXACIN	0.001	0.5
COLISTIN	2	2
TIGECYCLINE	1	2

Figure (5): The results of the experiment of antibiotic resistance

Examination of degradation rate

After determining the growth curves and the antibiotic resistance profile of the examined strains, samples were centrifugated for 30 min at 3220g, supernatant was filtered (using a syringe filter with 0.45 µm pore size) and sent to the Wessling Hungary Ltd. analytical laboratory to determine the residual amounts of pesticides. Analysis was performed according to the MSZ EN 15662:2018 standard for the determination of pesticide-residues using GC- and LC-based analysis.

RESULTS AND DISCUSSION

The effect on micro-pollutants on the growth rate of *P. aeruginosa*

Results of growth rate experiments of Control (untreated) group, Glyphosate group, S-metolachlor group, and Terbuthylazine group are summarized in Figure (6).

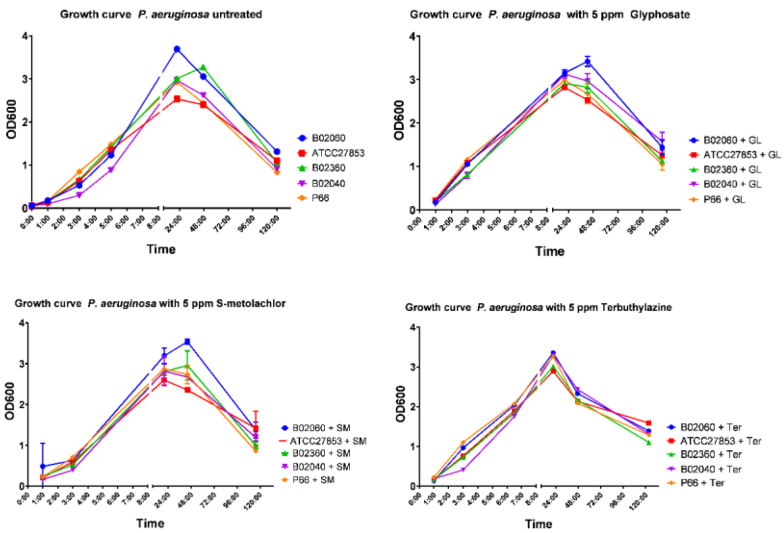

Figure (6): Growth curves of the examined strains of *P. aeruginosa* with different treatments

There were many similarities of these four sets of growth curves, for example, all tested strains were intensively developing in the first 24 hours, and then the inflection point occurred from 24 hours to 48 hours.

To give an overall evaluation of the 4 treatment, strain B02060 was detected as the most intensively growing strain in all groups with different treatments. Strain ATCC27853 had the lowest peak point comparing to other *P. aeruginosa* strains in all groups. Based on scientific sources, glyphosate could be utilized as a nutrient by various microbial species (Martinez et al., 2018) and S-metolachlor could also promote the growth of some plants as nutrition (Clewis et al., 2006). Thus, it is possible that the glyphosate and S-metolachlor might be used by *P. aeruginosa* as nutrients. At the same time, terbuthylazine may have slightly negative effect on *P. aeruginosa* strains. According to Tasca et al. (2018), the reason might relate to that the terbuthylazine is a chemical of emerging concern due to its persistence, and toxicity to aquatic organisms. Furthermore, the terbuthylazine has endocrine disruption capacity to wildlife and humans (Tasca et al., 2018).

Antibiotic resistance

Antibiotic resistance results of the examined strains (ATCC27853, B02040, B02060, B02360, and P66) in all settings (Control, Glyphosate, S-metolachlor, Terbuthylazine) for the nine examined antibiotics are shown in Figure 7. Average values with standard deviations are summarized in Figure (8). As supporting information, threshold values of antibiotic sensitivity (green line) and antibiotic resistance (red line) according to EUCAST are displayed.

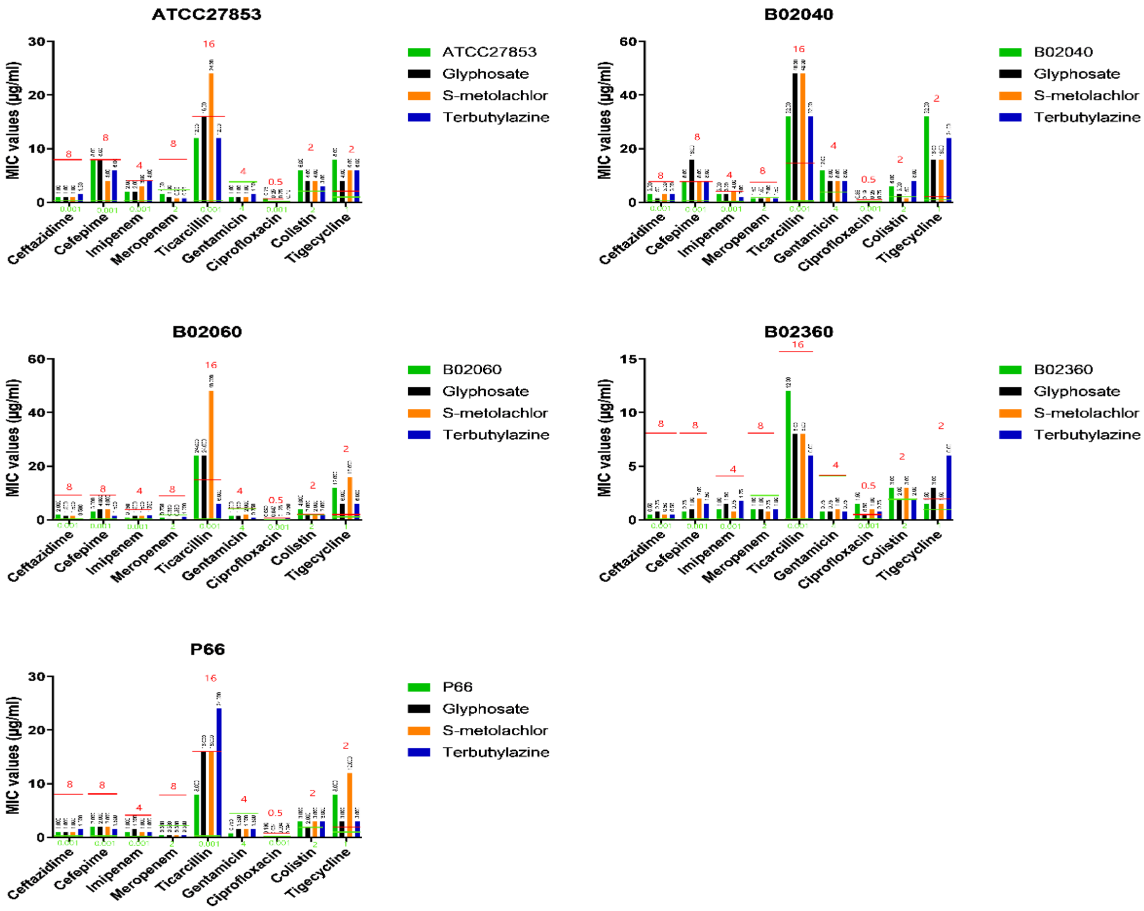

Figure (7): Antibiotic resistance of the examined *P. aeruginosa* strains ATCC27853, B02040, B02060, B02360, and P66 to antibiotic Ceftazidime, Cefepime, Imipenem, Meropenem, Ticarcillin, Gentamicin, Ciprofloxacin, Colistin, and Tigecycline respectively, under the condition of untreated, Glyphosate-treated, S-metolachlor-treated, and Terbuthylazine-treated.
Resistant threshold - red line; sensitive threshold - green line.

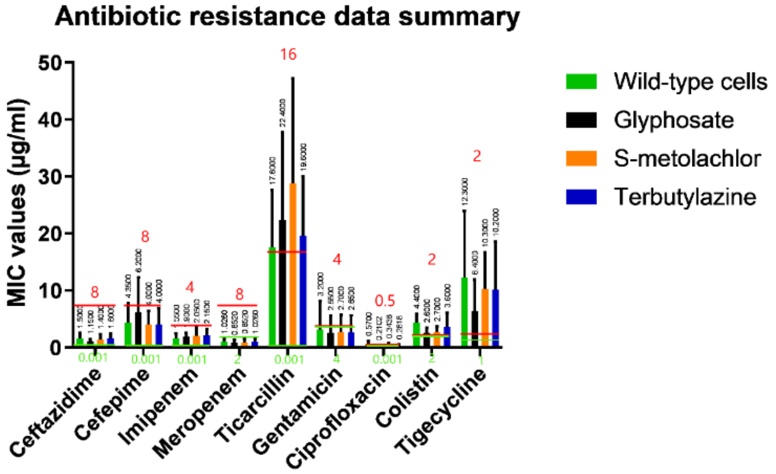

Figure (8): The average values of antibiotic resistance data of *P. aeruginosa* strains for different treatments

Based on the results displayed on Figure (7) and Figure (8), we can conclude that the vast majority of the examined *P. aeruginosa* were resistant to colistin irrespectively to their origin or treatment. For colistin resistance, antibiotic resistance is known and naturally occurs in some genera such as *Proteus*, *Serratia*, and *Burkholderia*, but not *P. aeruginosa*, which is well known to develop acquired resistance (Olaitan et al., 2014). In future experiments, it is crucial to evaluate the mechanism behind the detected colistin resistance (Gharaibeh and Shatnawi, 2019).

Tigecycline resistance was also detectable among untreated strains. Based on scientific literature, it can be a result of efflux-mediated mechanisms (Dean et al., 2003). The control cases were intermediate to ceftazidime, cefepime, imipenem, and were sensitive to meropenem in all the experiments. In control cases, nearly all strains were sensitive to gentamicin, with the exception of B02040 isolated from non-clinical sources. Overall, the control cases were resistant to colistin and tigecycline in all cases and were occasionally resistant to ticarcillin and ciprofloxacin.

Based on Figure 8, and according to the analysis of Figure 8, we could see some minor effects of the three tested pesticides on antibiotic resistance.

- Glyphosate caused a significant increase of MIC values on cefepime and ticarcillin and decreased the MIC values on tigecycline, however, but these slight changes had no effect on the interpretation categories of these antibiotics. There was no obvious change in the susceptibility to other antibiotics.
- S-metolachlor treatment caused an increased level of antibiotic resistance in one case only, (ticarcillin) and the effect was more pronounced than in the glyphosate and terbutylazine-treated groups. Other changes in susceptibility were not significant.
- In the terbuthylazine-treated group, the examined strains increased their resistance to ticarcillin just like in the S-metolachlor-treated group. However, the alteration in antibiotic resistance was insignificant.

Examination of the pesticide degradation

Comparing the residual amounts of pesticides with the initial concentrations (5 ppm) corrected with the abiotic loss measured in the non-inoculated control samples; we could calculate the rates of pesticide-biodegradation for S-metolachlor and terbuthylazine (Table 3). Due to technical problems, glyphosate degradation could not have been determined.

Table (3): The S-metolachlor and terbuthylazine degradation rates of the examined P. aeruginosa strains

P. aeruginosa strains	S-metolachlor average degradation rate (%)	Terbuthylazine average degradation rate (%)
1. B02060	34.0%	35.2%
2. ATCC27853	44.6%	54.2%
3. B02360	45.8%	21.3%
4. B02040	54.6%	39.0%
5. P66	35.7%	24.0%

According to our calculation, strain B02040 could degrade the highest rate of S-metolachlor during the experiment with an average degradation rate of 54.6%. Comparing to other strains, B02060 and P66 were weak degraders of S-metolachlor, the average of degradation rates reached 34% and 36%, respectively. Regarding terbuthylazine, degradation rates varied between 54.2% and 21.3%.
Overall, *P. aeruginosa* strains had a higher potential to degrade S-metolachlor than terbuthylazine, but there was no clear correlation between the origin and the degradation capability of the examined strains.
Considering the relatively low residual concentrations in the abiotic control samples, degradation assay should be repeated to get a more reliable view on the degradation capacity of *P. aeruginosa* strains.

CONCLUSIONS AND RECOMMENDATIONS

Nowadays, in the healthcare system infections caused by antibiotic-resistant bacteria mean a severe problem, threaten personal health, and have serious economic effects including the cost of therapy and all additional expendables. Treatment of resistant infections is associated with higher costs for second-line drugs, additional investigations, and longer hospitalization (Coast et al., 1996). Other indirect costs associated with AMR include productivity losses due to excess morbidity and premature mortality (Shrestha et al., 2018). Therefore, it is important to gain knowledge about emerging opportunistic bacteria, such as species *P. aeruginosa*, and the factors affecting its survival and antibiotic resistance profile. Antibiotic resistance could be developed by enhanced evolvability (Spiers et al., 2000), or acquiring from genotypic diversity (mutation or horizontal gene transfer) (Carmeli et al., 1999), normally the resistance will be increased continuously (Hughes and Andersson, 2017).
Based on our experiences there are cases that pesticides can enhance antibiotic resistance of *P. aeruginosa* which is in accordance with the explanation that environmental factors around bacteria increasing the selection of antibiotic resistant strains (Kurenbach et al., 2018). Strains that were used in the series of experiments were the same species, *P. aeruginosa*, but the origin of strains was different. It was reported that the different origins of isolation may cause the different phenotypical traits of *P. aeruginosa*, even if genomes are almost identical (Grosso-Becerra et al., 2014) that can be the reason of the slight differences in growth and antibiotic resistance.
In conclusion, based on the results and analyses in my thesis work, the micro-pollutants did affect species *P. aeruginosa*. According to our experiments, glyphosate and S-metolachlor did not inhibit the growth of the examined *P. aeruginosa* strains in the applied concentration (5 ppm). It is presumed that P. aeruginosa can utilize the examined pesticides as a carbon source, since *P. aeruginosa* strains could degrade S-metolachlor around 34-54% in 5 days.
By contrast, terbuthylazine may have a slightly negative effect on *P. aeruginosa* to limit its growth, but still *P. aeruginosa* strains could degrade terbuthylazine around 21-54% in 5 days. Altogether, we can conclude that the examined pesticide concentrations did not inhibit the growth of *P. aeruginosa*.

Based on the antibiotic resistance assay, there were only a few cases when the antibiotic resistance was increased by the examined pesticides.
The detected changes in MIC values were not significant, which is not supporting the findings of Plotkin et al. (2003). But still, we have found alterations and the reason for these differences should be clarified in future experiments with the application of a larger set of *P. aeruginosa* strains from various origins. In the future, another set of antibiotics should be also tested to get a better picture of this topic.
Our investigations prove that our knowledge is still limited on the sublethal effects of micro-pollutants. In the future it is important to reach deeper and extended data to identify the potential consequences arising from the application of pesticides.

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TL12.

ECOTOXICOLOGICAL EVALUATION OF BORON, ARSENIC, IODINE AND SELENIUM ON REPRODUCTION, MORTALITY AND AVOIDANCE OF FOLSOMIA CANDIDA (COLLEMBOLAN)

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Abstract: *Soil plays a significant role in the ecosystem. Soil pollutants have increased largely from a wide range of sources in the last decades. Trace elements accumulated in soil because of anthropogenic activities (e.g. industry, agricultural production, and health products) may pose a potential risk to environmental safety. Therefore, it is necessary to evaluate the ecotoxicological effects of trace elements on soil model animals. To evaluate the ecotoxicological impacts of four trace elements (As, B, Se, and I), the 28-days long tests based on the OECD guideline 232 had been executed with Folsomia candida (Collembola). The chosen trace elements were mixed with OECD artificial soil in the following concentrations [mg/kg]: 0, 0.0023, 0.0069, 0.0205, 0.0617, 0.185, 0.555, 1.66, and 5 for selenium; 0, 0.5, 7.5, 11.25, 16.875, 25.312, 37.969, and 56.953 for iodine; 0, 0.0002, 0.001, 0.005, 0.025, 0.125, 0.625, 3.125, and 15.625 for boron; 0, 0.01, 0.03, 0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 for arsenic. Meanwhile, 48-hours sublethal test (avoidance tests) had been carried out with Folsomia candida. Survival, reproduction, and avoidance behavior of F. candida were regarded as endpoints with the main ecotoxicological values (NOEC, LOEC, EC_{50}, and LC_{50}). The four trace elements all showed toxic effects on F. candida in different degrees and they had similar toxicity on the survival of F. candida, while boron showed higher toxicity on reproduction than the others. The survival of F. candida was more sensitive than reproduction to each element. Avoidance behaviour of F. candida was the most sensitive to boron, followed by arsenic and iodine, and finally selenium. In this study, boron and arsenic appeared to be potential risks in the natural fields.*

Keywords: *Environmental protection, trace elements, Folsomia candida, soil pollution, ecotoxicological toxicity*

INTRODUCTION

Trace elements are defined as elements occurring at low concentrations (mg/kg or less) in most soils, plants, and living organisms (He et al., 2005). Trace elements can be present in the environment naturally; some of them play influential roles in the growth of plants, animals, and humans as essential nutrients (Hooda, 2010). For example, copper (Cu) and boron (B) are essential for the growth of the plant. Selenium (Se) and iron (Fe) are necessary for the growth and health of animals and humans (He et al., 2005). The presence of trace elements can be natural and anthropogenic (He et al., 2005).

However, the increased amount of trace elements in the natural environment by human activities can generate a series of ecological and health problems (Hooda, 2010). Research by Wang et al. (2020) showed that trace elements were found to be high soil contamination risk (Wang et al., 2020). Their toxicity can affect the organisms' surrounding environment or directly affect the organisms through the food chain (Gupta and Gupta, 1998). It's worth remarking that trace elements appear in different amounts in different regions. For instance, the northeast of China is a typical selenium-deficient region, however, Enshi city in Hubei province which in the southwest of China has a wide range of selenium poisoning due to superabundant selenium in soil and drinking water (Yang et al., 2007).

According to the previous work, *Folsomia candida* is a standard test species to evaluate the ecotoxicological effects of trace elements in soil (Fountain and Hopkin, 2005). *F. candida* belongs to the order Collembolan that is a very common and abundant arthropod living in soil all over the world. *F. candida* is quite easy to maintain in the laboratory. Moreover, several official guidelines are available to this species, ISO guideline 11267 (ISO 11267 1999) and OECD 232 (OECD/OCDE 232, 2009) for the Collembolan reproduction test has been approved successfully for toxicity testing on soil contamination (Krogh et al., 2008). Therefore, evaluating the effects of soil contamination by using ecotoxicological testing on *F. candida* has evidence supporting and scientific values.

Therefore, this study aimed to evaluate the ecotoxicological effects of four trace elements (selenium, iodine, boron, and arsenic) on the survival and reproduction of *F. candida* according to the OECD guideline 232. The aqueous stock solutions containing four trace elements were sodium selenate decahydrate ($Na_2SeO_4{\cdot}10H_2O$), potassium iodide (KI), boric acid (H_3BO_3 or $B(OH)_3$), and sodium arsenate heptahydrate ($Na_2HAsO_4{\cdot}\ 7H_2O$) respectively. Also, the avoidance tests had been carried out to support the OECD tests and evaluate the results filed tolerance. Moreover, the endpoints were survival, reproduction, and avoidance behavior of *F. candida* supported by the main ecotoxicological values (NOEC, LOEC, EC_{50}, and LC_{50}).

MATERIALS AND METHODS

The *F. candida* used in our present study was bred on the laboratory culture mediums. The culture mediums had been contained in 9 cm diameter plastic dishes containing a mixture of plaster of Paris, active charcoal, and tap water in a ratio of 10:1:10. The culture medium in which *F. candida* lives were stored in the thermostat at a temperature 20 ± 0.2°C. The supply of water and food (baker's yeast) was added weekly and the Petri dishes were aerated during the process. To achieve accurate results of reproduction and mortality tests, the organisms had to be synchronized to gain the same age colony which according to the OECD guideline was 9-12 days old at the time of tests (OECD/OCDE 232, 2009).

For OECD reproduction tests, the aim was to evaluate the chronic toxic effects of the four trace elements on *F. candida*. Mortality and reproduction tests were applied based on the guideline OECD 232 (OECD/OCDE 232, 2009). The artificial OECD soil was composed of 5% air-dried and finely grand sphagnum peat, 20% kaolin clay, 74% air-dried industrial sand, and 1% calcium carbonate (OECD/OCDE 232, 2009). Selenium, iodine, boron, and arsenic were added as liquid solutions, which were diluted from their stock solutions respectively. The stock solutions used for preparing the test concentration were $Na_2SeO_4{\cdot}10H_2O$, KI, $B(OH)_{3,}$ and $Na_2HAsO4{\cdot}7H_2O$ for each trace element. The concentration of the mentioned stock solutions was all 1 g/L.

To have a valuable test concentration series: The range finding tests were applied for iodine, boron, and arsenic. It had been done earlier regarded as the range-finding test for selenium; therefore, it was not a part of this study. The range-finding test concentration series for iodine and arsenic were same: 0 mg/kg, 0.005 mg/kg, 0.05 mg/kg, 0.5 mg/kg, 5 mg/kg, and 50 mg/kg. The test concentration series for boron was: 0 mg/kg, 0.005 mg/kg, 0.05 mg/kg, 0.5 mg/kg, 5 mg/kg, and 25 mg/kg. The method for range-finding tests was the same as the reproduction tests based on the OECD guideline 232(OECD/OCDE 232, 2009). In the present study, the test concentrations [mg/kg] are 0, 0.0023, 0.0069, 0.0205, 0.0617, 0.185, 0.555, 1.66, and 5 for selenium; 0, 0.5, 7.5, 11.25, 16.875, 25.312, 37.969, and 56.953 for iodine; 0, 0.0002, 0.001, 0.005, 0.025, 0.125, 0.625, 3.125, and 15.625 for boron; 0, 0.01, 0.03, 0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 for arsenic. There were 10 replicates for the control and 5 replicates for each test concentration. About each experimental unit, there was 24.5 g

artificial soil mixed with a 5.5 ml test solution for every test concentration in one plastic, 100 ml cylinder pot. 10 synchronized *F. candida* juveniles were placed on the surface of mixed soil in each pot. According to the guideline, every test lasted for 28 days. During the experimental period, the temperature condition was 20°C (± 0.2°C) in the thermostat. Counting animals were on day 28 by flooding. Adult Collembolan was recorded as dead if not present in the extraction. The three trace elements tested in the present study were all operated according to the same experimental procedures described above. The method of the OECD reproduction tests was the same as the range-finding tests mentioned above based on the OECD guideline 232 (OECD/OCDE 232, 2009).
The avoidance tests were performed according to the method from Da Luz et al. (2004). The avoidance test lasted for 48 hours. There were 10 replicates for the control group and 5 replicates for each test concentration. The test concentrations were the same as in the OECD reproduction tests. In each experimental pot, there were two sides of artificial soil separately. One side was 24.5g artificial soil mixed with 5.5ml clean lab tap water, while another side was 24.5g artificial soil mixed with 5.5ml test solution. For each pot, 20 adult *F. candida* were randomly placed in the space between two sides of the soil. Food support and moisture control were not needed during the test. Similar to reproduction tests, the experimental temperature condition was 20°C (± 2°C). After 48 hours, the two sides of soil were separated and adults were counted by flooding.

STATISTICAL ANALYSIS AND GRAPHICAL PRESENTATION

R statistical program 3.6.1 (RStudio Team, 2019) was used to analyze data. The data were analyzed with the general linear model and the first lowest concentration significantly different from control was accepted as the lowest observed effect concentration (LOEC). In some cases, the concentration was transformed to log scale to reach normal distribution, but otherwise, the whole dataset met the requirements of normality according to the diagnostic plots (Residual variances, QQ plot, and Cook distance plot). LC_{50} (50% lethal concentration) and EC_{50} (50% effective concentration) values and confidence intervals were calculated with ToxRat® (ToxRat®Solutions.Gmbh, 2018). The avoidance tests were analyzed with paired t-test.

RESULTS AND DISCUSSION

OECD reproduction tests

As shown in Figure (1), the number of surviving *F. candida* adults was decreased significantly ($P < 0.001$, $t = -11.17$) in a concentration-dependent manner when exposed to arsenic-contaminated soil after 28 days. According to the graph, slightly fluctuation took place from 0 mg/kg to 8.1 mg/kg, the adult number significantly decreased at 0.01 mg/kg ($P < 0.001$, $t = -3.65$) and 0.1 mg/kg ($P < 0.001$, $t = -4.69$) respectively. Then there was no obvious change of adult number between the ranges of 0.3 mg/kg to 8.1 mg/kg. However, a sharp decrease in adult numbers occurred significantly at 24.3 mg/kg ($P < 0.001$, $t = -14.42$) where the adult number decreased to 0. Likewise, the number of juvenile decreased ($P < 0.001$, $t = -3.76$) in a concentration-dependent manner. The significant decrease took place at a concentration of 24.3 mg/kg ($P < 0.001$, $t = -3.8$) where the number of the juvenile was less than 8% of the control. The values of NOEC, LOEC, $LC_{50,}$ and EC_{50} were displayed in Table 1.
From Figure (2), the number of adults ($P < 0.001$, $t = -10.53$) decreased significantly in a concentration-dependent manner when exposed to boron-contaminated soil. The result of the juvenile was also visible in the graph ($P < 0.001$, $t = -3.75$). The number of survival adults was declined to 0 ($P < 0.001$, $t = -10.87$) at a concentration of 15.625 mg/kg, which had a significant difference compared with the control group. About the number of juvenile, it decreased sharply at 0.001 mg/kg ($P < 0.05$, $t = -2.2$). Fluctuation took place from 0.001 to 3.125 mg/kg, and the number of the juvenile at 3.125 mg/kg was lower than it was at 0.001 mg/kg. There were barely juveniles at the highest concentration of 15.625 mg/kg ($P < 0.001$, $t = -3.89$) where the number of the juvenile was only 2.77% of the control group. The values of NOEC, LOEC, $LC_{50,}$ and EC_{50} were displayed in Table (1). Because the difference from the control was not continuous, the NOEC and LOEC of boron were not dependable.

Figure (3) provides the results of the survival and reproduction of *F. candida* exposed to selenium-contaminated soil after 28 days. There were no significant differences (P > 0.05, t = -1.17) between the contaminated group and the control group in terms of adult mortality, and either there was no significant differences (P > 0.05, t = -1.41) between contaminated groups and the control group in terms of juvenile number. The values of NOEC, LOEC, $LC_{50,}$ and EC_{50} were displayed in Table 1. Because the difference from the control was not continuous, the NOEC and LOEC of selenium were not dependable.

Figure (4) shows the results of survival and reproduction exposed to iodine-contaminated soil after 28 days. The number of adult (P < 0.001, t = -4.6) decreased significantly in a concentration-dependent manner. Adult number decreased significantly (P < 0.001, t = -4.71) at 7.5 mg/kg comparing with the control, and then it decreased slightly at 11.25 mg/kg (P < 0.001, t = -3.93). Subsequently, the adult number decreased slowly from 11.25 to 37.969 mg/kg, reaching the lowest adult number at 37.969 mg/kg (P < 0.001, t = -6.28). At the highest concentration of 56.953 mg/kg, the number of adult decreased significantly (P < 0.001, t = -5.24). In another hand, the number of juveniles decreased significantly in a concentration-dependent manner (P < 0.001, t = -8.28). The number of juvenile decreased to 1 at the highest concentration of 56.953 mg/kg (P < 0.001, t = -9.53). The value of NOEC, LOEC, $LC_{50,}$ and EC_{50} can be found in Table 1.

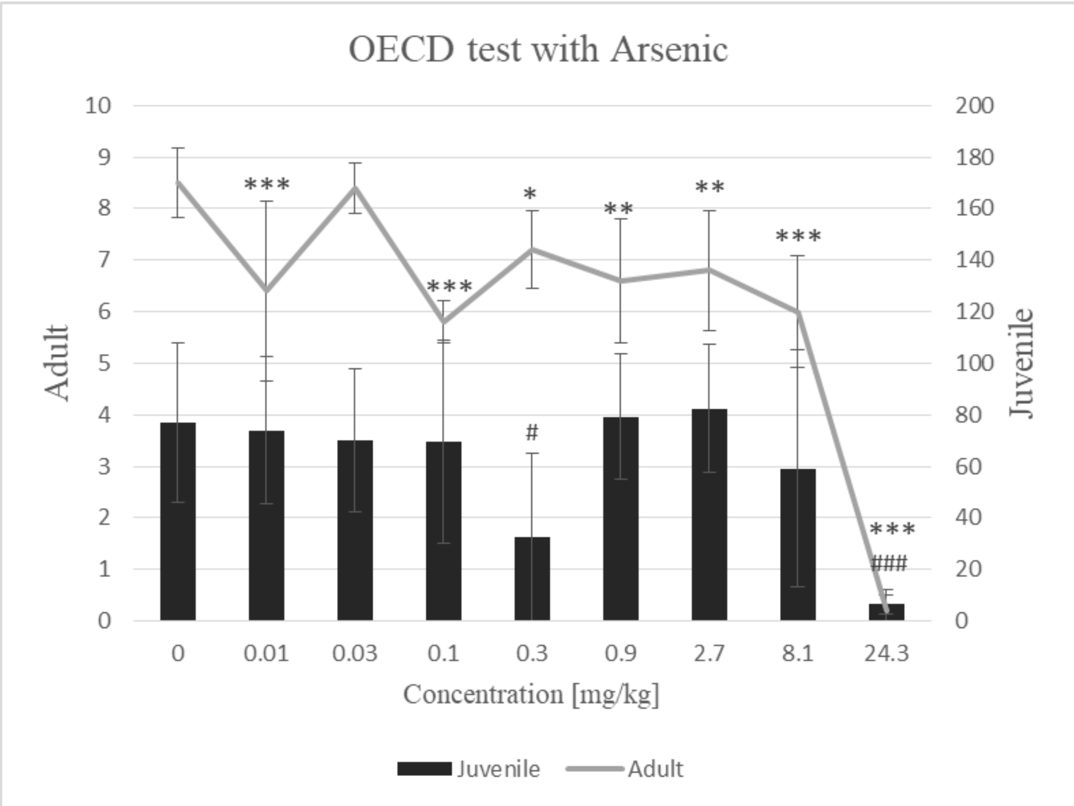

Figure (1): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to arsenic-contaminated soil after 28 days. (An asterisk “*” indicates there was a significant difference in mortality: “*” P ≤ 0.05, “**” P ≤ 0.01; “***” P ≤ 0.001. A pound “#” indicates there was a significant difference in reproduction: “#” P ≤ 0.05, “##” P ≤ 0.01; “###” P ≤ 0.001)

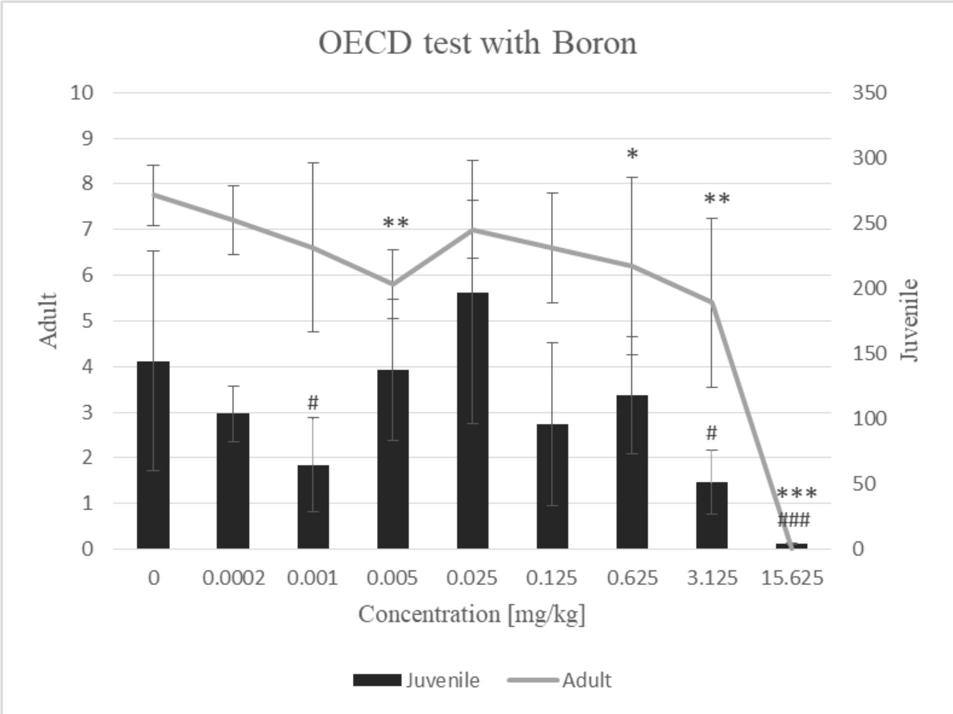

Figure (2): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to boron-contaminated soil after 28 days. (An asterisk "*" indicates there was a significant difference in mortality: "*" P ≤ 0.05, "**" P ≤ 0.01; "***" P ≤ 0.001. A pound "#" indicates there was a significant difference in reproduction: "#" P ≤ 0.05, "##" P ≤ 0.01; "###" P ≤ 0.001).

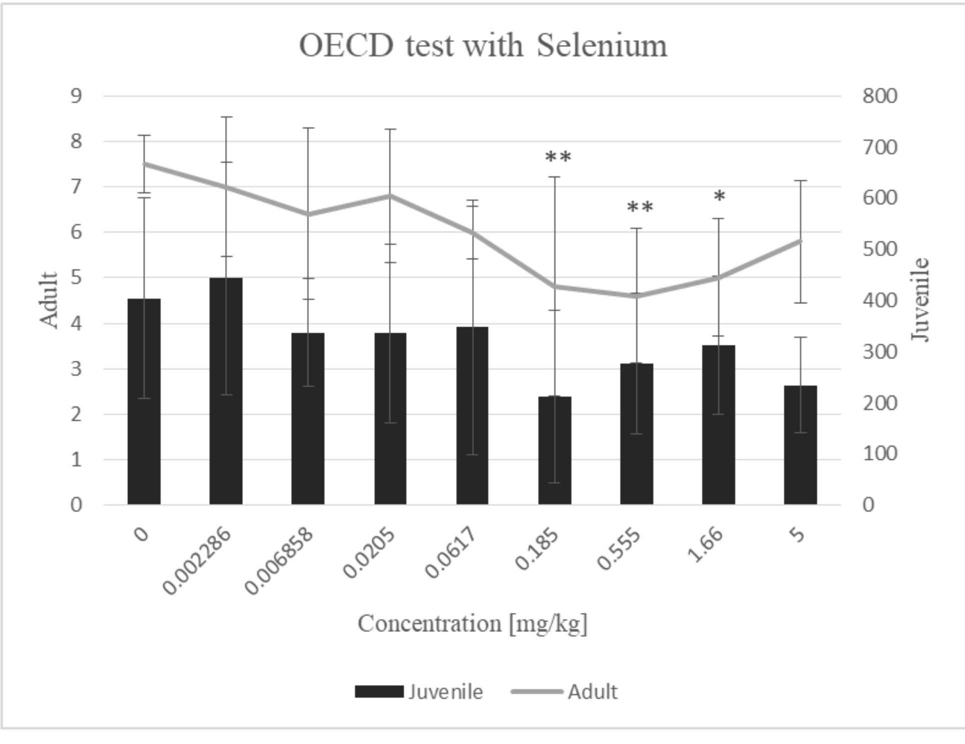

Figure (3): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to selenium-contaminated soil after 28 days. (An asterisk "*" indicates there was a significant difference in mortality: "*" P ≤ 0.05, "**" P ≤ 0.01; "***" P ≤ 0.001. A pound "#" indicates there was a significant difference in reproduction: "#" P ≤ 0.05, "##" P ≤ 0.01; "###" P ≤ 0.001).

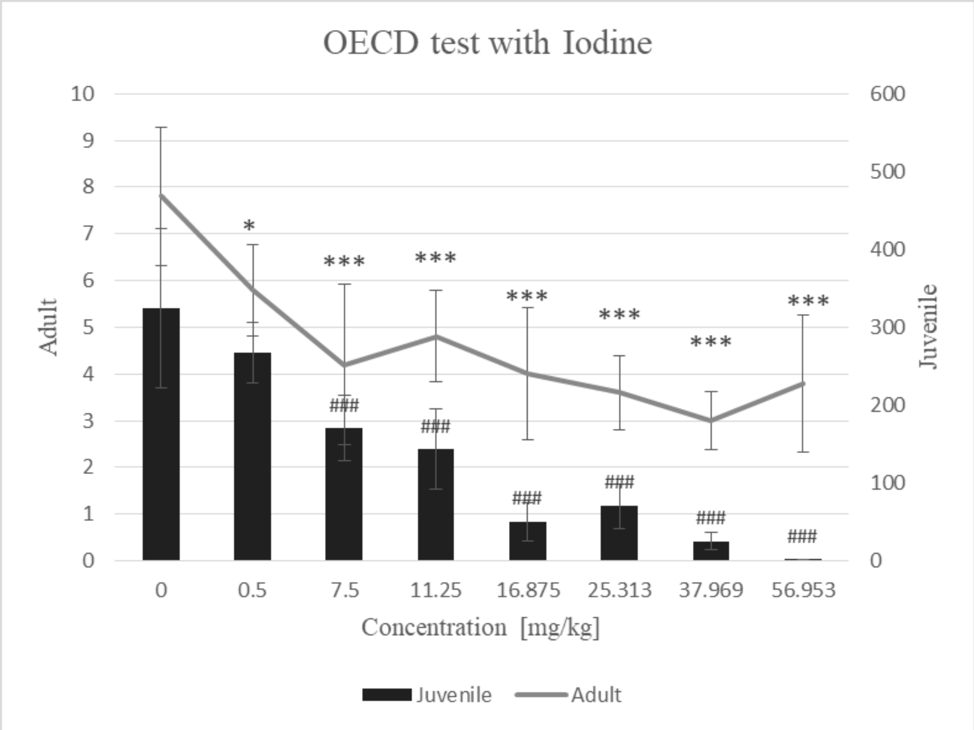

Figure (4): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to iodine-contaminated soil after 28 days. (An asterisk "*" indicates there was a significant difference in mortality: "*" P ≤ 0.05, "**" P ≤ 0.01; "***" P ≤ 0.001. A pound "#" indicates there was a significant difference in reproduction: "#" P ≤ 0.05, "##" P ≤ 0.01; "###" P ≤ 0.001).

Table (1) shows the detailed endpoints of OECD reproduction tests of four trace elements. The LC_{50} of arsenic and iodine was much lower than their EC_{50} respectively, which indicated that mortality was a more sensitive parameter than reproduction. It was possible in the case of springtails if the animals lay eggs before they died. On the other hand, the LC_{50} of selenium was marginally higher than its EC_{50}, therefore, the reproduction was more sensitive than survival in this case. About LOEC, the LOEC of arsenic, boron, and iodine on survival were all much lower than on reproduction, while the LOEC of selenium on survival was higher than on reproduction. Except for boron, the values of LC_{50} of arsenic, selenium, and iodine were quite close which were between 2.45 to 2.6 mg/kg. As for the EC_{50}, selenium had the lowest value which was less than 0.001 mg/kg. There was a four magnitude difference between boron and iodine and a five magnitude difference with arsenic. The value of EC_{50} of arsenic was 5 times higher than boron and 1.5 times higher than iodine.

Table (1): The LOEC, LC_{50}, and EC_{50} values with 95%-confidence intervals (CI) of the OECD

	Survival				Reproduction			
	NOEC	**LOEC**	**LC_{50}**	**95%-CI**	**NOEC**	**LOEC**	**EC_{50}**	**95%-CI**
As	0.03	0.1	2.595	n.d.	8.1	24.3	13.006	3.698-44.426
B	0.001	0.005	n.d.	n.d.	0.625	3.125	2.562	0.064-86.931
Se	0.0617	0.185	2.493	0.316-n.d	≥5	>5	<0.001	n.d.-n.d.
I	<0.5	≤0.5	2.452	0.045-7.138	0.5	7.5	8.792	2.672-29.612

The concentration was given in mg/kg. "n.d": not determined.

Avoidance tests

Figure (5) shows the result of avoidance test with boron. The y-axis presented the mean number of adult found in uncontaminated soil side. In some cases, the used concentrations caused mortality; therefore, the total number of animals could deviate from the originally introduced one. From the results, there was a significant avoidance behavior at 0.0002 mg/kg ($P < 0.01$, $t = 4.27$). Then, the avoidance behavior of *F. candida* adult was significant at 0.005 mg/kg ($P < 0.001$, $t = 10.63$), 0.025 mg/kg ($P < 0.001$, $t = 5.43$), 0.125 mg/kg ($P < 0.001$, $t = 7.09$), 0.625 mg/kg ($P < 0.001$, $t = 5.4$), and 3.125 mg/kg ($P < 0.05$, $t = 3.24$). The highest adult number at uncontaminated side was recorded at the concentration of 0.625 mg/kg.

As shown in the Figure (6), *F. candida* at contaminated side showed significant avoidance behaviors only at the concentrations of 1.66 mg/kg ($P < 0.001$, $t = 5.74$) and 5 mg/kg ($P < 0.05$, $t = 3.2$). The number of the adult at the uncontaminated side was the highest at 1.66 mg/kg, and then at 5 mg/kg.

According to the results in Figure (7), there were significant differences of avoidance behaviors at 0.5 mg/kg ($P < 0.01$, $t = 5.74$) and 56.953 mg/kg ($P < 0.05$, $t = 2.63$). And the number of adult at uncontaminated side at 0.5 mg/kg was more than it was at 56.953 mg/kg.

Figure (8) shows the results of avoidance test with arsenic. Avoidance behaviors were significant at 0.3 mg/kg ($P < 0.01$, $t = 4.12$), 2.7 mg/kg ($P < 0.05$, $t = 2.49$) and the highest concentration 8.1 mg/kg ($P < 0.01$, $t = 3.49$) respectively. Meanwhile, the results showed the highest adult number at the uncontaminated side was at the concentration of 8.1mg/kg, and then followed by 0.3 mg/kg, finally the lowest adult number was at 2.7 mg/kg.

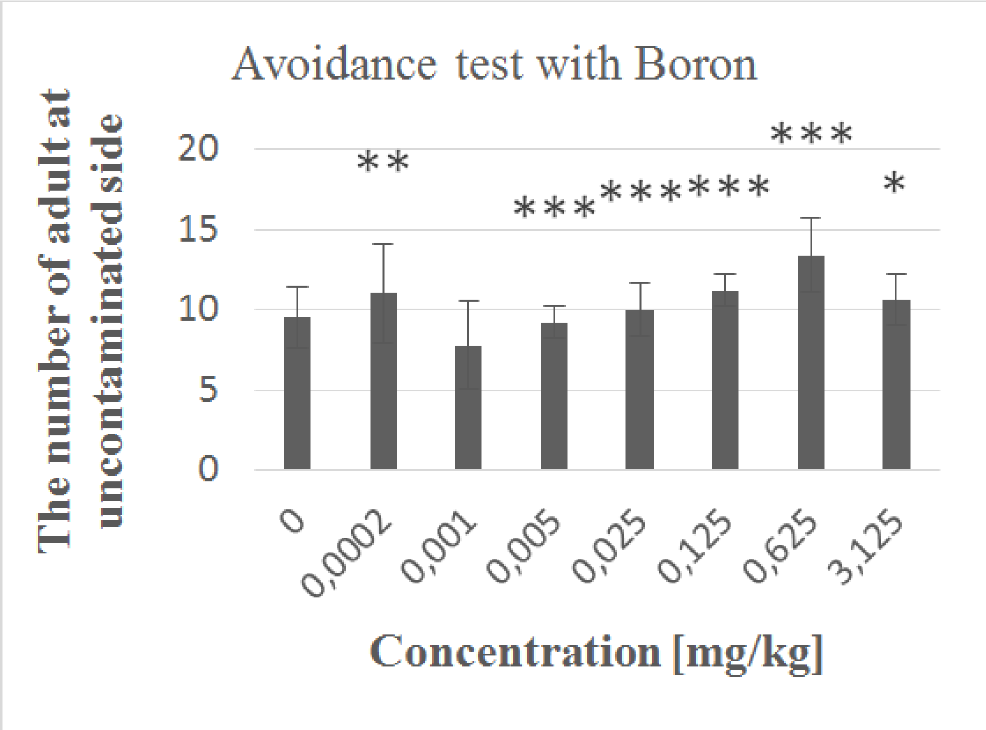

Figure (5): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and boron-contaminated soil after 48 hours. (An asterisk “*” indicates there was a significant difference: “*” $P \leq 0.05$, “**” $P \leq 0.01$; “***” $P \leq 0.001$)

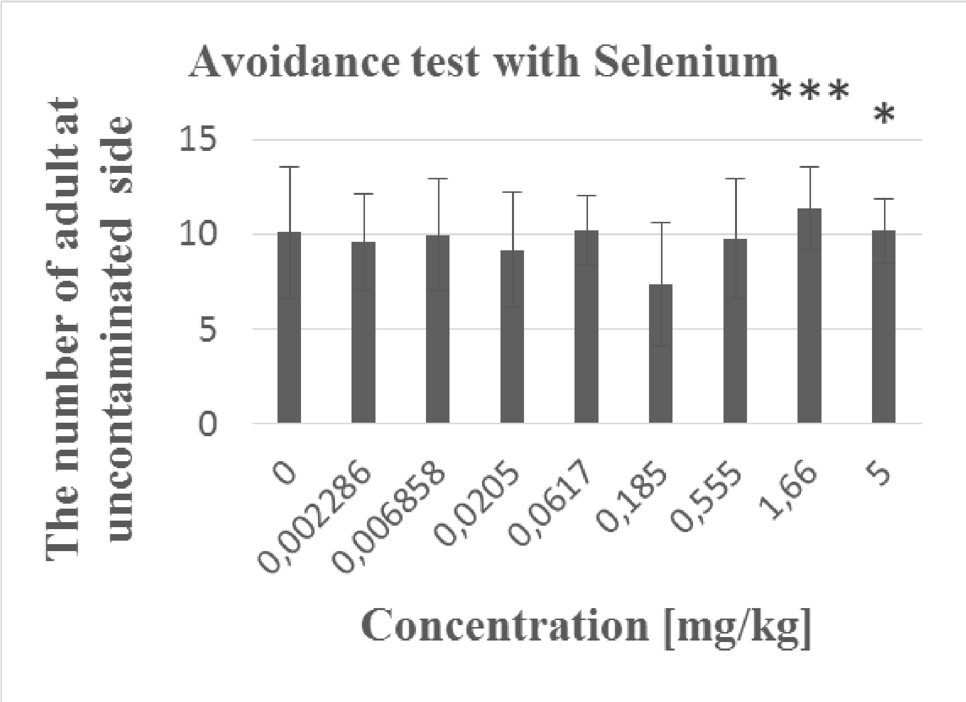

Figure (6): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and selenium-contaminated soil after 48 hours. (An asterisk "*" indicates there was a significant difference: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$)

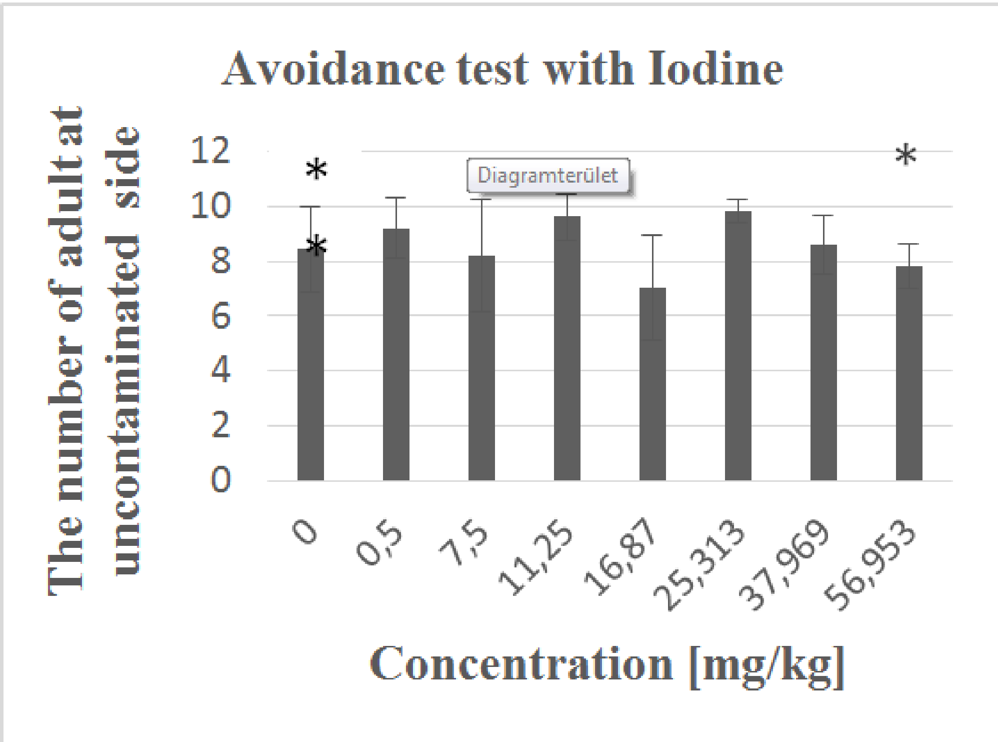

Figure (7): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and iodine-contaminated soil after 48 hours. (An asterisk "*" indicates there was a significant difference: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$)

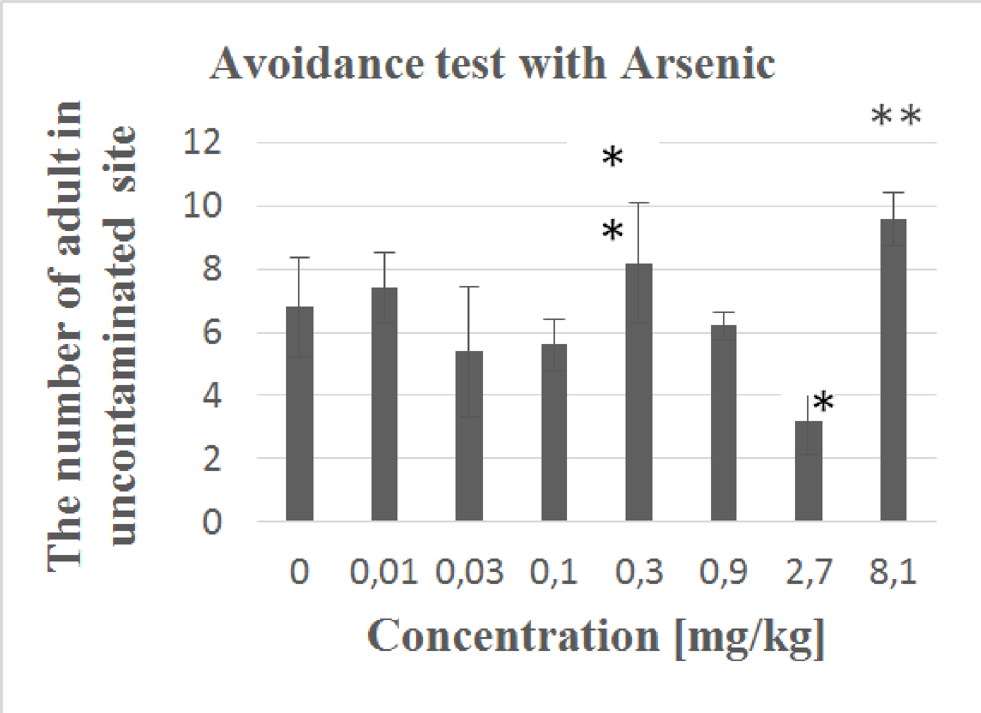

Figure (8): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and arsenic-contaminated soil after 48 hours. (An asterisk "*" indicates there was a significant difference: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$)

In the present study, the toxic effects of four trace elements were evaluated on *F. candida*, including two metalloid elements: arsenic and boron, and two non-metal elements: selenium and iodine. It had been well known that the reproduction and survival of *F. candida* had been the most frequently used endpoints to evaluate the toxicity of metal elements (Lin et al., 2019). The two-day long avoidance tests can indicate soil contamination as an early warning and a preliminary test to provide a reference for the OECD reproduction test and to trigger other tests (Bao et al., 2017; Da Luz et al., 2004; Lin et al., 2017). Besides, it could reflect on the environmental relevance of the data of OECD tests. In our study, arsenic, boron, selenium, and iodine all showed toxic effects on the survival and reproduction of *F. candida* in different degrees.

In terms of the reproduction test with arsenic, the LC_{50} (2.595 mg/kg) was one order of magnitude less than the EC_{50} (13.006 mg/kg). It indicated survival of *F. candida* was more sensitive than reproduction to arsenic. While Alves et al. (2018) found the EC_{50} of arsenic was 26.1 mg/kg on *F. candida* in tropical artificial soil (TAS), which was higher than the EC_{50} of arsenic in this study (Alves et al., 2018). Besides, Lin et al. (2019) found the LC_{50} of arsenic on *F. candida* was in the range of 320 to 1280 mg/kg in 10 different natural soils that were spiked from different regions in China (Lin et al., 2019). Arsenic-induced avoidance behaviors in *F. candida* occurred mostly at high concentrations, while avoidance behavior also occurred at the concentration of 0.3 mg/kg, which even lower than the LC_{50} (2.595 mg/kg) of arsenic. Thus, Collembolan could probably avoid slightly polluted soil in a natural field. Generally, the concentration of arsenic in uncontaminated soil is in the range of 0.2 - 40 mg/kg. A high level of 100 – 2500 mg/kg was found in the ambient soil around some copper smelters. Additionally, the concentration of arsenic had been found at a higher level (200-2500 mg/kg) than above in the soil of orchards because of the numerous arsenical pesticides applications (World Health Organization, 1996). So it would appear that arsenic might be a potential toxic risk in natural field, especially in contaminated regions.

About the reproduction test with boron, the LC_{50} value and its confidence interval were not calculated, the EC_{50} was 2.562 mg/kg with a wide range of confidence intervals (0.064-896.931). Therefore, we preferred to compare the sensitivity of survival and reproduction with their NOEC and LOEC values. The NOEC and LOEC of survival were three orders of magnitude less than NOEC and LOEC of reproduction respectively, which revealed survival was much more sensitive than reproduction to boron. Besides, boron showed higher toxicity on reproduction than arsenic and iodine in this study. Research by Krogh et al. (2008) found the EC_{50} of boric acid was 90 mg/kg on *F. candida* in the OECD artificial soil (Krogh et al., 2008). Also about boric acid, Amorim et al. (2012) found the EC_{50} was 54.5 mg/kg on *F. candida* in another type of soil (LUFA 2.2). The main differences between OECD soil and LUFA 2.2 soil are organic matter and clay content. Besides, Amorim et al. (2012) did not found avoidance behavior in boron-contaminated soil. Worthy be noticing, *F. candida* avoided boron-contaminated soil at almost all concentrations in this study, even when the concentration was lower than the LOEC (0.005 mg/kg) of survival, which could mitigate the toxic effects of boron. Because the *F. candida* could leave the contaminated area before toxic amounts of boron were uptaken. In nature, boron had been found to occur from 10 to 300 mg/kg with an average of 30 mg/kg in uncontaminated soils. While the level of concentration depends on the type of soil, amount of organic matter, and the amount of rainfall (World Health Organization, 1998). Based on the above description, our results showed boron had a potential toxic risk in nature.

Concerning the reproduction test with selenium, there were no toxic effects on the reproduction of *F. candida*. On the one hand, the EC_{50} value (< 0.001mg/kg) was not trustworthy, because the confidence interval was not determined by the program, and the NOEC (≥ 5 mg/kg) and the LOEC (> 5 mg/kg) were no less than the highest test concentration of selenium. What can be believed was that the EC_{50} of selenium might out of the range of test concentrations in this study. On the other hand, the toxic effect of selenium on mortality was a sensitive parameter from the results, and the LC_{50} was 2.493 mg/kg. While Kuperman et al. (2018) found in the natural soil Sassafras sandy loam (SSL), the LC_{50} of selenium was 12.7 mg/kg on *F. candida*, and 6.2 mg/kg on *Enchytraeus crypticus* (Kuperman et al., 2018). Speak about avoidance test with selenium; *F. candida* avoided only high concentrations. Generally, the concentration of selenium in soils is in the range of 0.005-1200 mg/kg, and the most common range is between 0.1-10 mg/kg. A high content of Se had been found in volcanic rocks (120 mg/kg) (Alexander, 2015). The available limitation analytical data of selenium was typically found in some foods in a range of 0.1-1.5 mg/kg, and < 0.1 mg/kg in most fruits and vegetables (World Health Organization, 1986). Although our results showed there existed toxic effects of selenium on *F. candida*, the concentration of selenium in nature seems to be a low potential toxic risk.

As for the reproduction test with iodine, the LC_{50} (2.452 mg/kg) was smaller than EC_{50} (8.792 mg/kg), which indicated reproduction was less sensitive than survival. The toxic evaluations of iodine on *F. candida* were rare. Irvine and Sikdar (1998) found the EC_{50} of iodine which from waste brine was less than 0.1 mg/kg on *Ceriodaphnia dubia*. The different EC_{50} values may be connected with inhabitations and individual variation (Irvine and Sikdar, 1998). In addition, Ilin and Nersesyan (2013) found the LC_{50} of I on *Eisenia fetida* was greater than 1000 mg/kg in dry soil and 740 mg/kg in wet soil (Ilin and Nersesyan, 2013). As for the avoidance behavior of *F. candida* to iodine, there were only two concentrations that induced the avoidance behaviors, which were at the lowest and the highest concentration respectively. With regard to the iodine in nature, the concentration of iodine had a huge variability from media to media and also from place to place on earth. Normal levels of iodine were in the range of 0.3-10 ppm. 0.5 ppm could be regarded as an average concentration in most parent materials. A concentration of 5 ppm had been adopted mostly in organic soils (Johanson, 2000). Based on the above data, our results showed that there exists a low potential toxic risk of iodine.

As described above, the survival of *F. candida* was more sensitive than its reproduction to each element. It implied individual *F. candida* was affected by contaminations firstly, and then followed by its reproduction ability under environmental stress. It was possible that the *F. candida* could lay eggs before they die because of the exposure. This symptom may be related to the physiological characteristics of Collembolan (Fountain and Hopkin, 2005). Likewise, Linares et al. (2007) found in some marine invertebrates, their survival was more sensitive than reproduction in undersea environmental stress (Linares et al., 2007). Besides, The LC_{50} value of arsenic, selenium, and iodine was extremely close, which expressed their toxicities were similar. However, their EC_{50} values were different. Various toxicity on reproduction may be related to the different properties of trace elements, as well as the dissimilar metabolic process in collembolans (Bao et al., 2017). Additionally, the variability of EC_{50} value and LC_{50} value of a specific trace element on *F. candida* might be related to the strong influence of soil properties, which contribute to a different bioavailability of each element in soil (Alves et al., 2018; Lin et al., 2019).

In brief, the results showed the avoidance behavior in *F. candida* was most sensitive to boron, followed by arsenic and iodine, and finally selenium. Previous researches by others, for example, Lin et al., (2017) found the *F. candida* avoided antimony (Sb) even at a concentration much lower than its LC_{50} (Lin et al., 2017). Bao et al., (2017) found *F. candida* only had avoidance behaviors at high concentrations of cadmium (Cd) which were much higher than its EC_{50} (Bao et al., 2017). Li et al., (2011) found *F. candida* avoided copper (Cu) at almost all concentrations (Li et al., 2011). These different avoidance behaviors of *F. candida* induced by different elements indicated that *F. candida* indeed can sense the presence of soil contaminations and had great variabilities in the avoidance behavior to different contaminations. Diverse avoidance behaviors might due to *F. candida* has different abilities of sense to different elements.

CONCLUSIONS AND RECOMMENDATIONS

To conclude, this paper achieved the aim of the study. The toxicity of each element on survival was similar, while boron showed higher toxicity on reproduction than the other three elements, besides, boron made *F. candida* showed the most avoidance behaviors among the four trace elements. Combined with official data, boron and arsenic had shown potential risks in natural field conditions. As a parameter in the ecotoxicological tests, survival appeared more sensitive than the reproduction of *F. candida* to every element in OECD reproduction tests. Ultimately, the whole four trace elements showed higher toxicities on the *F. candida* comparing with the previous studies by others. Many researchers have focused on this problem. The above conclusions further confirmed the importance of ecotoxicological evaluations of trace elements and gave an early warning of soil pollution caused by trace elements. This paper had a positive effect on the protection of the soil environment and it can provide help for further ecotoxicological researches. However, deeper researches should be continued on the toxicity of various elements to see long-term effects on different life-history parameters of *F. candida*. Also, the molecular mechanism of the toxicity should be revealed.

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TL13.

APPLICATION OF CLEANER PRODUCTION IN SOAP INDUSTRY FOR A HEALTHIER WORK ENVIRONMENT

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Abstract: *In the light of the increasing industrial impact on the environment, and since small industries have a cumulative effect that increases with time, the study examines the employment of Cleaner production in soap industry to achieve two goals: the first cutting back on waste produced, and the second reducing the use of toxic materials. Thus, it can contribute in the solution of chronic problems concerning the health and safety of workers and the environment as well. The study has taken place in two different developing countries, Syria and India. The current situation in every factory was evaluated considering the occupational health and safety of workers, and the influence on the environment. This includes the evaluation of energy consumption and the use of Cleaner Production alternatives. The required data is obtained originally from the direct observation of raw materials, production stages, production quantities and unsold products and from interviews with owners, engineers, and workers in the second place. It was found that waste from soap factories are not generated in large quantities, but their accumulation creates environmental and health troubles which can be alleviated by replacing chemical materials with natural ones, using hand storage and implementing cleaner production alternatives in the soap industry. This strategy can reduce waste created and energy used through benefiting from the sunlight, natural drying, reusing byproducts, which can rise the efficiency of production and management process. It also has a significant positive impact on the work environment, health and safety of workers in general.*

Keywords: *Byproducts, Cleaner Production alternatives, India, Occupational Safety Soap Industry, Syria*

INTRODUCTION

Soap industries have a significant impact on the environment, and this depends on the type of waste, the type of raw materials, which are industries generated wastes, effluent generation, heat generation, pollutants and greenhouse gases, noise generation etc. The cleaner production processes cut down on pollution to a great degree; using clean and environment- friendly technologies, and so this curtails using and releasing toxic materials. Moreover, non-renewable materials and energy are being replaced by renewable ones.

Evaluating the effect of Cleaner Production-Pollution Prevention (CPPP) is conducted by reducing the effect of chemicals on the workers’ health and safety. Through recognizing the relation between the workers’ safety and the environment we can determine the elements which lead us to reducing the risk of toxic materials on humans and the environment. (Karla R et., 2011)

In other words, the cleaner production is a method that aims for preserving the environment and health from pollution and saving its natural resources while treating the environmental problems at the source not after occurring, and this requires clean, environmentally friendly technology. (Hens et al., 2017)
So, we can say Cleaner Production-Pollution Preventive (CPPP) is the best technique for the environment and health protection and can replace chemical materials with natural materials.
Through searching in similar studies it was clear that the objective of this study is to determine the competitive priority that can stand for the organization's functions (Lin et al., 2007). Environmental performance, economic performance, social performance, the study of performance improving and cost control was studied by Xiong& Xiong (2010). In recent years, environmental problems and their effects have increased, and the environment is considered one of the most important topics in all countries of the world. This requires an increase in environmental awareness, including the production and product process (Pusporini & Vanany, 2017). The Cleaner production puts the whole-prevention strategy into the sustainable production processes, products and services to increase eco-efficiency and reduce human and environmental risks (Lin-xiu & Kun, 2008).
Through this paper we can recognize all soap manufacturing stages and all kinds of raw materials both natural and chemical, in addition to knowing its impacts on humans' health and environment, and Cleaner production options which makes this study unlike any other researches. It should be noted that I did a field study to complete this paper through interviewing owners, engineers and workers as well as direct observation. It began with determining the stages of manufacturing problems and the possibility of applying cleaner production. Various problems have been found throughout the study as Asian countries are some of the most polluted ones where the medium and small industries are produced and they lack energy efficiency in addition to their location near residential areas, which leads to the spread of environmental pollution in the neighbourhood. (Bantacut & Zulaikha, 2019)
Thus, research related to cleaner production helps to improve the production and environmental management practices.

MATERIALS AND METHODS

Authors listed the study into three groups: location and time, type and source of data, and research stages (Production process). We obtained my data as mentioned above from interviews and observation.

1. Location and Time:

This study was conducted in November 2019 at Al-Majd Factory for Soaps in Syria and in February 2020 at Remember India Cosmed Products in India.

2. Type and Source of Data:

Authors obtained my data as stated before from interviews and observations and we were deducted the scheme after recognizing the production line. As for the cleaner production, alternatives were obtained through interviews with employers.

3. *Research Stages:*

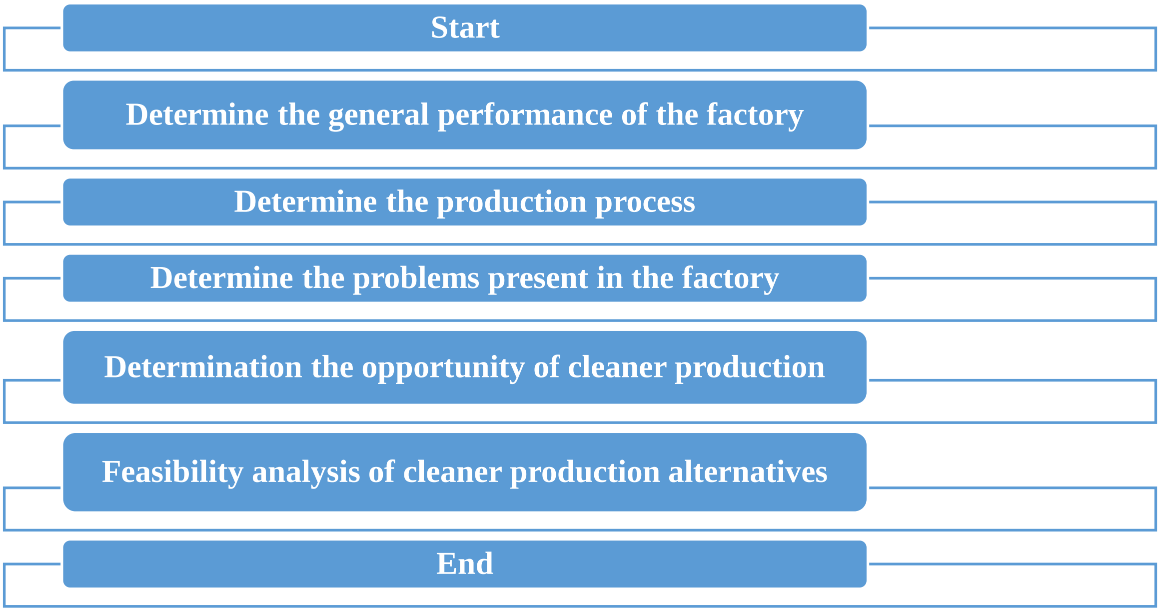

Figure (1): stages of the research
Source: (Bantacut&Zulaikha 2019)

A. Determine the general performance of the factory: In this stage, it was possible to know the general condition of the company from the date of construction, the number of workers, production capacity and management.
B. Determine the production process: Determined the sources of waste energy and water consumption based on the operating time of the machine.

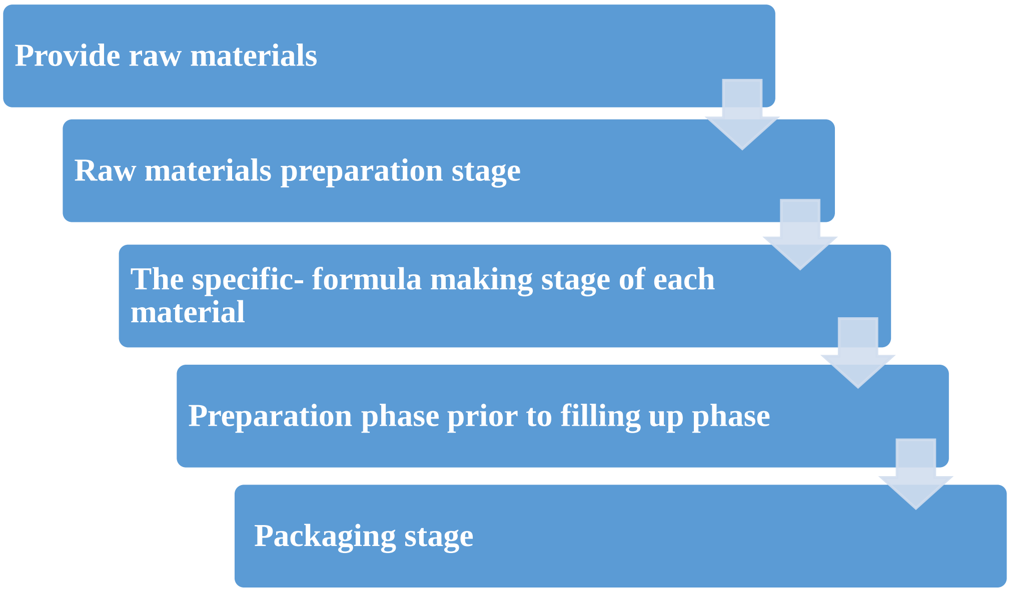

Figure (2) the production process

C. Provide raw materials
D. Those were olive oil, laurel oil fennei-flower, oleaeuropea, coconut oil and palm oil. There were chemicals such as toxapone to give the soap foam and other materials such as sulfonic acid (labsa), sodium carbonate (SODA) and sodium hydroxide (custic).

E. As for the source of the natural raw materials, they came from the same area of the factory, they were purchased from the inhabitants there, and the chemicals which were not manufactured locally were imported from abroad.
F. With regard to the effects of raw materials on the environment and people, they can be listed as follows:
G. Texabone (sodium dodecyl sulfate): It exposed humans and animals to injuries such as skin irritation and infections due to its ability to penetrate into the skin if it was used excessively. Therefore, preventive measures must have been taken and used in specific proportions and then wash the contacted skin quickly. On the other hand, it was considered safe that did not cause cancer.
H. Sulfonic acid (Labsa): there was a high risk of poisoning and high biological accumulations in the bodies of neighborhoods, which put their lives at risk.
I. Sodium carbonate (soda): It had benefits as it eliminated bacteria and removed spots and whitening, but it caused chemical burns. Therefore, preventive measures must have been taken.
J. Sodium hydroxide (Costic): also caused skin erosion and damaged the cornea, where it could have caused blindness and chemical burns.
K. Determine the problems present in the factory: it aimed to identify the problems in the detergent plant (soap) including raw materials and waste generation in each process. The data has been collected through interviews with workers and owners, and direct observation
L. Determination the opportunity of cleaner production: the use of magazines, previous studied and secondary data.

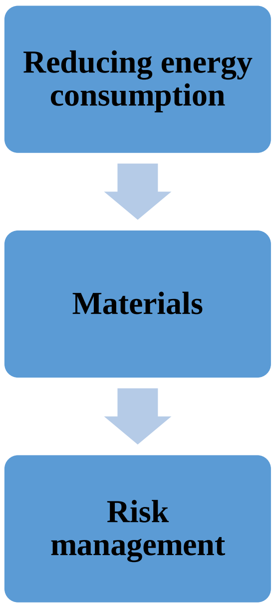

Figure (3): the opportunity production

E- Feasibility analysis of cleaner production alternatives: an analysis of all environmental, financial and technical aspects.

- Environmental: Reducing waste in the production process and avoiding the production of new waste
- Finance: by calculating cost benefits
- Technology: the compatibility of procedures and production with conditions in the industry and the efficiency of the process.

Also, the material standards consisted of the available equipment capacity, the efficiency of the use of raw materials, and the preservation of product quality. These standards were as essential as the availability of places, engine maintenance, standards for a safe work system for workers and the availability of human resources.

RESULTS AND DISCUSSION

Through this research we noticed that the environmental impact of soap factories might not be significant, but their progressive effect was large due to several factors.
This included: low level of technology, lack of trained workers, lack of space and lack of environmental protection (Erina & Andre 2016), and this was what soap factories suffered from. We noted that waste soap factories were not large, but their accumulation created an environmental problem, so there was a wide range of pollution prevention opportunities that could have been implemented and had financial advantages for the plant, as well as reducing environmental pollution.
These opportunities ranged from simple, low-cost improvements in housekeeping procedures to saving that could have been achieved from recovering chemicals used in the process, and potential products from waste (Sohair I & F.Zaher 1998).
By reducing the amount of waste produced or that could have been released, the company could cut down on waste disposal costs, improved worker safety, and reduced liability in the long run. Energy and material scales were crucial to reduce waste, as we could determine emissions and losses, and evaluated costs. Here are energy and material balance components:

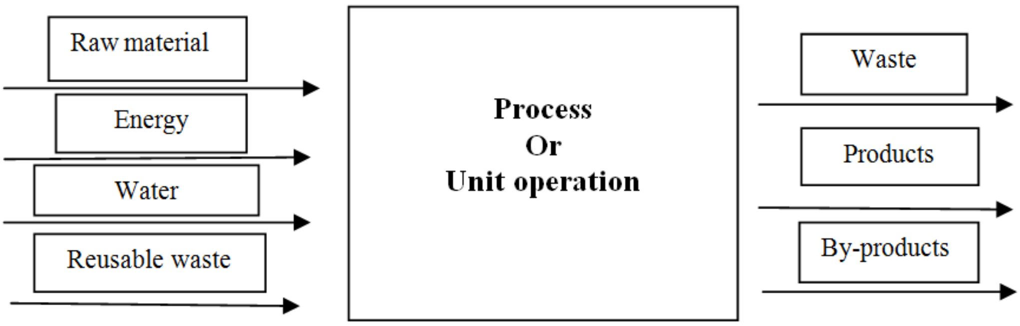

Figure (4):Components of material and energy balance
Source: waste Minimisation and Resource Conservation

Pollution prevention methods might have increased the efficiency of the production line and reduced costs, as they were consistent with the procurement process, raw materials and stock control.
Any resulting that could have changed the efficiency or expenses might have helped the company maintenance or improved its competitiveness in the market (Sohair I & F.Zaher 1998).
In light of these consequences, it was strongly recommended to follow the procedures of cleaner production through using the appropriate materials and technology, using renewable energy, reducing the process time as the energy consumption was proportional to the treatment time, and reducing / increasing the temperature as the heating and cooling process consumed the energy that changed the process current temperature of the product.
By reducing / increasing the set temperature, the power requirements would have been reduced due to the short temperature gradient (Cleaner Production Tips for Small and Medium Industries 2011).

Table (1): Unit Operation

In-put	Value	Out-put	Value
Raw Material	1000 Kg	Products	12000 pieces (900 Kg)
Energy	50 Kwh		
Reusable waste	100 Kg	Waste	27 Kg/d (3%)

Cleaner production options:

The availability of natural materials and hand storage was important to implement cleaner production in the soap industry, as soap waste was biodegradable and implementation of cleaner production succeeded in reducing the amount of waste resulting from manufacturing poor storage.

Table (2): Cleaner Production Options

Process	Problem	Current handling	Cleaner production options
Preparation stage	Size-related imperfections	Some mistakes occurred in size when cutting the formula into parts	Use the machine to set size to avoid these mistakes
Packaging stage	Waste packing boxes during the packing process	Manual packaging of soap	The packaging used for recycling is stored
Storage	A quantity of soap is damaged due to poor storage	No action Only store soap	Improve storage conditions by providing conditions such as: temperature and humidity to avoid stock damage

Table 3 Implementation of Cp in Soap Manufacturing Industry

Process	Investment/ Input	Benefits/ Output	Indian/ Syrian Soap Industry	CP Recommendations
Raw materials	• Selection of raw materials based on their properties • Material should be environment friendly	• Waste minimization • Better quality of product • Minimum consumption with maximum output	• 1000 kg	Selection of eco-friendly and non-toxic material
Energy Sources	• Environmental friendly and locally available energy source to be used	• Energy savings • Waste minimization	• Electricity (50Kwh)	• Saving of energy can be done as much as possible
Housekeeping	• It need proper planning and management practices with required goods	• Good and safe work place • Cleanliness and hygiene • Waste minimization	• 40 workers	Good housekeeping will reduce any losses during handling and storage
Waste Generation	• It is produced during production • It also can be a by-product	• Waste is an undesirable output	• 3% of total raw materials (27kg)	• Waste can be recycled • Processed again and can be a useful by-product
Product	• All the above mentioned process combine together to form product	• A product is the most desirable	• 300 boxes having 40soaps (75g per soap)	• Product can be modified like shape and size to minimize waste

CONCLUSIONS AND RECOMMENDATIONS

Through the case study, we find that the industries which perform preventive measures to preserve the safety of the workers, as they wear gloves and a periodic medical examination is carried out every six months and provide the appropriate treatment once any of that is needed and the appropriate healthy food is provided.

The research found various problems resulting from waste sources and inefficiencies that occur in the production and management process. Some of which are the lack of equipment and technology, the

lack of space, the use of lights during the day, the generation waste, the use of electric power, the lack of efficiency of workers and unsold products.
The detergent industry (soap) must have a special team to deal with the environmental aspects of reducing waste generated, applying cleaner production in an optimal way, using clean energy, rehabilitating workers, using technology more broadly, and providing space for all stages of manufacturing (Waste Minimizations and Resource Conservation).
By applying cleaner production technology, the waste ratio is halved from 3% (27,000 kg) to 1.5% or more from the previous percentage.
This strategy saves waste packaging and wasted soap packages, reduces costs and raises productivity. This technique cuts down the environmental risk and the cumulative negative impact of soap factories as well as it will be an environmentally friendly industry.

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TL14.

DEVELOPING SOIL PROTECTION TRAINING MATERIALS BASED ON EXPERIMENTAL PROCEDURES FOR AGE BETWEEN 10-14 IN HUNGARIAN PUBLIC EDUCATION

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Abstract: The choice of this study was inspired by recognition of gaps around the general knowledge and protection of soil. The poor knowledge of society today about soil protection puts the livelihoods of all life forms on Earth at risk. Loss of soil natural functions could have serious consequences in the future. Regeneration of soil as a non-renewable resource is too long to be defined on a human scale. The Food and Agriculture Organization of United Nations raised the awareness of the importance of healthy soil in connection of healthy, nutritious food production and importance of public awareness of soil protection. Therefore, it is necessary to integrate the basic theory and practice of soil protection into public consciousness, general and public education, and higher education too. Further generations will grow up without tangible knowledge and voluntarily or unintentionally destroy the essential foundation of our existence, the fertile soil. For this reason, it is important to thoroughly investigate the existing materials in the topic of soil protection in the National Core Curriculum and teaching materials. After a thorough research in the Hungarian Core Curriculum, it is clearly recognizable, that the topic of healthy soil is currently insufficient. The main question is, how to integrate more thoroughly the protection of this essential natural resource into education? My answer is in a precisely planned, practice oriented, age-appropriate educational material based on previously performed experiments, which experiments meant to provide the framework of the compiled teaching aid.

Keywords: *Awareness raising, Education, Healthy food, Healthy life, Soil protection*

INTRODUCTION

The 68[th] UN General Assembly in 2013 declared 2015 the International Year of Soils and December 5[th] for the World Soil Day. The Food and Agriculture Organization of the United Nations (FAO) aims to inform civil society and decision-makers, promote investment, make policy decisions, strengthen the Sustainable Development Goals and gather information on soil protection. Loss of soil natural functions could have serious consequences in the future. The status of soil health has a major impact on all living being and mankind on Earth. It not only affects the balance of biodiversity and natural communities, but food production, health and life of future generations as well. The FAO called attention to the importance of public awareness. So, it is a major issue to pay attention to awareness raising and education in the topic of soil health and its protection. Although soil education is already part of the primary school curriculum, but knowledge of soil protection provides a negligible opportunity for both students and teachers to acquire and transfer an understandable, tangible knowledge material. During the research for several textbooks used for education, we had the opportunity to observe these shortcomings. As a result, the knowledge of the rising generations can be said to be poor on the subject. Hungarian general and public education would be in great need of an illustrative aid, which clarifies, makes the importance of soil protection goals interesting and complements the curriculum with colorful, interesting, easy-to-interpret and processable information. The primary goal of my work is to help bridge the gaps in primary school curricula. Accordingly, a number of experiments demonstrating the physical, chemical, biological properties, functions, and degradation of soils were processed and described, all of which served as the basis for the preparation of worksheets, illustrations, and aids. The auxiliary material related to the completed curriculum is in close accordance with the requirements of the National Core Curriculum (NAT2020) and mainly provides professional help for its students and teachers in subject of natural science and biology year 5-7 thanks to its simple, understandable, illustrated design. It partly completes the suggestions of the recently released Hungarian Soil Protection Action Plan released by the Hungarian National Food Chain Safety Office (NÉBIH) as well. [1], [2], [3], [10].

MATERIALS AND METHODS

The teaching material

Based on the knowledge of soil science and soil protection, taking into account the methodological requirements of the National Core Curriculum, documentation of several experiments was prepared. This documentation package is the basis for compiling the teaching aids. For the teaching aid, in the thematic order of soil properties and soil protection knowledge, worksheets were prepared for each topic in an age-specific division. Worksheets are supported by a photo gallery for a more accurate understanding. We compiled the experimental worksheets in a way to meet the expectations that are interesting for the 10-14 years old students and make them suitable for the development of their knowledge. The requirements I set for the worksheets are as follows:

1. Fit into the curriculum and meets its requirements.
2. Develop the key competencies named by the curriculum requirement.
3. Contains integrated knowledge from other disciplines.
4. The compiled knowledge and practice should be age-appropriate.
5. The materials used are easy to obtain, could be found in any household and suitable for home and school experiments and can be used safely.
6. The knowledge is easy to understand, the practical instructions are easy to follow.
7. Experiments can be repeated at any time.
8. It takes into account the different abilities of the students, so that any student from the given age group can carry out the experiments without restrictions.
9. Interesting, eye-catching, thematically relevant illustration and helpful information.

The structure of the worksheets is based on the same schematic order. Each section has its on purpose to create and interesting, informative and complex teaching aid. The Figure1 illustrates the structure of worksheets. The description of each section is as follows:

Section 1: The illustration is closely related to the topic processed on the worksheet, which is informative in itself.

Section 2: The main and subtitle contains the exact title of the topic, using the terminology of the literature.

Section 3: The “Soil smarty” section contains a short and interesting description of the knowledge and facts related to the topic.

Section 4: The thematic text part contains the comprehensive knowledge of soil science and soil protection. It integrates different disciplines at the same time and gives a global picture of the contexts.

Section 5: Describes the tools and steps required to process the experiment.

Section 6: Introductory questions related to the experiment that need further processing.

Section 7: The "Did you know?" part contains additional interesting facts about the topic, which encourages further research, include curiosities related to experiments and also help to develop key competencies.

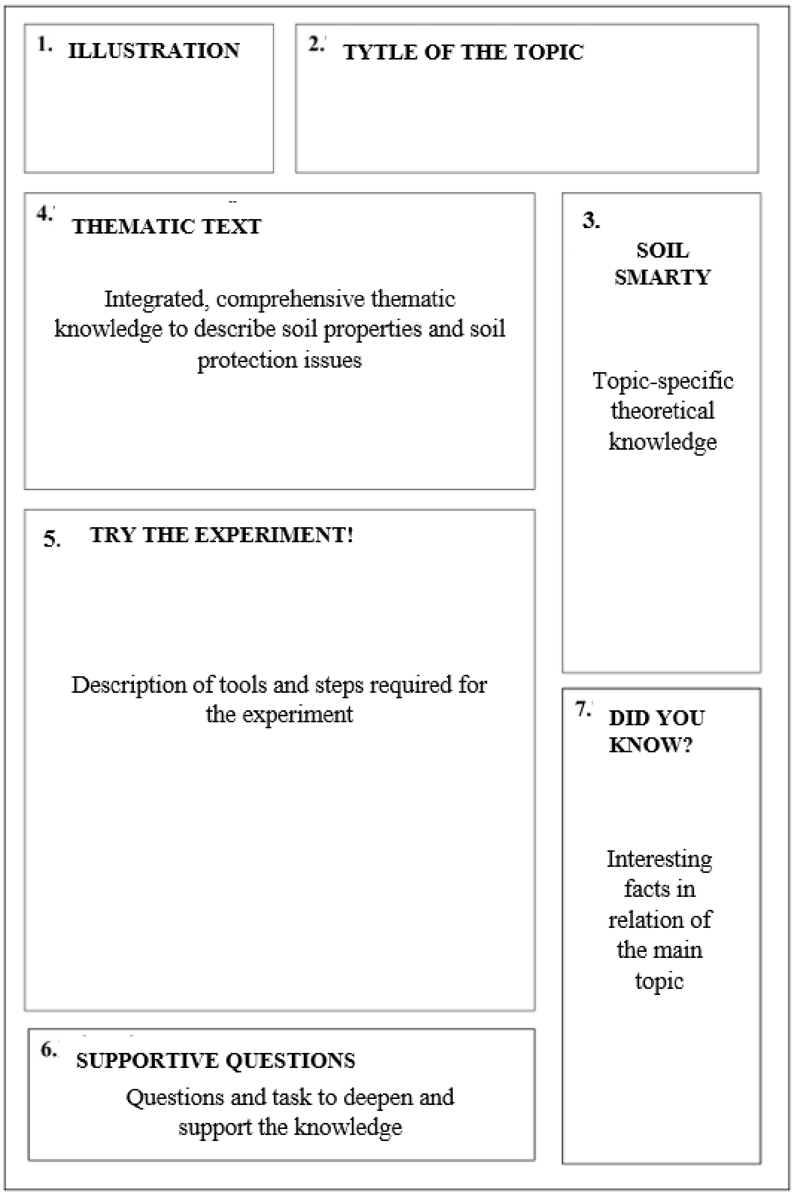

Figure 1: Schematic structure of the worksheets, own illustration

The performed experiments

We performed various experiments at home and in the Institute for Soil Sciences Centre for Agricultural Research in Budapest based on the expected knowledge of the target age group to compile the school aid. It was important during the preparation of the experiments to find material, tools and contaminators are available in a general household. In addition to presenting the physical, chemical and biological properties of the soil, soil degradation and soil pollution processes that destroy soil health were also examined. For the experiments, we used 3 soil types, which were chernozem loam soil, clay loam soil and sandy soil. The properties of the soils used are shown in Table 1, which data are from the database of the Institute for Soil Sciences Centre for Agricultural Research.

Table 1: Properties of the soils used for the experiments

Name of soil	Soil type	Soil level, humus (%)	pH value	Lime content (%)
chernozem soil form Nagyhörcsök	loam	level A, arable layer, humus 3%;	7,3	3-5 %
sandy soil from Őrbottyán	sand	level A, humus 0,9-1,1%;	7,5-8	3%
Clay loam soil from Tagyon	clay loam	level A, humus 2,16%	8,3	8,47%

Experiments showing the physical properties of the soil were carried out by examining the physical soil type with a so-called “kneading test”. The soil fractions were examined by sieving. Also, we examined the water permeability of the soils in the case of air-dry soils and the water storage function and water binding capacity of the soils. [4]

During the experiments showing the chemical properties of the soils, the lime content and organic matter content of the soil were determined. The lime content was determined with a 10% hydrochloric acid solution. The organic matter content was determined by examining the filtration capacity of quartz sands and sand with high humus content samples. A 20% solution of bio-fermented household vinegar is also suitable for the detection of lime content.

The observation of the biological properties of the soils was intended to show the visible and invisible soil life to the naked eye. To present the visible soil life, we demonstrated the activity of earthworms belonging to the annelid strain by constructing a “earthworm farm”. Earthworms mixed the layers of the already mentioned soil samples, so it provided a good mapping opportunity to observe the earthworm passages in a 10-liter vessel made of glass. Authors used 5 earthworms for the experiment, which were released after the end of the experiment. To illustrate the microscopic soil bacteria and fungi, we used chopped apples in 3 glass jars, two of which placed chernozem loam soil samples. The decomposition process of apple pieces placed on soil samples was illustrated under different conditions. Comparing the decomposition process of apple pieces stored on dry soil and wet soil sample showed that certain conditions are necessary for the operation of soil bacteria. On the wet soil sample the decomposition of fruit pieces took place partially, while in the other two cases it lagged and only shrunk, decomposition didn’t start.

We also, illustrated the degradation of the soil by water erosion modeling and demonstration of the deflation effects. Irrigation of vegetated and uncovered soils was used to demonstrate the erosion effects of water. The covered soil sample was captured by the roots and leaves of the vegetation, while the uncovered soil sample was largely washed out of the holding vessel. we used a 10-liter aquarium to illustrate the erosion effects of wind, where we also examined vegetation-covered and uncovered soil samples with a household hair dryer used to simulate wind movements. The granules of the covered soil did not move under the effect of the wind force, while the grains of the uncovered soil stirred up and mostly moved away.

Soil pollution and deterioration were illustrated by two series of 14-days experiments using materials also used in households. The first series of experiments was performed in Petri dishes using 5-5 seeds placed on 5 mg chernozem clay soil samples. Petri dishes could be replaced with small plastic or glass containers.

The seeds placed on the air-dry soil samples were contaminated with 7 ml of an aqueous solution of the materials generally found in any household, and then kept them moist by adding 2 ml of tap water every 3 days. For the entire duration of the experiment, a temperature of 22–25ºC was maintained in a place protected from daylight. In addition to the contaminated soil samples, a control group grown on a cotton wool basis and a not contaminated soil basis group was also observed. The type and percentage composition of the solutions are given in Table 2. The seeds used in the experiments were monocotyledonous cucumber and dicotyledon corn.

Table 2: Concentration and pH of contaminants used in the germination experiment

Contaminant	Solvent	Solution (%)
2 ml 20% Lidl biologically fermented vinegar (acetic acid, tap water)	5 ml H_2O (tap water)	6 % (V/V %) pH 2,6
2 ml 100% rapeseed oil	5 ml H_2O (tap water)	40 % (V/V %)
5 g Lidl iodized vacuum salt (NaCl, KI, E535)	50 ml H_2O (tap water)	10 % (m/V %) pH 6,5
0,5 g Baba soap (Sodium tallow, sodium palm kernel, water, Glycerin, Parfum, Sodium chloride, Tetrasodium EDTA, Tetrasodium etidronate, BHT, Geraniol, Hexyl cinnamon)	30 ml H_2O (tap water)	1,7 % (m/V%) pH10

As a result of the observation, there was no sign of germination in the case of seeds grown on saline-contaminated soil. In the case of seeds grown on soil contaminated with vinegar solution, the cucumber started to germinate on the tenth day, but significant deformation was observed on its cotyledons. In the case of soil sprinkled with soapy solution, the seeds began to germinate with a delay. In the oil-contaminated group, a seed germ was observed on a single seed, but all groups began to mold and by the end of the observation, the cucumber seeds began to decay. In the case of the soil-grown control group, the seeds began to germinate within a short time; however, several seeds began to mold. This is presumably due to the degrading function of the soil. In the cotton wool-grown control group, each seed grew strong cotyledons and roots.

The aim of the second 14-day experiment was to observe the most typical soil contamination effects, the consequences of inland water and soil dehydration on the soil and the development of plants growing in the soil. The experiment was carried out in transparent pots on 1-1 kg of soil below 2 mm grain size in a plant growth room at 22-24°C. For soil contamination we used Chernozem soil from Nagyhörcsök, and we used 3 types of soil to monitor the effects of dehydration and inland water. The characteristics of each soil type are shown in Table 1. The corn and cucumber seeds were pre-germinated in Petri dishes, and after the appearance of the root and buds, we planted them in the prepared pots filled up with soil close to the edges of the transparent pots. After placing the seeds in the soil, we watered the soils individually with 100 ml of tap water every three days for one week, when cotyledons developed in the case of cucumbers and leaves appeared in case of the corn seeds. As well as the development of the roots was clearly visible. The plants thus developed received contaminants that were the same as in the first 14-days germination experiment. The concentration was determined by the pH value and the estimated amount of substances used in the household. Compared to the experiment in the Petri dish, we reduced the concentration of soap and vinegar. We watered the plants once with 100 ml of the contaminating solutions as the initial step of the experiment. We examined the developmental differences of the groups contaminated with soap, vinegar, brine and oil solution compared to the growth of control plants irrigated only with tap water. Data on contaminants and the concentration used are given in Table 3.

Table 3: Concentration and pH of contaminants used in the pot experiment

Contaminant	Solvent	Solution (%)
10 ml 20% Lidl biologically fermented vinegar (acetic acid, tap water)	100 ml H_2O (tap water)	1,81 % (V/V %) pH 2,8
40 ml 100% rapeseed oil	100 ml H_2O (tap water)	28,6 % (V/V %)
10 g Lidl iodized vacuum salt (NaCl, KI, E535)	100 ml H_2O (tap water)	10 % (m/V %) pH 6,5
1 g Baba soap (Sodium tallow, sodium palm kernel, water, Glycerin, Parfum, Sodium chloride, Tetrasodium EDTA, Tetrasodium etidronate, BHT, Geraniol, Hexyl cinnamon)	100 ml H_2O (tap water)	1 % (m/V%) pH 9,8

After sprinkling with the contaminating solution, a spectacular plant stress response was observed at the sixtieth minute in the groups sprinkled with saline and vinegar solution. The plants drooped, slumped. On the seventh day of observation, in addition to the strengthening shoots and roots of the plants in the control group, the plants watered with vinegar and saline dried out and their roots thinned. The soil of the plants watered with the soap solution became lighter, the development of the plants was slower and the leaves turned yellow. The leaves of the plants watered with the oily solution turned brown in places, their development was delayed, and their roots thinned.
The harmful effects of inland water were studied with cucumbers on three types of soil. We placed the pots in which the plants grew on a larger, high-walled tray, which we then poured with 3 liters of tap water, and also sprinkled the plants with 100 ml of water each. Every three days, we watered the plants with 100 ml of water and made up the evaporated water from the tray. On the seventh day of the experiment, the cotyledons of the plants were yellowish and lagged behind in development compared to the plants in the control group.
In the study of dehydration, we also used three types of soil and examined the growth of cucumber in each case. The plants from the seventh day did not receive water supply. By the end of the experiment, their cotyledons began to sag and their development was slower than in the control group.

RESULTS AND DISCUSSION

The aim of the performed experiments was to compile the worksheets written in Hungarian in addition to gaining experience. In addition to the worksheets, students are also given an illustrative guide as a second page next to each worksheet to check each step of the experiments performed.

Authors divided the colourful, eye-catching experimental worksheets in the following topics:

1. Soil Physical Properties, Soil Particle Size Worksheet and Guide
2. Physical properties of soil, Determination of physical soil types I. worksheet and guide
3. Physical properties of soil, Determination of physical soil types II. worksheet and help
4. The Soil Drainage worksheet and guide
5. The Inland Water Worksheet and Guide
6. The Workshop and Guide to Soil Erosion
7. Worksheet and Guide to Soil Filtering
8. The Germination and Soil Contamination worksheet and guide based on your own illustration and source material used
9. Worksheet and guide on Basic (Alkaline) pH and Soil Pollution
10. Worksheet and guide on Soil Acidity and Soil Pollution
11. The Salination worksheet and guide
12. The Oil Pollution Worksheet and Guide
13. Worksheet and Guide to Soil Biological Properties, Soil Creatures

Each worksheet contains sequential knowledge, but can also be used individually and also, contains at least one of the performed experiments. The proposed experimental procedures list ordinary tools used in everyday life, so they can be easily performed in the students' homes without the need for a laboratory or school environment. Topics suggested for class 5-6 are included in the worksheets on soil physical properties, and topics suggested for class 7 are included in worksheets on soil chemical and biological properties. As a demonstration of the result, we represent the Salination worksheet and guide out of the 13 prepared teaching aids in Figure 2.

Figure 2: Salination worksheet and guide, own illustration [5], [6], [7], [8], [9]

The presentation of saline soil with a photo illustration taken in the Hortobágy National Park by Attila Szilágyi helps to understand the topic [6]. The natural and anthropogenic causes of saline soils are presented in the description. In the Soil Smarty section, the most commonly found salt and sodium is listed and detailed. The Try the experiment section, invites students to observe the plant irrigated with saline and its consequences based the experiments that have already been performed. The illustration and explanation in the guide help to follow up and understand the experimental steps. In the Did you know? Paragraph students can get familiar with the typically saline areas of Hungary with the help of a blind map of Hungary depicting saline soils. The questions at the end of the worksheet serve to deepen the curriculum.

In terms of future usage of the teaching aid package authors plan to offer it to primary school teachers as a trial version of soil protection teaching material. Therefore, actively working teachers can get involved into the final piece of this study. Furthermore, future plans include the compilation of a video material on the same topics, which will help with further illustration, support the development of digital competencies, and promote a better understanding of the topics. The ready worksheets and video package then will be available for free to use for students and teachers to support and understand soil, soil protection, consequences of soil degradation and its connection with healthy life.

CONCLUSIONS AND RECOMMENDATIONS

Experiments have revealed that soil, as a primary resource, forms an indispensable unit with the spheres surrounding it. The complex functioning of the soil is fragile and even the slightest intervention can destroy decades of soil-building work, which can cause invaluable damage to the functioning of nature as well as to future generations. During the preparation of my study, we were fortunate to study in more depth the versatility and importance of soil for terrestrial life forms. Authors have personally experienced the detrimental consequences of adverse anthropogenic influences.

Furthermore, authors understood that soil protection has become extremely important these days. It is not enough to publish the importance of this in the form of scientific dissertations, but to illustrate and show useful practical steps that can be taken to protect it in an interesting and comprehensible way for as many age groups as possible. This requires an initial step that provides direction for dedicated professionals who can be involved in putting the idea into practice. The main goal with the worksheets is for the growing generations to understand the importance of preserving natural values, including the protection of soils, and to treat their environment responsibly. In this study, the target age group is 10-14 years old, primary school student class 5-7. However, knowledge of the soil and soil protection demonstration material can be extended to other age groups, adapted to the expectations of the age group. As a result, it can be incorporated into the playful basics of pre-school education in the same way as it can be integrated into secondary school curricula or higher education units. Furthermore, it can be prepared in the same way as an understandable learning opportunity for adults. These goals are opportunities for future soil protection efforts. The tools and materials suggested for use in the experiment sections are necessity of everyday life, so they are not tied to space, time, or expensive equipment. Based on the worksheets and aids, most people can try out the experiments on display. In addition to a vision based on accuracy, technical knowledge, and prepared professional foundations. Becoming an Environmental engineer how to represent solutions based on logical reasoning on a simple cost-effective way. This study contains a realization of a vision for a healthier future that holds many new opportunities for the future.

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TL16.

IMPACT OF GLYPHOSATE HERBICIDE ON SOME SOIL BIOLOGY AND FUNCTION

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Abstract: *Soil microbiotas regulate the bioprocesses in soil that are essential for plant growth, soil health and sustained productivity. Despite an increasing concern of consequences of using vast amounts of Glyphosate-based herbicides in agroecosystems, their potential effects on non-target soil organisms and soil functioning are mostly unknown. Application of herbicides may not affect the overall size of the soil microbiotas but selectively can effect on specific microbial groups which may result in changing the balance of bioactivity and consequently nutrient availability, disease incidence and plant growth. Glyphosate is a broad-spectrum, non-selective and post-emergence herbicide, used as an active ingredient in several weed killing products since 1970s. Glyphosate is also vulnerable to microbial degradation and its main degradation product, aminomethylphosphonic acid, is strongly adsorbed to soil solids. For these reasons, Glyphosate has generally been regarded as an environmentally safe herbicide. In the current study, N_2-fixing soil microbial populations and their activities were determined in microcosms under the stress of Glyphosate herbicide were applied at 3 doses based on the active ingredients and incubated for 10 weeks at 28°C. Results indicated that: Application of Glyphosate had negative impact on symbiotic N_2-fixation by legumes in rotation and application of a post-emergent herbicide caused significant changes in the microbial community and the activities of some hydrolytic enzymes in the rhizosphere. Impacts of Glyphosate on rhizosphere microorganisms and activities were investigated. Information on the effects of herbicide application on useful soil microbiotas and their bioprocesses is necessary for the successful crop production. A particular practices may have the desired result in one situation but have little effect in another because biological communities respond to the interaction of multiple factors including food sources, physical habitat, moisture, and impacts of historical land use. Therefore, before a new product is applied to a field soil, it should be tested on a limited area and the results should be monitored in comparison to an untreated one.*

Keywords: *Herbicides, Glyphosate, soil bioprocesses, alfalfa rhizosphere*

INTRODUCTION

Soil is a natural environment required for the growth and development of plants, consists of a mixture of organic and inorganic components occurring in gaseous, aqueous and solid states. Genetically and environmentally, soils differ significantly; they tend to perform the function of natural reservoirs of water and nutrients necessary for a suitable development of the plant root system and soil microbiomes. Soil has the capacity of retaining various pollutants, such as pesticides; and therefore, it

functions as a pollution absorber. They contribute to the contamination of the food chain, which can potentially threaten human health.
The goal of managing the soil biology is to improve biofunctions, e.g., forming and stabilizing soil structure, cycling nutrients, controlling pests and disease, and degrading or detoxifying contaminants. New climatic change and agricultural practice have led to an emerging pressure from weeds and phytopathogens, which complicate farming practice and have resulted in the increased use of agrochemicals worldwide. The term "Pollution" has many definitions, one being the presence of a substance in the environment whose chemical composition or quantity prevents the functioning of natural processes and produces undesirable environmental and health effects [1]. Pollution prevention is defined as the reduction of pollutants at the source. Moreover, the technological advancement has also given rise to new pollutants which are increasing at an alarming rate and are above the self-remediating ability of the environment. There is an urgent need to find technologies that would reduce these rates/pollution levels to risk-free status in quick and easy manner [2]. Soil microbiomes are very important for agroecosystem function and sustainability due to their contributions to nutrient cycling and to soil structure maintenance [3, 4].
Soil microbiomes habitat, their activity and interactions with each other are interdependent and they may vary even within a micro-scale. The most important soil microbiomess are: bacteria, fungi, algae, protozoa and nematodes, because their metabolism is associated with the flow of energy and the circulation of elements in ecosystems in the term of biogeochemical cycles.
Microbial properties like microbial diversity and biomass are useful for predicting changes in the soil functioning and for providing an integrated and relevant vision of soil quality. Little information is currently available for preventing the loss of microbial diversity and activity through the use of sustainable farming practices from the first cultivation year. Plants are able to select their own microbiome based on their specific root exudates or some other strategies such as root physical structure and mineral nutrients preference [5].
Activities of soil enzymes and the amount of hormones produced by soil microbiomes are important indicators of soil quality and its health because of their immediate response to natural or anthropogenic changes in soil. The analysis of microbial distribution in soil environment is a key factor determining the optimal conditions for the crop development and productivity.
The exposure to pesticide has detrimental effects on health and might contribute to an increased risk of long-term diseases, including cancer and neurodegenerative diseases, reproductive and developmental disturbances, and emerging risks such as developmental neurotoxicity and immunotoxicological effects [6]. Soil organisms can be grouped by size [7], or by functions as described by Wardle [8].
Herbicide use is a vital component of modern agriculture; in particular under reduced till systems. With increased adoption of stubble retention and reduced till practices and the introduction of new herbicides, herbicide use will remain as an essential practice in the near future. Application of herbicides may not affect the overall size of the microbial pool but selectively affect specific groups of microbiotas which may result in altering the balance of biological activity (soil biological health) and consequently nutrient availability, disease incidence and plant growth.
Glyphosate is one of the most widely applied herbicides globally but its persistence in seawater has not been reported. The primary function of herbicides is to protect agricultural crops from infestation with weeds and to prevent arable soil from being overgrown by plant cover indigenous to the ecosystem. The chemicals known as herbicides are mainly synthetic organic compounds with broad molecular configurations having as a common property the ability of selectivity killing or inhibiting the growth of plants. The effects of 2,4-D, a potent herbicide, on the rhizosphere associated N-ase, N_2-fixing bacteria and plant growth characters are little understood [9]. Glyphosate, a widely used broad spectrum, non-selective, post-emergence herbicide has been shown to reduce soil bacterial populations in field soils [10]. Bayoumi [11] studied the sensitivity of three strains of *R. leguminosarum* bv. *viceae* comparing with strains of *R. leguminosarum* bv. *phaseoli, R. leguminosarum* bv. *trifolii,* and *R. loti* to 8 herbicides. Among them, Paraquat was the most toxic one. Bayoumi et al. [12] reported that Acetochlor was the least inhibitor on tested *Rhizobium* strains. Bayoumi & Kecskés [13, 14, 15] in pot experiments established that microsymbionts were more sensitive to Trifluralin than macrosymbionts, whereas are sensitive to Paraquat, which reduced the size, number and dry weight of root-nodules, phytobiomass, height, and total N-content / plant. Eberbach & Douglas [16] found Paraquat and Trifluralin affected the nodulation potential of *Rhizobium*-legume. Quantitative and representative

recovery of microbiomes from environmental soil samples is essential in understanding ecosystem function. Microbial communities play essential roles in the earth's ecology. A powerful concept in modern biology is that of the ecosystem. Soil enzymes are present in important cycles, such as C (invertase), N (urease and protease), and P (phosphatase) cycles. Soil enzyme activities are used as indices of microbial activity (Bergstrom et al., 1998) and react quickly to environment change. The biological activity in soil under the impacts of herbicides is an important issue. The present review is a comprehensive presentation of the studies discussing the state of art on the activity of soil enzymes and microorganisms. Hydrolytic enzymes activities (phosphatase, β-glucosidase), oxidoreductase activities (dehydrogenase) and indole acetic acid production, were used as measures of soil perturbation by Benitez et al. (2004). The hypothesis in this study is to estimate the effects of applied Glyphosate on (1) survival of some symbiotic N_2-fixing bacteria before and after herbicide application and (2) activities of some soil enzymes.

MATERIALS AND METHODS

The soil samples used for the present study were collected from a non-cultivated field (without previous treatments) of the experimental station at Szent István University, Gödöllő, Hungary. The soil of the sampling area was sandy brown forest of general properties are given in Table (1).

Table (1): Physical and chemical analysis of used soil

Different parameters	Value of contents
pH_{KCl}	5.33
K_A	24.6
Total Salt %	0.03
$CaCO_3$ %	0.01
Humus %	1.22
NH_4-N mg/kg	1.69
NO_3-N mg/kg	3.08
AL-K_2O mg/kg	107
AL-P_2O_5 mg/kg	106.1
Oxalate Fe mg/kg	789
Na mg/kg	36
Mg mg/kg	203
Zn mg/kg	6.9
Cu mg/kg	2.7
Mn mg/kg	36
SO_4 mg/kg	4.8

The first portion was stored at 4°C for enzymatic activities and microbiological analyses. The second portion was stored at cool room for plant-microbe interaction. Herbicides Sys 67 B 80%, Dikamin D 40%, Ro-Neet 6E 74%, Gliaka 20%,, Linuron 50%, Sys 67-ME 80%, Gramexone A 25%, and Treflan 26% were used in the *in vitro* while Glyphosate used *in vivo* experiments. The characterisations of the tested herbicides are mentioned in Table (2).

Eight herbicides were subjected to establish their side-effects on the growth rate of 8 *S. meliloti* strains in yeast mannitol broth (YMB) medium using microfermentor technique [12, 15] at 5 concentrations of active ingredients (0.1, 1.0, 10.0 and 100 mgl^{-1}) of each herbicide. The experiment was designed to detect the interactions between the herbicides amendment and the biological N_2-fixation in brown forest soil as well as the potential activities of some soil enzymes in alfalfa rhizosphere soil. The study was conducted in pot experiment predicated to low and high herbicide doses. The herbicide was applied to the soil before (post-emergence) and after (pre-emergence) plantation and plant inoculation in two separated sets of pots.

Table (2): Some characteristics of herbicides used in laboratory and greenhouse investigations

Group	Common name	Active substance	Field Recommended
Aliphatics	Gliaka	Glyphosate (20%)	1.2 l
Bipyridylims	Gramexone A	Paraquat (25%)	3.5 l
Carbamates	Sabet 72 EC	Cycloate (72%)	4.0 l
Dinitroanilines	Treflan	Trifluralin (26%)	3.5 l
Phenoxys	Dikamin D	2,4-D (40%)	2.4 l
	Sys 67 B	2,4-DB (80%)	2.0 kg
	Sys 67-ME 80	MCPA (80%)	2.0 kg
Substituted Ureas	Linuron 50	Linuron (50%)	2.0 kg

Generally, the following experiments of predicating the impacts of herbicide on the plant-microbe interactions were carried out using one Hungarian cultivar of alfalfa, three new isolated strains of *S. meliloti* (GHF-162, GHF-281 and GHF-3153), one standard strain (GHR-94) and one herbicide Glyphosate at 3 concentrations (Table 3). Sterile plastic pots of 3 Kg capacity were used, filled with 2 kg sterile (steamed 100°C for 1 h on three consecutive days) brown forest soil. Seeds of alfalfa (*M. sativa)* were surface-sterilized and germinated in the pots. The post-emergence or pre-emergence application of the Glyphosate was applied to soil accordance with the fact that one hectare containing 3.6 x 10^6 kg soil, 10^4 m^2 (surface area), 0.25 m (depth) and 1.44 kg m^{-3} (soil density). The commercial formulation of the tested herbicides diluted with sterile distilled water to the appropriate concentration of each one according to its active ingredient (a.i.) on top of 25 cm of field soil.

Table (3): Herbicide treated doses

Active ingredient (a.i)	Rate (liter a.i/ha)	Relevance to field application rate
Glyphosate	0.60	½ Recommended
	1.20	Recommended
	2.40	2 x Recommended

Seeds were selected for healthy and uniformity without any injury, and surface sterilized with 70% ethanol followed by acidified 0.2% $HgCl_2$ for 5 minutes, and thoroughly washed in several changes of sterile distilled water. Seeds were then soaked for 8 h at room temperature in sterile distilled water (soaking water was changed every 2 h) and then seeds were germinated on sterile wetted filter paper in large Petri dish for 72 h in the dark at 28°C according to Franco & Vincent (1976). Seedling rhizosphere was inoculated with 10 ml of a suspension of biofertilizer *Sinorhizobium* inoculum (GHR-94, GHF-162, GHF-281, or GHF-3153) prepared as follows: each one of the five biofertilizers *S. meliloti* strains was grown in YMB for 48 h at 28°C, to give a final cell concentration 2.5 x 10^8 cell capacity ml^{-1}, using haemocytometer for calibration. Seedlings were watered with sterile tap water when required, and the plants grown under natural illumination (14 h) at around 28 ± 2°C.

At the end, plants were carefully uprooted, and washed several times in tap water for farther investigations. Data recorded / plant as follows: Height of plant shoot in cm, number of root nodules. Dry weight of plants biomass and nodules were determined after oven dried at 75°C to a constant weight and the values were expressed as g $plant^{-1}$ and mg root nodules $plant^{-1}$. Total N-content (mg $plant^{-1}$) was measured using micro-Kjeldahl method as a criterion of N_2-fixation [17]. Therefore, all visible plant fragments were carefully removed from the soil sample prior to the enzyme assays. The potential activities of some enzymes in the alfalfa rhizosphere and soil bulk were measured under the stress at different concentrations of herbicides, and inoculated different biofertilizers strains of *S. meliloti*.

The following enzymes were determined: Dehydrogenase activity was determined according to García et al. [18]. Dehydrogenase activity is expressed as μg of INTF per gram dry soil. Protease activity on N-α-benzoyl-L-argininamide (Protease-BAA) was measured using the method of Nannipieri et al. [19]. Protease activity is expressed as μmol of NH_4^+-N released per gram dry soil per hour.

Phosphatase activity was carried out using the method of Tabatabai & Bermner [20] and the phosphatase activity is expressed as μmol of PNP per gram dry soil and incubation time (hour). β-glucosidase activity is expressed as μmol of PNP per gram dry soil and incubation time (hours) after applying the method of Masciandaro et al. [21].
The experiments were conducted in three replicates and some statistical analyses are carried out to determine the efficiency of the application of herbicide on the soil biology.

RESULTS AND DISCUSSION

Pesticides are xenobiotic molecules necessary to control pests in agriculture, home, and industry. However, water and soil can become contaminated as a consequence of their extensive use. Therefore, because of its eco-friendly characteristics and efficiency, bioremediation of contaminated sites is a powerful tool with advantages over other kinds of treatments [22].

Selection of bacterial strains and herbicides

Table (4) summarizes the relative growth rates and the tolerance ability of the *S. meliloti* strains toward the potent effect of herbicides in compared with control growth of the strains in herbicide-free broth medium. These investigations showed that herbicide MCPA had no harmful influence on *S. meliloti* strains GHR-94, GHF-162, GHF-281, and GHF-3153. However, the other strains were tolerated the herbicide-amended YMB up to 10 mgl^{-1}. Herbicides Paraquat and Trifluralin were highly toxic to the all tested strains and their effect was rapid. The investigated strains can be grouped in three groups according to the effect of Paraquat, first group contained tolerant strains to 10 mgl^{-1} (GHR-94, GHF-162, GHF-281 and GHF-3153), the second group included the tolerant strains to 1 mgl^{-1} (GHF-1141 and GHF-353), the third group of strains tolerant 0.1 mgl^{-1} (GH-130 and GHF-2100). Herbicides Linuron, Cycloate and Glyphosate were toxic to all strains but at 100 mgl^{-1}. But their effects on the growth rate of strains were more gradual compare with the effect of Paraquat and Trifluralin. Herbicides 2,4-D, 2,4-DB, had stimulated effect on the growth of the strains GHR-94, GHF-162, GHF-281 and GHF-3153 throughout all investigated concentrations. These herbicides stimulated the growth rates of the all tested strains at different patterns at 1 mgl^{-1} and 10 mgl^{-1} but at 100 mgl^{-1}, strains GH-130, GHF-1141, GHF-2100, and GHF-353 were inhibited. The results indicated that the most tolerant strains of *S. meliloti* were GHR-94, GHF-162, GHF-281 and GHF-3153. Also, most of statistically significance values as regard between the relative growth rate of the different investigated strains and the low and high of herbicidal concentrations.

Table (4): Tolerance of the selected strains to concentrations of herbicides

Strain	Herbicides (mg/l)							
	Glyphosate	*Paraquat*	*Cycloate*	*Trifluralin*	*2,4-D*	*2,4-DB*	*MCPA*	*Linuron*
GHR-94	*100*	*10*	*100*	*10*	*> 100*	*> 100*	*> 100*	*100*
GH-130	*100*	*0.1*	*100*	*1*	*> 100*	*> 100*	*10*	*100*
GHF-162	*100*	*10*	*100*	*10*	*> 100*	*> 100*	*> 100*	*100*
GHF-1141	*100*	*1*	*100*	*1*	*> 100*	*> 100*	*10*	*100*
GHF-281	*100*	*10*	*100*	*10*	*> 100*	*> 100*	*> 100*	*100*
GHF-2100	*100*	*0.1*	*100*	*1*	*> 100*	*> 100*	*10*	*100*
GHF-353	*100*	*1*	*100*	*1*	*> 100*	*> 100*	*10*	*100*
GHF-3153	*100*	*10*	*100*	*10*	*> 100*	*> 100*	*> 100*	*100*

Effect of the applied herbicide on symbiotic relationship

Depending on the received results, and in addition, to determine the impacts of Glyphosate (selected herbicide) on the effectiveness (dry matter and total N content), „saprophytic competence" and competitiveness to evaluate the nodulation potential of the strains, *in vivo* experiment was conducted in a pot model under several factors. This investigation was done to show the influence of application

of different factors such as herbicide applied in post- and pre-emergence of young alfalfa seedlings inoculated with the selected biofertilizer of *S. meliloti* strains (GHR-94, GHF-162, GHF-281 and GHF-3153). The results showed that reduction in nodulation was usually associated with reduced plant growth caused by herbicidal injury. The double of field recommended applied dose of herbicide in pre-emergence phase reduced the morphological characterization and plant dry weight of the plant as well as the symbiotic properties. These investigations show that herbicide Glyphosate had no harmful influence on the investigated *S. meliloti* strains if used as recommended. Generally, herbicide is presently used for establishment of alfalfa. The herbicide at three applied rates (0.5x, x and 2x, x = FRD) did not cause any visual phytotoxicity to the plant crop. Glyphosate had significantly improved the number and size of nodules especially those plants inoculated by the new isolated strains (GHF-162, GHF-281 and GHF-3153) compared with standard strains GHR-94.
Table (5) shows the impacts of soil treated by Glyphosate on the nodule numbers formed by different strains of biofertilizer.
In Table (5), results indicated a significance differences between the controls plants grown in soil free from herbicide treatment and those plant grown in soil treated with herbicide at post-emergence at 0.5x dose applied to the soil. But when the herbicide was applied as pre-emergence, no statistical differences were recognized throughout all treatments.
The nodule formation at post-emergence was more than at the pre-emergence condition. Only one case showed statistically difference between the plant inoculated by all biofertilizers and Glyphosate at 0.5x under post-emergence. The results indicated that the new isolated strains were more effective to modulate the plant than the standard strain (GHR-94) did. Here, the nodulation potential of the strains in decreasing order can be GHF-3153 > GHF-281 > GHF-162 > GHR 94.

Table (5): Impacts of post- and pre-emergence of Glyphosate on nodule number (number $plant^{-1}$) of alfalfa root inoculated by different biofertilizers

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
GHR-94	*0*	*25*	*25*
	0.5x	*33**	*31*
	x	*29*	*28*
	2x	*27*	*25*
GHF-162	*0*	*34*	*34*
	0.5x	*42**	*36*
	x	*39*	*32*
	2x	*35*	*30*
GHF-281	*0*	*40*	*40*
	0.5x	*47**	*42*
	x	*42*	*41*
	2x	*40*	*37*
GHF-3153	*0*	*44*	*44*
	0.5x	*51**	*46*
	x	*47*	*45*
	2x	*43*	*41*
S.D. (*P* = 0.05)		*6.733*	*6.970*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

Table (6) shows the relationship between the application of Glyphosate and the nodule dry weight, under the artificial inoculation by different biofertilizers. It was found that, the nodule dry weight in the case of post-emergence was more than those in the pre-emergence, and the results showed a statistically differences in the nodule dry weight on at the 0.5x level of herbicide applied dose. Similar results can be obtained based on the nodule dry weight in which the efficiency of the strain towards the effect of Glyphosate in decreasing order is GHF-3153 > GHF-281 > GHF-162 > GHR 94.

Table (6): Impacts of post- and pre-emergence of Glyphosate on nodule dry weight (mg plant^{-1}) of alfalfa root inoculated by different biofertilizers

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
GHR-94	*0*	*132*	*113*
	0.5x	*163**	*138**
	x	*145*	*134*
	2x	*128*	*107*
GHF-162	*0*	*172*	*144*
	0.5x	*201**	*169**
	x	*196*	*157*
	2x	*175*	*132*
GHF-281	*0*	*174*	*146*
	0.5x	*204**	*172**
	x	*182*	*154*
	2x	*174*	*139*
GHF-3153	*0*	*197*	*163*
	0.5x	*226**	*191**
	x	*217*	*179*
	2x	*196*	*163*
S.D. (P = 0.05)		*27.203*	*20.940*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

The effect of Glyphosate on the plant growth can be shown in Table (7), which summarized the action of the herbicide on the plant height. The results of the present study indicated that the plant inoculated with different biofertilizers increased the plant height significantly under the post-emergence treatment at all treated doses (0.5x, x, and 2x of FRD). While, under the effect of pre-emergence treatments, the plant height was increased but not statistically differences with the control treatment. The biofertilization of the plant with the new isolated strains of *S. meliloti* improved the plant height than the standard strain GHR-94. The effective strain was GHF-3153.

Table (7): Impacts of post- and pre-emergence of Glyphosate on plant height (cm) of alfalfa root inoculated by different biofertilizers

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
Uninoculated	*0*	*40.1*	*40.1*
	0.5x	*46.5**	*39.1*
	x	*47.2**	*41.1*
	2x	*44.2*	*38.3*
GHR-94	*0*	*52.3*	*46.2*
	0.5x	*61.4**	*51.1*
	x	*60.3**	*54.8*
	2x	*58.7**	*49.5*
GHF-162	*0*	*55.7*	*51.2*
	0.5x	*64.3**	*57.9*
	x	*62.7**	*55.3*
	2x	*61.3**	*52.6*
GHF-281	*0*	*59.6*	*54.3*
	0.5x	*70.5**	*61.3*
	x	*68.5**	*57.8*
	2x	*65.2**	*54.8*

GHF-3153	*0*	*61.7*	*56.4*
	0.5x	*74.5**	*64.7*
	x	*70.4**	*60.4*
	2x	*68.6**	*57.6*
S.D. (P = 0.05)		*5.75*	*4.69*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

Table (8) explains the beneficial inoculation of the plant by the biofertilizer strains of *S. meliloti*. The plant dry weight is a fact of the symbiosis take place and the process was efficient. This means that the biological nitrogen fixation was happened, and the plant can accumulate the dry matter.
The application of the Glyphosate at post-emergence to the soil significantly increased the plant dry matter compared with the control. At pre-emergence application, it was significant differences when the plant inoculated by GHF-162.

Table (8): Impacts of post- and pre-emergence of Glyphosate on plant dry weight (g plant^{-1}) of alfalfa root inoculated by different biofertilizers

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
Uninoculated	*0*	*3.4*	*3.4*
	0.5x	*3.6*	*3.5*
	x	*3.4*	*3.2*
	2x	*3.1*	*3.2*
GHR-94	*0*	*3.7*	*3.7*
	0.5x	*4.1**	*3.8*
	x	*4.7**	*3.9*
	2x	*4.5**	*3.7*
GHF-162	*0*	*3.9*	*3.9*
	0.5x	*4.4**	*4.2**
	x	*4.5**	*4.2**
	2x	*4.3**	*4.1**
GHF-281	*0*	*4.1*	*4.1*
	0.5x	*4.5**	*4.2*
	x	*4.7**	*4.3*
	2x	*4.6**	*4.3*
GHF-3153	*0*	*4.2*	*4.2*
	0.5x	*4.8**	*4.4*
	x	*4.7**	*4.3*
	2x	*4.5**	*4.3*
S.D. (P = 0.05)		*0.308*	*0.235*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

The present study showed that there were no statistically differences between the post- and pre-emergence application of herbicide in term of plant dry weight. The results indicated that GHF-3153 is the most effective strain. The concept of total N content/plant gives an idea about the N_2-fixed by the association between the symbiotic partners as shown in Table (9). It was found that the nitrogen accumulated in plant after inoculation with biofertilizers was increased compared with the control uninoculated plants. Results were indicated that the strain GHF-3153 is the most effective strain and economically can be industrially formulated as biofertilizer with high potent to increase the plant growth, plant dry matter and able to fix the atmospheric nitrogen more than the other investigated strains.

It was found that the application of herbicide at the x and 2x of FRD increased significantly the amount of nitrogen accumulated in the plant; also the amount of nitrogen measured in plant grown at post-emergence application of herbicide was higher than the amount measured at pre-emergence. In pre-emergence, the application of double FRD was significantly increased the amount of nitrogen in the plants. The results directly demonstrated high amount of nitrogen content in the plant and indirectly showed the ability of the strains to multiply and survive in the soil.

Table (9): Impacts of post- and pre-emergence of Glyphosate on total N content (mg N plant^{-1})

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
Uninoculated	*0*	*214*	*214*
	0.5x	*236*	*233*
	x	*273*	*256*
	2x	*299**	*285**
GHR-94	*0*	*278*	*278*
	0.5x	*317*	*305*
	x	*366**	*346**
	2x	*379**	*369**
GHF-162	*0*	*315*	*315*
	0.5x	*338*	*324*
	x	*389**	*383**
	2x	*401**	*389**
GHF-281	*0*	*394*	*394*
	0.5x	*445*	*399*
	x	*468**	*462**
	2x	*477**	*475**
GHF-3153	*0*	*427*	*427*
	0.5x	*487*	*466*
	x	*501**	*496**
	2x	*527**	*502**
S.D. (P = 0.05)		*73.330*	*67.112*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

Effect of the applied herbicide on the function of some soil enzymes

In the second part of the alfalfa *S. meliloti* strains in symbiotic relationship in the presence or absence of herbicide enhanced soil enzyme activities in the rhizosphere soil. In this study, soil treated with Glyphosate at 0.5 x had greater dehydrogenase, protease, β-glucosidase and phosphatase activities than soil treated with higher concentrations (Tables 10-13).

Table (10) indicates that the inoculation promoted the dehydrogenase activity and enhanced the enzyme activity at post-emergence over the activity at the pre-emergence. The results showed that application of Glyphosate at x and 2x of FRD were more statistically significant with the control. The inoculation of plants with GHF-3153 more effective to enhanced the activity of the enzyme.

Table (11) shows that the biofertilization of plants with *S. meliloti* strains promoted the protease activity and enhanced the enzyme activity at post-emergence over the activity at the pre-emergence. Similar mode of interactions between the symbionts and the application of herbicide were observed in case of determining the activity of protease. The results showed that application of Glyphosate at x and 2x of FRD were more statistically significant with the control and the incoculation of plants with GHF-3153 more effective to enhance the activity of the enzyme. Statistically, significant differences between the FRD of the herbicide at higher doses and the enzyme activity were found.

Table (10): Impacts of post- and pre-emergence of Glyphosate on the activity of dehydrogenase (μg INTF/g soil) in the rhizosphere

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
Uninoculated	*0*	*70,4*	*70,4*
	0.5x	*84,3*	*71,8*
	x	*91,6*	*80,7*
	2x	*115,8**	*100,6**
GHR-94	*0*	*88,1*	*88,1*
	0.5x	*117,3*	*97,5*
	x	*128,5**	*105,4*
	2x	*157**	*134,5**
GHF-162	*0*	*97,9*	*97,9*
	0.5x	*124,7*	*104,3*
	x	*144,9**	*127,1**
	2x	*178,9**	*144,7**
GHF-281	*0*	*104,1*	*104,1*
	0.5x	*147.3**	*133,1**
	x	*186,9**	*165,7**
	2x	*201,1**	*178,5**
GHF-3153	*0*	*117,9*	*117,9*
	0.5x	*159,5**	*138,9**
	x	*194,1**	*154,4**
	2x	*212,8**	*177,9**
S.D. (P = 0.05)		*40.07*	*29.08*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

Table (12) illustrates that the *S. meliloti* strains promoted the phosphatase activity and increased the enzyme activity at post-emergence over the activity at the pre-emergence. Similar results were found as in the cases of dehydrogenase and protease that the effect of the plant inoculation with different strains of *S. meliloti* of interactions between the symbionts and the application of herbicide were observed in case of determining the activity of phosphatase. These results showed that application of Glyphosate at x and 2x of FRD were more statistically significant with the control and the incoculation of plants with GHF-3153 more effective to enhance the activity of the enzyme.

Table (13) shows that the activity of β-glucosidase activity in the rhizosphere of the alfalfa plants were inoculated by different strains of *S. meliloti* was increased with the increasing the concentrations of the Glyphosate, especially at higher concentrations which show a statistically significant. The strain GHF-3153 proved to be ecological important for increasing the activities of the soil enzymes and reduce the toxicity of the herbicide. Also, the selection of the *S. meliloti* strains for the monitoring the effect of herbicide applied in the field of symbiotic N_2-fixation was successful and suitable for applied in the field after industrial process to be commercial for market.

Many pesticides are used around the world to protect crops against insects, fungi, weeds, and other pests [23]. In addition to their agricultural application, they are also used for preserving wood and controlling home garden pests [24].

Adegas et al. [25] mentioned that the intensive use of Glyphosate has resulted in the selection of seven resistant species: ryegrass (*Lolium multiflorum*), buva (*Conyza bonariensis, C. canadensis, C. sumatrensis*), bittergrass (*Digitaria insularis*), caruru- palmeri (*Amaranthus palmeri*) and the wing-foot grass (*Eleusine indica*). To some extent, our results are in agree with the results of Aynalem1 and Assefa [26] when they investigate the effect of Glyphosate on the Rhizobia Isolated from Nodules of *Vicia faba*.

Table (11): Impacts of post- and pre-emergence of Glyphosate on the activity of protease (μmol NH_4^+-N/g soil/h) in the rhizosphere

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
Uninoculated	*0*	*1,39*	*1,39*
	0.5x	*1,47*	*1,25*
	X	*1,66*	*1,43*
	2x	*1,87**	*1,65**
GHR-94	*0*	*1,54*	*1,43*
	0.5x	*1,88**	*1,67**
	X	*1,94**	*1,74**
	2x	*2,2**	*1,98**
GHF-162	*0*	*1,63*	*1,45*
	0.5x	*1,97**	*1,89**
	X	*2,06**	*1,95**
	2x	*2,28**	*2,03**
GHF-281	*0*	*1,71*	*1,57*
	0.5x	*2,05**	*1,83**
	X	*2,34**	*1,99**
	2x	*2,65**	*2,07**
GHF-3153	*0*	*1,76*	*1,66*
	0.5x	*2,03*	*1,90**
	X	*2,45**	*2,01**
	2x	*2,61**	*2,25**
S.D. (P = 0.05)		*0.335*	*0.233*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

Herbicides can exert collateral effects on soil microbiotas and important functions such as N cycling, decomposition, hydrolytic enzyme activities, etc. Some of these compounds represent a source of N to microbial communities through mineralization. Soil microbiotas regulate a majority of processes in soil that are essential for plant growth, soil health and sustained productivity.

A large, diverse and active soil microbiotas could provide soil conditions for sustainable crop production through (i) crop residue decomposition and improvement of nutrients, (ii) preventing aggressive phytopathogens taking hold and improve plants ability to withstand disease effects, (iii) reducing the loss of inorganic fertilizers through erosion and leaching by short-term immobilization (iv) stabilizing soil structure and (v) reducing the reliance for agrochemicals and reduced persistence of pesticides in soil and thus less off-site impacts.

Application of herbicides may not affect the overall size of the soil microbiotas but selectively affect specific groups of microbiota which may result in altering the balance of biological activity and consequently nutrient availability, disease incidence and plant growth. Non-target effects of herbicides could be either positive or negative.

Herbicides are widely used in agriculture to control unwanted organisms including weeds. However, they can also threaten the environment and human health through spray drift, runoff, leaching, disposal, spills, and leakage [26, 27]. Thus, their dissipation, elimination, and distribution behaviors under environmental conditions must be comprehensively assessed, especially in the soil and aquatic sediments (Davis et al. 2005) that tend to be the environmental compartments most at risk for herbicide accumulation.

Management of herbicides usage that cause reversible inhibitions is difficult, as reaching a balance between high herbicide efficiency and minimum non-target effects requires a better understanding of herbicide-microbial communities-environment interactions.

Table (12): Impacts of post- and pre-emergence of Glyphosate on the activity of phosphatase (μmol PNP/g soil /h) in the rhizosphere

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
Uninoculated	*0*	*54,3*	*44,4*
	0.5x	*76,3*	*67,3*
	x	*89,1**	*78,9**
	2x	*107,6**	*92,6**
GHR-94	*0*	*74,7*	*56,6*
	0.5x	*67,4*	*66,1*
	x	*100,1**	*86,3**
	2x	*115,7**	*92,3**
GHF-162	*0*	*88,9*	*74,6*
	0.5x	*97,6*	*87,5*
	x	*114,5*	*99,6**
	2x	*132,1**	*112,1**
GHF-281	*0*	*89,8*	*78,6*
	0.5x	*103,5*	*84,5*
	x	*132,1**	*104,1**
	2x	*144,3**	*115,3**
GHF-3153	*0*	*95,7*	*88,5*
	0.5x	*115,6*	*104,6*
	x	*134,5**	*123,3**
	2x	*156,3**	*134,2**
S.D. (P = 0.05)		*26.67*	*22.39*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

Table (13): Impacts of post- and pre-emergence of Glyphosate on the activity of β-glucosidase (μmol PNP/g soil /h) in the rhizosphere

Biofertilizers	*Herbicide dose*	*Emergence*	
		Post-	*Pre-*
Uninoculated	*0*	*114*	*98*
	0.5x	*123*	*115*
	x	*148*	*132*
	2x	*178**	*154**
GHR-94	*0*	*167*	*145*
	0.5x	*183*	*163*
	x	*212**	*189**
	2x	*241**	*203**
GHF-162	*0*	*177*	*145*
	0.5x	*197*	*165*
	x	*223**	*189*
	2x	*254**	*214**
GHF-281	*0*	*179*	*156*
	0.5x	*201*	*189*
	x	*254**	*221**
	2x	*266**	*235**
GHF-3153	*0*	*174*	*162*
	0.5x	*204*	*187*
	x	*277**	*211**

	2x	*287**	*267**
S.D. (P = 0.05)		*48.9*	*41.6*

Values are means of three replicates, and values labelled with * shows statistically significance with the control (untreated) plants

Different soil microbial populations and their activities were determined in a microcosms of 3 kg capacity under the stress of 8 herbicides were applied at 3 doses based on the active ingredients and incubated for 10 weeks at 25°C. Results from these investigations on the effects of a single application of selected herbicides in alfalfa growing soils indicated that: Some of herbicides currently used in alfalfa soils have a negative impact on key groups of microorganisms; Most of the negative effects were reversible partly or fully within 10-weeks after herbicide application. Some herbicides caused a significant shift in bacteria : fungi ratio, reduced the rate of cellulose-decomposition; Some herbicides applied had negative impact on symbiotic N_2-fixation by legumes in rotation and application of a post-emergent herbicide caused significant changes in the rhizosphere microbial activity and the composition of microbial community as well as the activities of some hydrolytic enzymes. It was found that Glyphosate causes activation in soil urease and invertase soil enzymes but suppression of phosphatase [29].

Finally, this study was designed to assess the effect of Glyphosate on growth of alfalfa sinorhizobial isolates in vitro and on their N_2-fixation performance. Hence, four selected isolates were isolated using plant-soil trap method from soil samples collected from farm lands. Those isolates were morphologically characterized using YMA medium and authenticated as nodulating sinorhizobial isolates using sand culture. These isolates were treated with 0.5X, X and 2X herbicide doses. The result showed that almost all isolates were not affected. For *in vivo* experiment, alfalfa seedlings in brown forest soil culture were inoculated with four relatively *in vitro* test tolerant isolates. The inoculated isolates were treated with half, double and field recommended concentration of Glyphosate,. Thus, experimental plants almost all showed normal nodulation and N_2-fixation performance as compared to the control.

CONCLUSIONS AND RECOMMENDATIONS

The consistent application of agrochemical herbicides has been reported to impact negatively on human health, environment, and food safety, and facilitated the emergence of weed resistances. A generalized relationship between injury level and yield response can be established in weed-free experiments by observing the injury patterns which result from the application of various rates of herbicides. Glyphosate did not significantly interfere in plant growth with a gradual reduction in height increase relative to the control, with increasing Glyphosate doses. Post-emergence increase total N content, total root-nodules, root-nodule dry weight, plant height, plant dry weight and all investigated soil enzymatic activities at all applied doses. It was observed that there is still a need for research evaluating the physiological suitability of specific soybean cultivars to determine the tolerance of the crop to different mechanisms of action in order to minimize the frequent use of herbicides and weed control strategies, good performance and productivity of the crop. This is promising result to identify and obtain agrochemical resistant inoculum for more N_2-harvest under no-tillage agriculture system, but further actual field experiment will be required.

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TL17.

ECOTOXICOLOGICAL EVALUATION OF THE TOLERANCE OF SOME PLANT GROWTH-PROMOTING RHIZOBACTERIA TO ENVIRONMENTAL METAL POLLUTANTS

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Abstract: *The major N_2-fixing systems can play a significant role in improving the fertility and productivity of low-N soils. The plant growth-promoting rhizobacteria (PGPR) have received the most attention and have been examined extensively. Under heavy metal stress, soil microorganisms including PGPR have developed many strategies to evade the toxicity generated by the various heavy metals. In the present study, some environmental factors such as heavy metals (Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}), Al^{3+} were studied at different concentrations (0, 20, 40, 80 and 160 µm) on the ecophysiological properties and their growth using microfermentor technique as well as their tolerances to various hydrogen ion concentration. The results showed that the stress factor suppresses the growth characteristics of the studied PGPR including symbiotic Sinohizobium strains related to R. leguminosarum, R. phaseoli, R. trifolii, and R. loti as well as Sinorhizobium meliloti in comparison with non-symbiotic N_2-fixer Azotobacter and Pseudomonas as PGPR strains which are differentially react with the stress factors at high concentrations. However, the tolerance of several investigated strains, distributed among Sinorhizobium meliloti strains: GH-130, GHR-94, GH-97 and GH-07 are sensitive to the stress of 160 µm for heavy metals effects in comparison with Azotobacter and Pseudomonas strain. None of the strains can tolerate 160 µm of Cd^{2+}, $Pb^{2+}$and Cu^{2+}. The most toxic metal was Cd^{2+}, followed by $Pb^{2+}$and Cu^{2+}. The ecophysiological tolerant Sinorhizobium and Azotobacter as well as Pseudomonas putida strains are suggested to be the ideal solution for the improvement of soil fertility and the rehabilitation of reclaimed soils and are an important direction for future soil protection and quality research. Hence, a better understanding of N_2-fixing bacteria ecophysiological responses to different intrinsic stresses factor is very important to improve crop production by harnessing the growth of the N_2-fixing bacteria process.*

Keywords: *heavy metals, aluminium, pH, relative growth rate, tolerance, N_2-fixing bacteria, Azotobacter, Pseudomonas*

INTRODUCTION

Heavy metals are well known for their toxicity and become significant environmental pollution with a continually rising technology and public outcry to ensure the safest and healthiest environment. Contamination of the environment by heavy metals has resulted in the manifestation of heavy metal tolerant microorganisms in the soil polluted with metals. In addition, such heavy metals, once in the soil, accumulate preferentially in the parts where the plant roots are aggregated and in the forms that are easily available to plants. These heavy metals are then absorbed by the plants thus, ultimately entering the food chain. Microorganisms use different kinds of mechanisms related to resistance and detoxification of heavy metals [1] thus play prominent part in biogeochemical cycling of harmful heavy metals leading to the remediation of metal contaminated environments [2]. Abo-Amer [3] demonstrated that among *Azotobacter* isolates extracted from the soil contaminated with wastewater, 10 strains exhibited considerable degree of resistance to the heavy metals like Co, Ni, Zn and Cu. The soil microbial community faces extremely high pressure due to adulteration of soil by a range of toxic materials including heavy metals along with other organic contaminants of wastewater, sewage sludge etc. The addition of heavy metals in several forms in the environment results in significant alterations of the microbial diversity and activities, thus directly affecting the soil fertility [4]. Some of the heavy metals are necessary for microbial growth and biochemical reactions in very low concentrations in the cell. However, as the heavy metal concentration increase it becomes largely toxic to microorganisms thereby leading to disturbance in vital ecological processes [5]. Pollution of soil with heavy metals is known to have a negative effect on microbial activities. Metal toxicity is of great environmental concern because of their bioaccumulation and non-biodegradability in nature. Today, heavy metal pollution has become one of the serious issues of concern amongst all environmental crises. Heavy metals and aluminium are one of the major sources of environmental pollutants and exist in soil as free metal ions, soluble metal complexes, exchangeable metal ions, organically bound metals, precipitated or insoluble compounds like oxides, carbonates and hydroxides or they may form a part of silicate materials [6]. Heavy metal arise in soil by repeated applications of sewage sludge, municipal wastes and animal slurries, activity of mining and smelting industries, impurities in fertilizers and decomposition of air pollutants by burning of fossil fuels and various industrial activities [7]. Heavy metals such as Cu, Fe, Mn, Ni and Zn are essential for plant growth and are important constituents of many enzymes, whereas metals such as Al, As, Cd, Cr, Hg, Pb, Sb and Se are nonessential and toxic above certain threshold levels [8]. An increase in metal concentration also influences the soil microbial properties, especially respiration and enzymatic activity that serve as good indicators of metal pollution. Several studies have shown a negative relationship between heavy metal concentration and microbial activities, such as respiration [9], [10], mineralization [11].

A total of 120 *Azotobacter* sp. were isolated from soils irrigated with wastewater (contaminated soils) and groundwater (uncontaminated soils). These isolates were screened for resistance to heavy metals. The size of the *Azotobacter* population in heavy metal contaminated soil was lower than that in uncontaminated soil. Of the *Azotobacter* isolates, 64 that were recovered from contaminated soil exhibited high resistance to heavy metals (Hg^{2+}, Cd^{2+}, Cu^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+} and Pb^{2+}) compared to 56 isolates from uncontaminated soil. The majority of *Azotobacter* isolates from contaminated soil showed multiple-resistance to different metal ions. All isolates failed to grow at pH less than 6 [4].

The study thereby highlighted the possible utilization of such bacterial isolates for the bioremediation of metal-contaminated system. Studies by Joshi and Juwarkar [12] revealed that a heavy metal-resistant strain of *Azotobacter* spp. possess a high tendency of binding with Cd and Cr both under in vitro as well as in vivo conditions, and thereby consists of significant control of their uptake by wheat plants raised in heavy metal polluted soils. Resistance to heavy metals in *Azotobcter* spp. is demonstrated to be provided by plasmids [13]. But, in case of *Azotobacter* species particularly, prior to the entry of heavy metal into the cell, they face an encounter with extracellular polymeric substances, which are reported to be produced in large amounts by this bacterium. Extracellular polymeric substances thus clearly play the role of first barrier by chelating the metal ions and restricting their access into the bacterial cells. In addition to its beneficial impact on plant growth promotion, *Azotobacter* is also known to be associated with the suppression of pathogenic diseases of plants. Several examples are present in the literature advocating the importance of disease suppression by different species of *Azotobacter.*

The utilization of PGPR in agriculture is continuously increasing as it offers an effective tool to replace the use of chemical fertilizers, pesticides and other harmful supplements [14], [15], [16]. Growth promoting substances are produced in huge quantities by the action of these rhizosphere microorganisms that directly or indirectly influence the overall morphology and physiology of the crops. Recent advances in the field of sustainable development relies on the use and diversity of PGPR, their colonizing capability and the mechanism of action that may be used to facilitate their application as a dependable element in the management of sustainable agricultural system [15], [16].

MATERIALS AND METHODS

Bacterial strains

- *Sinorhizobium meliloti* strains: GH-130, GHR-94, GH-97 and GH-07
- *Azotobacter* strains: Azot-99 and *Azotobacter* sp
- *Pseudomonas* strain: *Pseudomonas putida*

Aluminium and heavy metal salts:

- Zinc chloride ($ZnCl_2.6\ H_2O$)
- Copper chloride ($CuCl_2$)
- Lead chloride ($PbCl_2$)
- Cadmium chloride ($CdCl_2.2.5H_2O$)
- Aluminium sulphate (Al2(SO4)3

Growth of bacteria in heavy metal treated culture

Using the microfermentor technique, the following investigations were done to determine the relative growth rate (%) of the investigated *Sinorhizobium*, *Azotobacter* and *Pseudomonas* bacterial strains when the cultural broth media contaminated with heavy metals (Zn, Cu, Pb, Cd) and Al or the pH of the cultural broth media adjusted to be 7; 6.2; 5.2 and 4.5.

Azotobacter bacterial strains were used to measure their growth under different heavy metal stress. A synthetic medium was used (according to Ueda et al. [17]) which contained 1.0 g K_2HPO_4, 0.2 g $MgSO_4$-$7H_20$, 0.1 g $CaCO_3$, 0.2 g NaC1, 5 mg $Na_2MoO_4.2H_20$, and 10.0 g sucrose per litter of distilled water. In flasks of 150 ml capacity, each, containing 45 ml of the culture broth medium containing different concentration (0, 20, 40, 80, 160 μM) of each tested heavy metal ($ZnCl_2$, $CuCl_2$, $PbCl_2$, $CdCl_2$) and $Al_2(SO_4)_3$). Each flask of the polluted broth medium was inoculated with 5 ml of *Azotobacter* bacterial suspension with the cell density $1X10^6$ CFU/ml. Then the flasks were shaken continuously (150 rotation per min) at 28°C. After 96 hours incubation, aliquots were transferred in a sterile manner to test tubes and the optical density (O.D.) at a wavelength of 650 nm was determined. Experiments were carried out in triplicate.

Sinorhizobium strains were grown and maintained on yeast extract mannitol agar medium (YEMA). *Yeast extract mannitol (YEM) agar and broth for Sinorhizobium (g/L): 0.4 yeast extract, 10 mannitol, 0.1 NaCl, 0. $MgSO_4.7H_2O$, 0.2 K_2HPO_4, 15 agar 1000 ml distilled water, pH 6.8. 3 g $CaCO_3$ is added for buffering. Medium was prepared according to Kleczkowska et al.* [18].

Culture medium used in this investigations were YEMA and in broth (YEMB) without agar. Maintenance of sinorhizobial strains was carried out on YEMA + 3 g/l $CaCO_3$. Incubation temperature was at 28°C. A suspension of $1X10^6$ cell forming unit/ml (CFU/ml) of inoculum (in physiological saline of 0.85% NaCl) of each of *Sinorhizobium* strains was used in the following *in vitro* experimental work and the measurement was completed at the logarithmic stage of reproduction. Similar procedure was carried as in case of *Azotobacter* strains. The incubation time was 48 hours.

For *Pseudomonas* strain, similar technique was carried out as mentioned in case of *Azotobacter* and *Sinorhizobium* strains, but the medium was King's B (*(g/L):* (20 g proteose peptone, 1.5 g $MgSO_4.7H_2O$, 1.5 g KH_2PO_4, 15 g agar, 10 ml glycerol, pH 7.2), broth medium. *Pseudomonas* strain was maintained on King's B agar medium. The incubation time was 48 hours. At the end of each experiment, the relative growth rate (%) was calculated as following: (the inhibition or the stimulation of the growth X 100)/the control.

Growth of bacteria in different pH of culture medium

Similar microfermentor technique was used but the pH of the cultural media was adjusted to be 7 as control, 6.2; 5.2 and 4.5. All cultural media were adjusted with diluted HCl (0.1 N) or diluted NaCl (0.1 N). Relative growth rate (%) was calculated as before.

STATISTICAL ANALYSIS AND GRAPHICAL PRESENTATION

The experimental work was used completely randomized design with three replications. Measurements presented throughout this study represent average values from triplicate analyses. In Figures, error bars represent the standard deviation of these measurements. The averages and standard deviation calculated and the significant differences were calculated at the 5% of probability level. The results are represented by the values of the mean of three replicates. All data were subjected to one-way analysis of variance. Data were processed by analysis of variance (ANOVA) and Fisher's protected least significant differences (LSD at 5%)

RESULTS AND DISCUSSION

The effects of aluminium and heavy metals on some useful soil microorganisms are shown in the Figures from 1 to 6 as well as useful some statistical analysis (Tables from 1 to 12). The results of microfermenting technique showed that zinc chloride at various concentrations (20, 40, 80, and 160 μM) had influencing effects on the relative growth rates of various plant growth-promoting rhizobacteria as shown in Figure (1). It was found that low concentrations had stimulated effects on some rhizobacteria such as GH-130, GH-97 (*Sinorhizobium*), Azot-99, Azotobacter sp. and *Pseudomonas putida*. While the concentractions 80 and 160 μM reduced the relative growth rate in all strains except *Pseudomonas putida*.

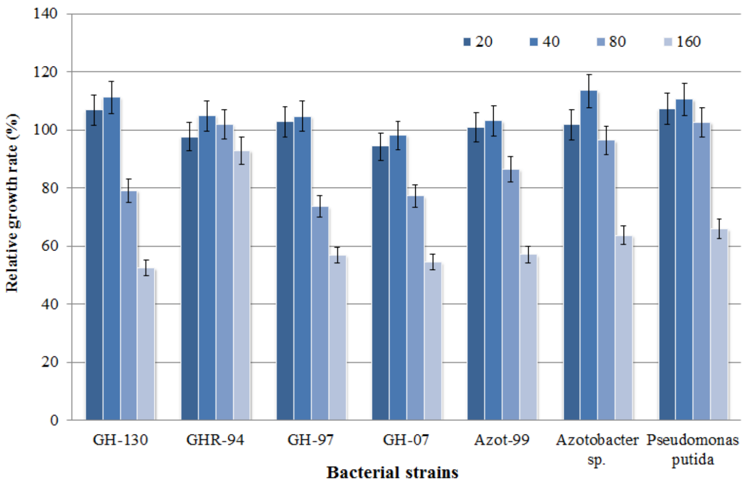

Figure (1): Effect of zinc chloride on the relative growth rates of various plant growths promoting rhizobacteria

Data in Table (1) shows the correlation matrix between the concentrations of zinic chloride in regards to the relative growth rates of the investigated strains. The interaction was significant between the 20 and 40 μM $ZnCl_2$.

Table (1): Correlation matrix between the concentrations of zinc chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.7730*	1		
80	0.1359	0.4464	1	
160	-0.2516	0.0492	0.737	1

Table (2) shows the correlations between the relative growth rates of the investigated strains in regards to the applied of different concentrations on the relative growth rates of used rhizobacteria. High correlations were found between the strains GH-130 and GH97, GH07 (*Sinorhizobium*), Azot-99, *Azotobacter* sp. and *Pseudomonas putida*. Also, these correlations can be found between GH-97 and GH-07 (*Sinorhizobium*), Azot-99 and *Azotobacter* sp. Similarlly, it was found between GH-07 and Azot-99, *Azotobacter* sp. and *Pseudomonas putida*. Similar correlation was found between Azot-99 and *Azotobacter* sp. as well as *Pseudomonas putida*. All correlations there were ovwer 0.9000.

Table (2): Correlation matrix between the concentrations of zinc chloride and the relative growth rates of various plant growths promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.7097	1					
GH-97	0.9930*	0.6347	1				
GH-07	0.9971*	0.7501	0.9812*	1			
Azot-99	0.9814*	0.7739	0.9537*	0.9925*	1		
Azotobacter sp	0.9480*	0.8858	0.9051*	0.9681*	0.9792*	1	
Pseudomonas putida	0.9209*	0.8378	0.8703	0.9475*	0.9781*	0.9808*	1

Figure (2) shows that copper chloride was more inhibiting the relative growth rates of the tested rhizobacterial strains. The highest concentration of copper chloride had toxic effect on GHR-94 GH-97 GH-07 and Pseudomonas putida than other strains.

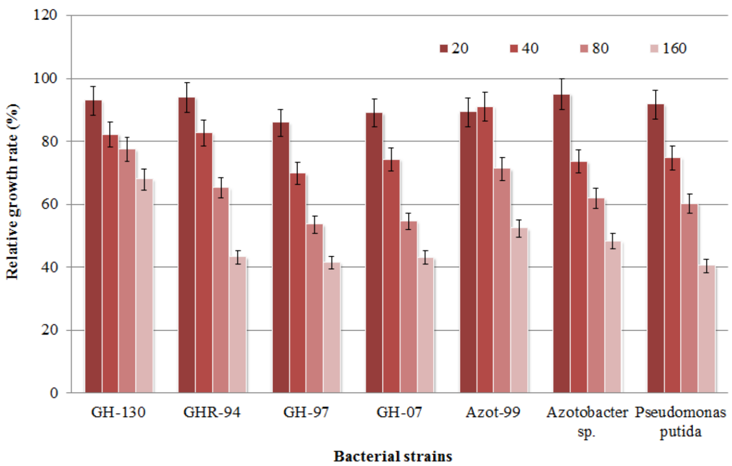

Figure (2): Effect of copper chloride on the relative growth rates of various plant growth-promoting rhizobacteria

Table (3) shows the correlation matrix between the concentrations of copper chloride applied to the broth media and their effects on them. The best correlation was foud between 160 and 80 µM $CuCl_2$.

Table (3): Correlation matrix between the concentrations of copper chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.1990	1		
80	0.4671	0.7917	1	
160	0.2941	0.5012	0.8683*	1

Table (4) illustres the correlation matrix between the plant growth-promoting rhizobacteria in regads to $CuCl_2$ concentrations. The best corrlations were found between GH-130 and other strains except with the strain Azot-99. Similar seults were found between GHR-94, Gh-97 and GH-07 and other strains. Good correlations were found berween Azot-99 and *Pseudomonas putida* as well as between *Azotobacter* sp. and *Pseudomonas putida*.

Table (4): Correlation matrix between the concentrations of copper chloride and the relative growth rates of various plant growths promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9752*	1					
GH-97	0.9868*	0.9801*	1				
GH-07	0.9766*	0.9802*	0.9983*	1			
Azot-99	0.8892	0.9684*	0.9127*	0.9241*	1		
Azotobacter sp	0.9952*	0.9644*	0.9931*	0.9848*	0.8713	1	
Pseudomonas putida	0.9936*	0.9935*	0.9926*	0.9883*	0.9340*	0.9879*	1

Figure (3) domentstrates that 20 µM of lead chloride can increase the relative growth rates of tested microorganisms except the *Pseudomonas putida* strain. Also, it was found that by increasing the concentration the relative growth rates were decreased.

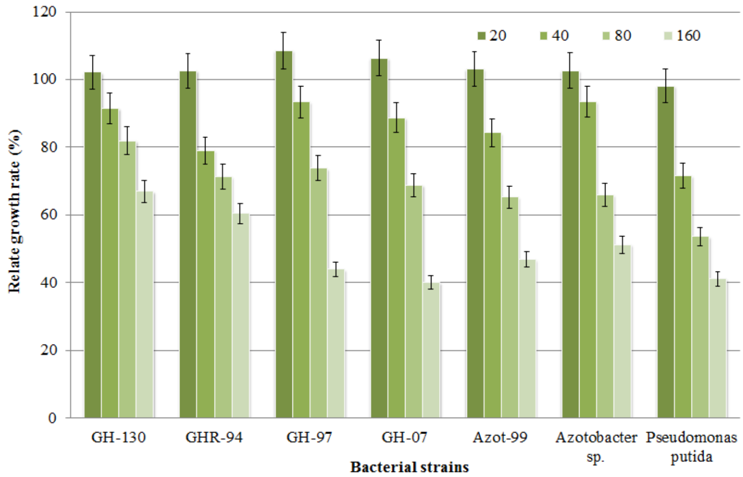

Figure (3): Effect of lead chloride on the relative growth rates of various plant growth-promoting rhizobacteria

Table (5) shows that the correlation between the concentrations of $PbCl_2$ was low. The best correlation was found between 20 and 40 µM.

Table (5): Correlation matrix between the concentrations of lead chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.7045*	1		
80	0.5216	0.6864	1	
160	-0.2141	0.1832	0.6779	1

Table (6) shows the correlation matrix between the plant growth-promoting rhizobacteria in regads to $PbCl_2$ concentrations. The best corrlations were found between all tested plant growth promoting rhizobacteria GH-130, GHR-94, GH-97, Azot-99, *Azotobacter* sp. and *Pseudomonas putida* under the application of lead chloride concentrations.

Table (6): Correlation matrix between the concentrations of lead chloride and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9545*	1					
GH-97	0.9973*	0.9303*	1				
GH-07	0.9993*	0.9436*	0.9993*	1			
Azot-99	0.9945*	0.9697*	0.9874*	0.9926*	1		
Azotobacter sp	0.9749*	0.9165*	0.9773*	0.9790*	0.9836*	1	
Pseudomonas putida	0.9687*	0.9917*	0.9505*	0.9617*	0.9871*	0.9566*	1

Figure (4) shows the effect of cadmium chloride on the investigated rhizobacterial strains under microfermenting technique. Regarding to the relative growth rates of tested bacteria, it was found the effect of cadmium chloride more toxic than copper chlorids.

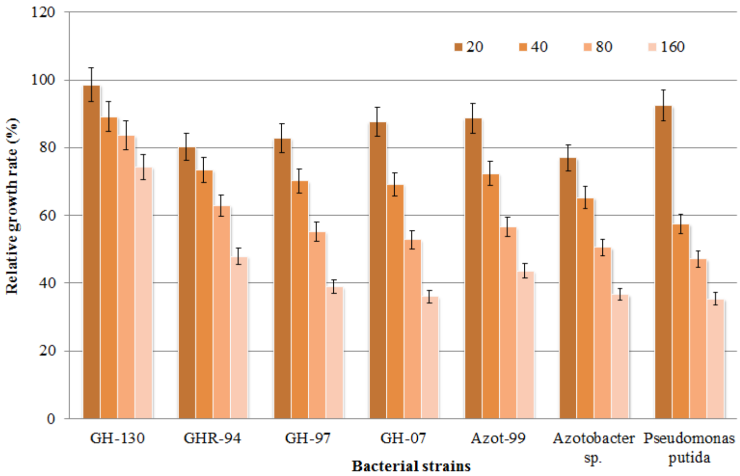

Figure (4): Effect of cadmium chloride on the relative growth rates of various plant growths promoting rhizobacteria

Table (7) shows the correlation matrix between the applied concentrations of cadmium chloride and their effects on the growth of the tested rhizobacteria. The best correlation was found between 160 and 40 and 80 µM.

Table (7): Correlation matrix between the concentrations of cadmium chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.4274	1		
80	0.5260	0.9616*	1	
160	0.5968	0.9215*	0.9874*	1

Table (8) shows the correlation matrix between the plant growth-promoting rhizobacteria in regads to $CdCl_2$ concentrations. The best corrlations were found between all tested plant growth-promoting rhizobacteria GH-130, GHR-94 (except with *Pseudomonas putida*), GH-97, Azot-99, *Azotobacter* sp. and *Pseudomonas putida* under the application of lead chloride concentrations. Most of correlation matrix was laid over 0.9900.

Table (8): Correlation matrix between the concentrations of cadmium chloride and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9819*	1					
GH-97	0.9933*	0.9933*	1				
GH-07	0.9965*	0.9817*	0.9968*	1			
Azot-99	0.9928*	0.9758*	0.9945*	0.9992*	1		
Azotobacter sp	0.9917*	0.9902*	0.9996*	0.9975*	0.9964*	1	
Pseudomonas putida	0.9613*	0.8941	0.9352*	0.9602*	0.9629*	0.9383*	1

Figure (5) demonstrates the effect of aluminium sulphate on the tested plant growth-promoting rhizobacterial strains under microfermenting technique. Regarding to the relative growth rates of tested rhizobacteria, it was found the effect of aluminium sulphate less toxic than cadmium and copper chlorids. Lower concentration (20 µM) can be act as lettel stimulationg doses to increase the relative growth rates of the investigated strain except the strain of *Pseudomonas putida*, while 40 µM increases the relative growth rates of GH-130 and *Azotobacter* sp. The highest concentration (160 µM) acts as inhibitor for all strains except GH-130.

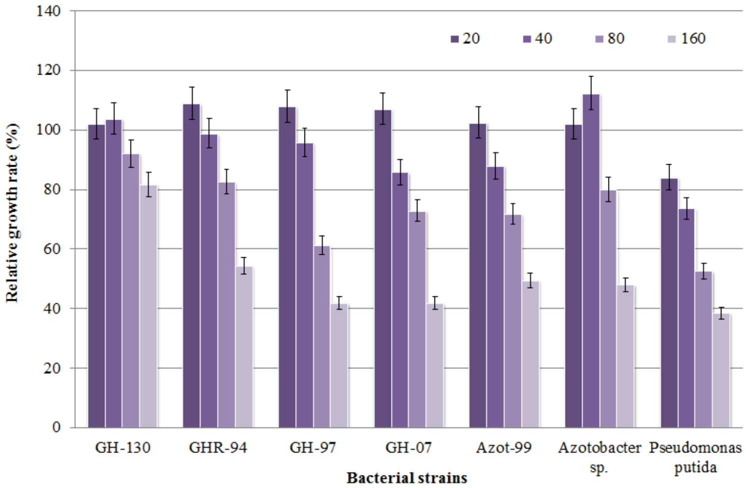

Figure (5): Effect of aluminium sulphate on the relative growth rates of various plant growth-promoting rhizobacteria

Table (9) illustrates the correlation matrix between the applied concentrations of aluminium sulphate and their effects on the growth of the investigated soil hizobacteria. The best correlation was found between 40 and 80 µM. This correlation was not completely significant.

Table (9): Correlation matrix between the concentrations of aluminium sulphate on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.5651	1		
80	0.5359	0.7520	1	
160	0.1980	0.5213	0.8232*	1

Table (10) demonstrates the correlation matrix between the soil useful microbiomes as plant growths promoting rhizobacteria in regads to aluminium sulphate concentrations. The best corrlations were found between all tested plant growth-promoting rhizobacteria GH-130, GHR-94, GH-97, Azot-99, *Azotobacter* sp (with an exception with GH-07), and *Pseudomonas putida* under the application of lead chloride concentrations. Most of correlation matrix was laid between 0.9200 and 0.9900.

Table (10): Correlation matrix between the concentrations of aluminium sulphate and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9677*	1					
GH-97	0.9610*	0.9704*	1				
GH-07	0.9218*	0.9888*	0.9597*	1			
Azot-99	0.9463*	0.9926*	0.9826*	0.9952*	1		
Azotobacter sp	0.9951*	0.9486*	0.9289*	0.8911	0.9156*	1	
Pseudomonas putida	0.9537*	0.9754*	0.9988*	0.9709*	0.9894*	0.9196*	1

Figure (6) shows the relative growth rates of the investigated soil microbiomes as plant growth promoting rhizobacter at different pH values. It was foud that all the investigated soil microbiomes grow well at pH 7, while at pH 6.2 reduced the relative growth rates of GH-94, GH-07 Azot-99 and *Pseudomonas putida*. At pH 5.2 the relative growth rates of the rhizobacteria *Azotobacter* sp. was stimulated. *Pseudomonas putida* strain at pH 4.5 was reduced significantly in comparison with other strains.

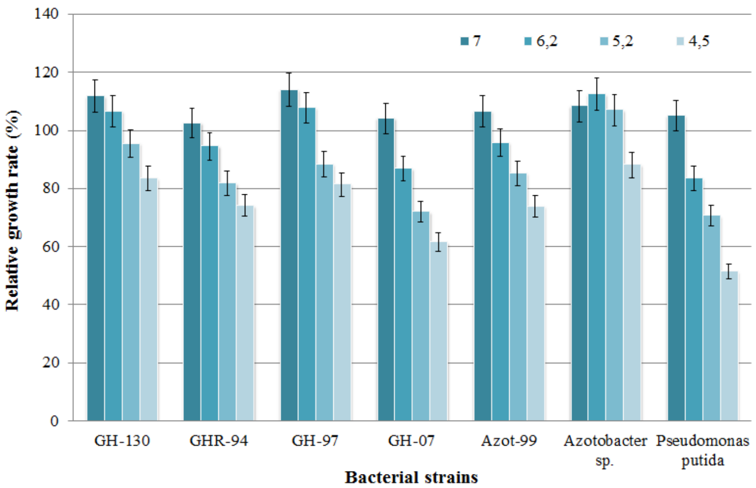

Figure (6): Effect of hydrogen ion concentrations on the relative growth rates of various plant growth-promoting rhizobacteria

Table (11) shows the correlation matrix between the pH values and their effects on the growth of the investigated soil hizobacteria. The best correlation was found between 5.2, 4.5 and 6.2. These correlations at these pH values were completely significant.

Table (11): Correlation matrix between hydrogen ion concentrations on the relative growth rates of various plant growth-promoting rhizobacteria

	7	6.2	5.2	4.5
70	1			
6.2	0.7454	1		
5.2	0.5652	0.9466*	1	
4.5	0.6124	0.9636*	0.9281*	1

Table (12) demonstrates the correlation matrix between the soil useful microbiomes as plant growths promoting rhizobacteria in regads to their relative growth rates at different pH values. The best corrlations were found between all tested plant growth promoting rhizobacteria with GH-130 (with the exception with *Azotobacter* sp.), GHR-94 (except with the strain *Azotobacter* sp.), GH-97 (except with the strain *Azotobacter* sp.), GH-07 (except with the strain *Azotobacter* sp.), and Azot-99 (with the exception with *Azotobacter* sp).

Table (12): Correlation matrix between hydrogen ion concentrations and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9859*	1					
GH-97	0.9767*	0.9930*	1				
GH-07	0.9651*	0.9913*	0.9724*	1			
Azot-99	0.9879*	0.9937*	0.9742*	0.9925*	1		
Azotobacter sp	0.8720	0.7784	0.7706	0.7199	0.7987	1	
Pseudomonas putida	0.9734*	0.9826*	0.9536*	0.9921*	0.9963*	0.7725	1

CONCLUSIONS AND RECOMMENDATIONS

Consequently, the strains selected in the present study can be used as a biomonitor of these heavy metal polluted soils. Moreover, it could be used as a bioremediator, taken into consideration their use in all soil as biofertilizer purposes. None of the strains can tolerate 160 µm of Cd^{2+}, $Pb^{2+}$and Cu^{2+}. The most toxic metal was Cd^{2+}, followed by $Pb^{2+}$and Cu^{2+}. The ecophysiological tolerant *Sinorhizobium* and *Azotobacter* as well as *Pseudomonas putida* strains are suggested to be the ideal solution for the improvement of soil fertility and the rehabilitation of reclaimed soils and are an important direction for future soil protection and quality research.

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TL18.

EFFECT OF SOME HERBICIDES ON SOIL MICROBIAL POPULATION, SOIL ORGANIC MATTER AND DEHYDROGENASE ACTIVITY

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Abstract: *A balanced agroecosystem is dependent on soil microbial biomass and population which are integral parts of the soil environment. The effect of five herbicides (bromoxynil, paraquat, glyphosate, linuron, and acetochlor) on soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), soil microbial population, soil organic matter, and dehydrogenase activity was assessed over a period of four weeks. The herbicides were applied at recommended rates to brown forest soil which obtained from the garden of RKK and contained in 250 g plastic pots. The results demonstrated that bacterial, fungal, actinobacterial; phosphate solubilizing, and cellulose-decomposing populations were decreased upon treatment with herbicides when compared to the control. The study showed that the herbicide bromoxynil caused suppression in microbial activity and biomass by exerting a toxic effect on them. The result illustrated a significant reduction in the percentage of soil organic matter after the application of the herbicide to soil samples. Soil organic matter then increased after continuous application from the second to the fourth week of treatment. Herbicide treatment caused a significant decrease in dehydrogenase activity when compared to the control soil samples. The obtained results indicated that soil samples treated with bromoxynil and paraquat had the lowest dehydrogenase activity after the 4th week of treatment, while soil samples treated with glyphosate followed by linuron and acetochlor had the highest dehydrogenase activity when compared to control treatment. This study indicated a significant response of soil microbial activity to herbicide treatment and increased adaptation of the microbial community to the stress caused by an increase in the concentration of the herbicides over weeks of treatment.*

Keywords: *soil microbial biomass, microbial population, herbicides, dehydrogenase activity, soil C and N*

INTRODUCTION

The intensive use of herbicides in agricultural soils in the world is a matter of environmental concern. Herbicides are chemical compounds that are used to kill unwanted plants. These are used to clear waste grounds, industrial sites, railways and farm lands. Prior to the wide spread use of chemical herbicides, cultural controls such as altering soil pH and fertility levels were used to control weeds. Mechanical control was also used to control weeds (Kelogg et al., 2000).
Application of pesticides and other chemicals used in agriculture affects the vital functions and population dynamics of soil microorganisms. Microorganisms are a heterogeneous group of organisms whose enzymatic systems comprise 60—90% of the total metabolic activity of the soil. Population size, enzymatic activity and biodiversity of certain systematic and physiological groups of

microorganisms may serve as bioindicators of changes taking place in the soil following herbicide application (Miloševiã et al., 1995; Konstantinoviã et al., 1999). Results of our studies have shown that generally, herbicides tended to reduce the total number of soil microorganisms 7 to 30 days after application. After that period, the numbers of microorganisms in the treated variants reached the level of the control variant while on the 90th day after application the numbers of microorganisms in the variants with clomazone (Command) and alachlor + linuron (Linuron S-50) were increased in relation to the control. Herbicide application reduced the number of *Azotobacter*. The reduction was large in the period of 30 days after herbicide application. The largest rates of reduction occurred 14 days after application, 62% in the case of Rival and 78% in the case of Linuron S-50 (Miloševiã and Govedarica, *2002)*

Paraquat (Gramoxone) is the most highly acutely toxic herbicide to be marketed over the last 60 years. Yet it is one of the most widely used herbicides in the world and in most countries where it is registered, it can be used without restriction. It is used on more than 100 crops in about 100 countries. Gramoxone, manufactured by Syngenta, is the most common trade name for paraquat, but the herbicide is also sold under many different names by many different manufacturers. Paraquat is used as an herbicide, desiccant, defoliant and plant growth regulator. It is also used for controlling broadleaf weeds and grasses in more than 100 different crops, including plantations. Again, it is used as a pre-harvest defoliant or desiccant on crops such as cereals, cotton, beans, hops, sugar cane, pineapple, soy, potatoes, and sunflowers; and as a post-harvest desiccant to speed up removal of spent plants such as tomato plants. Furthermore, it is used for weed control in non-agricultural areas such as roadsides, airports, around commercial buildings, drains, irrigation ditches, and waterways. Presently, it is the second highest-selling weed killer globally and is available in a 20% solution form and that needs to be diluted before agricultural use. (Arts et al., 2006; Saravu et al., 2013) Natural ways of controlling pests and pathogens of crops which are friendly to the environment have been forfeited by farmers for synthetic herbicides as the synthetic herbicides provide quick solutions to pests or pathogens problems as noted by (Bandala et al., 2007). The use of paraquat as herbicide of choice in this study was as a result of it being the most widely used herbicide to control broad-leaf weeds and grasses especially in the study area.

The pollution of plant, soil, surface and ground-water by pesticides involves a serious risk to the environment and also to human health due to direct exposure or through residues in food and drinking water. The use of agricultural chemicals inevitably raises questions about the fate of the active substance and its degradation products in the environment as well as their effects on eco-logically sensitive areas close to agricultural fields (Triantafyllidis et al. 2009, Łozowicka et al. 2012).

Processes like chemical degradation, degradation by soil microorganisms, sorption and binding by organic and mineral components, uptake by plant roots and volatilization determine pesticide behaviour in soil. Evaluation of the persistence in topsoil is fundamental in the assessment of the fate and behaviour of all chemical substances, including active ingredients in plant protection products.

Paraquat [1,1-dimethyl-4,4-bipyridinium dichloride] is total vegetative control herbicide that is strongly adsorbed to soil. It also undergoes metabolism and degradation under a range of conditions. Paraquat is stable in acidic or neutral solutions, but is hydrolyzed at pH>12. It undergoes photolysis in aqueous solution to form N- methybetaine of isonicotinic acid, and subsequently methylamine hydrochloride (Slade, 1965).

Glyphosate [N-(phosphonomethyl)glycine] is a broad-spectrum, non-selective, post-emergence herbicide that control most of the annual and perennial weeds by inhibiting aromatic amino acids biosynthesis (tyrosine, tryptophan, and phenylalanine) involved in protein synthesis (Battaglin *et al.*, 2005).

Besides, it also inhibits 5-enolpyruvylshikimic acid synthase *via* the shikimic acid pathway (Franz *et al.*, 1997), which is ubiquitous in microorganisms (Bentley, 1990) that link primary and secondary metabolism (Carlisle and Trevors, 1988). Most of the living organisms (excluding plants) lack this pathway, and are thus unaffected directly by glyphosate. Glyphosate also acts as a competitive inhibitor of phosphoenolpyruvate, which is one of the precursors to aromatic amino acid synthesis.

Toxicity, persistence and selectivity are important characteristics of herbicides (Konstantinoviã et al., 1999). The systemic and post-emergence herbicides 2,4-D and glyphosate are commonly used to control a broad spectrum of weeds in crops and pastures worldwide (Zabaloy et al., 2008).

Studies on herbicide effects on microbial diversity and community structure are fairly new because methods for evaluating these microbial characteristics are new (Johnsen et al. 2001). Most studies on herbicide effects on soil microorganisms have focussed on one or two herbicides at a time (Wardle and Parkinson 1990, Haney et al. 2000). While results of these studies indicate that herbicides applied at recommended rates generally do not have significant effects on soil microorganisms, evaluating only a few herbicides at a time limits comparison amongst herbicides on their relative effects on soil microbial ecology. In addition, traditional methods of evaluating effects of herbicides on microorganisms have focussed on microbial biomass and functional parameters such as carbon and nitrogen mineralization (Johnsen et al. 2001). However, the diversity or structure of the soil microbial community may be altered markedly even if total biomass or carbon and nitrogen metabolism appear unaffected by the herbicide (Johnsen et al. 2001).

Several scientific investigations have suggested the importance of preserving soil fertility and quality, and consequently soil microbial population. As the soil microorganisms are very sensitive to low concentrations of contaminants and rapidly response to soil perturbation, they are considered as an indicator of soil pollution (Shen *et al.,* 2005).

Soil microorganisms are an important link in soil-plant-herbicide-fauna-man relationships. They take part in herbicide a) degradation, their activity, number and diversity may serve as b) bioindicators of changes in soil biological activity following herbicide application and, finally, some microbial species may be used as c) bioherbicides. Microorganisms are efficient decomposers of aliphatic and hydroxyl compounds, but they decompose aromatic substances at a slower rate.

In general, herbicides affect microbes indirectly, causing physiological changes, increased enzymatic production or, when applied in high doses, death of susceptible groups of microorganisms. Soil microbiological population uses herbicides and their metabolites as sources of biogenous elements.

It has been noticed that certain groups of microorganisms (primary population) start to decompose herbicides a few days after their arrival. On the other hand, the so-called secondary population, which produces induced enzymes, decomposes herbicides while these are passing through a period of adaptation.

Pesticide decomposition is affected by the size and composition of microbiological population. Microorganisms are a highly heterogeneous group, including aerobes and anaerobes, heterotrophs and autotrophs or saprophytes, symbionts and parasites. Certain microbial species may decrease or increase the toxic action of herbicides. Most soil microorganisms are capable of decomposing herbicides, using them most frequently as sources of biogenous elements. Experiments have shown that microbes may use atrazine as a source of carbon or nitrogen.

The soil is a highly complex system. It is also dynamic, on account of microorganisms whose enzymes take part in most syntheses and decompositions. The number, enzymatic activity and biodiversity of microorganisms may serve as indicators of soil fertility, as well as indicators of all changes taking place in the soil as an ecological system. Herbicides adsorb on cell surface, affecting ion transport. They affect the metabolism inside the cell by binding to amino and sulfide groups. In the course of these processes, changes take place in the oxidoreduction level of soil and, depending on the chemical composition and dose of herbicide; the microorganism concerned may be killed. Bioaccumulation mitigates the toxic effect of herbicides.

Nutrient availability to plant is strongly influence by organic and inorganic amendments that usually increase the amounts of soil organic C, N and other nutrients. Organic matter added to soil by incorporating plant material, animal residues, manure, sewage sludge, or municipal waste. Amendments not only influence soil fertility directly, but can also affect the composition and activities of soil microorganisms. The use of selected parameters, such as enzyme activities, organic matter, physical properties, structure and formation of humic substances, together with agronomic and chemical properties, has provided additional information about functioning and productivity of degraded soil-ecosystems under appropriate regeneration practices.

The application of herbicides is commonly used in agricultural systems, as it is a practical and efficient method for weed control. However, after being applied, herbicides may follow different paths in the environment, and the soil its main destination (Oliveira and Brighenti, 2011). Among the soil components, soil organic matter (SOM) has been reported to be an important factor in the retention of herbicides, the reduction of soil profile mobility and the potential for environmental decontamination (Tejada and Benítez, 2017). In addition to increasing the microbiological activity of soils, SOM also accelerates the biodegradation processes of herbicides (Gómez et al., 2014). Thus, changes made by organic matter (OM) in the soil can decrease the mobility of herbicides in the soil profile and play an important role in the management of environmental pollution due to the use of these molecules (Rojas et al., 2013). The retention of cations is the main process that interferes in the behavior of the herbicides in the soil by the SOM, favoring the reduction of leaching and the availability of the herbicide molecules to degradation.

The bioavailability of herbicides is inherent to absorption processes and weed control, as well as the availability of products to microbial degradation and their reduction as a contaminant in the environment. Herbicides can be retained by both the organic fraction and the mineral fraction of the soils (Elkhattabi et al., 2007).

Total organic carbon (TOC) is the carbon (C) stored in SOM. Organic carbon (OC) enters the soil through the decomposition of plant and animal residues, root exudates, living and dead microorganisms, and soil biota. SOM is the organic fraction of soil exclusive of non-decomposed plant and animal residues. Nevertheless, most analytical methods do not distinguish between decomposed and non-decomposed residues. SOM is a heterogeneous, dynamic substance that varies in particle size, C content, decomposition rate, and turnover time (Edwards et al., 1999). The bioavailability of the herbicides in the soil depends on the particular characteristics of each molecule. When the molecules have higher retention in SOM, such as in the case of non-ionic and neutral herbicides, due to the greater affinity with the hydrophobic components of SOM, there is a lower availability for weed absorption and control.

Natural and anthropogenic factors may affect the soil enzyme activities directly or indirectly. Among anthropogenic factors, pesticides are of primary importance due to their continuous entry into the soil environment. Herbicides are one of the major groups of pesticides, which include substances or cultured biological organism used to kill or suppress the growth of unwanted plants and vegetation in order to minimize the cultivation cost as well as to sustain high yield. A number of herbicides have not only been introduced as pre- or post-emergence weed killer (Ayansina and Oso, 2006) but also leave unwanted residues in soil, which are ecologically harmful (Riaz et al., 2007). Preferred herbicides should not only have good efficacy, but also poses minimum adverse effects to crop, ecology and environment (Hoerlein, 1994). Herbicide-desiccated cover crops may impact microbial activity. Since microorganisms play a considerable role in soil processes that directly affect soil quality (Paul **&** Clark, 1989), the effect of this approach on parameters of biological soil quality is one of most important factors that should be investigated.

Soil dehydrogenase enzyme activity is used as index of microbial activity (Bergstrom et al., 1998) and reacts quickly to environment change (Sparling, 1997). One of oxidoreductases activities is dehydrogenase which is used to measure soil perturbation (Benitez et al., 2004). The use of herbicides is important for controlling weeds in crops. However, they can present impacts on soil properties, such as biological properties (Pertile et al, 2020). Herbicides are used in large quantities in modern agriculture to control undesirable plant species within a field. The increased application of herbicides leads to increased chemical concentrations in soil, altered soil reactions and potential adverse effect on non-target organisms. Repeated application of herbicides may involve a risk of reduced or altered soil microbial activities. In the environment enzymes may play important and different roles at least in three cases: as main agents in charge of either the transformation and/or degradation of compounds polluting the environment and the restoration of the polluted environment; as reliable and sensitive tools to detect and measure the amount and concentration of pollutants before, during and after the restoration process; as reliable, easy and sensitive indicators of quality and health status of the environment subjected to the restoration process (Rao et al., 2014).

Herbicides are biologically active compounds and an unintended consequence of its application may lead to significant changes in microbial populations and activities influencing microbial ecological balance affecting soil fertility as microbial community plays crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth (Pandey *et al.*, 2007), and hence occupy a unique position in biological cycles in terrestrial habitat. On the other hand any action of the chemicals altering the life functions of soil organisms could indirectly affect soil enzyme activity. Agrochemicals often stimulate or decrease the growth of soil microbial population and thus may alter the enzyme activity. Also these chemicals may exert some physiological effect on living organisms. An agrochemical may also modify the inter relationship between the particular group of organisms and thus influence the amount and type of enzyme produced. When agrochemicals are released into the environment about 1% reaches the target organism while remaining 0.99% interferes with local metabolism or enzymes activities (Ramudu et al. 2011).

Effects of glyphosate, paraquat, trifluralin and atrazine on soil enzymatic activities of dehydrogenase, phosphatase and urease in one soil were studied (Davies and Greaves, 1981). It was reported that when recommended doses were used, enzymes activities were not affected by the herbicides. The wide range of soils used with greatly differing enzyme activities, and varying assay conditions like temperature, pH, and substrate concentrations were responsible for contradictory results on effect of herbicides on soil. So, serious attempts should be made, possibly by judging the effects against those natural stresses or against the background of natural variation for assay of soil enzymes. It is proposed that effects of natural stress can be used to judge the relative importance of herbicide induced change.

Methods employed for assessing soil enzyme activities are proposed as sensitive to ecosystems perturbations and agricultural practices (Rao et al., 2014). A number of studies have used soil enzymes as an indicator for investigating the potential effects of agrochemicals on soil function (Riah et al., 2014).

The prolonged intensive and indiscriminate use of herbicides adversely affected the soil biodiversity, agricultural sustainability, and food safety, bringing in long-term harmful effects on nutritional security, human and animal health. The research work provides an improved understanding of the impacts of application of herbicides on agricultural soil microbiotas management for food and nutritional security. There is a growing awareness about the importance of soil for sustaining crop production and providing beneficial microbial ecosystem services. It is important to evaluate the effects of herbicide on soil microbiotas and their activities. Addition of herbicides to the soil can have both positive and negative impacts upon different soil microbial community. Depending on the balance of these impacts, which can be either direct or indirect, alterations to soil biogeochemical cycling may evolve. Direct effects include the herbicides being toxic to the microbes, which can result in a reduction of microbial biomass, and thence soil heterotrophic respiration and activity of organic matter decomposing and nutrient-cycling microbes as well as PGPR In contrast, herbicide addition can directly benefit soil microbes by providing a resource to support their growth.

The aims of this study were to observe the interaction between herbicides and soil microorganisms may be of practical significance because of possible inhibition in microbial activities contributing to soil fertility. Hence, the effects of herbicides on soil microbial communities addressing the apprehensions about the environmental impacts of herbicide use. Therefore, there is a need to assess the effects of theses herbicides on special soil microorganisms. The purpose of this work was to evaluate the effect of five herbicides were used at the field recommended dose and to investigate their impacts on soil microbial biomass carbon and nitrogen, soil microbial population such as total aerobic heterotroph bacteria, filamentous fungi, actinobacteria, cellulose-decomposers and phosphate solubilizing microbiota as well as soil organic carbon, total nitrogen content and the potential activity of dehydrogenase enzyme during four weeks of incubation under dark laboratory conditions.

MATERIALS AND METHODS

Soil sample Collection: The top soil samples were collected at the depth of 0-25 cm from the garden of the Rejtő Sándor Faculty of Light Industry and Environmental Engineering. It was then put into a sterilized polythene bags and were taken to the Microbiological Environment and Soil Biotechnology laboratory of the Institute of Environmental Engineering and Natural Sciences for preparation, treatments and isolation. The soil samples (Table 1) were then made free of large stones and plant debris using 4.0 mm mesh sieve and stored at 4°C before processing. The soil collection was done at one week interval for a period of four weeks.

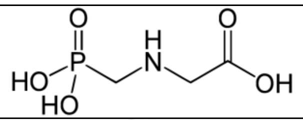
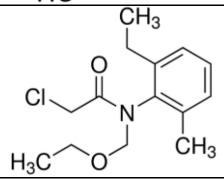
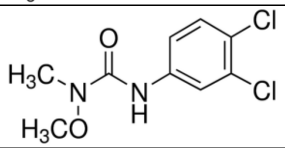
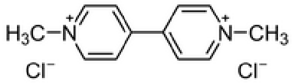
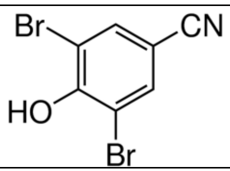
Experimental Design: Completely randomized block design was used for carrying out the experiment. Each treatment was done in three replicates.

Table (1): Some physicochemical properties of soil

Investigated parameters	**Units**	**Results**
pH_{KCL}		7.13
EC	µS/cm	483
Σsalt	m/m %	0.040
$CaCO_3$	m/m %	32.34
H	m/m %	3.34
Total N	mg/kg	1930
NH_4-N	mg/kg	18.76
NO_3-N	mg/kg	13.69
AL-p_2O_5	mg/kg	51.7
AL-K_2O	mg/kg	169
AL-Na	mg/kg	37.2
KCl-Mg	mg/kg	146
KCl-S	mg/kg	14.1

Preparation of Herbicides: Bromoxynil, Paraquat, Glyphosate, Linuron and Acetochlor were used in this study. The general information about the five herbicides is present in Table (2)..

Table (2): General information about the used herbicides

Investigated herbicide	**Herbicide class**	**IUPAC name**	**Chemical structure**
Glyphosate	Organofoszfor (glicin)	2-[(Foszfonometil)amino]-ecetsav	
Acetochlor	Monocarboxylic acid amide	2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	
linuron	Phenylureas	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea	
Paraquat	Bipyridylium	N, N′-dimetil-4,4′-bipiridinium-diklorid	
Bromoxynil	Nitrile	3,5-Dibromo-4-hydroxybenzonitrile	

The mode of action of the used herbicide Glyphosate is protein synthesis inhibitors, for Paraquat is cell membrane disruptors. The mode of action of for Linuron and Bromoxynil is photosynthesis inhibitor.

The herbicides used in the experiment were commonly used in the agricultural fields, which were obtained from a local agricultural store. The herbicides recommended applied rates were presented in Table (3).

The preparation for concentration of the five herbicides for soil application was prepared according to the manufacturer's recommendations. Each of the concentrations has three replications.

Table (3): The herbicides recommended applied rates

Herbicide	Field recommended applied dose
Glyphosate	2.6 – 3.4 l/ha
Acetochlor	1680 g/ha
Linuron	3.9 – 5.4 L/ha
Paraquat	2.0 – 5.0 l/ha
Bromoxynil	20 L/ha

Soil Treatment: The soil was collected in plastic trays. Herbicide solutions were sprayed on the soil surface of the trays using a greenhouse sprayer. About 250 g soil samples were collected from each herbicide treated tray and replaced in a plastic pot. Pots were incubated under laboratory dark conditions for 1, 2, 3 and 4 weeks after herbicide application. The soil was watered as required to maintain field capacity for about 45%.
The soil samples were treated with field recommended concentration of the herbicide. Polythene bag was used to cover the soil surface for each sample so as to avoid the chemical drifting to the next neighbouring pots thus, avoiding contamination during the incubation time. The control pots were not treated or applied herbicide.
The effect of the five herbicides in the tested brown forest soil were analyzed in response to total organic carbon, microbial biomass-C and N, soil dehydrogenase activity and microbial enumeration with respect to control soil (without treatment) in triplicates at regular intervals 7th, 14th, 21st and 28th day after treatment for a period of 4 weeks individually.

Soil Organic Carbon: Total organic carbon (TOC) was analyzed by dichromate ($K_2Cr_2O_7$) oxidation and titration with ferrous ammonium sulphate (Walkley & Black, 1934). Soil organic carbon in different herbicides treated and control soil samples were determined by partial oxidation method (Walkley and Black, 1934) through titration against 1N $(NH_4)_2Fe(SO_4)_2.6H_2O$ using diphenylamine indicator. In this reaction C is oxidized by the dichromate ion and the excess of dichromate ion is then back titrated with ferrous ion. TOC was determined in the herbicide treated soil and control soil samples.

Total nitrogen content: Determine the total nitrogen was done on the basis of the wet oxidation of soil organic matter and soil samples using sulfuric acid and digestion catalyst and conversion of organic nitrogen to the ammonium form. According to the method of Bremner (1960), total nitrogen content in soil treated with different herbicides and control soil samples was determined by semi-micro Kjeldahl method.

Enumeration of aerobic heterotrophic bacteria: Enumerations of aerobic heterotroph bacteria were done through serial dilution. Culturable bacteria were enumerated by the plate count method. Five gram soil sub-samples were suspended in 45 ml sterile 0.85% NaCl. Soil suspension was shaken in an orbital shaker (300 rpm, 20 min). Serial dilution technique was used. Serially diluted in 9 ml sterile saline and 0.1 ml of each 10^{-4} and 10^{-5} dilutions were plated on the surfaces of different plate media according to the types of microbial counts. Plates were incubated for 7 days at 28°C for fungal and actinobacterial growth, and 2 days at 37°C for counting the aerobic heterotroph bacterial growth, cellulose-decomposers as well as the phosphate solubilizing microbes. The number of cultivable microorganisms was expressed as $\log_{10}$ CFU g^{-1} soil.

Isolation and Counting of Microorganisms: Isolation of bacteria: The inoculated nutrient agar plates with suspension of herbicide-treated soil were then after 20 minutes for stability, the plates were incubated at 37°C for 24-48 hours. After growth was observed, bacterial colonies counted and sub-culturing was done for the isolation of pure culture. The pure cultures were then subjected to Gram's staining so as to identify it and characterized based on their morphological and microscopic features using Bergey's manual as described by [Cheesbrough, 2006].

Isolation of fungi: The counting and isolation of fungi was performed using serial dilution. The soil sample solution (dilution 10^{-4}) was then pour on a Potato Dextrose Agar (PDA) medium plates prepared under sterilized conditions and was spread using a sterilized glass rod. The inoculated plates were then incubated in incubator with temperature about 28°C for 5 to 7 days. After incubation period, on the presence of visible growth, the fungal colonies were counted and pure cultures were then obtained through sub-culturing. Identification and characterization of the fungal species were done based on their morphological and microscopic characters analysis by using taxanomic guides and standard procedures as described and done by (Rohilla and Salar, 2012).

Isolation of actinobacteria: Counting and isolation as well as characterization of the actinobacteria were carried out using Arifuzzama et al. (2010) method. Actinomycetes isolates were characterized based on cultural characteristics, staining reactions, biochemical tests and enumerated using starch-casein agar (Hunter-Cevera and Eveleigh, 1990). Streptomycin 40µl/ml and griseofulvin 50 µl/ml were used to prevent bacterial and fungal contaminants (Alharbi *et al.*, 2012). Rose Bengal agar supplemented with 50 µl/ml of streptomycin was used for fungal count (Alef and Nannipieri, 1995).

Microbial Biomass

Soil samples were stored at 28±2°C for a week to stabilize respiration, subsequently used for further analysis. Microbial biomass-C (MB-C) and MB- nitrogen (MB-N) in herbicide treated as well as control soil sample was determined by fumigation extraction method (Vance *et al.,* 1987) through back titration against 0.04N $(NH_4)_2Fe(SO_4)_2.6H_2O$ using ferroin indicator.

Determination of dehydrogenase activity

Dehydrogenase activity was measured following reduction of 2,3,5-triphenylotetrazolium chloride (TTC) to red-coloured triphenyl formazon (TPF), which were determined spectrophotometrically (Alef and Nannipieri, 1995). Three grams of soil sub-samples were weighed in a 50 ml polypropylene centrifuge tube and mixed with 4ml phosphate buffer (pH 7.6) and 1 ml 3% 1,3,5-triphenyiltetrazolium chloride (TTC) solution. Tubes were incubated for 24 h at 37°C in the dark. After incubation, triphenylformazan (TPF) formed by reduction of TTC was extracted with 10 ml acetone. Tubes were shaken in an orbital shaker at 300 rpm for 30 min, centrifuged (2000 rpm, 5 min), and the supernatant was filtered with filter paper. Blanks without the addition of TTC were carried out in the same manner. The concentration of TPF was determined by spectrophotometrical measurements at 485 nm, and the results were expressed as mg TPF g^{-1} soil 24 h^{-1}.

All experiments were conducted in triplicates, with randomized complete block design. All values reported are the arithmetic means of the three determinations expressed on an oven-dried soil basis. Statistical analyses were performed using ANOVA to determine the significant differences between the treatments at $P = 0.05$.

RESULTS AND DISCUSSION

Pesticides have been used for centuries in agricultural products to protect plants from damages such as weeds, plant diseases, and insects. These chemicals showed beneficial effects for crops; however, the main problem of pesticides is a high level of pesticide residues remaining, which cause food poisoning (Chung 2018; Huang et al. 2020). Pesticides could be classified based on these properties and can be categorized into various classes such as insecticides, herbicides, fungicides, and various other substances, the most used pesticides in agricultural products (Yadav and Devi 2017).

In agricultural systems, weeds often pose a major threat to crop yields and herbicides are the largest class of pesticides used. Therefore, there has been considerable interest in the side effects of these chemicals on non-target organisms, including soil useful microorganisms.

The majority of studies on this field have focussed on herbicide effects on microbially mediated processes, e.g. nitrification, denitrification, soil respiration, and soil enzyme activity. One aspect which has received little attention is the influence of herbicides on the soil microbial biomass. Two studies appear to have involved assessment of the soil microbial biomass following application of various herbicide solutions, e.g., Soulas et al. (1984) and Duah-Yentumi and Johnson (1986).

Although the microbial biomass has been largely neglected in herbicide studies, it plays an important role in the soil ecosystem in acting as a highly labile pool of nutrients which are rapidly cycled and are directly responsible for regulating the nutrient levels available for plants.

Effects of herbicide application on soil total organic carbon: The variation in organic carbon (OC) content was exhibited with respect to different herbicide treated soil. Figure (1) shows the effects of five herbicides on total organic carbon in brown forest soil. It was found that the application of herbicides significantly increased the TOC by increasing the incubation time over the control samples. Paraquat contaminated soil samples was the least TOC improvement while Linuron and Acetochlor were the most pollutant herbicides which increase the TOC.

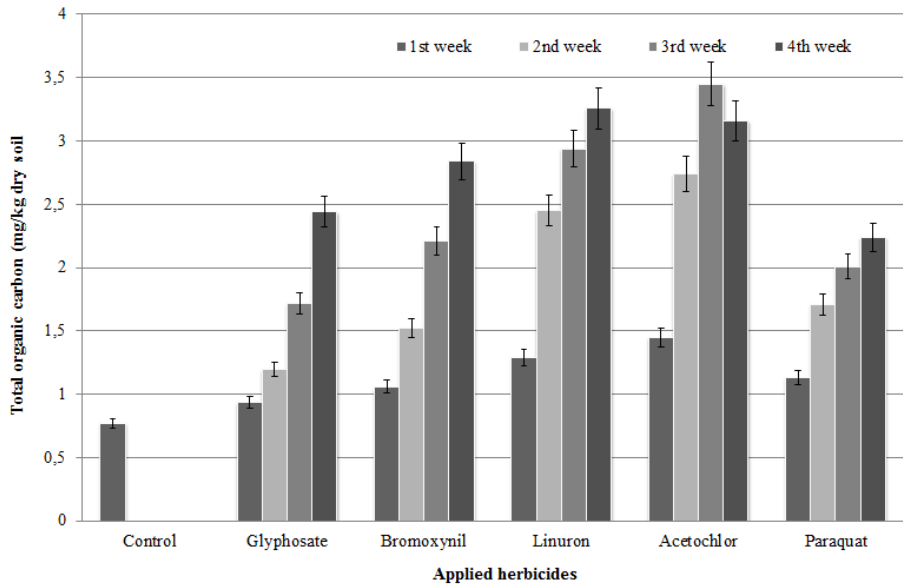


Figure (1): Effects of different herbicides on total organic carbon in brown forest soil

There was significant reduction in percentage OC level after the application of herbicides, although OC increased after continuous application from the 14^{th} day of glyphosate treatment (Sebiomo *et al.*, 2011). Further, the fate of herbicides is greatly affected by the presence of soil organic matter by aiding their disappearance (Ayansina and Oso 2006).

Determination of total nitrogen content in soil treated with herbicides: Figure (2) illustrates that the effects of tested herbicides on total nitrogen content in brown forest soil were improved the TNC of the tested soil samples over the control. This improvement was significantly positive with the control herbicide-free soil. Also, Paraquat was the least improver herbicide. The highest amount of TNC was recognized after three weeks incubation. After the 4^{th} week of incubation, it was found that the amount of TNC was decreased but higher than the amount of TNC in control samples.

Microbial biomass carbon and nitrogen under herbicide effects: The microbial biomass-C showed a decline trend from 7^{th} day to 28^{th} day of incubation in all herbicide treated soil. Further, the variation in microbial biomass-C with respect to different herbicides and days after treatment was statistically significant (*p*<0.001).

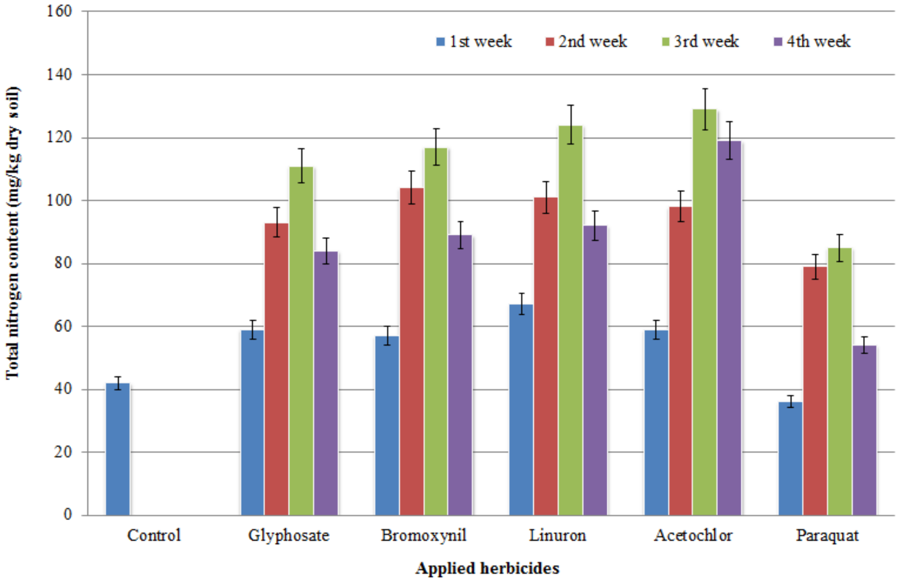


Figure (2): Effects of different herbicides on total nitrogen content in brown forest soil

Figure (3) demonstrates the effects of applied herbicides on microbial biomass carbon in brown forest soil. All incubation periods except the 4th week increased the MBC. Acetochlor was the most MBC improver and Parquat was the least improver. The increasing in MBC in all cases were significant with the control soil.

Herbicides affect various soil microbial processes, inhibit decomposition, which depends upon the type and rate of application that can alter the microbial biomass quantitatively and qualitatively in both short-term and long-term (Anderson et al., 1981). Besides, herbicides affect non-target microorganisms by interfering with vital processes such as respiration, photosynthesis and biosynthetic reactions as well as cell growth and division and molecular composition (De Lorenzo et al., 2001). Increase in MB-C in some herbicide treated soil may be due to the fact that some of the herbicides acting as the source of nutrients, in which case they significantly affect microbial growth and multiplication.

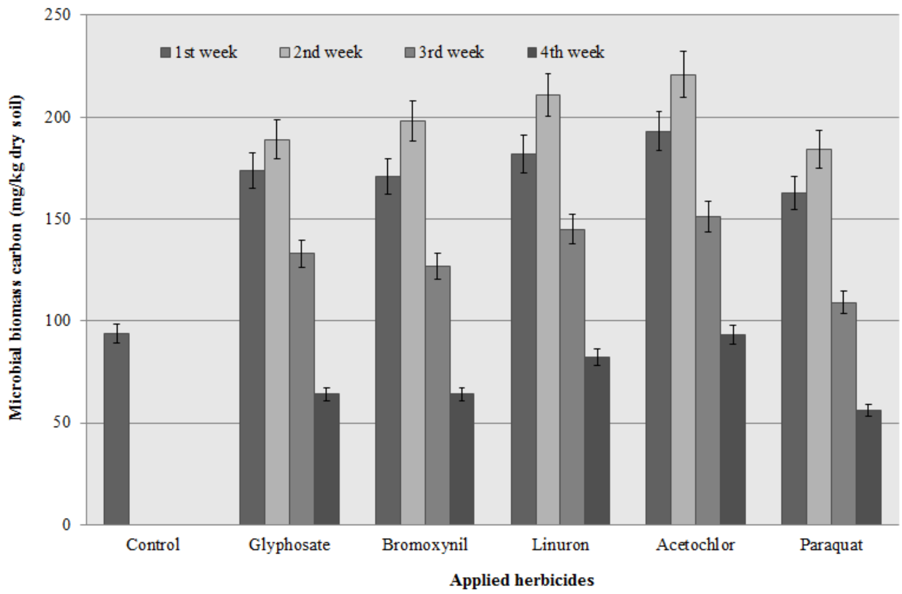


Figure (3): Effects of different herbicides on microbial biomass carbon in brown forest soil

However, the effect of herbicides is usually short-term and minor, when compared with natural, spatial and temporal variation in soil microbial biomass. The biomass of microorganisms is one of the important properties of ecological studies, which can be related to parameters describing microbial activity and soil health. Short-term effects in response to the use of herbicides are related to disturbances of chemical and biological balance in soil, and are known to selectively suppress the activity of soil microorganisms. The decrease in MB-C may be due to the adsorption of small amount of pesticides on organic matter that mask the effects of these agrochemicals on soil microbial biomass, and subsequently led to lysis of microbial cells (Jayamadhuri and Rangaswamy, 2005).

Figure (4) shows the effects of applied herbicides on microbial biomass nitrogen in brown forest soil. The highest MBN was observed after the 2nd incubation period. The MBN values were decreased gradually by the increasing the incubation time. Paraquat was the most toxic herbicide while the Acetochlor was the most improver herbicide.

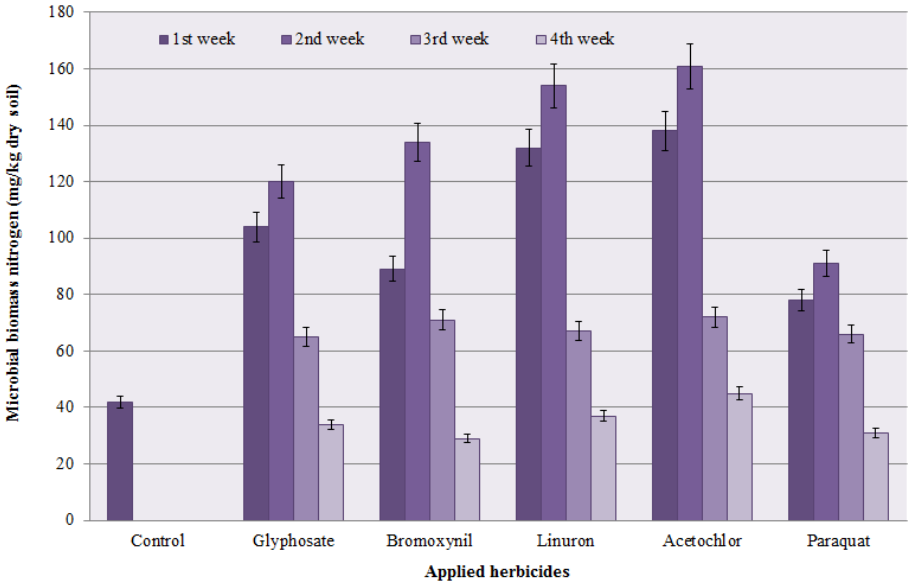


Figure (4): Effects of different herbicides on microbial biomass nitrogen in brown forest soil

Impacts of herbicides on the potential activity of dehydrogenase: The effects of applied herbicides on dehydrogenase activity in brown forest soil were recorded as in Figure (5). The increasing of the incubation time and application of herbicides decreased the activity of dehydrogenase. The highest reduction of the potential activity of dehydrogenase was recognized at the 4th incubation week. Paraquat inhibited the enzyme activity more than the other herbicides and Linuron was the least inhibitor.

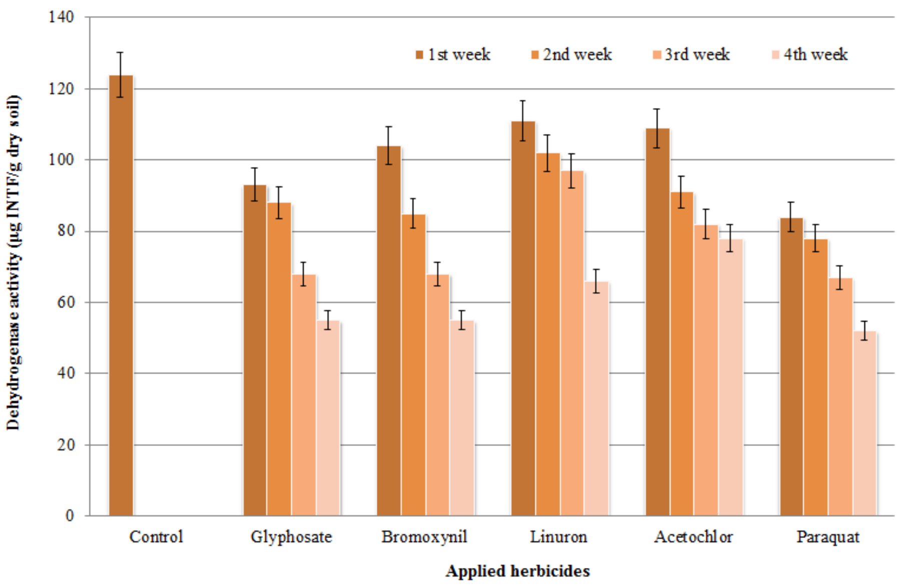


Figure (5): Effects of different herbicides on dehydrogenase activity in brown forest soil

Microbial enumeration in soil treated with herbicides: Unintended consequence of herbicides applications may be the reduction of sensitive populations and/or stimulation of certain microbial groups with or without detriment to co-existing microbial populations that may compete for available resources (Microorganisms are a highly heterogeneous group, including aerobes and anaerobes, heterotrophs and autotrophs or saprophytes, symbionts and parasites).

The abundance and distribution of different groups of soil microorganisms were enumerated and expressed in terms of colony forming units per gram soil (CFU/g). Differences could be observed between the different herbicide treated soil profiles based on microbial enumeration. Besides, the variations in microbial population in different herbicides treated soil were presented in terns of log_{10} transformed of CFU/g soil.

Figure (6) shows the effects of applied herbicides on total aerobic heterotrophic bacterial count in brown forest soil. It was found that all herbicides except Acetochlor decreased the total aerobic heterotroph bacteria. Also, the results in Figure (6) show that Paraquat is the great inhibitor of aerobic bacterial count followed by Bromoxynil and Glyphosate.

Figure (7) informs that the effects of applied herbicides on total filamentous fungal count in brown forest soil had the similar tendency as it was found in case of aerobic bacterial counts. Acetochlor was the least effective against the activity of the filamentous fungi in the soil treated with the herbicides while Paraquat followed by Bromoxynil and Glyphosate were the most effective herbicides to reduce the total count of the filamentous fungi in the treated soil.

The fate of the herbicide residues in soil is a matter of great concern since they would persist on top soil (Ayansina *et al.*, 2003), accumulate to toxic level and become harmful to microorganisms, and changes in nutrient levels (Wang *et al.*, 2008). Some microorganisms have the ability to degrade herbicide, while some others were adversely affected depending upon the application rate/dose and type of herbicide used (Ayansina and Oso, 2006; Sebiomo *et al.*, 2011). Thus, the effects of herbicides on soil microbial population may be either stimulating or depressive depending on the agrochemicals (type/formulation and concentration), mode of application, groups of microorganisms and environmental conditions. Besides, herbicides decomposition is frequently faster in soils that contain high organic matter, presumably because of more vigorous microbial activity. Use of herbicides can reduce total microbial populations in soil (Greaves *et al.*, 1976), where some researchers attribute to reduced input of organic residues. Various studies have revealed that herbicides can cause qualitative and quantitative change in soil microbial populations (Ayansina and Oso, 2006; Pampulha *et al.*, 2007; Latha and Gopal, 2010).

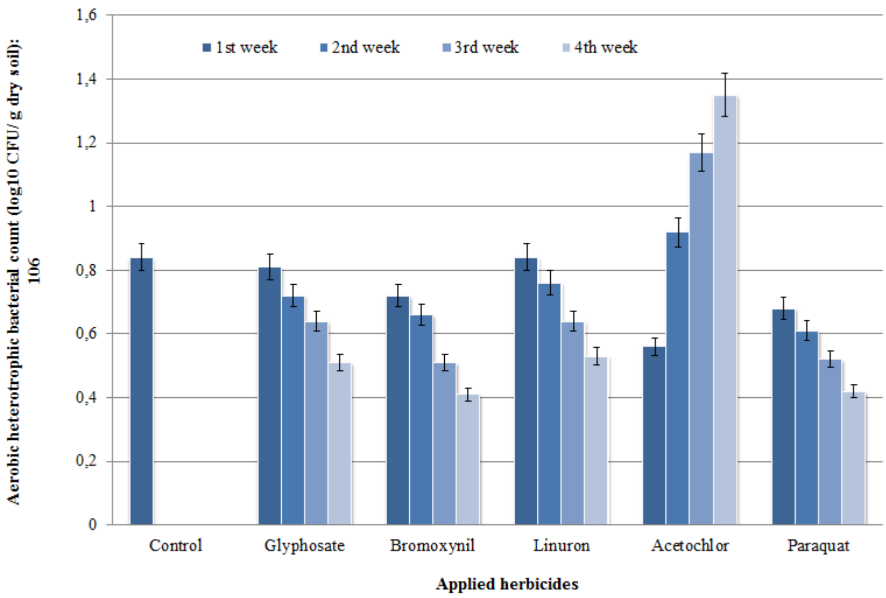


Figure (6): Effects of different herbicides on total aerobic heterotrophic bacterial count in brown forest soil

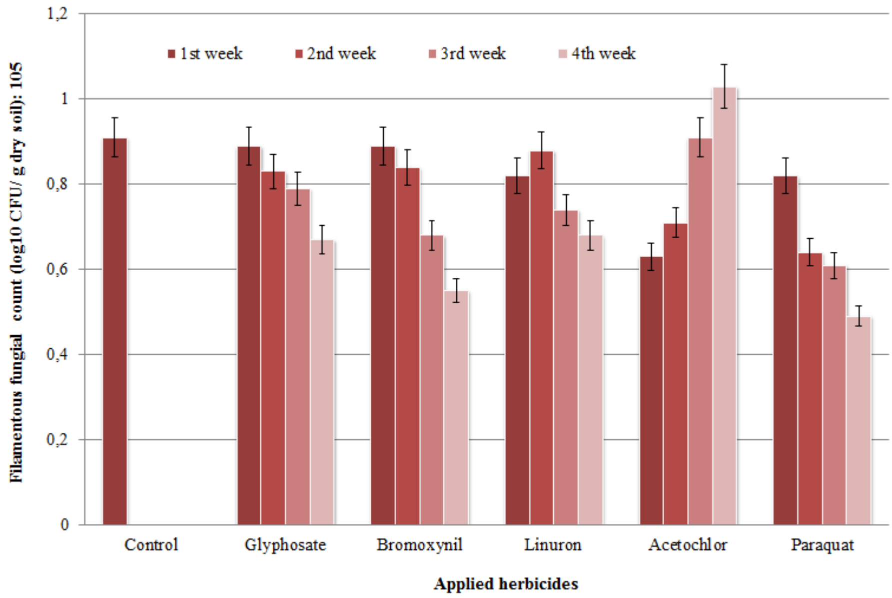


Figure (7): Effects of different herbicides on total filamentous fungal count in brown forest soil

Figure (8) describes the effects of applied herbicides on total actinobacterial count in brown forest soil. It was found that Paraquat and Glyphosate were the most effective herbicides against the total count of actinobacteria. The reduction the actinobacterial count was recognized by increasing the incubation time except in case of treatment of the soil by Bromoxynil which improve the count number of actinobacteria followed by Acetochlor.

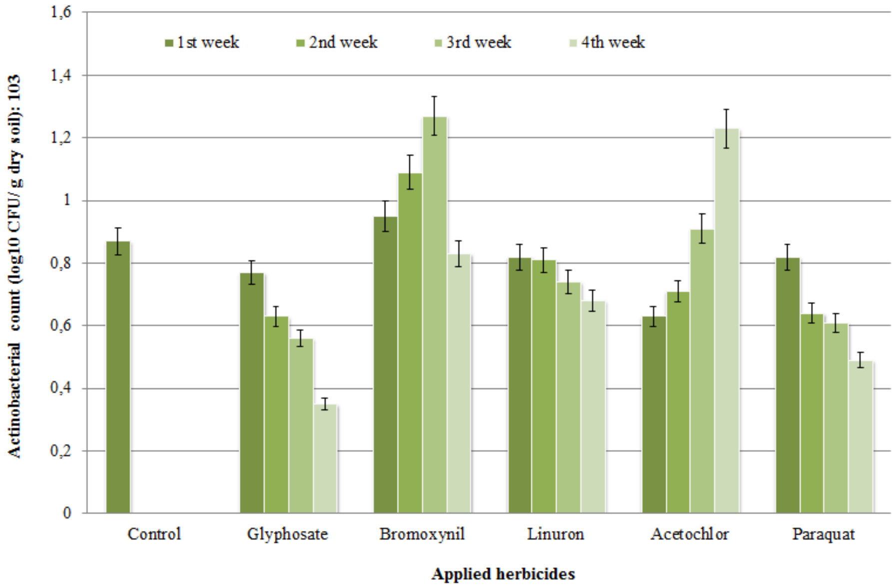


Figure (8): Effects of different herbicides on total actinobacterial count in brown forest soil

Figure (9) demonstrates the effects of applied herbicides on total count of cellulose-decomposers in brown forest soil. Comparing with the control herbicide free soil, it was found that Acetochlor was the herbicide which increases the counting number of the cellulose-decomposers followed by Glyphosate, whereas the Bromoxynil showed its ability to reduce and inhibit the cellulose-decomposers followed by Paraquat and Linuron. The number of the cellulose-decomposers was very low at the 1st and 2nd incubation time and the increasing in the counting was gradually increased but less than the control.
Figure (10) shows the effects of applied herbicides on total count of phosphate solubilizing microorganisms in brown forest soil. It was found that the counting of phosphate solubilizing microbes at 1st and 2nd incubation time were lower that the control, while the 3rd and 4th incubation time showed the increasing in the amount of this functional microbial group. Paraquat demonstrated

its ability to reduce strongly the enumeration of this group while the Acetochlor and Linuron increased the population of this functional microbial group.

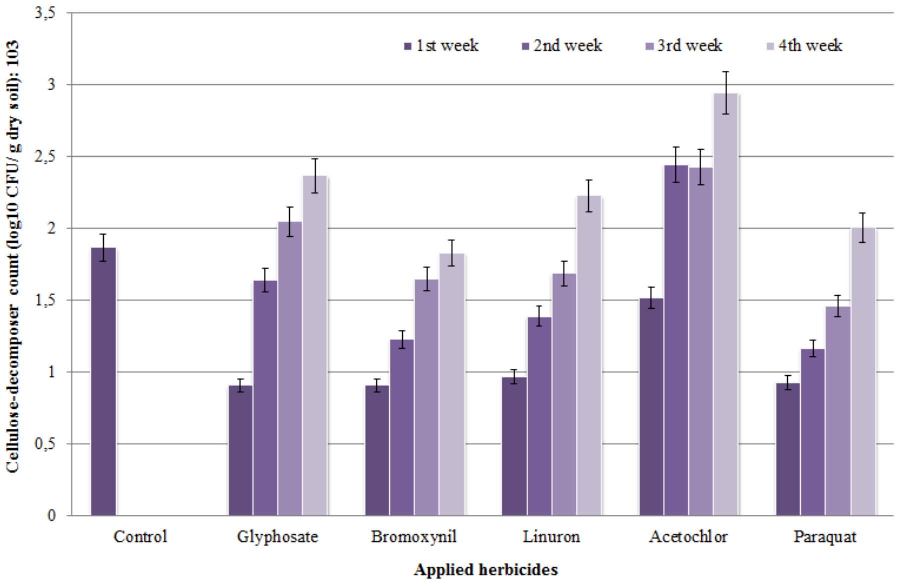


Figure (9): Effects of different herbicides on total count of cellulose-decomposers in brown forest soil

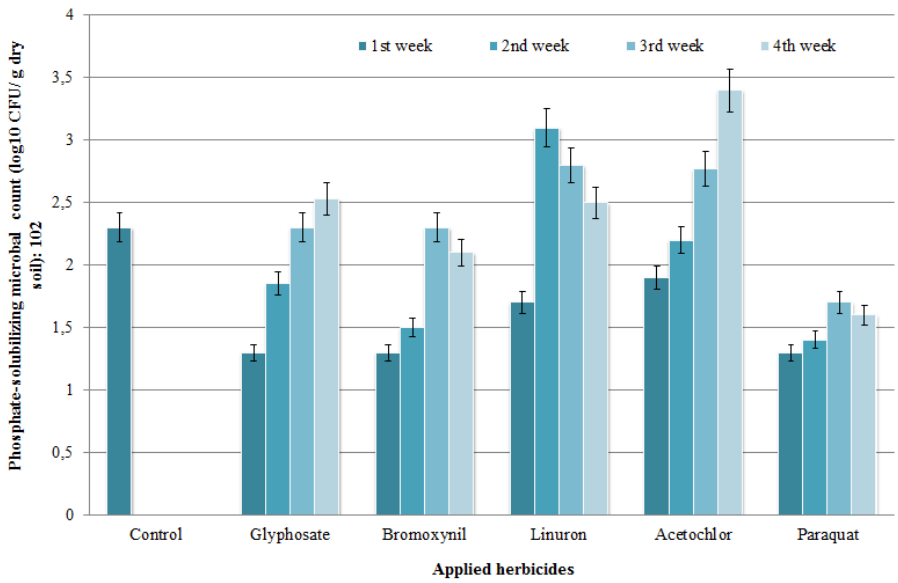


Figure (10): Effects of different herbicides on total count of phosphate solubilizing microorganisms in brown forest soil

The initial rise in microbial counts in herbicides treated soil may be due to their ability to temporarily mineralize and use the herbicides as energy source. There exists positive correlation between microbial population and soil organic matter, and the variation in microbial activity represents the capacity of soil microorganisms to respond to the inputs of herbicides in soil (Sebiomo *et al.*, 2011). However, the fungal population (unlike bacteria) took more time to recover from the detrimental effect caused by herbicides. Besides, herbicides can influence fungal count directly or indirectly by affecting the interaction of fungi with other microorganisms (Wardle and Parkinson, 1990; Araujo *et al.*, 2003). The order of inhibition of butachlor on soil microorganisms is bacteria > actinobacteria > fungi. Higher rate of pyrazosulfuron application impaired microbial parameters, enzyme activity to a greater extend and had a long lasting negative effect on soil fertility. These xenobiotic compounds force the microbial biomass to direct a large part of its energy budget into reducing mineralization activity. Paraquat is also known to be bounded strongly and coherently to soil components including clay

minerals and organic matter, therefore limits the access of microorganisms to paraquat in soil (Isenring, 2006).
Adsorption of paraquat rapidly decreases the bioavailability of herbicide in soil, and the capability of adsorption to deactivate paraquat application. Some microbial species are capable of metabolizing paraquat as a source of carbon (Imai and Kuwatsuka 1989). The presence of glyphosate may cause changes in microbial population as well as overall microbial activity (Wardle and Parkinson, 1990). Glyphosate is degraded primarily by microbial metabolism. The degradation of glyphosate is slower in soil with a higher adsorption capacity. Degradation rate was also affected by specific soil microbial community (Carlisle and Trevors 1988), and also vary considerably in different soils. Some microbes may use herbicides as a source of carbon and energy. Glyphosate is an organophosphate that can be used as a source of P, C and N by either gram-positive or gram-negative bacteria, and hence increase in bacterial abundance and biomass (Zabaloy *et al.,* 2008) and fungal count (Araujo *et al.,* 2003; Ratcliff *et al.,* 2006). The increases population of actinomycetes and fungi after glyphosate treatment was observed (Araujo *et al.,* 2003). Certain microbes (fungi and actinomycetes) are able to metabolize xenobiotics like pesticides, and thus have the ability to flourish and multiply following an initial transient decrease in number. Actinomycetes showed a significant increase in glyphosate treated soil with time, which indicated that actinomycetes may use glyphosate as nutrient and energy source (Araujo *et al.,* 2003).

CONCLUSIONS AND RECOMMENDATIONS

The herbicides in the present study were applied once at the beginning of each experiment. Therefore, changes in soil microbial biomass C, N and diversity as well as the soil organic carbon, total nitrogen content and potential activity of dehydrogenase with time indicated whether the effects were reversible or not. The results showed that significant effects on microbial biomass C, N and diversity, with some exceptions, occurred within the first week or two, and started to recover thereafter.
Organic C in brown forest soil has the same pattern which was observed for microbial biomass C because soil organic C is the primary substrate for soil microorganisms.
In summary, single applications of herbicides at recommended rates did not significantly or consistently affect soil microbial biomass C and N or bacterial diversity. However, shifts in microbial community structures were sometimes evident where microbial biomass C or N and diversity were not different. Different doses of paraquat at different WAA affect soil microbial populations as well the chemical components of the soil. So, the effects of paraquat on soil microbial populations and chemical components depended on the concentrations used and the duration of application. It also determines the type of microbes present in the soil by getting rid of those that cannot withstand its effects, in this way allowing only the resistible species.

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TL19.

EFFECT OF FOUR HERBICIDES ON SOIL ENZYME ACTIVITY IN BROWN FOREST AGRICULTURAL SOIL

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Abstract: *Soil enzymatic activity assays are only one way to measure the soil ecosystem status. The technique is quite simple and produces reproducible results, and is nowadays of practical importance because the influence of herbicides, as well as soil fertility management, can be measured. Especially the search for urease inhibitor is of particular interest in order to reduce ammonia losses from soils. Soil enzymes have been reported as useful soil quality indicators due to their relationship to soil biology, being operationally practical, sensitive, integrative, easy to measure, and described as "biological fingerprints" of past soil management. Herbicides are biologically active compounds, and an unintended consequence of their application may lead to significant changes in biological activities influencing microbial ecological balance affecting soil fertility. The fate of herbicides applied in agricultural ecosystems is governed by the transfer and degradation processes, and their interaction with soil microorganisms. The increasing reliance of sustainable agriculture on herbicides has led to concern about their ecotoxicological effects influencing enzyme activities, which may serve as indicators of soil quality. The effects of herbicides (glyphosate, paraquat, trifluralin, and 2,4-D) on soil enzyme activities (β-glucosidase, amylase, invertase, cellulase, protease, and urease as well as phosphatase and aryl-sulfatase) were assessed over a period of four weeks. There was a significant reduction in organic carbon with time. Herbicide treatments resulted from variation in enzyme activities, while the highest activity was recorded for control soil. Only paraquat and glyphosate at field recommended dose were found to be more inhibit the enzyme activities and 2,4-D was the least inhibited herbicide. The study suggested that the herbicides cause a transient impact on enzyme activities associated with the type of herbicides at recommended field application rate.*

Keywords: *Soil enzymatic activity, herbicides, sustainable agriculture, brown forest soil*

INTRODUCTION

A powerful concept in modern biology is that of the ecosystem. An ecosystem is the sum total biotics of all types living in a defined area in addition to those portions of the non-living or inorganic environment with which they interact. In a micro-ecosystem, conditions may be very different from the general situation e.g., the limitation in availability of nutrition status, aeration, moisture and other ecological factors, which can be different from one micro-ecosystem to others. One of the major concerns in today's world is the pollution and soil contamination. Global herbicide usage has increased in the past three decades in response to declining reliance on mechanical cultivation for weed control. Questions have been raised about the potential for increased herbicide use to negatively affect soil organisms and impair the services they provide for crop production. Natural and anthropogenic factors may affect the soil enzyme activities directly or indirectly. Among anthropogenic factors, pesticides are of primary importance due to their continuous entry into the soil environment. Herbicides are one of the major groups of pesticides, which include substances or cultured biological organism used to kill or suppress the growth of unwanted plants and vegetation in order to minimize the cultivation cost as well as to sustain high yield. A number of herbicides have not only been introduced as pre- or post-emergence weed killer (Ayansina & Oso, 2006) but also leave unwanted residues in soil, which are ecologically harmful (Riaz *et al*., 2007). Preferred herbicides should not only have good efficacy, but also poses minimum adverse effects to crop, ecology and environment (Hoerlein, 1994).

The benefits of organic matter in soil are well known and have been widely studied. The use of organic waste as soil amendments is an efficient strategy to restore degraded land (Ros *et al*., 2003), especially in developing countries, when the disposal of organic wastes is becoming a serious problem. Soil resilience defined as the ability of the soil to recover after disturbance. The related term soil quality signifies the capacity of the soil to produce healthy and nutritious crops, resist erosion, and reduce the impact of environmental stress on plants. For the assessment of soil perturbation, enzymatic activities and biochemical indexes have been proposed (Benitez *et al*., 2004).

Nutrient availability to plant is strongly influence by organic and inorganic amendments that usually increase the amounts of soil organic C, N and other nutrients. Organic matter added to soil by incorporating plant material, animal residues, manure, sewage sludge, or municipal waste. Amendments not only influence soil fertility directly, but can also affect the composition and activities of soil microorganisms. The use of selected parameters, such as enzyme activities, organic matter, physical properties, structure and formation of humic substances, together with agronomic and chemical properties, has provided additional information about functioning and productivity of degraded soil-ecosystems under appropriate regeneration practices (Masciandaro & Ceccanti, 1999).

The use of enzymes for environmental purposes has more and more increased with the time due to specific and peculiar properties of this class of proteins. Hence arable soil is often amended with agrochemicals to increase agricultural productivity. In transforming the natural ecosystems into agricultural ecosystems characterized by a low biodiversity, as well as the intensive development of farming systems, resulted in a large-scale application of crop protection chemicals especially pesticides such as insecticides, fungicides and herbicides. Sustainable agriculture involves optimizing agricultural resources and at the same time maintaining the quality of environment and sustaining natural resources and the need for substantial increase in agricultural production is an urgent problem particularly in the less developed areas of the world and further increase in agrochemical use can be foreseen. The use of herbicides, as an effective practice for controlling weeds in crops, has increased in the agricultural systems. Therefore, studies assessing the effect of herbicides on soil biological properties are important for evaluating soil quality and health. In addition, soil biological properties are more effective as indicators of soil quality than physical and chemical properties as they often show a faster response to an environmental impact. As important and responsive biological properties, soil microbial biomass and enzyme activities are frequently recommended for evaluating the effects of herbicides on the soil environment.

Within the biological diversity components of agriculture, weeds are model for exploring management options relying on the principle of eco-intensification in arable lands. Weeds can decrease crop production, contribute to farmland functional biodiversity and are strongly associated with the generic issue of pesticide use. Soil enzymatic activities are essential components of all biospheric nutrient recycles and, therefore, are important as test bioindicators in any ecotoxicological assessment involving herbicides. Soil enzymatic processes are an integral part of soil quality and soil health, and a better understanding of these processes is needed. Enzymes play a main role in bioremediation, the process in which enzymes are used to target, transform and remove specific pollutants in wastes. The role of soil enzymes may range from natural processes such as intrinsic bioremediation as in the transformation of pollutants to less toxic compounds in a contaminated environment (Gianfreda & Bollag, 2002), to manipulation of the contaminated environment by addition of specific chemical or biochemical additives or to bioaccumulation. All processes are occurring in living cells and involved in the removal, enzymatic degradation of organic pollutants, and accumulation and bioprecipitation of metal ions (Demarche *et al*., 2012).

The solution of the problems related to the measurement of soil enzyme activities requires future research. Indeed it is still difficult to precisely identify which factor would virtually operate soil biochemical processes and to compare soil enzyme data obtained from different regions or climate zones (Karaca *et al*., 2011). The development of soil enzyme index usable as a reliable measure of soil fertility and health is still a key research priority that requires new approaches, ideas and technologies (Burns *et al*., 2013).

Research in soil proteomic, metabolomic and transcriptomics studies is probably the most important challenge that soil enzymologist ever have faced. As clearly stated by Burns et al. (2013) the ‘‘pressing demand to understand the relationships between genetic diversity and community structure and function’’ may be solved by studying soil extracellular enzymes being these latter ‘‘an ideal model system for linking microbial identity to specific and critical ecosystem processes. Among the soil components, soil organic matter (SOM) has been reported to be an important factor in the retention of herbicides, the reduction of soil profile mobility and the potential for environmental decontamination (Tejada & Benítez, 2017).

Sessitsch *et al.* (2004) studied the effects of glufosinate and metazachlor on activities of invertase, alkaline phosphatase and urease as well as aryl sulfatase in the rhizosphere of oilseed rape. The results indicated that oilseed rape rhizosphere bacteria were affected by the genetic modification as well as by the herbicide application. At senescence, invertase, urease and alkaline phosphatase activities were significantly enhanced in the rhizosphere.

Herbicide-desiccated cover crops may impact microbial activity. Since microorganisms play a considerable role in soil processes that directly affect soil quality (Paul & Clark, 1989), the effect of this approach on parameters of biological soil quality is one of most important factors that should be investigated. Soil enzymes are present in important cycles, such as C (invertase, cellulase, β-glucosidase), N (urease and protease), P (phosphatase) S (aryl sulphate) cycles. Soil enzyme activities are used as indices of microbial activity (Bergstrom *et al*., 1998) and react quickly to environment change (Sparling, 1997). Both Glyphosate and Paraquat were reported to cause activation in soil urease and invertase soil enzymes but suppression of phosphatase (Sannino & Gianfreda, 2001). Hydrolytic enzymes activities (phosphatase, β-glucosidase), oxidoreductases activities (dehydrogenase) and indole acetic acid production, were used as measures of soil perturbation by Benitez *et al.* (2004). Enzymes such as esterase and aryl sulfatase may also be indicators of the potential for hydrolytic catabolism of several families of soil-applied herbicides. Both studies demonstrated that herbicide-desiccated cover crops enhanced descriptors of biological soil quality, namely microbial biomass, microbial populations, and soil enzyme activities. These factors can potentially affect the availability of plant nutrients, organic matter transformations, and the fate of pesticides in the environment (Locke *et al*., 1995), all of which are important in the development of sustainable agricultural systems.

The use of herbicides is important for controlling weeds in crops. However, they can present impacts on soil properties, such as biological properties (Pertile *et al*, 2020). Herbicides are used in large quantities in modern agriculture to control undesirable plant species within a field. The increased application of herbicides leads to increased chemical concentrations in soil, altered soil reactions and potential adverse effect on non-target organisms. Repeated application of herbicides may involve a risk of reduced or altered soil microbial activities.

In the environment enzymes may play important and different roles at least in three cases: as main agents (as isolated, cell-bound or immobilized enzymes) in charge of either the transformation and/or degradation of compounds polluting the environment and the restoration of the polluted environment; as reliable and sensitive tools to detect and measure the amount and concentration of pollutants before, during and after the restoration process; as reliable, easy and sensitive indicators of quality and health status of the environment subjected to the restoration process (Rao *et al*., 2014).

Herbicides are biologically active compounds and an unintended consequence of its application may lead to significant changes in microbial populations and activities influencing microbial ecological balance affecting soil fertility as microbial community plays crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth (Pandey *et al*., 2007), and hence occupy a unique position in biological cycles in terrestrial habitat. On the other hand any action of the chemicals altering the life functions of soil organisms could indirectly affect soil enzyme activity.

Agrochemicals often stimulate or decrease the growth of soil microbial population and thus may alter the enzyme activity. Also these chemicals may exert some physiological effect on living organisms. An agrochemical may also modify the inter relationship between the particular group of organisms and

thus influence the amount and type of enzyme produced. When agrochemicals are released into the environment about 1% reaches the target organism while remaining 0.99% interferes with local metabolism or enzymes activities (Ramudu *et al*. 2011).
Effects of glyphosate, paraquat, trifluralin and atrazine on soil enzymatic activities of dehydrogenase, phosphatase and urease in one soil were studied (Davies & Greaves, 1981). It was reported that when recommended doses were used, enzymes activities were not affected by the herbicides. The wide range of soils used with greatly differing enzyme activities, and varying assay conditions like temperature, pH, and substrate concentrations were responsible for contradictory results on effect of herbicides on soil.
So, serious attempts should be made, possibly by judging the effects against those natural stresses or against the background of natural variation for assay of soil enzymes. It is proposed that effects of natural stress can be used to judge the relative importance of herbicide induced change.
Methods employed for assessing soil enzyme activities are proposed as sensitive to ecosystems perturbations and agricultural practices (Rao *et al*., 2014). A number of studies have used soil enzymes as an indicator for investigating the potential effects of herbicides on soil function.
The aim of this study was to qualified the potential effect of four herbicides glyphosate, 2,4-dichlorophenoxyacetic acid (2,4-D), trifluralin and paraquat to brown forest soil type at a recommended field applied rate on total organic carbon and some soil enzymatic activities

MATERIALS AND METHODS (11 Bold)

Materials

Soil characterisation

The physicochemical properties of the brown forest soil sample are presented in Table (1). Topsoil (0–200 mm) was collected from the pesticide non-treated area in the garden of the Rejtő Sándor Faculty of Light Industry and Environmental Engineering and passed through a 4-mm sieve.

Table (1) Characterizations of used soil sample

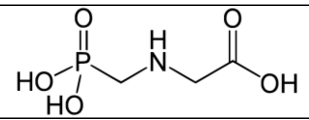
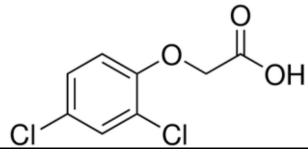
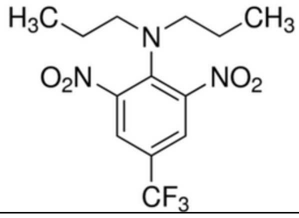
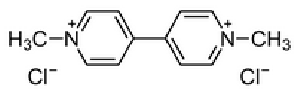
Investigated parameters	Units	Results
pH_{KCL}		7,13
EC	µS/cm	483
Σsó	m/m %	0,040
$CaCO_3$	m/m %	32,34
H	m/m %	3,34
Totat N	mg/kg	1930
NH_4-N	mg/kg	18,76
NO_3-N	mg/kg	13,69
AL-p_2O_5	mg/kg	51,7
AL-K_2O	mg/kg	169
AL-Na	mg/kg	37,2
KCl-Mg	mg/kg	146
KCl-S	mg/kg	14,1

Soil samples were collected during the dry summer fallow period; so that all soil samples were have < 25% water holding capacity at sampling and during storage of bulk soil. Four weeks prior to experimental setup, sub-samples of soil were air-dried under the laboratory condition (25±3°C) for 1 week, then it homogenized and sieved (4 mm).

Herbicides

The four herbicides in Table (2) glyphosate, 2,4-dichlorophenoxyacetic acid trifluralin and paraquat were dissolved in water. The soil was spiked with a sufficient volume of the diluted herbicide to bring the soil moisture to 45% of the maximum water holding capacity (WHC) and the herbicide concentration to the required level as recommended field applied rate.

Table (2) IUPAC names and chemical structures of the four used herbicides

Used Herbicide	Herbicide Class	IUPAC name	Chemical structure
Glyphosate	Organophosphorus (Glycine)	2-[(Phosphonomethyl)amino]acetic acid	
2,4-D	Phenoxycarboxylic acids	2-(2,4-Dichlorophenoxy)acetic acid	
Trifluralin	Dinitroaniline	α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toiuidine	
Paraquat	Bipyridylium	N,N′-dimethyl-4,4′-bipyridinium dichloride	

Applied Methods

Experimental setup and herbicide treatment

The laboratory pot experiments were laid out to investigate the ecotoxicological effects of four herbicides on total organic carbon and the soil enzymatic activities. Fresh soil samples were air dried, sieved through a 4 mm sieve and placed in polyethylene pots. Before use, soil samples were kept for air dry environment at room temperature (25±3°C) for 24 hours. The four herbicides were used as commercial formulations and added to soil at recommended field application rate as shown in Table (3).

Table (3) The field applied dose of the four herbicides used in the pot experiment

Herbicide	Field applied dose
2,4-D	2.6 - 3.4 l/ha
paraquat	0.28 - 1.12 kg/ha
trifluralin	0.35 - 0.5 kg/ha
glyphosate	2.0 – 5.0 l/ha

The applied herbicides solutions to the soil samples were added as a part of the moisture required to adjust the soil to 45% of their water holding capacity. The herbicidal solution was sprayed onto the soil surfaces by means of a syringe that dispensed very small droplets.
Pots of 250 g of untreated control and herbicide amended soil samples were incubated at 25±3°C for 4 weeks at dark. Loss of water by evaporation was compensated daily to avoid dryness. Treatments and control were conducted in triplicates in a full randomized block design.

Determination of Dynamics of organic carbon

Total organic carbon (TOC) was analyzed by dichromate ($K_2Cr_2O_7$) oxidation and titration with ferrous ammonium sulphate (Walkley & Black, 1934). In this reaction C is oxidized by the dichromate ion and the excess of dichromate ion is then back titrated with ferrous ion. TOC was determined in the herbicide treated soil and control soil samples.

Study the effects of herbicides on soil potential enzymatic activities

Following incubation and from each pot, 5 g of the soil sample received 50 mL of distilled water and was shaken for 30 min to obtain homogeneous soil slurries. For enzyme assays, the potential activities of eight enzymes involved in organic matter breakdown and nutrient cycling were: amylase, invertase, cellulase, β-glucosidase, protease, urease, and phosphatase as well as aryl sulfatase. Soil enzyme potential activities were determined using spectrophotometrical (Zhao *et al*., 2015) techniques.

- **Amylase activity**

The potential activity of amylase in soil treated with different herbicides was determined using the procedure described by Somogyi (1952) and Roberge (1978) by taking starch as substrate. Amylase activity was determined by using phosphate buffer (pH = 5.5, 10 mL) and substrate (10 mL 1% starch solution). After incubation at 37°C for 24 h, the content of maltose was measured at 508 nm.

- **Invertase activity**

The potential activity of invertase was determined by spectrophotometric method (Ross, 1983) by using sucrose as substrate. Invertase was determined by using phosphate buffer (pH = 5.5, 15 mL) and substrate (5 mL 8% sucrose solution for invertase). The amount of glucose was measured at 508 nm after incubation at 37°C for 24 h.

- **Cellulase activity**

The potential activity of cellulase was measured as following: 5 g soil was placed in a 50-ml Erlenmeyer flask and incubated with 0.5 ml toluene and 20 ml buffered 2% CMC at 30°C for 24 h. After incubation, the suspension was mixed well and centrifuged three times at 17,390g for 10 min. A portion (10 ml) of the supernatant was transferred into a 50-ml plastic centrifuge tube and treated with K-saturated cation-exchange resin (2 g). The mixture was shaken for 30min, and the supernatant was analyzed for reducing sugars by the Somogyi-Nelson method (Deng & Tabatabai, 1994). The blue solutions developed for the soil extracts obtained from the CMC-treated soils were centrifuged once as described above before colour measurement at 710 nm. cellulase activities was expressed as mg glucose kg^{-1} soil.

- **β-glucosidase activity**

The potential activity of β-glucosidase was calculated as following: Two ml of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of 50 mM *p*-nitrophenyl-β-D-glucopyranoside (PNG) were added to 0.5 g of soil sample. The rest of the method was the same as for phosphatase activity (Masciandaro *et al*., 1994). The PNP absorbance was measured at 400 nm. β-glucosidase activity is expressed as μmol of PNP per gram dry soil and incubation time (hours).

- **Protease activity on N-α-benzoyl-L-argininamide (Protease-BAA)**

The potential activity of protease was determined as following: Two ml of phosphate buffer (pH 7) and 0.5 ml of 0.05 M N-α-benzoyl-L-arginamide (BAA) substrate were added to 0.5 g soil sample. The mixture was incubated at 37°C for 90 min and then diluted to 10 ml with sterile distilled water. The release of ammonium was measured in the same way as for urease (Nannipieri *et al*., 1980). Protease activity is expressed as μmol of NH_4^+-N released per gram dry soil per hour.

- **Urease activity**

The potential activity of urease under the stress of herbicides treated soil was determined by titration method using 0.005 N H_2SO_4 with boric acid indicator (Tabatabai & Bremner, 1971, 1972).

- **Phosphatase activity**

The potential activity of phosphatase was done as following: Two ml of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of 0.115 M *p*-nitrophenyl phosphate (PNP) were added to 0.5 g of soil sample and incubated at 37°C for 90 min. The reaction was stopped by cooling to 2°C for 15 min., then 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added and the mixture was centrifuged to 4000 rpm for 5 min. The reaction product was filtrated and the filtrate (*p*-nitrophenol) was analyzed calorimetrically at 398 nm (Tabatabai & Bermner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH. The PNP absorbance was measured at 398 nm. Phosphatase activity is expressed as μmol of PNP per gram dry soil and incubation time (hour).

♦ **Aryl-sulfatase activity**

The potential activity of aryl-sulfatase (µmol nitrophenol/g dry soil/h) was determined according to Tabatabai & Bremner (1969). The activity was measured by absorption of p-phenol at 400 nm after incubation with PNP sulphate.

Statistical Analysis

All experiments were conducted in triplicates, with randomized complete block design. All values reported are the arithmetic means of the three determinations expressed on an oven-dried soil basis. Statistical analyses were performed using ANOVA to determine the significant differences between the treatments at $P = 0.05$.

RESULTS AND DISCUSSION

Herbicides are specific regarding to their toxic level. However, the application of several chemicals may lead to synergy and development of toxic effects hazardous for humans and the ecosystem. Herbicides may cause acute and genetic toxicity which are perilous for the biota inhabiting the ecosystem. In order to increase the crop yield in agricultural areas, synthetic pesticides including insecticides, acaricides, fungicides, and herbicides were widely applied to control harmful organisms as well as in modern agricultural production, herbicide application is a regular practice. The problems caused by the increased application of herbicides call for multidisciplinary approach. Incorrect and indiscriminate application of herbicides affects negatively the health of humans, plants and animals. Particularly hazardous are the poorly degradable herbicides whose persistence may lead to long-term accumulation.

Effect of tested herbicides on soil organic carbon

The variation in OC content was exhibited with respect to different herbicide treated soil (Figure 1). There was significant reduction in OC level after the application of herbicides, although OC decreased after continuous application from the 7th day of glyphosate treatment (Sebiomo *et al.,* 2011) as well as the other herbicides too. However, the herbicides decomposition is frequently faster in soils with high OC, presumably due to vigorous microbial activity. Further, the fate of herbicides is greatly affected by the presence of soil organic matter by aiding their disappearance (Ayansina & Oso 2006).

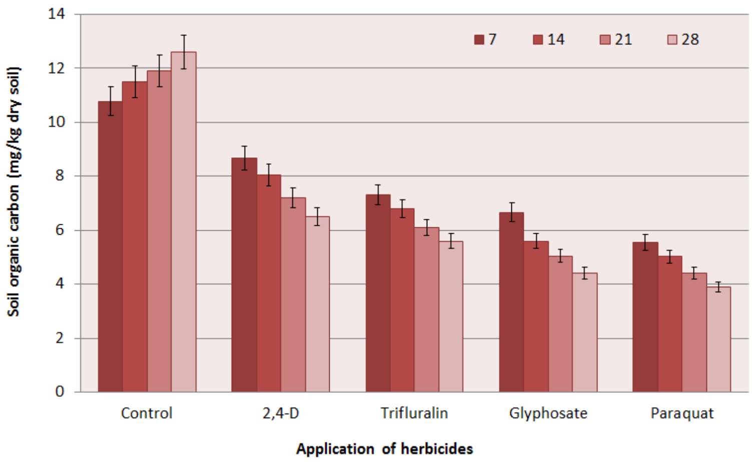


Figure (1): Effect of various herbicides on soil organic matter in brown forest soil

Effect of tested herbicides on soil enzyme activity

The amylase and invertase, cellulase and ß-glucosidase activities have been selected for their importance in soil C cycle. Besides, the protease and urease activity was selected for their involvement in soil N cycle. However, soil phosphatase and aryl-sulphatase are the two enzymes involved in the biogeochemical cycles of P and S, respectively. A close perusal of data indicates that significant difference exist between different herbicide treatments and periods of study. Among the treatments

there was significant difference, from the data the activity of the enzymes under different treatment can be grouped under three categories i.e., only stimulation or only inhibition and both stimulation and inhibition.
The results showed that in comparison with the control soil samples, it was found that paraquat was the most harmful herbicide on the activity of amylase in the brown forest soil, followed by glyphosate, and trifluralin. The 2,4-D had the lowest effect herbicide on amylase activity. Also, it was found that by increasing the incubation period, the activity of amylase decreased (Figure 2).

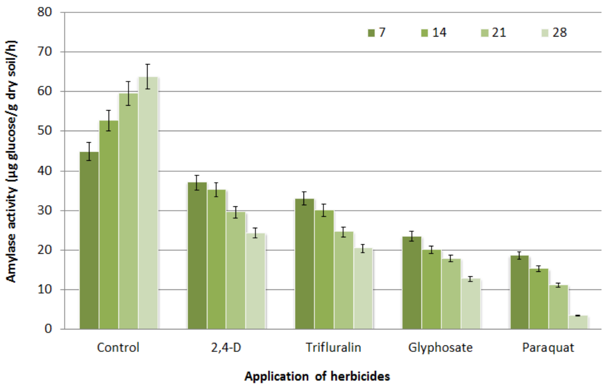


Figure (2): Effect of various herbicides on amylase activity in brown forest soil

Figure (3) indicated that the effect of applied herbicides on the activity of invertase in the brown forest soil had the same tendency as in case of amylase. The 2,4-D hormonal herbicide had the lowest impact on the invertase enzyme.

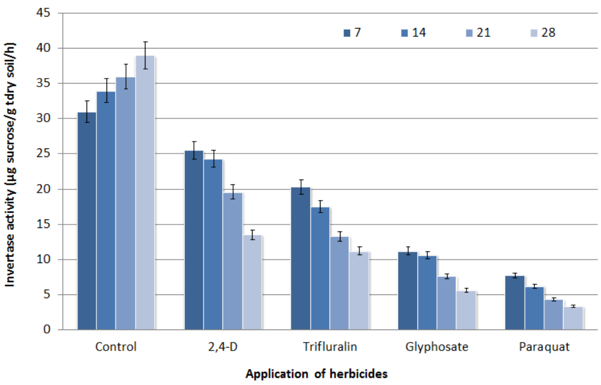


Figure (3): Effect of various herbicides on invertase activity in brown forest soil

In Figure (4), it was found that the differences between the effects of herbicides were reduced and the significant differences between the incubation periods in case of 2,4-D was absent. Paraquat was the more toxic herbicides and the activity of cellulase at the 3rd week was the highest under the stress of paraquat.

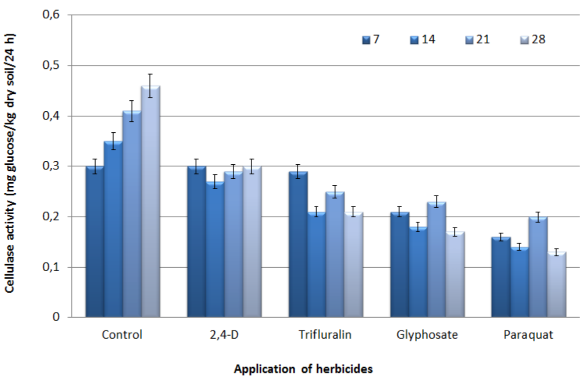


Figure (4): Effect of various herbicides on cellulase activity in brown forest soil

The effects of various herbicides on ß-glucosidase activity in brown forest soil are shown in the Figure (5). It was found that the activity of the enzyme is significantly differences among the variation of the incubation period in comparative with the control soil samples. Also the paraquat was the most toxic herbicide and the least toxic herbicide was the hormonal herbicide 2,4-D.

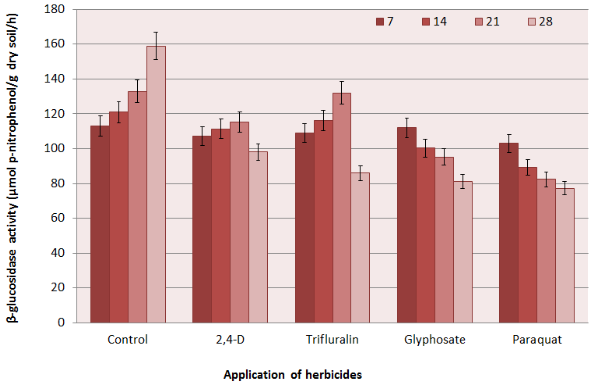


Figure (5): Effect of various herbicides on ß-glucosidase activity in brown forest soil

Figure (6) illustrates that the enzyme protease was more affected by the applied herbicides at the 4th week of the incubation, but in some cases the enzyme was stimulated by the application of the herbicides during the 1st, 2nd and the 3rd week of the incubation in comparison with the control soil samples.

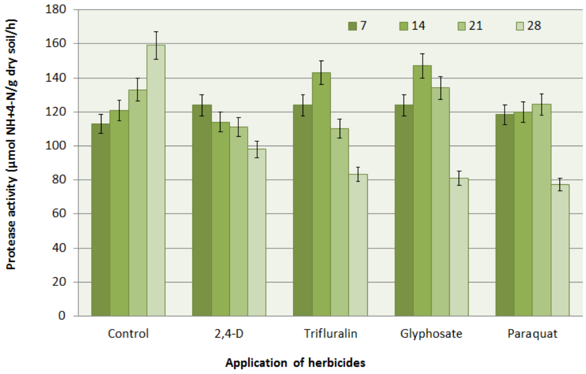


Figure (6): Effect of various herbicides on protease activity in brown forest soil

Effect of various herbicides on urease activity in brown forest soil samples were clearly shown in the Figure (7). The results demonstrated that all applied herbicides were inhibiting the activity of the enzyme in all incubation periods.

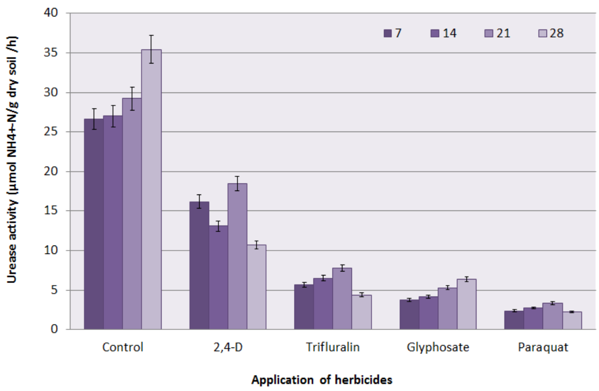


Figure (7): Effect of various herbicides on urease activity in brown forest soil

The most toxic herbicides were paraquat followed by glyphosate and trifluralin. The least effect on the urease enzyme activity was shown when the soil samples treated with hormonal herbicide 2,4-D.

Figure (8) shows that the effect of various herbicides on phosphatase activity in brown forest soil samples. The enzyme activity was decreased by increasing the incubation period. All herbicides were affected the activity of the enzyme in comparison with the control soil samples. Paraquat was shown as the most toxic herbicide and 2,4-D is the least toxic effect.

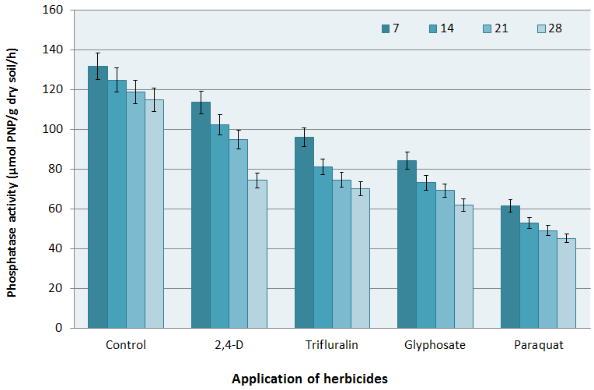


Figure (8): Effect of various herbicides on phosphatase activity in brown forest soil

Figure (9) shows the results of the effect of various herbicides on aryl-sulphatase activity in brown forest soil samples during four weeks of incubation in dark condition. It was found that the activity of the enzyme was increased over the control during the 3rd and 4th weeks of incubation when the soil samples treated with glyphosate significantly, and at the 3rd week of incubation when the soil samples treated with 2,4-D and paraquat. Meanwhile, all herbicides applied to the soil samples significantly reduced the activity of the enzyme.

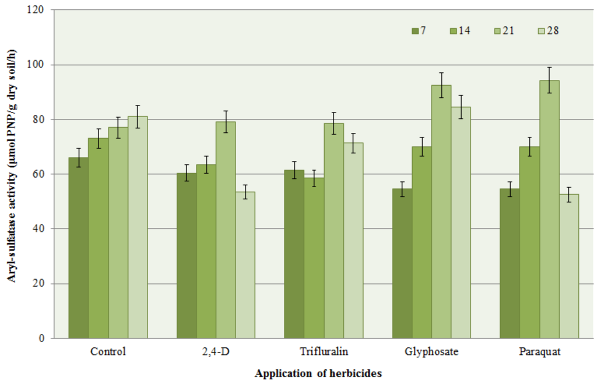


Figure (9): Effect of various herbicides on aryl-sulphatase activity in brown forest soil

Weed management is an integral part of crop production, and herbicides continue to be the most common weed management tool in most cropping systems. Because herbicides are usually applied when crops are absent or at early growth stages, most of the spray solution contacts soil. These chemicals may affect non-target soil organisms, including microorganisms. Herbicide-induced changes in abundance, diversity and activity of soil microbial communities may, in turn, influence microorganism-mediated processes that are important to sustainable agriculture, e.g., recycling of plant nutrients and maintenance of soil structure. While results of these studies indicate that herbicides applied at recommended rates generally do not have significant effects on soil microorganisms, evaluating only a few herbicides at a time limits comparison amongst herbicides on their relative effects on soil microbial ecology (Lupwayi *et al.*, 2004).

Based on the overall enzyme activity they can be further classified broadly into two classes i.e., stimulation or inhibition. Similar results have been reported by Cerevelli *et al*. (1975) for substituted urea herbicides. They were of the opinion that the soil enzymes are protected against inhibition action of herbicide as the incubation time increases the inhibition activity decreases. The inhibition, of enzyme activity by the herbicide could be the direct effect of herbicide on urease activity and also due to competitive and non-competitive inhibition. The increased enzyme activity in these treatments at latter stages with time may be due to different reasons i.e., the herbicide effect on microbial population may get stabilized after some time and the herbicides themselves are adsorbed irreversibly on soil colloids with increase in time resulting in decreased inhibition. The partial degradation of the herbicide with time in soil may also be another factor for decrease in inhibition. The recovery from inhibition may also be due to enzyme secreted by plants. The detracting effect of herbicides towards all microbes and enzyme activities decreased with time and this may also be due to microbial population and enzyme activities after initial inhibition due to microbial adaptation to these chemicals or due to their degradation.

Latha & Gopal, (2010) studied the effects of herbicides and found an inhibition in the enzyme activity particularly due to application of substituted urea herbicides. Kavitha *et al.* (2011) observed that application of herbicide disturbs and alter the biological equilibrium in the soil, and lower the microbial population and hence enzyme activity. Vandana *et al.,* (2012) reported that soil enzyme activities increased from 0 to 60 days after transplanting of the crop irrespective of the treatment to the soil as the root volume increases the production of soil enzymes increase. Sireesha *et al*., (2012) reported that lower level of herbicide application increased enzyme activity and vice versa. Abbas *et al*.(2015) reported that there was 30% reduction in urease activity, 36% inhibition in dehydrogenase activity and 34% decline in alkaline phosphatase activity with bromoxynil herbicide application due to decrease in microbial population. Baboo *et al.*, (2013) reported that the enzyme activities of amylase, invertase, protease, urease and dehydrogenase were affected by the herbicides butachlor, pyrazosulfuran, paraquat and glyphosate over a period of four weeks, the herbicides caused transient impact on microbial populations and enzyme activities associated with the type of herbicides at recommended field application. Rasool & Reshi, (2010), reported that pesticide application affects activities of different soil enzymes differently while the activities of urease and asparaginase were inhibited and the activities of dehydrogenase, protease and amidase activities were stimulated in response to pesticide treatments on the other hand phosphatase exhibited a highly variable response to different concentration of pesticide as the phosphatase activity was influenced by pH. Weaver *et al. (*2004) reported inactivation of most soil enzymes, because of herbicide attachment on the active site of enzyme. Increasing trend in the activity of urease was observed from day 15 to day 60. This reduction in inhibitory effect was due to soil microbe's recovery with the passage of time due to which urease activity increased. Singh (2014) reported that high dose of pendimethalin herbicide proved deleterious for soil enzymes as compared to low and medium doses. High doses inactivate the enzymes irreversibly hence during the lag period the enzyme activity is decreased and at later stage recovery of the microbes and synthesis of new pool of enzymes by plant as well as microbes.

The sharp increase in urease enzyme activity at 30 days of applied treatment coincides with the active growth stage of the crop, enhanced root activity and release of extracellular enzymes like urease into soil which resulted in higher rate of mineralization of nutrients in the soil. The increase in urease activity may also be due to luxuriant root proliferation and large amount of leaf fall leading to nutrient rich environment, which was more conducive for proliferation of the micro flora for enhanced enzyme synthesis (Reddy *et al*., 2011). Increased dosage inhibits the survival of microbe due to osmotic stress created by herbicides, and the soil enzyme activity was influenced by native soil ecosystem and the type of herbicide applied (Bharathi *et al*., 2011). Increase in microbial population was responsible for sharp increase in urease and dehydrogenase activity of soil enzymes. The activities of these enzymes were significantly and positively correlated with organic carbon, fungal, bacterial and actinobacterial population in the soil. The application of recommended dose of nitrogen through organic sources specifically by farm yard manure, press mud increases the enzyme activity at different days of incubation. This is because addition of organic sources acts as good source of carbon and energy to heterotrophs by which their population increases there by increase in soil enzyme activity (Rai & Yadav, 2011). The possible reason for the decrease in soil invertase activity may be attributed to the cellular destruction caused by the toxic substances in herbicides (Perucci & Scarponi, 1994). Recovery of enzyme activities after the initial inhibition could be due to growth of microbial population after adaptation or most probably due to increased availability of nutrients and degradation of herbicides. The increase in soil dehydrogenase activity in herbicides treated soil from 7th to 28th day of incubation might be due to the increase in microbial community composition with the capability of utilizing the herbicides as carbon source (Sebiomo *et al*., 2011).

The effects of herbicides (paraquat and glyphosate) on soil organic carbon and enzymatic activities (amylase, invertase, protease and urease) were assessed over a period of four weeks. There was significant reduction in organic carbon with time. Herbicide treatments resulted variation in enzymatic activities, while highest activity was recorded for control soil. The study suggested that the herbicides cause transient impact on enzyme activities associated with the type of herbicides at recommended field application rate (Baboo *et al*., 2013).

CONCLUSIONS AND RECOMMENDATIONS (11 Bold)

Herbicides are biologically active compounds, and an unintended consequence of its application may lead to significant changes in microbial populations and activities influencing microbial ecological balance affecting soil fertility. The fate of herbicides applied in agroecosystems is governed by the transfer and degradation processes, and their interaction with soil microorganisms. The increasing reliance of sustainable agriculture on herbicides has led to concern about their ecotoxicological effects influencing microbial populations and enzyme activities, which may serve as indicators of soil quality. In this study, the application of herbicides influenced the soil enzyme activity differently. From the present study it can be emphasized that the type of herbicide, its concentration and its time of exposure influence soil enzyme activities. Hence great care is required while applying herbicide to soil.

The enzyme activities are considered to be sensitive to chemical pollutants/agrochemicals and have been proposed as potential indicators for measuring the degree of pollution of contaminated soil

The study confirmed that the herbicides (2,-D, trifluralin, paraquat and glyphosate) may alter the affects the different soil enzyme activities. Since the investigations were performed *in* vitro in pot experiment, and the effects of herbicides are highly transitory, it is particularly difficult to explain a change of soil enzyme activities in response to certain factors or to establish the cause-effect relationships between the herbicide treatments and the various components contributing to the variation in overall soil enzyme activities. Among the applied herbicides, paraquat was the most toxic one.

Further task, it is necessary to strengthen the scientific basis of modern agriculture, because herbicides may be advantageously used only if their persistence, bioaccumulation, and toxicity in agroecosystem are strictly controlled.

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TL20.

SURVEY OF LICHENS BIODIVERSITY IN DIFFERENT AREAS OF CSÓR VILLAGE, HUNGARY

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Abstract: *The bioindication is an important agent today and in the future, as what could give a clearer picture of the atmosphere around us. Lichens play as bioindicator. Lichens do not tolerate the sulfur dioxide content of the air. This urban or industrial pollution is polluted air are often called "lichen deserts". So where the lichens absence, there is SO_2 in the air. For this purpose, 58pictures of lichens were taken in 4 different areas of Csór village: 1) Mátyás Király utca (street), 2) Hadi út (road), 3) Vízmű telep (Waterworks Plant), and 4) Dózsa György köz (interval).. Study areas were selected to include a wooded, green area, a less populated, not very busy area, and parts with more traffic in the village. First location was the garden of a family house, where most varieties of fruit trees are found are full with lichens. Second area was examined a small part of a wooded area were one or two or many lichen colonies are found. Third location was a part of the waterworks site is already a wooded area. The fourth area was the garden of another family house. There are no fruit trees in this area, only two pieces of ornamental trees. It was the first area where the lichens found on the fence. Based on the photos, morphology, colour, diameter, and extent of the lichens were examined. In addition, most dominant types were investigated. Lichens of different shapes and sizes in the four areas were examined. The investigations showed that mostly yellow and white leafy lichen are there. Yellow plate lichen and map lichen were also found. Exceptions are the third and fourth locations, where the yellow-leafed lichen outside the trunk of the tree on the stones on the side of the building and on the boards of the bench and fence. The sizes of the lichens are different. Their surface area is very varied. Because Csóron does not contain SO_2 in the air, lichens are present everywhere in an undamaged state. The most dominant species in the studied areas was the yellow-leafed lichen. Lichens are used to study global climate change, air quality. Attention must be paid to its use in the pharmaceutical industry due to its antibiotic and antiseptic effect.*

Keywords: *Lichens, air quality, distribution, Csór village, morphological investigation*

INTRODUCTION

Lichens were already known before our time and were probably used them. Several ancient written relics prove this, although it was not mentioned as lichen, but we can recognize different types of lichens from the description of the species. From later sources in which they are already mentioned and studied as lichens, we can see that these complex organisms have been used both in medicine (e.g. Icelandic lichen, bearded lichen) and in art (extracted dye) and many other fields. Lichens are one of the most peculiar groups in the living world. The word ―lichen‖ was termed by Theophrastus in 300 BC. It is a Greek word which means superficial growth on the bark of the trees. Lichen is a combination of two organisms one is unicellular green alga and another one is fungus, sometimes in place of alga, cyanobacteria is also present (Stace, 1991). Alga and fungus helps each other in surviving, the algal part is known as photobiont as it contain photosynthetic pigment which provide food for the whole organism with the help of sunlight and other part is known as mycobiont this provides structural support to the organisms (Stace, 1991). There were lots of controversies about the relationship between alga and fungus. For the first time a Swiss botanist Schwendener in 1867 demonstrated the dual nature of lichen thallus after this there is a great discussion started on the nature of relation between the fungus and the algae. It was proposed that the nature is parasitism because of the haustarium of fungus enter in the algae and draw out nutrients for its growth, but later this theory was discarded because in parasitism the organism which is dominant kill the weaker one but in this case the algae does not die. Then this is called helostism because in helostism master and slave relationship is appears and in this case fungus is the master and the algae is slave, some scientists called it endosaprophytes because fungus is saprophyte (which feeds on dead and decade organisms) it is observed in some lichen thallus the algae is dead and the fungus feed on them (Stace, 1991).

Some lichenologist called the relationship mutualism in which both the organisms help each other without any harm and in lichen alga provide food and nutrition for growth and fungus provide protection and structural support to the whole organism, therefore relation is called symbiotic in which two organisms living together without killing each other (Ahmadjian, 1995). Lichen is very complicated organism, mostly it contains 90% of fungus and the remaining is the alga, this ratio can be varying from species to species. With the help of information technology and computer applications all the informative data which is collected during identification is compiled and stored in an application in the form of digital library through which one can easily retrieved the data for future use. This helps the taxonomists in identification, characterization and in quick classification of lichens (Singh et al., 2019).

Morphology: In morphology of the outer most surfaces was observed (Figure 1), the upper surface and the lower surface of lichen thallus were observed deeply under dissection microscope. The morphological characters are divided into two parts, characters of thallus and characters of fruiting bodies, firstly the thallus were grouped according to their growth forms (leprose, crustose, foliose, squamulose, dimorphic, fruiticose), thallus shape (irregular, circular), size, colour, texture (smooth, rough, warty), upper surface is observed for the presence of finger like projections (isidia), granular structure (soredia), fine powder (pruina), black dots (pycnidia) and whitish decorticated areas (pseudocyphellae).

The branching pattern length and breadth of marginal lobes, presence of hair like structure (cilia) has to be noted. The morphology of fruiting bodies has to be studied separately. In case of apothecia, shape (rounded or stretched), size, attachment (stalked or not) colour and texture of the margin and disc, shape of the disc (concave or convex) are observed. In case of perithecia its colour, shape, size and the position of its opening (ostiole, apical or lateral); single or grouped has to be noted. The lower surface of only foliose lichens can be seen as it is absent in crustose lichens. While dimorphic and fruticose lichens do not show dorsiventral differentiation. The colour of lower surface, presence of any pores (cyphaellae, pseudocyphellae), presence or absence of rihizines (root like structure) their colour, distribution, branching, abundance are to be noted.

Lichens are also observed inside a closed UV chamber at 254 and 365 nm to see whether the lichen has florescence characteristic (yellowish and bluish colour) or not because some lichens shows florescence due to the presence of substance called lichexanthone.

Figure (1): The vertical section of lichen thallus

Their bodies were formed by a symbiosis of fungal filaments and photosynthetic organisms. Over the millions of years, coexistence has resulted in such a close relationship between certain fungi and algae that they have become self-sustaining organisms with a specific body structure, specific metabolism. The concept of lichen has been most accurately described so far in the work of Hawksworth and Honegger, the most complex, complete formulation to date: ecologically obligatory, stable, mutualistic coexistence of the population of green algae and / or cyanobacterial cells, the photobionate. To date, approximately 650 compounds have been detected. The lichen material of some species is medicinal. Icelandic lichen is used in cough candy as herbal tea. There are also lichens that are suitable for textile dyeing. Today, lichen is also used by the perfume industry, and the lichen in it is used to preserve the scent. In addition, in some species, the lichen material acts as a UV filter or is responsible for the colour of the colony, such as in the yellow lichen. An interesting feature of lichens is that they are sensitive to increases in the SO_2 content of the air, i.e. they are narrowly tolerant to this environmental factor. They disappear from places where the air is polluted with SO_2. They are also called indicator organisms because their absence indicates high SO_2 content in the air. Air pollution is well illustrated by lichen maps made in big cities and around industrial estates.

Bioindicators are living things that somehow respond to changes in the environment. This may mean that they disappear or that more of some of their types appear in a particular area. With these we can examine the change of the environment, the degree of pollution. To study such processes, it is preferable to study living organisms in nature because all environmental changes can be detected in them, as they are most closely related to the environment. Advantage of bioindicator It is very good for monitoring the environment as they are closely related to nature. We can get a general picture of the area we are looking at. We can see the relationship of living beings to their habitat. This is a quick, very simple method. Disadvantage of bioindicator is that we cannot compare it to any standard here. There is no given method for how to examine a living thing.

Identification: Identifying lichens is much more difficult than identifying vascular plants. Each lichen thallus is a complete microscopic world with unique characteristics separating it from the other lichens. Lichens are classified based on the fungus and fungal features. When identifying lichens, keep in mind that one species of fungus can have two different forms if paired with two different "photobionts". It is not common but it does happen. In order to identify lichen to species, lichenologists use common household chemicals and some not-so-common chemicals to test the colour reaction of the unique compounds found in the structure of the lichen, as well as using a lichen key to distinguish between species. Although a few of the chemicals are common, such as bleach and iodine, others are not as easy to get and are costly and dangerous. However, just about anyone can use a botanical identification key and a hand lens to identify the genus of lichen and appreciate their collection. Even if you are not interested in identifying lichens, they are still interesting and amazing organisms to look at with the naked eye as well as under a hand lens or microscope. Realizing the roles lichens play in our environment will give you a greater appreciation of the world around you.

Types of lichens: Lichens are the organisms which grow in diverse condition and at all phytological regions of the world (Boustie and Grube, 2005). Their growth is very slow, they grow few millimetres or centimetres in a year (Table 1).
Types of lichens, differentiated on the basis of size, growth forms and substrate on which they grow.
Lichens are categorized in different types on the basis of their size, substrate in which they grow and on the basis of their growth forms.

Based on size: On the basis of size lichens are of two types: Microlichens and Macrolichens (Awasthi, 1988; 1991; 2007).

- **Microlichens:** These are the lichens which are small in size and are not easily defined because their physical characters cannot be seen easily without microscope by naked eyes.
- **Macrolichens:** These are the lichens which are well defined and can easily observe by naked eyes.

Table.1 Types of lichens

Based on size	1. Macrolichens
	2. Microlichens
Based on growth forms	1. Crustose
	2. Foliose
	3. Fruticose
Based on substrate on which they grow	1. Corticolous
	2. Ramicolous
	3. Legnicolous
	4. Saxicolous
	5. Musicolous
	6. Terricolous
	7. Follicolous

Based on growth forms: Lichens which are grow on bark of the trees are called corticolous, which grows on twigs called ramicolous, which grows on dead wood logs are called legnicolous, which grows on rock and boulders are called saxicolous, grows on moss called musicolous, grows on soil called terricolous and on evergreen leaves called follicolous. They are also growing under water but not only in water or in ice, they always required substrate for growth (Mishra *et al.*, 2016).
Based on substrate on which they grow: On the basis of physical appearance lichens are of three types.

- **Crustose:** These are the lichens in which thallus is closely attached to the substratum without leaving any free margin and it is very difficult to collect it. Most of the times these lichens are collected along with its substrate, in these lichens rihzines and the lower cortex is absent.
- **Foliose:** These are the lichens in which thallus is loosely attached to the substratum leaving free margins, they appears like a leaf and therefore also called leafy lichens.
- **Fruiticose:** These are the lichen in which the thallus is attached only at one point and the rest of the thallus growing hanging and erect like a fruit and therefore they are called fruiticose lichens.

Living spaces of lichens

1. **Epiphytic species**
    - bark dwellers (corticol)
    - branch dwellers (ramikol)
    - leaf dwellers (epifil)
2. **Terrestrial species**
    - rock dwellers (saxicol)
    - soil dwellers (terricol)
3. **Artificial grouping of lichens (External appearance)**
    - Bark-settled lichens: Writer's lichen, Map lichen
    - Leafy lichens: Yellow lichen, Lung lichen, Yellowish lichen
    - Bushy lichens: Bearded lichen, Oak lichen, Oak lichen, Reindeer lichen, Common funnel lichen

Importance of identification of lichens

Lichens has been used for many purposes in India as well as in all over the world because lichens have tremendous properties in them due to the presence of various natural compounds known as primary and secondary metabolites they have varied chemistry and produced many polyketide derived phenolic compounds such as depsides and depsidones which are not known to other group of plants (unique with respect to higher plants), therefore lichens are used for the preparation of natural dyes, crude drugs for medicines, perfumery, agrochemicals (Banerjee, 2002; Kumar and Upreti, 2008), also evaluate air quality, climate change, to detect accumulation of heavy metals. Lichens are the most significant indicators of air pollution and ecosystem health (Upreti and Pandev, 1994; Wolseley *et al.*, 1994; Upreti, 1995; Sloof, 1995; Mistry, 1998; Vokou *et al.*, 1999). Many lichens have economic applications, including uses in traditional medicines (Richardson, 1991; Gonzalez-Tejero *et al.*, 1995; Upreti and Negi, 1996), these properties makes lichens very popular among the scientists for future research, to take the benefit of these properties correct identification of lichens is necessary because these properties are specific for specific species. There are many properties which are not explored yet because of identification, which is very difficult task due to the complex structure of lichens which consumes lot of time. Lichens are very interesting organisms as they contain huge properties and most of them are left untouched. Exploration of these properties is only possible when lichens are identified correctly, properly, easily and on time.

Methods used for identification of lichens

Lichens contain two organisms together (alga and fungi), both the organisms have different species of alga and fungi in different ratio. Lichens are very sensitive to environment and geographical conditions therefore their properties are changed according to the environment in which they found due to this, clear and correct identification of lichens is very difficult, thus the taxonomist used different methods for the identification of lichens, these methods were used from so many years and are modified day by day to get more information or knowledge about lichens.

Three major approaches are used for the identification of lichens, Microscopic approaches are based on physical characters of lichens, Chemical approaches exploit the chemical compounds of lichens to identify them while advanced molecular approaches used to identifying lichens on the basis of their genetic make-up. There are different approaches of identifying the lichens are in Table (2).

Table (2): Methods used for identification of lichens

Microscopic Approaches	Morphology
	Anatomy
Chemical Approaches (chemotyping)	a) Colour spot test
	b) TLC
	c) HPTLC
	d) HPLC
Molecular Approaches	a) PCR genotyping
	b) DNA Barcoding

Relationship between lichens and sulfur dioxide

Since the mid-1800s, we have read studies from several countries that have shown that the disappearance of lichens is primarily caused by the appearance of SO^2. These are the first records in which SO_2 is blamed. There are many reasons why lichens disappear with such contamination. One such reason is that lichens absorb the amount of water and nutrients they need for life from the air and not from the soil. Due to their low chlorophyll content, their ability to recover is limited. Lichens do not have an outer protective layer that would prevent contaminants from entering the plant immediately, so these substances can easily enter all the way to the inside of the plant and accumulate these toxic substances within themselves. This can be solved by other plants by deciduous trees, but this does not occur in lichens, so the parts damaged by the toxic substance or anything else cannot be removed.

In this study, we examined the appearance and distribution of lichens in some areas of Csór village, by comparing the gardens of family houses and wooded areas. Lichens play an important role in nature and in people's lives. Their importance in biomonitoring, environmental protection and nature conservation increases their importance. Therefore, lichens are increasingly used to assess endangered natural habitats. Also, lichens are used for environmental impact assessments and are used to monitor disturbances in nature, especially those that have an exceptionally high and increasing number of chemical pollutants. Lichens are sensitive to air pollution but mostly to SO_2pollution.

Sulfur dioxide is found in the air in cities where industrial activities take place. Fortunately, Csór village is not found in this atmosphere. The settlement of lichens depends on the air quality, which is manifested in the fact that if the air quality improves, the lichens can settle back in that place. Also, the second aim of this study was to know how much of SO_2 in the Csór village atmosphere influences the

appearance of lichens. In addition to the photographs, we determined the different characteristics (location, type, etc.) and properties of the lichens found in the study areas. Morphologically, we wrote a type description of them and described in more detail the relationships between SO_2 and lichens.
Th is paper gives an account on the discovery of the lichen types in Csór village, Hungary. We provide data on population size and types of the Hungarian lichen flora.

MATERIALS AND METHODS

For the studying the distributions of lichens in some areas in the village Csór, Hungary, we used only the morphological approach and it was documented by the pictures of lichens in 4 different areas of Csór: 1) Mátyás Király utca (street), 2) Hadi út (road), 3) Vízmű telep (Waterworks Plant), and 4) Dózsa György köz (interval). We tried to select the areas to include a wooded, green area, a less populated, not very busy area, and parts with more traffic.
The first location was the garden of a family house, where most varieties of fruit trees are found. Pictures were taken for all of them in their entirety and then up close the lichens.
Second location, we examined a small part of a wooded area. Almost everywhere there were one or two or many lichen colonies.
The third location was very similar to the second. As this part of the waterworks site is already a wooded area.
The fourth location, we visited the garden of another family house. There are no fruit trees in the area, only two pieces of ornamental trees. It was the first place we found lichens on the fence. Based on the photos we took, we looked at the type, morphology, colour, diameter, and extent of the lichens to see how many lichens could be seen on each tree. In addition, we looked by area to see which type was the most dominant there.
At the end, we collected the information about the average of the annual data on SO_2 as atmospheric pollutant concentrations (μg/m^3) from the website of the National Environmental Information System (OKIR). Based on the extracted data, we made a diagram of the change in SO_2 concentration broken down by years. We then compared the data with the amount of lichens photographed that were found. Our aim is to examine the values issued by OKIR, whether they are correct, or whether we come to a different conclusion due to the amount of lichens in the areas.

RESULTS AND DISCUSSION

Plant biomonitoring of trace element air pollution, including the use of lichens, often is affected by local variation in specimen concentrations; this variation has been termed relative local uncertainty (RLU). These RLUs consist of variations that arise from biological and microclimatic variability, and from sample handling and analysis procedures (Wolterbeek and Bode, 1995). Relative local uncertainties affect biomonitoring programs because RLUs must be accounted for when comparing results between sample sites. If the differences in the results of the monitoring between the sample sites are not greater than the RLU they should not be considered biologically significant and should not be used to support a conclusion of different levels of air pollution.
Intensified field work and application of new techniques for studying the distribution of lichens in a local region, we obtained the following observations in the Csór village.
Lichen investigation in Csór village Beak
• Csór village is located (Figure 3) in Fejér county, in the Székesfehérvár district.
• Region: Central Transdanubia
• Its location. s. 47 ° 12 ′ 14 ″, k. h. 18 ° 15 ′ 25 ″
• It is located between Székesfehérvár and Várpalota cities. The border of Veszprém and Fejér counties is one kilometers away.
• It is surrounded by the Bakony in the north and Sárré in the south.
• Csór village has special water base and extremely high quality water (karst water).
• A karst spring from a hill next to the waterworks supplies water to Székesfehérvár and the local residents. But there are other springs that are slightly warm water.

Regarding to the distribution and appendence of lichens in the village, our results documented that we collect 58 pictures of different lichen specimens.

Figure (3) The Location of the Csór village

Observations and distributions of lichens in the first location

Figure (4): First examined area: 8041 Csór Mátyás Király utca (street) 23

The observations and collected specimens from the first location are shown in the Figures (5A-5G). In Figure (5), the observation of lichens in part A showed the yellow-leafed lichen and the white-leafed lichen can be found in several places on the tree. Their diameter: 2-5 cm. The most dominant is the yellow-leafed lichen.

Figure (5A) Observations of lichens in first location – part A

In Figure (5), the observation of lichens in part B showed that white and yellow leaf lichens and yellow plate lichens can be observed alternately on the pear tree. Their diameter: 2-6 cm. The most dominant is the white-leafed lichen.

Figure (5B) Observations of lichens in first location – part B

In Figure (5), the observation of lichens in part C showed that the white-leafed lichen and the yellow plate lichen can be observed alternately on the cherry tree. Length: 1-12 cm. The most dominant is the yellow plate lichen.

Figure (5C) Observations of lichens in first location – part C

In Figure (5), the observation of lichens in part D showed that the yellow and white plate of lichens can be found in several places on the walnut tree. In some places they form a whole colony. Diameter: 1-8cm. The most dominant is the yellow plate lichen.

Figure (5D) Observations of lichens in first location – part D

In Figure (5), the observation of lichens in part E showed that the white-leafed lichen is found to a greater extent on the vinegar tree. The surface of one or two branches is completely covered. Length: 1-30 cm. The most dominant is the white-leafed lichen.

Figure (5E) Observations of lichens in first location – part E

In Figure (5), the observation of lichens in part F and part G showed that there are also 3 different lichens on the cherry tree. Yellow plate lichen, white leaf lichen and yellow leaf lichen. Their diameter: 1-5 cm. The most dominant is the yellow-leafed lichen.

Figure (5F) Observations of lichens in first location – part F

Figure (5G) Observations of lichens in first location – part G

Observations and distributions of lichens in the second location

Figure (6) shows the location of the second collection area.

Figure (6): Second examined area: 8041 Csór Hadi út (Road)

It was found that four different species of lichens in the wooded area. They were mostly in a combination between yellow mixed with grey coloured lichens, and in somewhere you can find also slightly white lichens (Figures 7A- 7C). Those were the yellow and grey leaf lichens, are characterized by the yellow plate lichens, and the map lichens, respectively. The diameter of the yellow lichen is 2-5 cm. The most dominant was the yellow and map lichen shaped.

Figure (7A): Collections of the white, yellow and grey lichens on the branches of trees

Figure (7B): Collections of the yellow, yellowish-green and grey lichens on the branches of trees

Figure (7C): Collections of the yellow, yellowish-green and grey lichens on the branches of trees

Observations and distributions of lichens in the third location

Figure (8) shows the location of the third collection area.

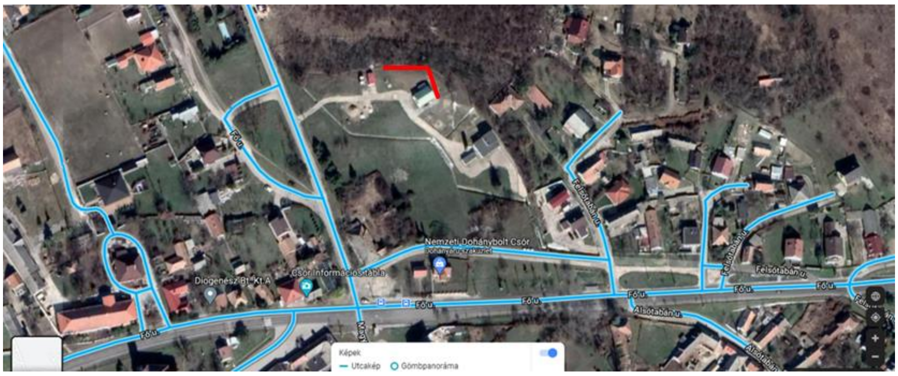

Figure (8): Third examined area: 8041 Csór Waterworks

During the observation and collection of lichen specimens, it was the first place that we found lichens growth on a rock. In addition to the many small yellow and greenish-yellow lichens, we also found yellow plate lichens. These observations can be shown in Figures (9A – 9D).

Figure (9A): Lichens collections. Growth of lichens on the rock surfaces from the third examined area: 8041 Csór Waterworks

It was found that the first tree where it was photographed is related to white map lichen group instead of yellow lichen. The second picture was taken of a broken tree branch. It has yellow and white leafy lichens on it. Their edges are curled (Figure 9B).

Figure (9B): Lichens collections from the third examined area: 8041 Csór Waterworks Plant

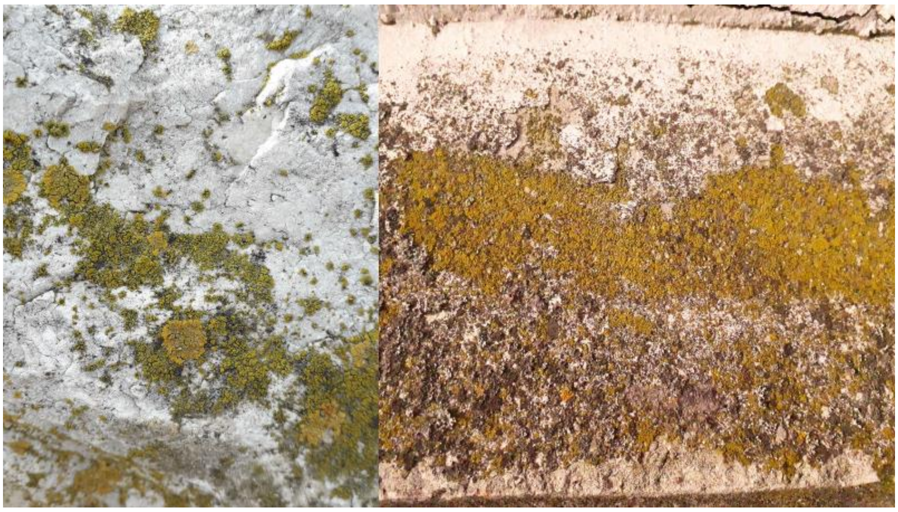

Figure (9C): Lichens collections from the third examined area: 8041 Csór Waterworks

Figure (9D): Lichens collections from the third examined area: 8041 Csór Waterworks

Observations and distributions of lichens in the fourth location

Figure (10) shows the location of the fourth collection area.

Figure (10): Fourth examined area: 8041 Csór Dózsa György köz (interval) 5

On the bench of a tree, many colonisations of lichens can be found, yellow, small grey and white leaf lichens alternated. The observation could be documented by photograph yellow-leafed lichens on the planks of the fence. The most dominant was the yellow-leafed lichen (Figures 11A and 11B)..

Figure (11A): Lichens collections from the fourth examined area

There is only one tree in the fourth study area. The observation showed that the truck of the tree has yellow plate lichen on one side and white and yellow leaf lichen on the other side. The most dominant: yellow plate lichen.

Figure (11B): Lichens collections from the fourth examined area

Figure (11C): Lichens collections from the fourth examined area

Figure (11D): Lichens collections from the fourth examined area

General characterization of leaf, plate, map lichen (Yellow and white leaf lichen)

- Golden-yellow, whitish at the bottom, with erect-edged colonies frillate, lobed, rosette-shaped, broad, flat, wrinkled, almost concave.
- Extremely rich in form.
- Its colonies are mostly circular.
- Found on both bark and calcareous rocks.
- Nitrophilic (nitrogen-loving) along cities.
- Tolerates air pollution well.
- Their batteries lie loosely on the socket and can be easily detached.
- The substrate can be: rock, bark, soil.

Yellowish lichen

- The battery is greenish or straw yellow.
- Older or damaged parts are greyish-green or greenish-black, fragmented.
- The size of the palm-sized, circular, cross-wrinkled, thick colonies of rounded lobes.
- The back is black with a glowing stripe on the edge.
- Lives on deciduous, rarely coniferous trees or mossy rocks.
- Damaged specimens are found in air-contaminated areas.

Map lichen

- Its colonies of greenish-yellow fields are located in a sub-colony.
- Its square, black apothecia (apothecium = cup-shaped fruiting body of some fungi and lichens with concave hoses at the top) show a map-like pattern.
- It forms mostly on silicate rocks.
- The substrate can be: rock, bark, anthropogenic substrate.
- Some species live inside the bark.
- The battery may be cohesive, cracked, granular, warty, lined.

Sulfur dioxide

- SO_2 is a toxic, colourless gas with a typical odour.
- Converts to sulfuric acid on contact with water.
- Main cause of acid rain.
- SO_2 has natural and anthropogenic sources.
- Natural sources include pollution from oceans, forest fires, and volcanoes.
- Anthropogenic sources can be coal and oil burned in power plants and households, but metallurgy, sulfuric acid production and elemental sulfur processing also play an important role.

Physiological effects

- SO_2 is harmful to humans and animals if inhaled.
- When adsorbed on wet airway mucosa, it is irritating due to its acidic pH.
- Once in the bloodstream, it converts hemoglobin to sulfhemoglobin, inhibiting oxygen uptake. In clean air, the blood count is restored.
- Acute irritation of the nose, throat and lungs, may cause coughing, discharge and asthma attacks.
- At free atmospheric concentrations, these do not occur.
- In chronic cases, SO_2 respiratory diseases e.g. can cause bronchitis.

Effects on the ecosystem

- SO_2 forms sulfuric acid, sulfuric acid with the humidity of the air, which damages the living world.
- The main component of acid rain, which damages trees and can destroy entire forests.
- Lichens show the presence of SO_2 as a bioindicator because they do not develop in its presence.
- It can be established that there is no SO_2 in the atmosphere in Csór village (Figure 12). Since 2013 up to 2019, the concentrations of SO_2 in the village were zero, because there is, no industrial works are carried out in the village.
- This is also due to the high quality of the air. This is also due to the ubiquitous lichens. The concentrations of SO_2 in the atmosphere in the Csór village during 2020 and 2021 were not determined.

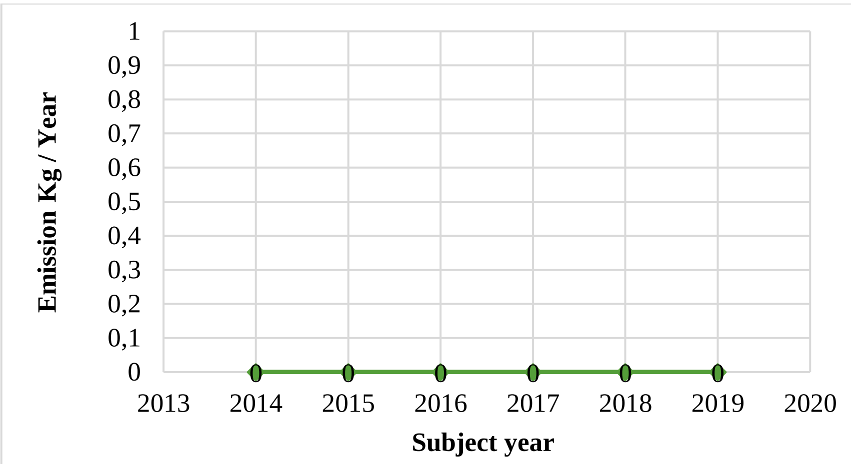

Figure (12): Atmospheric content of SO_2 in Csór village, Hungary

According to recent estimations, lichens comprise about 18 500 species (Boustie and Grube, 2005; Feuerer and Hawksworth, 2007). Since 1983, the name of lichen refers to its mycobiont (Voss et al., 1983). The fungal partners are mostly (98%) Ascomycota (Gilbert, 2000; Honegger, 1991) and the others belong to the Basidiomycota and anamorphic fungi.

Approximately 21% of all fungi are able to act as a mycobiont (Honegger, 1991), thus lichens form the largest mutualistic group among fungi. Only 40 genera are involved as photosynthetic partners in lichen formation: 25 algae and 15 cyanobacteria (Kirk et al., 2008). The photobionts in approximately 98% of lichens are not known at the species level (Honegger, 2001) Lichens are able to survive in extreme environmental conditions; they can adapt to extreme temperatures, drought, inundation, salinity, high concentrations of air pollutants, and nutrient-poor, highly nitrified environments (Nash, 2008), and they are the first colonizers of terrestrial habitats (pioneers). In addition, both fungal and algal cells in the lichen thallus are known for their ability to survive in space (Sancho et al., 2007). Interactions between the symbiotic partners partially explain this spectacular success of lichens in unusual environments (Backo and Fahselt, 2008). Nevertheless, many lichens are very sensitive to various air pollutants, especially nitrogen-, sulfur- and heavy metal-based compounds; therefore they are widely used as bioindicators (e.g., Fernández-Salegui et al., 2007; Glavich and Geiser, 2008; Sheppard et al., 2007). Xanthoparmelia mougeotii is a usnic acid containing, small foliose, sorediate, parmelioid lichen species with a pantemperate distribution. In Europe it occurs in most countries especially in cool, humid, “suboceanic” habitats, mainly on acidic rocks, and rather sporadic in the continental areas, e.g. isolated populations in Slovakia (near Strečno) or in Ukraine (Crimea). Recently another isolated Central European population was discovered in the Zemplen Mts (NE Hungary), approx. 200 km far from the Slovak population (Matus, et al., 2017). Lichens have been used in many areas, are used today and will continue to benefit many in the future. They used to be used for paint making as well: they got the so-called French purple with which silk and wool were dyed red, but the dye was not lightfast. In Egypt, lichens have also been used in the preparation of mummies, probably due to their antibiotic effect. Nowadays, the lichen research observed for surveying nature is becoming more and more widespread. Lichens are used to study global climate change, air quality, and biodiversity. Attention must be paid to its use in the pharmaceutical industry due to its antibiotic and antiseptic effect.

SUMMARY

Pollution of the environment is a very big problem today, although there are instruments that can be used to measure the amount of pollutants, it still does not provide an accurate answer to how the organisms present in the environment react with pollutants. Therefore, bioindication, especially with organisms in cities, will be important in the future, as what could give a clearer picture of the world around us than these living things. At the beginning of the documentation, as we mentioned, lichens also play a bioindicator role. Lichens do not tolerate the SO_2 content of the air if less than the limit value. This urban or industrial pollution is deadly to them, which is why cities with polluted air are often called “lichen deserts”. So where the lichens became extinct, there is SO_2 in the air. For the examinations required for this investigation, authors took pictures of lichens in four different areas of Csór village (Mátyás Király utca ((street), Hadi út (road), Vízmű telep (Waterworks Plant), Dózsa György köz (interval)), and made a total of 58 pictures of the collected lichen specimens. authors tried to select the areas to include a wooded, green area, a less populated, not very busy area, and parts with more traffic. The first location was the garden of a family house, where most varieties of fruit trees are found. Authors photographed all of them in their entirety and then up close the lichens. Second area, authors examined a small portion of a wooded area. Almost everywhere there were one or two or many lichen colonies. In the third location, it was very similar to the second. As this part of the waterworks plant is already a wooded area. The fourth location, we visited the garden of another family house. There are no fruit trees in the area, only two pieces of ornamental trees. It was the first place we found lichens on the fence. Based on the photos we took, authors looked at the type, morphology, colour, diameter, and extent of the lichens to see how many lichens could be seen on each tree. In addition, authors looked by area to see which type was the most dominant there. Authors saw lichens of different shapes and sizes in the four areas examined. Mostly yellow, grey and white leafy lichen; we photographed yellow plate lichen and map lichen. Authors found lichens on tree trunks as well as on broken tree branches. The third and fourth locations are exceptions, where we found the yellow-leafed lichen outside the trunk of the tree on the stones on the side of the building and on the boards of the bench and fence. The sizes of the lichens are different. They were very small and there were also nice adult specimens. Their surface area is very varied. Because the atmosphere of

Csór village does not contain SO_2 in the air, lichens are present everywhere in an undamaged state. In our opinion, the most dominant species in the studied areas was the yellow-leafed lichen.

CONCLUSIONS AND RECOMMENDATIONS

Lichen biomonitoring using in situ specimens for air quality analysis requires sampling controls to minimize within–site variability. By uploading the pictures of the lichen specimens in this application, and comparing with the atmospheric air composition, results almost all the possible and nearest out comes (all about the lichens- photos, their classification and other information). This kind of approach should also be used for lichens to overcome the expense and consumption of time in identification of lichens. All the valuable data of the collected lichen samples can also be stored in the form of digital library and applications such as ―picture this‖ can be created and easily used by a person to get information about any lichen simply by clicking the photo of unknown one. Finally, this one can retrieve the sequence from database for future research.

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TL21.

EMISSIONS OF GREENHOUSE GAS AND AIR POLLUTANT FROM DIFFERENT ECONOMIC SECTORS IN THE EUROPEAN UNION

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Abstract: *The article focuses on influences of economic development on gas emissions and air pollutants effects in EU-28. The study analyses emissions of different kinds of greenhouse gases (CO_2, CH_4, N_2O, HFC, PFC, NF_3_SF_6), acidifying gas (SO_X, NO_X, NH_3), ozone precursors (CO, NMVOC) and particulate matter (PM_{10}, $PM_{2.5}$) resulted by economic activities of different economic sectors including the household during period of 2010-2018. Total emissions of some selected economic sectors plus households issuing greenhouse gas and acidifying gas concentrations significantly respectively reduced by 10.6% and 15.2% in EU-28. In spite that the trends of SO_X and CO emissions have sharply respectively decreased by about 38.4% and 21.1%, but the NF_3_SF_6 has increased by 18.8% for the period of 2010-2018 in EU-28. Also, household activities have respectively increased N_2O and HFC emissions by 5.8% and 6.6% for the same period in EU-28, which emphasizes the importance of the considerable force to decrease gas emissions and air pollutions in order to follow the sustainable economic development accompanying with sustainable environment as well as remaining and protecting the natural environment. The database in this article was selected from the Eurostat online database published in 2020. The paper focuses on analysing the different economic sectors, creating gas emissions and air pollutions. Therefore, the main object of this study is to give an overview about the gas emissions and air pollutions cumulative pressures deriving from growth and change of economic sectors, which influenced on the EU-28 environment. The environmental conservation needs for introducing new technologies and strategies to mitigate the gas emissions and air pollutants in order to avoid danger air emissions causing global warming and air pollution as well as to maintaining and improving the environmental conservation, protection and management.*

Keywords: *Environmental conservation, GDP growth, Global warming, Household, Particulate matter, Sustainable development*

INTRODUCTION

Climate change, biodiversity loss and degradation of ecosystems are interdependent and pose a systemic global threat to human society, including significant societal challenges, threatening economic and social stability, public health and well-being [1]. Climate change and environmental degradation are two of the most serious threats to the European Union and the wider world [2]. Economic development and population growth are the main drivers of increased resource use, environmental damage and degradation. The global/European socio-economic and political context is changing rapidly and has potential implications for the ability of Europe to meet its environmental and sustainability goals [3]. Harmful environmental changes are taking place in an increasingly globalized, industrialized and interconnected world, with a growing world population and unsustainable production and consumption patterns [4].

Since 1950, the world's population has tripled to 7.5 billion; the number of people living in cities has quadrupled to more than 4 billion; economic output has expanded 12-fold, matched by a similar increase in the use of fertilizers (e.g., nitrogen and phosphate fertilizers); and primary energy use has increased 5-fold. These world developments continuously increase threat for the environment. The global population is estimated to increase by almost one third to 10 billion by 2050 [5]. Therefore, for providing a decent life and well-being for mankind by 2050, without further compromising the ecological limits of our Earth planet and its benefits. Also, this is one of the most serious challenges and responsibilities humanity has ever faced [6].

Millions of people in both developing and developed countries prematurely die annually, because of long-term exposure to air pollutants, although air quality measures generally have positive results in many places of the world [7]. Air pollution is one cause of premature deaths from environmental factors in Europe but it also has considerable economic impacts. This increases medical costs and reduces economic productivity due to the ill health of workers. Air pollution also harms soil, crops, forests, lakes and rivers. Pollutants even damage houses, bridges and other built infrastructure [8]. Therefore, there is an urgent need for reducing levels of air pollution globally [7].

In general, climate change is the most important atmospheric issue (4), worldwide, greenhouse gas emissions continue to rise every year. This global challenge requires a global response [9]. Scientific evidence suggests that man-made greenhouse gas emissions, especially carbon dioxide (CO_2) emissions, are a factor contributing to global climate change. This global climate change negatively impacts our Earth planet [10]. Efforts to reduce disaster risk and at the same time adapt to a changing climate have become a global and European priority [11].

Nowadays, the world is confronted by many environmental challenges, e.g., tackling climate change, preserving nature and biodiversity and promoting the sustainable use of natural resources. The inter-relationship between an economy and a society on one hand and their surrounding environment on the other hand is a factor for many of these challenges and underlies the interest in sustainable growth and development, with positive economic, social and environmental outcomes [12].The sustainability includes the environment friendly technology and productivity of industry and agricultural industry in general [13]. Moreover, climate friendly technology became promoted in order to mitigate and avoid danger gas emissions causing climate change and global warming at international level [14]. At European level, the European Union environmental and climate policy landscape aims to address the short-, medium- and long-term time horizons through a range of policies, strategies and instruments that increasingly connect the environmental, social and economic dimensions of sustainable development (Figure 1).

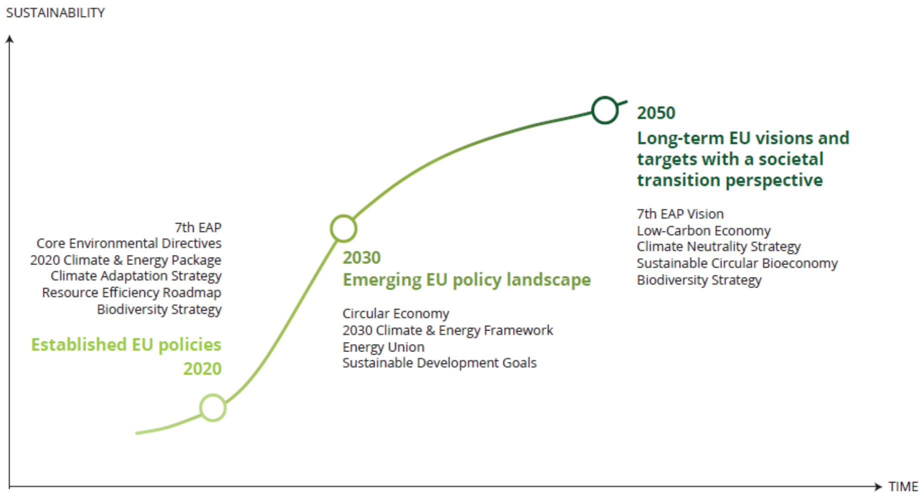

Figure 1: The emerging EU environmental and climate policy landscape

Source: European Environment Agency (EEA). The European environment - state and outlook 2020. Knowledge for transition to a sustainable Europe. EEA, *Luxembourg: Publications Office of the European Union*, 2019, p. 66 [5]. Accessed at: 10.05.2021.

Nevertheless, environmental and climate policymaking is increasingly driven by long-term sustainability goals, as embedded in the European Union's Seventh Environment Action Programme (7th EAP) 2050 vision (i.e. the low-carbon economy, the circular economy and the bioeconomy), the 2030 agenda for sustainable development and the Paris Agreement on climate change [5].

SOME ISSUES IN GAS EMISSION AND AIR POLLUTION: CLIMATE CHANGE EFFECTS

Air pollution is a global threat leading to large impacts on human health and ecosystems [15]. Their impact is reflected in a range of measures from local to regional and global. Also, air pollution is a complex problem that poses multiple challenges in terms of management and mitigation. Sectors such as transport, industry, agriculture and waste management all contribute to air pollution in general [16]. Therefore, human activities and economic performance (e.g., energy consumption, industrial process, power plants, transportation, agricultural activities and waste management) are the main source of gas emission and environmental pollution. Nowadays, changing climate patterns, economic globalization, economic development, population growth, increasing use of natural resources and rapid urbanization are putting pressure on the environment, on ecosystems and biodiversity, as never before [4].
Further, climate change is one of the main global environmental concerns remaining one of the most serious challenges; threats to humanity's future, and mitigation is necessary to avoid or prevent those challenges and threats. The climate is changing globally and in Europe. Land and sea temperatures are increasing; precipitation patterns are changing, generally making wet regions in Europe wetter, particularly in winter, and dry regions drier, particularly in summer; sea ice extent, glacier volume and snow cover are decreasing; sea levels are rising; and, climate-related extremes such as heat waves, heavy precipitation and droughts are increasing in frequency and intensity in many regions [17].
On one hand, air pollution is both an environmental and a social problem, as it leads to a multitude of adverse effects on human health, ecosystems, the built environment etc. [16]. Therefore, air pollutants can affect ecosystem and biodiversity, harm human health and contribute to climate change. Also, many of air pollutants can cause a variety of serious environmental impacts such as nutrient depletion in soils and acidification of water bodies. Here, we can state that, air quality is an important issue for public health, the economy and the environment [18]. On the other hand, climate change will continue for many decades to come, having further impacts on ecosystems and society [17]. Climate change is a global problem that people are faced with in this century. Every country in the world will be affected in some way by climate change [19]. The observed changes in climate are already having wide-ranging impacts on ecosystems, economic sectors and human health and well-being in Europe [17]. So, climate change is one of the largest threats to human and ecosystem health as well as to global environmental changes and to achieving sustainable development; it's exerting multiple and interlinked problems and pressures on the environment that are having adverse impacts on environmental, social and economic sustainability [20].
Furthermore, agriculture has multiple impacts on the environment, climate and human health. Unsustainable farming practices lead to pollution of soil, water, air and food and over-exploitation of natural resources [8]. Agricultural activities remain a major source of pressure on the environment, it is the sector where the need to balance the three dimensions of sustainable development (economy, environment and society) is most evident in general. Also, agriculture and food consumption are identified as one of the most important drivers of environmental pressures and effects, especially habitat change, occupying large land areas, using huge quantities of water, climate change and toxic emissions [21]. Therefore, these effects cause, serious environmental problems related to food production and consumption such as climate change, water pollution, deforestation, land degradation, eutrophication of water bodies, freshwater usage, and loss of habitats and biodiversity [22]. Nevertheless, biodiversity conservation, climate change mitigation and the global shift towards renewable energy sources (bioenergy) are the main crucial environmental challenges in the agriculture sector [23]. On one hand, the main concern for the food and agricultural sector today is to provide enough quantity and quality foods, to meet the needs of population growth in an environmentally, economically and socio-culturally sustainable way [24].
On the other hand, the most important goals of sustainable agriculture integrate economic sustainability and profitability; environmental concerns; and, social acceptability [25]. In addition,

climate change affects agriculture in a number of ways; changes in temperature and precipitation as well as weather and climate extremes are already influencing crop yields and livestock productivity in Europe [26].

From the above mentioned, we can conclude that under increasing numbers of population and economic development, environmental problems, pressures and challenges are becoming more complicated due to excessive use of natural resources. All human activities designed and implemented for the economic activities and economic development of a country and the social needs would have directly or indirectly effect on the environment [27]. In general, European Union environment and climate policies have delivered substantial benefits over recent decades [5]. Therefore, in 2018, greenhouse gas emissions in the European Union decreased by 21% compared with level of 1990, putting the European Union on track to surpass its target for 2020, which is to reduce greenhouse gas emissions by 20% by 2020 and by 40% by 2030 compared with 1990 [28]. Nevertheless, Europe faces persistent problems in areas such as biodiversity loss, resource use, climate change impacts and environmental risks to health and well-being [5], which needs for introducing new policies, technologies and strategies for mitigating the climate change impacts as well as to maintaining the environmental conservation and protection. Finally, since spring 2020 a decrease in many social and economic activities has been for the period of pandemic (COVID-19) led to a decrease in emissions and subsequent levels of certain air pollutants, e.g., the use of vehicles declined during lockdowns and this led to lower nitrogen dioxide (NO_2) concentrations in many cities across Europe [8].

AN OVERVIEW OF EMISSION TRENDS IN EUROPEAN UNION

EEA [29] mentioned that greenhouse gases were emitted through both natural processes and human activities. The primary natural greenhouse gas in the atmosphere is water vapour. However, human activities release large amounts of other greenhouse gases, which increase their atmospheric concentration and enhance the greenhouse effect, thus warming the climate (global warming potential). The main sources of greenhouse gases from human activities, namely burning of fossil fuels (coal, oil and gas) in electricity generation, transport, industry and households (CO_2); agriculture [methane (CH_4) from livestock and manure]; land use change and deforestation (CO_2); land filling of waste (CH_4 from decomposition); combustion of fossil fuels, agricultural, industrial activities [nitrous oxide (N_2O)]; and, use of industrial fluorinated gases (F-gases) [29].

EEA [30] stated that air pollutants are caused by human activities and natural sources; they may be either emitted directly or formed in the atmosphere. They have a number of effects on health, the built environment and the climate [30], Therefore, air pollution damage the environment [acid rain and acidification [nitrogen oxides (NO_X), sulphur oxides (SO_2), ammonia (NH_3)] causes damage mainly to soil, water and forest], cause human diseases (lung and heart disease), and impact on climate (climate change and global warming) [16]. Carbon monoxide (CO) is one of the air pollutants that leads to smog, and in combination with other pollutants and sunshine, it takes part in the formation of ground-level ozone precursors. And also, exposure to CO reduces the capacity of the blood to carry oxygen. In general, some non-methane volatile organic compounds (NMVOC) e.g., benzene and 1.3-butadiene are directly hazardous and harmful to human health, as well as, this pollutant contributes to forming hazard and harmful ground-level ozone precursors. Also, particulate matter (PM_{10} and $PM_{2.5}$) are considered as potential pollutants that cause economic losses and health consequences on society [31].

Based on the above, it can be concluded, human activities, economic development, economic progress and population growth are the main driver of increased resource use and environmental damage. Moreover, the environmental economic analyses became very actual, because of the negative influences of the human activities and the performances of the economies of the world economy, including the EU-28 member states on the nature [32].

This present paper consists of the main points which are as follows: impacts of economic growth on the environment, (e.g., economic activities and economic development); influences of socio-economics on the environmental conservation (e.g., greenhouse gases emission); and, key indicators of environmental quality (e.g., air quality and climate change) in European Union member states. In this study the differences among different air emissions (gas emissions and air pollutants) have been overviews based on their economic sectors and economic branches for the period of 2010-2018 [33] in

EU-28. In addition, the data relevant to EU-28 in fields of gas emission and air pollution (greenhouse gases, acidifying gas, ozone precursors and particulate matter) resulted by some selected economic sectors or economic branches including the household activities as outlined in Table (1) [33].
The main economic sectors or economic branches (industries/economic activities) used in this study, which are follows namely Agriculture, forestry and fishing; Fishing and aquaculture; Mining and quarrying; Manufacturing; Manufacture of food products (beverages and tobacco products); Manufacture of textiles, wearing apparel, leather and related products; Manufacture of paper and paper products; Manufacture of chemicals and chemical products; Manufacture of basic pharmaceutical products and pharmaceutical preparations; Manufacture of coke and refined petroleum products; Manufacture of basic metals; Manufacture of rubber and plastic products; Manufacture of other non-metallic mineral products; Manufacture of computer, electronic and optical products; Manufacture of electrical equipment; Manufacture of motor vehicles, trailers and semi-trailers; Manufacture of furniture (other manufacturing); Electricity, gas, steam and air conditioning supply; Water collection, treatment and supply; Sewerage, waste management, remediation activities; Construction; Wholesale and retail trade (repair of motor vehicles and motorcycles); Transportation and storage; Air transport; Accommodation and food service activities; Information and communication; Telecommunications; Computer programming, consultancy, and information service activities; Publishing activities; Financial and insurance activities; Activities auxiliary to financial services and insurance activities; Real estate activities; Professional, scientific and technical activities; Advertising and market research; Architectural and engineering activities (technical testing and analysis); Administrative and support service activities; Employment activities; Public administration and defence (compulsory social security); Education; Human health and social work activities; Arts, entertainment and recreation; Other personal service activities; Activities of membership organisations; Other service activities; and, Households activities (Table 1). Different types of air emissions (gas emissions and air pollutants) used in this study, which are as the followings (Table 1) [33]:

GREENHOUSE GASES

GHG: Greenhouse gases (CO_2, N_2O in CO_2 equivalent, CH_4 in CO_2 equivalent, HFC in CO_2 equivalent, PFC in CO_2 equivalent, SF_6 in CO_2 equivalent, NF_3 in CO_2 equivalent), Air emissions accounts by NACE Rev. 2 activity [NACE Rev. 2 (NACE_R2) activity = European Classification of Economic Activities]; NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2018, in Percentage (%).
CO_2: Carbon dioxide, NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2018, in %.
CH_4: Methane, NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2018, in %.
N_2O: Nitrous oxide, NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2018, in %.
HFC: Hydrofluorocarbones (CO_2 equivalent), NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2018, in %.
PFC: Perfluorocarbones (CO_2 equivalent), NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2018, in %.
NF_3_SF_6: Nitrogen trifluoride and sulphur hexafluoride (CO_2 equivalent), NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2018, in %.

ACIDIFYING GASES

ACG: Acidifying gases [SO_X in Sulphur dioxide (SO_2) equivalent, NO_X in SO_2 equivalent, NH_3 in SO_2 equivalent], NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2017, in %.
SO_X: Sulphur oxides (SO_2 equivalent), NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2017, in %.
NO_X: Nitrogen oxides, NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2017, in %.

NH_3: Ammonia, NACE_R2: different economic activities, UNIT: Tonne, 2010=100, 2010-2017, in %.

OZONE PRECURSORS

CO: Carbon monoxide, NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2017, in %.

NMVOC: Non-methane volatile organic compounds, NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2017, in %.

PARTICULATE MATTERS

PM_{10}: Particulate matters with a diameter of 10μm or less PM_{10} (Particulates < 10μm), NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2017, in %.

$PM_{2.5}$: Particulate matters with a diameter of 2.5μm or less (Particulates < 2.5μm), NACE_R2: different economic activities, UNIT: Tonne, 2010 = 100, 2010-2017, in %.

Table (1) shows greenhouse gas concentrations were significantly reduced in EU-28, the decreasing trend of the total greenhouse gas emissions reached 10.7%, except in case of NF_3_SF_6 concentrations were increased by 22.3% for the period of 2010-2018. Meanwhile, all acidifying gas concentrations were reduced in EU-28, except in case of NH_3 concentrations were increased about 3%, the extent of the reductions varied considerably within cities and countries, however reductions exceeding 15% were observed in EU-28 (Table 1). Also, in case of ozone precursors; CO and NMVOC concentrations were reduced by 17.8% and 8.9% respectively. As well as, in case of particulate matter; PM_{10} and $PM_{2.5}$ concentrations were also decreased in EU-28, it reached up to 12.9% and 15.3% respectively. While, in case of total emissions plus households issuing greenhouse gas and acidifying gas concentrations significantly respectively reduced by 10.6% and 15.2% in EU-28. In spite that the trends of SO_X and CO emissions have sharply respectively decreased by about 38.4% and 21.1%, but the NH_3 and NF_3_SF_6 have increased by 2.4% and 18.8% respectively for the period of 2010-2018 in EU-28. In particular, greenhouse gases, acidifying gases, ozone precursors and particulate matter concentrations were significantly reduced with considerable share of the households, except in cases of N_2O and HFC concentrations were slightly increased by 5.8% and 6.6% respectively for the period of 2010-2018 (Table 1). In general, sectors such as manufacture of basic pharmaceutical products and pharmaceutical preparations; construction; transportation and storage in general; and, air transport in particular; and, employment activities have implemented considerable gas emissions and air pollutions for the period 2010-2018 as listed in Table (1), therefore, such economic activities, needs for introducing more technologies and strategies for mitigating the gas emissions in order to avoid danger gas emissions causing global warming and air pollution as well as to maintaining and improving the environmental conservation, protection and management.

Moreover, there has been a progressive decoupling of gross domestic product (GDP) and greenhouse gas emissions since 1990, with an increase in GDP of about 50% alongside a decrease in emissions of almost 24% over a 25 year period (1990-2015) [34], while, in the present study a decrease in greenhouse gas emissions by 10,7% over a 8 year period (2010-2018), these decreases were a combined result of policies, economic factors and climatic conditions in general [34]. Also, these decreases were due to growing shares of renewable, less carbon intensive fuels, improvements in energy efficiency, significant reductions in the residential sector and the recession of 2008-2009 [35, 20]. Generally, almost all European Union Member States reduced their emissions compared with 1990 and thus contributed to the overall positive European Union performance [34]. Also, over the past 50 years, the level of environmental protection in most parts of Europe has improved measurably. Emissions of specific pollutants to the air, water and soil have generally been reduced significantly. These improvements are to a substantial degree a result of the comprehensive environmental legislation established across Europe, and they are delivering a range of direct environmental, economic and societal benefits, as well as more indirect ones [36].

Table 1: Emission of Greenhouse gases (GHG); Carbon dioxide (CO_2); Methane (CH_4); Nitrous oxide (N_2O); Hydrofluorocarbones (HFC); Perfluorocarbones (PFC); Nitrogen trifluoride and sulphur hexafluoride (NF_3_SF_6); Acidifying gases (ACG); Sulphur oxides (SO_X); Nitrogen oxides (NO_X); Ammonia (NH_3); Carbon monoxide (CO); Non-methane volatile organic compounds (NMVOC); and, Particulate matter (PM_{10} and $PM_{2.5}$) by some selected sectors (economic activities - NACE rev.2/industries/economic branches) in EU-28 between 2010-2018 [Tonne], 2010 = 100%

Sectors (economic activities/industries/economic branches)	GHG	CO_2	CH_4	N_2O	HFC	PFC	NF_3_SF_6
Agriculture, forestry and fishing	1.5	-0.8	0.6	3.9	9.2	351.3	-34.1
Fishing and aquaculture	2.9	3.0	12.9	16.0	4.0	-82.8	-36.0
Mining and quarrying	-16.0	-14.9	-18.0	11.0	13.6	135.2	-59.8
Manufacturing	-10.0	-9.3	-7.5	-46.0	-7.0	-1.0	-31.3
Manufacture of food products; beverages and tobacco products	-2.3	-3.0	13.0	18.0	1.2	-79.0	2.8
Manufacture of textiles, wearing apparel, leather and related products	-16.6	-15.8	-23.8	-24.5	-33.8	-30.2	-11.4
Manufacture of paper and paper products	-13.2	-12.7	-2.1	7.9	-65.3	56.9	46.0
Manufacture of chemicals and chemical products	-7.9	-2.5	-2.6	-55.0	-29.0	22.3	13.9
Manufacture of basic pharmaceutical products and pharmaceutical preparations	15.6	19.0	4.0	-11.5	2.2	398.0	30.0
Manufacture of coke and refined petroleum products	-13.0	-12.8	-21.0	-20.0	-52.5	22.4	12.0
Manufacture of basic metals	-15.0	-14.0	-15.0	-28.0	-64.0	-46.7	-42.6
Manufacture of rubber and plastic products	-7.3	-10.0	-37.6	-73.2	27.0	27.1	-7.9
Manufacture of other non-metallic mineral products	-9.0	-8.3	5.2	-20.8	-40.9	-67.8	-86.5
Manufacture of computer, electronic and optical products	11.0	10.8	-18.5	-11.4	42.3	12.6	-28.0
Manufacture of electrical equipment	4.9	-15.0	-23.1	-9.0	35.0	-49.0	-35.9
Manufacture of motor vehicles, trailers and semi-trailers	-8.0	-4.5	-52.0	12.0	-42.8	-47.6	63.4
Manufacture of furniture; other manufacturing	-6.7	-8.2	-2.3	-7.0	34.0	-50.4	-21.7
Electricity, gas, steam and air conditioning supply	-22.4	-23.0	-13.0	-15.8	-26.9	-69.0	-6.0
Water collection, treatment and supply	-3.0	-6.0	-2.5	2.9	37.1	-60.4	74.5
Sewerage, waste management, remediation activities	-14.0	-0.4	-22.0	-1.3	84.9	-87.0	104.2
Construction	4.2	3.0	-2.1	24.0	21.3	-90.0	-8.4
Wholesale and retail trade; repair of motor vehicles and motorcycles	-11.0	-10.5	-27.4	18.0	-14.0	-75.9	-29.0
Transportation and storage	5.4	5.2	12.6	20.2	3.5	-84.6	207.7
Air transport	13.5	13.6	2.1	7.8	-4.7	-55.9	-13.4
Accommodation and food service activities	-15.2	-16.0	-34.5	-7.1	-10.0	-77.0	-39.9
Information and communication	-8.8	-10.2	-26.0	5.0	18.0	-17.4	35.0
Telecommunications	-23.6	-25.0	-68.9	-5.9	2.9	-15.0	12.5
Computer programming, consultancy, and information service activities	8.0	5.8	24.7	24.1	39.9	7.6	52.4
Publishing activities	-16.1	-16.4	-34.9	-4.8	-8.0	-51.2	36.9
Financial and insurance activities	5.3	4.5	-17.0	26.3	25.9	-35.0	-2.4
Activities auxiliary to financial services and insurance activities	-9.0	-12.2	-33.6	-9.1	36.0	-14.9	-25.6
Real estate activities	-21.0	-22.0	-10.0	-9.5	9.6	-28.6	-49.0
Professional, scientific and technical activities	-19.0	-21.3	-32.0	-12.1	23.0	102.2	15.1
Advertising and market research	-27.1	-28.5	-27.6	-16.0	4.9	-24.5	7.3
Architectural and engineering activities; technical testing and analysis	-15.6	-17.2	-34.0	-5.8	17.0	93.7	25.0
Administrative and support service activities	6.9	5.9	-39.9	17.4	36.1	-10.2	-64.6
Employment activities	14.2	10.6	-2.7	7.2	69.9	46.0	-78.4
Public administration and defence; compulsory social security	-19.0	-20.0	-14.3	-24.0	16.0	-38.0	3.9
Education	-17.0	-18.1	-13.6	-6.0	33.0	-17.9	31.3
Human health and social work activities	-2.6	-2.3	-23.4	-15.8	21.5	92.0	1.0
Arts, entertainment and recreation	-11.4	-12.0	-25.0	-12.0	19.0	-36.0	-29.0
Other personal service activities	-8.6	-9.1	-38.0	-3.3	13.8	-18.0	-32.0
Activities of membership organisations	-17.9	-18.7	-8.6	-13.1	10.0	2.8	-19.3
Other service activities	-10.7	-13.0	-25.0	-7.0	14.0	-94.0	-28.7
Total - all NACE activities	*-10.7*	*-12.0*	*-8.7*	*-1.4*	*-2.5*	*-5.2*	*22.3*
Households activities	-9.9	-10.4	-11.5	5.8	6.6	-35.2	-31.3
All NACE activities plus households	*-10.6*	*-11.6*	*-8.8*	*-1.1*	*-0.8*	*-5.3*	*18.8*

Source: Own calculations based on Eurostat [33].
Eurostat. Database by themes – Environment (env) – Emissions of greenhouse gases and air pollutants (env_air) – Air emissions accounts (env_air_aa) – *Air emissions accounts by NACE Rev. 2 activity* (env_ac_ainah_r2). *Eurostat* [Database Online], Luxembourg, 2020. Last update: 24.02.2020 [33]. Available from: https://ec.europa.eu/eurostat [Accessed at: 21.10.2020].

ntinued of Table (1)

Sectors (economic activities/industries/economic branches)	ACG	SO_X	NO_X	NH_3	CO	NMVOC	PM_{10}	$PM_{2.5}$
Agriculture, forestry and fishing	1.7	-27.6	-5.2	3.1	-6.4	1.0	-3.6	-11.2
Fishing and aquaculture	1.2	-31.9	4.1	-60.3	-9.0	-1.4	-2.9	-1.8
Mining and quarrying	-24.0	-50.0	-13.0	6.5	8.3	-19.0	-16.0	-25.0
Manufacturing	-22.9	-29.4	-20.5	12.3	-20.0	-11.0	-4.6	-4.3
Manufacture of food products; beverages and tobacco products	-14.0	-17.9	-13.0	-2.1	-4.0	12.9	6.9	2.0
Manufacture of textiles, wearing apparel, leather and related products	-38.2	-66.5	-19.8	-9.6	-27.9	-13.6	-27.0	-25.6
Manufacture of paper and paper products	-1.7	-4.9	-2.5	17.0	4.6	-6.0	5.6	7.0
Manufacture of chemicals and chemical products	-14.0	-25.0	-23.3	16.7	-8.0	-10.3	-0.2	-1.0
Manufacture of basic pharmaceutical products and pharmaceutical preparations	1.0	-19.0	7.0	35.9	2.0	-11.0	3.7	4.0
Manufacture of coke and refined petroleum products	-38.0	-44.2	-20.0	-1.0	-18.0	-23.0	-20.1	-16.2
Manufacture of basic metals	-12.9	-12.0	-14.9	-18.4	-21.5	-10.7	-11.4	-10.0
Manufacture of rubber and plastic products	-19.5	-33.4	-6.5	37.0	-19.0	-3.2	-17.0	-18.0
Manufacture of other non-metallic mineral products	-29.0	-29.7	-29.8	-5.1	-22.5	-2.3	-10.2	-11.0
Manufacture of computer, electronic and optical products	-12.2	-43.0	-8.4	10.0	-12.0	-26.0	-9.7	-13.0
Manufacture of electrical equipment	-31.0	-54.9	-26.7	8.1	-37.1	-20.0	-21.0	-22.4
Manufacture of motor vehicles, trailers and semi-trailers	-28.0	-27.1	-29.3	35.0	2.9	-13.8	-13.0	-16.9
Manufacture of furniture; other manufacturing	-15.6	-33.7	-15.0	9.9	-22.0	-24.3	-0.4	-0.3
Electricity, gas, steam and air conditioning supply	-51.0	-60.0	-33.8	12.0	2.7	-22.0	-43.8	-34.8
Water collection, treatment and supply	-17.4	-15.8	-14.0	-66.8	-26.0	-19.6	-27.0	-30.1
Sewerage, waste management, remediation activities	-16.9	3.5	-26.2	-10.6	-23.0	-12.3	-4.9	-9.5
Construction	-24.6	-48.0	-23.6	-13.9	8.0	-14.0	-18.5	-23.0
Wholesale and retail trade; repair of motor vehicles and motorcycles	-25.0	-30.0	-25.0	-23.0	-43.0	-16.0	-22.0	-29.0
Transportation and storage	-12.0	-17.0	-9.7	-5.0	-24.0	-15.8	-14.9	-16.7
Air transport	21.5	27.9	21.0	10.0	-2.9	3.0	9.4	9.9
Accommodation and food service activities	-14.7	-23.4	-11.9	-34.2	-33.7	-23.2	-12.5	-15.5
Information and communication	-27.5	-28.9	-27.0	-29.0	-43.3	-37.0	-21.9	-27.2
Telecommunications	-36.2	-19.5	-37.2	-45.0	-51.0	-34.7	-33.5	-38.5
Computer programming, consultancy, and information service activities	-11.0	5.2	-12.4	-18.0	-35.0	-18.3	-3.7	-9.2
Publishing activities	-35.0	-45.6	-34.1	-36.0	-51.2	-57.0	-40.5	-44.0
Financial and insurance activities	1.8	-25.0	4.5	-29.0	-32.9	-26.0	-3.8	-9.6
Activities auxiliary to financial services and insurance activities	-21.5	5.5	-25.0	-26.6	-42.0	-31.3	-19.0	-25.5
Real estate activities	-26.0	-46.2	-23.5	-28.1	-30.9	-10.9	-22.3	-26.1
Professional, scientific and technical activities	-32.0	-6.0	-34.1	-19.0	-37.0	-29.0	-36.0	-41.7
Advertising and market research	-37.8	-32.9	-38.4	-28.3	-39.0	-34.4	-42.7	-46.0
Architectural and engineering activities; technical testing and analysis	-31.0	-34.0	-30.6	-21.8	-37.1	-30.1	-38.0	-44.5
Administrative and support service activities	0.1	7.2	0.2	-11.0	-26.7	-23.0	-22.0	-30.0
Employment activities	3.9	40.0	-1.4	7.6	-7.4	8.2	3.9	2.9
Public administration and defence; compulsory social security	-36.5	-57.2	-33.2	-16.0	-39.9	-22.0	-33.0	-39.4
Education	-25.1	-38.0	-20.0	-19.0	-42.6	-27.1	-31.9	-33.0
Human health and social work activities	-3.9	-21.6	1.0	7.4	-15.0	-14.0	-2.7	-7.8
Arts, entertainment and recreation	-24.6	-23.0	-30.0	-5.0	-26.9	-29.7	-25.0	-30.0
Other personal service activities	-19.0	-11.0	-19.9	-20.0	-24.0	-2.2	-11.7	-11.8
Activities of membership organisations	-36.0	-26.3	-37.6	-18.7	-21.9	-23.0	-4.0	-4.3
Other service activities	-26.2	-16.9	-27.0	-20.5	-24.3	-4.9	-8.8	-9.0
Total - all NACE activities	*-15.4*	*-39.7*	*-16.0*	*3.0*	*-17.8*	*-8.9*	*-12.9*	*-15.3*
Households activities	-13.5	-18.5	-13.2	-9.7	-23.4	-19.0	-15.5	-15.7
All NACE activities plus households	*-15.2*	*-38.4*	*-15.6*	*2.4*	*-21.1*	*-12.4*	*-14.0*	*-15.5*

Source: Own calculations based on Eurostat [33].
Eurostat. Database by themes – Environment (env) – Emissions of greenhouse gases and air pollutants (env_air) – Air emissions accounts (env_air_aa) – *Air emissions accounts by NACE Rev. 2 activity* (env_ac_ainah_r2). *Eurostat* [Database Online], Luxembourg, 2020. Last update: 24.02.2020 [33]. Available from: https://ec.europa.eu/eurostat [Accessed at: 21.10.2020].

Since the 1970s, a broad range of environment legislation has been put in place. This now amounts to the most comprehensive modern set of standards in the world [36]. Therefore, the European Union has acted at a number of levels to reduce exposure to air pollution and harmful emissions in order to improve air quality and mitigate the gas emissions in Europe, through the European Union's legislation and directives, through regulations and decisions, through policies and strategies, through cooperation with sectors and organisations responsible for air pollution, through authorities and non-government organisations at national, regional and international level [37, 38]. European Union environmental policies address a range of environmental and resource use challenges, such as climate change and energy use, air pollution and transport, biodiversity and land use, waters, waste, and sustainable consumption and production [39].

Cutting air pollution and reducing greenhouse gas emissions almost always go hand in hand. Curbing water and soil pollution would benefit nature. Reducing resource use and moving to a circular economy would also reduce pollution [8].

Further, the European Union environment action programme - referred to as the 7th EAP - was adopted by Decision 1386/2013 of the European Parliament and Council in November 2013 [40] under the title 'Living well, within the limits of our planet'; it guides the European Union's environment policy up to 2020. The programme draws on a number of strategic initiatives, including the resource efficiency roadmap, the biodiversity strategy and the low carbon economy roadmap [41].

Furthermore, in 2014, the European Council agreed on the 2030 climate and energy policy framework for the European Union and endorsed new targets on greenhouse gas emissions, renewable energy and energy efficiency for 2030. In 2015, the European Union adopted an Energy Union Strategy to ensure that Europe has secure, affordable and climate-friendly energy and achieve its climate and energy goals for 2030 [35]. The European Union was the first major economy to submit its Intended Nationally Determined Contribution (INDC) to the Paris negotiations in March 2015: a binding, economy-wide domestic emissions reduction target of at least 40% by 2030 compared with level of 1990 [42]. Also, the European Union has articulated a long-term goal for 2050 of reducing Europe's greenhouse gas emissions by 80% to 95% compared with level of 1990 [35]. More recently, in 2020 the European Commission presented a proposal to raise the European Union's ambition to reducing net greenhouse gas emissions by at least 55% below 1990 levels by 2030, as well as to set Europe on a responsible path to becoming climate neutral by 2050 [43, 44].

In general, European air quality policy has achieved considerable successes in the past in reducing air pollution [18]. Nevertheless, an effective action to reduce the impacts of air pollution requires a good understanding of the sources that cause it, as well as up-to-date knowledge of air quality status and its impact on humans and on ecosystems [16]. Overall, building on its climate change mitigation policies and greenhouse gas emissions reductions over recent decades, the European Union aims to become the world's first climate-neutral economy [45]. Moreover, under the European Green Deal, Europe's ambition to become the first climate-neutral continent will need to be carried out by all sectors of the economy, as well as by European Union bodies and institutions [46]. So, on one hand, achieving climate neutrality will help Europe contribute to reach the Paris Agreement objectives. On the other hand, a new long-term strategic vision for 2050, which the European Commission set out in November 2018 [47], provides a range of challenging but feasible pathways for the transition towards climate neutrality. These pathways would all necessitate shifts in many areas of society, not least towards the intensified generation of low- to zero- carbon-based energy, also considerable savings in energy consumed [45].

Ultimately, as earlier mentioned, climate change and the loss of biodiversity and associated ecosystem services pose a systemic global threat to human society [1]. Therefore, climate change represents one of the biggest challenges for mankind in the 21st century and taking action to address it is one of the top priorities for the European Union [48], and also, mitigation is necessary to avoid those challenges and threats.

However, the European Union's environment and climate policies aim to protect the environment and minimize risks to climate, human health and biodiversity. This is achieved both by mainstreaming sustainability considerations into broader economic and social initiatives and with specific policies for the environment and climate change [28].

CONCLUSIONS

This present study overviews some selected economic activities and air emissions status putting pressure on environment in European Union member states during the period of 2010-2018. In general, climate change impacts are projected to increase in future years, which may result in major environmental changes as well as economic and social difficulties. The scientists mostly agree that further increase in the emission of harmful greenhouse gases will result in global warming and would cause more damage than ever before in the climate system and that is a problem we need to solve.

For recent decades, changes in climate have caused impacts on human and natural systems, such as human diseases and environmental problems. Therefore, an effective response and action to the climate change problem at global level requires both a concerted international response and national efforts to reduce greenhouse gas emissions and air pollutants effects. Nevertheless, the European Union has decreased emissions by 10.70% between 2010 and 2018 and thus contributed to the overall positive performance of European Union. Also, the European Union has a range of policies to reduce emissions, promote clean energy and energy efficiency, and stimulate Europe’s transition to a low-carbon economy.

Finally, climate change can result negative processes for human society, ecosystems and the natural environmental. The global warming and air pollution will lead to narrower the natural life places, highly impact on climate through the climatic changes, and also, reduced the environmental quality and human health especially by increasing the respiratory diseases. Therefore, reaching climate stabilization of the world economy must be totally transformed in a climate friendly and sustainable manner, as well as the policy makers and decision makers should change the technological and strategical development based on the environmentally friendly way and stimulates innovation in clean technologies such as renewable sustainable energy resources and energy efficiency in order to mitigate air emissions, improve the air quality and promoting sustainable economic growth. Also, people and nations should even more concerned with environmental issues and conservations, and act in environmentally friendly ways.

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TL22.

POSSIBILITIES TO ENSURE SUSTAINABILITY AND A GREEN STRATEGY IN HUNGARIAN ROAD TRANSPORT

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Abstract: *Greening transport plays a key role in fulfilling climate goals and carbon neutrality. The sector is responsible for one fifth of greenhouse gas emissions in Hungary. Electric transport creates the possibility of zero emissions, so the Green Strategy can be implemented by supporting the spread of electric cars and implementing the Green Bus program. Our goal is to realize climate strategy, green transport, sustainability by taking into account the financial possibilities. Financial support is needed to put transport on less polluting fundaments. It can involve introducing a new type of tax or supplementing existing taxes. Based on the polluter pays principle, this can be mileage or consumption based. At the same time, the increase in fuel costs for heavy duty vehicles reduces competitiveness, so compensation is needed for the sustainable operation of hauliers and delivery companies.*

Keywords*: Ensure Sustainability, Green Strategy, Hungarian, Road Transport*

INTRODUCTION

The main objective of Green Strategy is to create the conditions for a sustainable growth model, which will enable Hungary to become a climate-neutral country by 2050 without the burden of transition being mainly being caused by top polluting countries. We must work together with the world's biggest polluters to tackle the cross-sectorial effects of climate change, and our long-term task is to reduce greenhouse gas and pollutant emissions while maintaining the competitiveness of the economy. Accomplishing this goal requires all emitter sectors to adapt to changing circumstances. Climate protection goals can only be reached together. 98% of transport's greenhouse gas emissions come from the road sub-sector; hence this field already requires well defined measures for today.
Electromobility and other alternative technologies are expected to become more prominent in the next decade, which could underpin the decarbonisation of road transport in the long run. Greening urban transport will play a significant role in the Green Strategy. The decarbonisation process requires the involvement of all stakeholders, from automobile industry to industrial pollutants. [1, 2] Transport contributes to air pollution in several ways. Vehicles emit harmful substances that endanger health (organic compounds, CO, NO_2, SO_2 and the PM and ozone formed from them), as well as greenhouse gases (CO_2) being not harmful, but causing climate change. Vehicle emissions cause direct health problems in areas with heavy traffic and spread from there to areas not affected by industry and transport, while increasing concentrations of greenhouse gases are leading to global warming.

The multi-directional pollution effect can be mitigated globally by reducing road transport emissions. Achieving net zero emissions is most important for Hungary, situated in the Carpathian Basin because atmospheric pollutants are difficult to clean from the atmosphere. . Rainy-windy weather would be needed for natural purification, however, climate change, on the other hand, is bringing a decrease in the amount of precipitation to Hungary. These factors lead to the need for Hungary keeping water within the country and attaining the lowest possible pollutant concentration are the two main goals.

CLIMATE STRATEGY AND ENVIRONMENTAL PROTECTION

The biggest challenge for transport in the 21st century is to meet the increased demand for mobility while minimizing its damage to the environment. Among the environmental impacts of transport, we can find harmfulness for health, the effects on climate change, traffic noise, accidents and the consequences of traffic jams. A large-scale increase in transport demand and the alignment of the climate strategy are possible with measures in

- meeting the requirements of sustainable development,
- using technical developments,
- increasing the share of environment-friandly transport sub-sectors and
- by advantageous combination of different modes of transport and
- by carrying out planned and conscious environmental management activities.

Green, environmental-friendly, sustainable operation can also increase efficiency with lower specific energy consumption or less material consumption.

SUSTAINABILITY

The Brundtland Commission has developed the most widely used concept of sustainable development: “Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs” (Brundtland World Commission on Environment and Development, 1987). The direction of decisions has shifted over time from the primary emphasis on environmental issues to a common focus on environmental responsibility, social awareness and economic profitability in development.

Based on the above factors, a transport project can be considered "sustainable" if:

(1) contributes to economic development,
(2) meets the transport needs of society,
(3) is in accordance with natural laws and human values.

In addition, it is necessary to take into account:

(4) the degree of sustainability is sensitive to the economic, social and environmental conditions of the project site,
(5) the appropriate definition of "sustainable transport projects" for the "long-term approach" should cover the whole life cycle, from concept to operation, maintenance and the recycling / reuse phase.

Sustainability is worth considering at the planning stage, as at the evaluation and decision-making point of projects:

- decision-makers have a major influence on the future sustainability and environmental performance of the project,
- implementation of the principles of sustainability and climate strategy becomes more effective at the planning stage than as part of the ex-post evaluation,
- integration of sustainability objectives into the decision-making process can support the most cost-effective outcome for all criteria considered,
- after identifying project impacts, the multi-criteria approach can be used, for example, to weight the strength of impacts,
- ranking of alternatives helps to select the most sustainable project proposal.

IMPACT OF ROAD TRANSPORT POLLUTANTS

Road transport is partly responsible for urban air pollution, which endangers the health of many people. Higher concentrations of harmful substances increase the risk of cardiovascular and respiratory diseases, cancer and adverse birth events (low birth weight, preterm birth) and also result higher mortality rates with reducing the number of healthy life years. Pollutants emitted by vehicles, according to WHO (World Health Organization) estimates, claimed the early deaths of 4.2 million people worldwide in 2016.

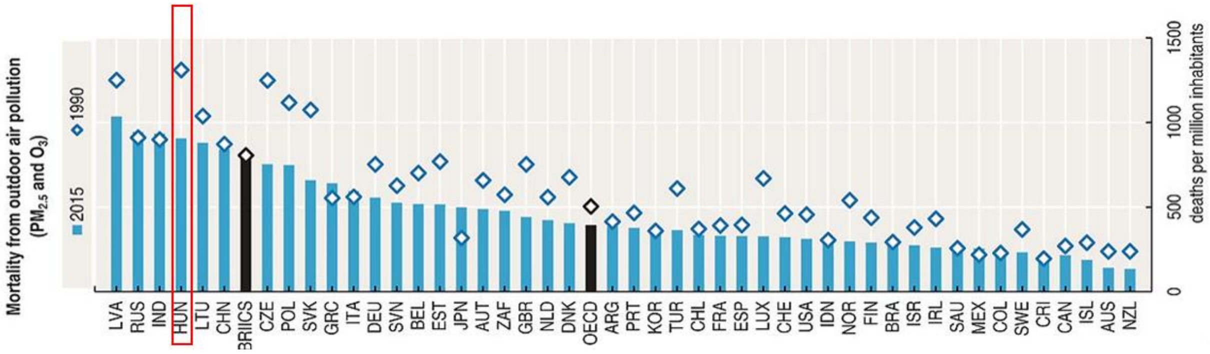

Figure (1): Deaths due to air pollution per 1 million people in OECD countries, plus China and India in 1990 and 2015 [3]

The harmful effects of air pollution on health in the Carpathian Basin are exacerbated; therefore it is necessary to reduce emissions as much as possible, involving all sources, including road transport. Due to the topography of Hungary, the purification of air pollutants is inhibited and photochemical reaction takes place to result the formation of $PM_{2.5}$ and ground-level ozone (O_3). Their starting materials are NOx and SO_2. Emission can be cut down directly by efforts to decrease the concentration of harmful substances, but it can also be aimed by supporting the purchase of vehicles with battery- or hydrogen-based electric propulsion.

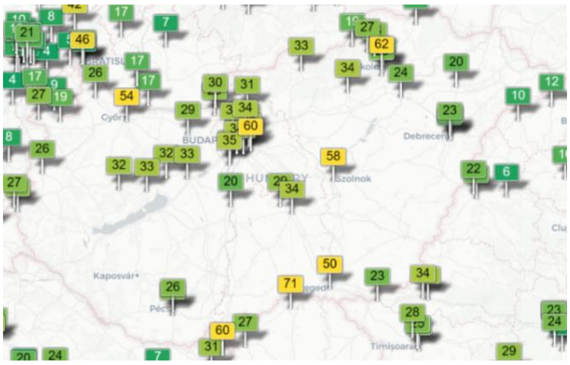

Figure (2): Air pollution in Hungary: A visual map of the real-time air quality index (13/03/2020) [4]

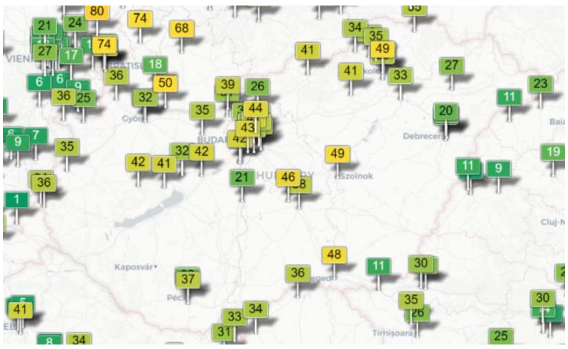

Figure (3): Air pollution in Hungary: A visual map of the real-time air quality index (30/04/2020) [5]

Figures show that the air quality index has fallen from 60 to a low-risk value below 50 (adequate level) in Budapest during the one and a half months of traffic stoppages, but has risen from 30 to 40 in rural areas. There was neither a sufficient amount of rain nor windy weather during this period of time. Concentrations of NO_2 from transport emissions were decreased by 19% during lockdown, but concentrations of PM_{10} increased by 3% over the same period. The source of PM_{10} is mainly residential heating; a small amount comes from transport only.

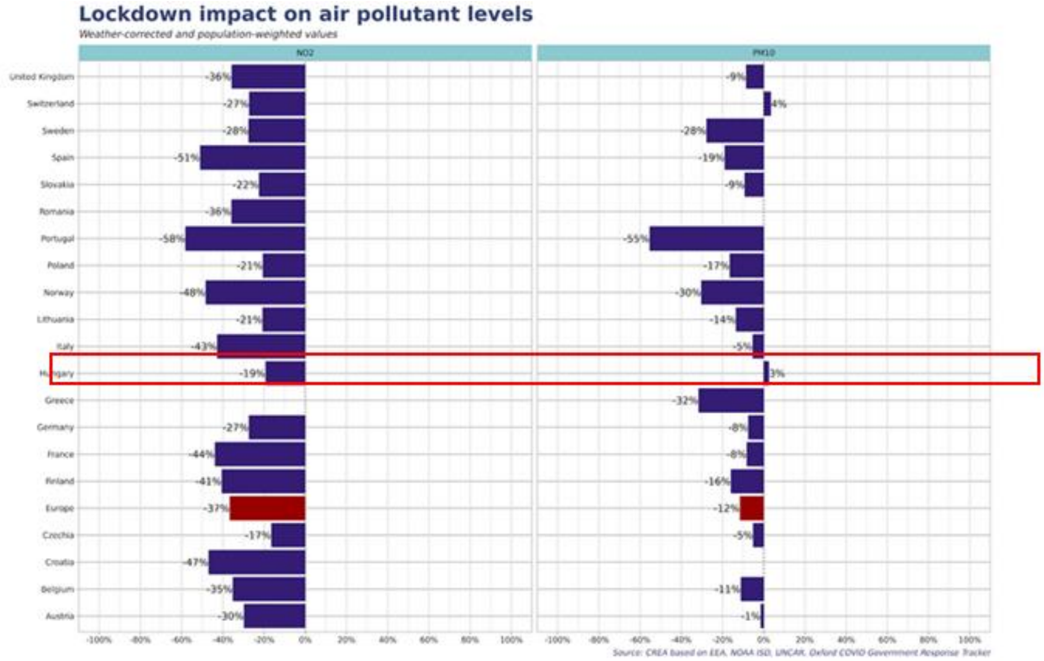

Figure (4): Changes in NO_2 and PM_{10} level in European countries compared to the same period of the previous year [3]

A decrease in the concentration of small particulate matter in the atmosphere is accompanied by a decrease in the amount of precipitation (raindrops would condense on the surface of the dust). In March and April 2020, less precipitation fell than the average of previous years.

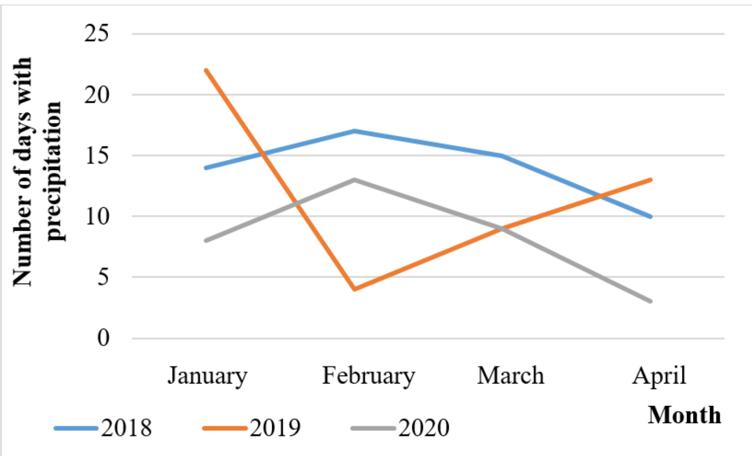

Figure (5): Changes in the number of rainy days in the period January-April, 2018-2020. years

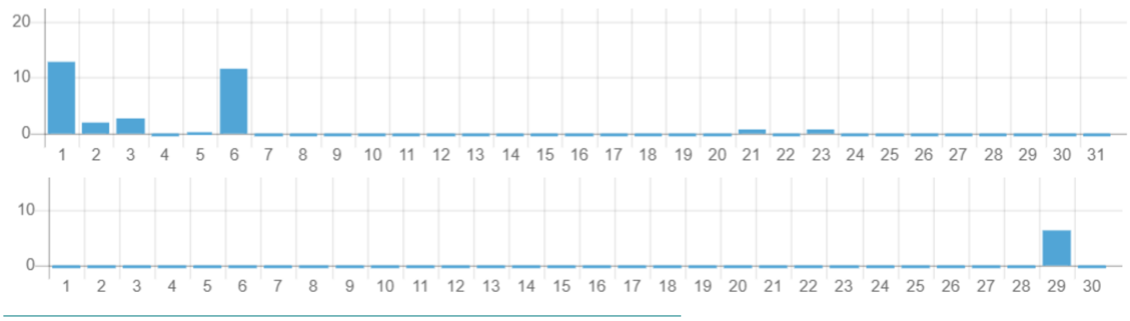

Figure (6): Precipitation, March and April 2020 (mm)

An increase in the concentration of carbon dioxide in the troposphere has no detrimental effect on human health, but as a greenhouse gas it contributes to the increase of the temperature of atmosphere and water and the acidification of the oceans. The melting of ice shelves and rise of more frequent forest fires, stronger hurricanes, and more variable and unpredictable weather can all be attributed to climate change. In Hungary the rise in temperature shifts the conditions in a more volatile and drier direction. The lockdown in transport has also led to a reduction in CO_2 emissions, but this will only be reflected in a small reduction in atmospheric CO_2 concentrations in the future.

PROJECT PROPOSALS FOR TRANSPORT GREENERING

KTI Institute for Transport Sciences Non Profit Ltd has compiled a list of project proposals for the possibilities of greening transport. Selected project proposals that offer different solutions to the problems that arise in road transport, so that, as from a menu, they can be selected and adopted for the given situation, as follows:

1. Performing the greatest reduction is possible by placing mobility on an electrical basis, especially if the electricity required for this is at the same time of green origin, so both go hand in hand with the goals of climate neutrality.

Examples of such solutions are of electric public transport vehicles. Here, mainly trolleybuses and electric buses can be considered as developed technology. High-capacity articulated trolleybuses are now able to move on a few km with their battery, without an overhead line. In addition to public transport, electric propulsion may also be required for service vehicles, taxis and luggage carriers, which circulate in cities. At the same time, the purchase of electric cars can be selectively supported, with the help of a tax rebate, credit, and even the regulation on the electrification of vehicles leased or used by companies.

For the post-epidemic period, the Hungarian government has developed a fundamentally new program to support electric cars. The budget of the tender was HUF 3 billion and started on June 15, 2020, and has been repeated in 2021 as well. An additional HUF 2 billion could be requested for taxis. For new passenger cars with a list price of less than HUF 11 million, all-electric cars receive HUF 2.5 million in state gratuity, while in the price category between HUF 11 million and HUF 15 million there is a state subsidy of HUF 0.5 million.

2. In addition to electric propulsion, the fuel cell solution is also environmentally friendly and contributes to greening. Hydrogen-powered technology is already available for buses and cars, but in very small amount. If the supply of hydrogen-powered vehicles, the availability of filling stations and domestic energy targets also point in this direction, the purchase of these vehicles also will need to be supported.
3. The third group of emission reductions includes support for community travel. The options are: making urban public transport more attractive, developing agglomeration public transport and expanding P+R systems. High passenger capacity has lower specific emissions, reducing both the load on road traffic and the likelihood of congestion. If public transport is placed on an electric fundament, greening is even greater. Transport-sharing is also possible at the car level; the necessary applications are already available.

4. The fourth group of options aims at reducing the concentration of pollutants in the exhaust gases. Restricting the use and import of vehicles with out-of-date technology, tightening the conditions for periodic roadworthiness tests, stepping up roadside inspections or filtering out extremely polluting vehicles will help to eliminate pollution sources. Old-style buses, used only for sightseeing, would also require stricter regulations. In Hungary, the average age of vehicles is high and not all owners can pay the maintenance costs. Another option is attitude formation. By mastering the eco-driving technique, fuel consumption can be minimized, while monitoring the condition of the vehicle allows for early detection and management of problems.
5. The fifth project group includes the possibilities of traffic management and traffic reduction. Here we can mention the introduction of recovery restrictions, either selectively or completely. The creation of low-emission zones, the expansion of no-drive areas and the restriction of public parking are also among the possible options.

At the same time, it is not only urban transport that can be greened by organization. With the involvement of the other sub-sectors, freight transport can be diverted to rail, with roads only need to be loaded from the house to the transhipment nodes.

6. The sixth group of proposals is to divert road users to use bicycles, scooters or other zero-emission solutions. This requires the expansion and improving the safety of urban cycle paths, of which we have seen many good examples recently. Increased urban cycling traffic during the epidemic period also showed that cycling would have a raison d'être if their safe transportation was resolved.

ECONOMIC CONSIDERATIONS

Public revenues from road transport are intended for the development, maintenance and operation of the road network, including the reduction of externalities caused by road users. At the same time, there is hardly any environmental and additional expenditure in the budget that is specifically designed to reduce the externalities of transport. Existing expenditures, on the other hand, always require compliance with increasingly stringent environmental regulations, such as the design of a new road, which requires very strict environmental conditions, which obviously have significant additional costs when it enters the implementation phase and is included in transport expenditure. However, there are only a few specifically environmental interventions on existing road infrastructure (e.g. some noise walls). Expenditures on road network lags behind revenues specifically collected from road users, but if expenditure on public and rail transport is interpreted solely as a reduction in the external effects of road transport, expenditures already far exceeds revenues. Revenues from fuel for transport greening and environmental protection can be obtained in the following ways:

- introduction of a new type of tax
- raising excise duty
- raising other taxes or fees

Environmental tax

An environmental tax on fuel is justified in some form, as the combustion of fuel produces greenhouse gases and several local pollutants (e.g. particulate matter PM10, NOx) that pollute the environment, deteriorating the health of those living in the environment and thus compensating for externalities, which should be compensated by the state. The most appropriate form of taxation to offset the pollution of road transport is a tax on fuel, as the harmful effect is proportionate to its consumption. A new tax could be paid by producers (or importers) or rather the distributors and they would account it further to the final users (as it works in case of excise tax or VAT).

Excise tax

It is more appropriate to limit the scope to distributors, because the collection of taxes is also simpler and producers are usually foreign, they could not be taxed directly. Distributors, on the other hand, are also subject to excise duty and VAT, as they also collect these taxes built into the price of fuel. In Hungary, the rate of excise duty has never been at the forefront of Europe. The largest Central European carriers, such as Poland, Lithuania, Romania, Bulgaria, also have the lowest rates of excise

duty. As of January 1, 2011, Hungary has also introduced the institution of excise tax refund for commercial gas oil, which is already in practice in some other EU member states (Belgium, France, Italy, Slovenia), with which the net excise tax level came closer to the EU minimum. For the purposes of the Directive, the minimum tax value includes all taxes other than excise duties on energy products other than VAT (Article 4 (2)). In Hungary, such a tax is the stockpiling fee, which in the case of diesel has been 3.883 HUF per litre since June 2020, but also includes carbon taxes and other environmental taxes. It would be useful if there would be a 3-4 HUF per litre earmarked environmental tax as part of the (increased) excise tax. The institution of commercial diesel rebates is an important opportunity to remain competitive, as smaller Central European countries could suffer large revenue losses if, in addition to private fuel tourism, carriers optimize their refuelling in the lower tax burden country (in addition it is much easier for them and they decide on larger volumes). Thus, in these countries it is also an economic compulsion to keep the excise tax at a minimum level, which can be achieved with a discount on commercial diesel even in the case of a higher normal excise tax rates. In Hungary, the amount of the commercial diesel price used to be set just above to the EU minimum tax level (0.33 EUR per litre) [7]. Our proposal is to keep this policy but it is also recommended to increase normal tariff of the excise tax of gasoline by 15%, while excise tax of petrol should be increased much less. At the same time discount for commercial diesel should be increased with the same price difference in HUF in order not to maintain current excise tax tariff. There is a moving part (10 HUF per litre for gasoline and 5 HUF for petrol) of the tax if the oil price is under 50 USD per barrel and it is set every quarter of a year. This moving part of the tax should rather be introduced already under 60 USD per barrel price, while the value of it and the update period should be more sophisticated (e.g. 0.8 HUF/USD oil price difference under the limit and every month).
In 2019, according to the Hungarian Tax Authority (NAV)'s fuel turnover statistics, the turnover of petrol and 4.5 billion litres of diesel were nearly 2 billion litres, so a tariff increase of HUF 1-1 per litre would increase the budget's excise tax revenues by HUF 5.6 billion (plus approximately HUF 1 billion VAT).
The purpose of the excise tax is currently according to the regulations:

- providing revenues necessary for the performance of public finance tasks,
- operation of a competition-neutral tax system,
- Hungary's participation in the European Union as a Member State, with special regard to the resulting legal harmonization obligations in the field of excise duties.

Thus, neither the financing of transport infrastructure nor environmental reasons can be found among the objectives of the excise tax, although it is obvious that since this indirectly results in additional expenditures of the state in proportion to increased fuel consumption (~ increase in road traffic), it seeks a targeted and proportionate source. The toll proportional to mileage currently only applies to lorries, their use of motorways and trunk roads, where the toll is not levied on all vehicles and kilometres driven. At the same time, we can say that there is a distinction according to the environmental category in the case of truck tolls. From the introduction on 1 July 2013 until 2018, EURO-III lorries have already been able to receive the best environmental rating in terms of tolls, and since 2019 only lorries with at least EURO-V environmental rating have been awarded. In all cases, at least 90% of the mileage was in the best environmental category, i.e. no significant additional revenue was associated with it, but hopefully indirectly facilitated the transition to a less environmentally damaging technology for trucks.
A tax adjustment of HUF 1 per litre for petrol and HUF 2 per litre for diesel, which better reflects the relative proportions of environmental pollution and would be more in line with each other, would mean additional annual revenue of HUF 9.4 billion (plus approx. HUF 1.5 billion HUF VAT) in addition to fuel turnover in 2019. The double revenue compared to the previous version seems to be sustainable even in the case of the 2 HUF per litre petrol and 4 HUF per litre diesel increase. Excise duty is also, in fact, a special tax levied on energy products, such as fuels, which are presumed to incur some additional health and environmental costs for the state due to their use. The objectives of the excise tax do not include the financing of transport infrastructure or environmental reasons, although it is clear that since these indirectly result additional expenditures, so the government must seek a targeted and proportionate source in proportion to the increased fuel consumption (i.e. traffic too).

Vehicle tax of trucks

The toll proportional to the mileage currently only applies to lorries, their use of motorways and trunk roads, i.e. the toll does not apply to all vehicles and kilometres driven. At the same time, we can say that there is a distinction according to the environmental category in the case of truck tolls. Act LXXXII of 1991 on vehicle tax rules vehicle taxation and company car tax as well. The tax was collected by the municipalities, they could keep 40% of the amounts collected, and the rest had to be transferred to the central budget. From the introduction on 1 July 2013 until 2018, EURO-III lorries have already been able to receive the best environmental rating in terms of tolls, and since 2019 only lorries with at least EURO-V environmental rating have been awarded. Since 2020, the vehicle tax is 100% central revenue. The lower limit of the toll reduction can be raised to EuroVI types. Overall, the best tax base for carbon taxation is to determine fuel consumption, the local pollutant capacity, which also depends on the technology used, but the type of fuel alone is less suitable. In the case of lorries, the tax base is the unladen weight of the lorry in the official register, increased by 50% of its payload, in the case of a tractor it is twice the unladen weight of the lorry, increasing the semi-trailer) with half the positive difference between the maximum permissible laden mass and the unladen mass of the tractor.

The rate of motor vehicle tax is HUF 850 for every 100 kilograms of the tax base started in the case of a truck, tractor, and bus with an air spring or equivalent suspension system, otherwise it is HUF 1,380. There is a 20% tax reduction for EURO-III rated lorries (30% for semi-trailer tractors) and a 30% tax reduction for EURO-IV or better lorries (50% for semi-trailer tractors). The EU-wide minimum level of motor vehicle tax is set out in Directive 1999/62/EC (Eurovignette)[6], broken down by number and distribution of axles and permissible gross vehicle weight. Items have remained unchanged since the entry into force of the Directive in 1999, and even in the previous Directive 93/89/EEC the itemized amounts remain unchanged (from 1 January 1995), then in ECU (the predecessor of the euro). The methodology for setting the European minimum tax level is deficient in several respects. It’s only known description appeared in the Official Journal of the EU on 20.03.1991, when its proposal, submitted in 1987, was amended by the Commission. According to this, the method of calculating the minimum car tax rate is

minimum vehicle tax = 57.6% x 0.15 (total road infrastructure cost – excise tax paid).

In the formula, the total infrastructure cost was calculated on the basis of a Dutch study in 1989. The breakdown of the vehicle tax by category of vehicle is allegedly published by the UK Department for Transport but not indicated. However, it is well-known the correlation the pavement degradation depends with the fourth power from the axle load, so obviously the heaviest categories cause the highest infrastructure costs. In the background calculations of the Directive 1999/62/EC [6], the infrastructure cost of excise duty was found to have been paid for all lorries with a total permissible weight of 12 tons, so a vehicle tax was set only above this limit. The coefficient of 0.15 meant that only 15% of the infrastructure cost not paid by excise duty was planned to be paid as an annual motor vehicle tax. This was planned to be raised to 25% but it was cancelled and in fact it was decreased to 8.6%. In the final directive, lorries with air suspension or equivalent suspension received an additional category discount according to the permissible gross vehicle weight. The values found have remained modest compared to the given methodology and have not even increased in euros in 25 years (!), so the cost of infrastructure is only covered by a declining extent of the excise duty and the vehicle tax, while tolls are playing an increasingly important role. As the EU and Hungarian vehicle tax bases are different and cannot be converted directly, it is difficult to take assess how inconsistent the Hungarian tax rates are with the EU minimum in the higher categories. In most categories, Hungarian tax rates are well above the EU minimum tax thresholds below 30 tonnes. In heavier vehicle categories, where the tax rate expressed in euros may be below the minimum due to the rising euro exchange rate, so the application of the EU minimum may be required, either by repeating EU tariffs in the national law or simply by referring to the Directive.

This would maintain the progressivity of the tax, and even the more categories the EU minimum would have to apply, the more progressivity would be achieved, so the heaviest categories would contribute more proportionally. As pointed out above, the methodology for established EU tax minima is repeatedly outdated and hard to explain, how they were created and why they should be used.

However, this has been the source of law and in any case it is fairer in the progressivity of the tax (at least above 15 tonnes where it is really important) than domestic law that applying only a linear tax rate. We propose to keep the linear tax rates with giving 20-30% discount only to EURO V and VI categories. We also propose to use directly the European minimum rates, if the national tax rate would be lower than that (but 100-120% of the European minimum depending on the environmental categories).

Toll discount (13%) for large users

Under the Eurovignette Directive [6], Member States have the option of applying a reduction of up to 13% to the infrastructure charge. Hungarian Toll Act (Act LXVII of 2013), which forms the legal basis of the Hungarian toll system also contains the possibility of a toll reduction, however, there is no detailed regulation of this (it is not clear under what conditions and to what extent it can be used), i.e. even if the possibility provided by law is available, carriers cannot use it yet. At EU level, however, there are Member States that make use of this possibility and have introduced this tariff reduction. In Slovenia, you can get a 10% discount at a fleet level of € 30,000 net per month, while in the Czech Republic you can get a maximum discount of over 13,000 euros per calendar year per vehicle, while in Slovakia you can get a maximum 9% discount (under 12 tons: 11%) over 50,000 km per calendar year per vehicle. From a business point of view, granting a discount may seem like a good solution, but from an environmental point of view, it seems bad at first glance. At the same time, the moderate rate of the discount does not encourage over-consumption, so we can only expect a loss of budget revenue. However the introduction of the large user discount could be economically and environmentally compatible with the Hungarian toll system, if only the trip on the expressway network were included in the discount, thus encouraging using motorways, which would normally be even more expensive but it has lower environmental impact per vehicle, than trunk roads.

SUMMARY

Due to Hungary's topography, the harmful substances emitted by road traffic, which are dangerous to health, can be cleared out from the atmosphere very slowly, mainly due to rainy and windy weather. However, this is becoming less frequent, and the length of low rainfall periods is also increased by the decrease in the concentration of small particles. As a result, the country's climate development strategy needs to be based on two basic pillars:

1. Reducing the amount of pollutants emitted as much as possible,
2. Gaining financial support for transport greening.

Reducing emissions from road transport can help the first pillar, this means primarily the replacement of diesel-powered buses by buses with electric drive, but the development of the trolleybus network also arises. For this, it is necessary to compare the cost of vehicle acquisition, the construction of the overhead line and the battery charging system, as well as the long-term maintenance and operating costs in a separate expert study. Today's modern trolleybuses can cover a few kilometers without overhead lines, so continuous line construction is not a prerequisite for placing on the line. The trolleybus is a proven, reliable means of transport, has a large passenger capacity (see articulated version) and is more cost-effective than electric buses with current technology. If, in the absence of better data, we start from the Hungarian Tax Authority (NAV)'s 2019 fuel turnover statistics and assume an environmental tax of HUF 2 per litre, then we can count on a source of HUF 9.4 billion per year. We recommend that the support should be implemented on a normative basis. The different forms of support can be summarized, such as the already announced intention to support from the government, the support from the environmental tax, and the possible EU support. Based on market conditions, the amount of environmental tax aid per vehicle should be reviewed at least every two years. All petrol / diesel users could pay, including transport companies, the polluters. From this, we protect the health of the entire living population traveling in larger cities. In public transport there are used approx. 8500 buses, take part, of approx. 2500 of which are the city buses. Companies providing road public transport are typically municipally owned, state-owned, but some companies also have private subcontractors. The vehicle fleet is extremely variable in composition and age. A heavily polluting bus is common, which in principle should not even travel, but without these the service provider would not be able to provide the required number of vehicles and there would not be a budget available for costly repairs. There are two options to choose from, with either a heavily incense bus running or nothing running instead. Replacement of the vehicle fleet is only possible in the long run and with continuous renewal, on a scheduled basis and the resource requirements are extremely high. By using electric buses and trolleybuses, urban, local emissions can be reduced to zero, and air pollution can be noticeably improved in terms of PM10 and NOx.

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EFFECT OF DIFFERENT CHELATING AGENTS AND BIOINOCULANTS ON FRACTIONAL DISTRIBUTION OF CADMIUM AND NICKEL IN SPIKED SOIL AFTER HARVEST OF INDIAN MUSTARD (*BRASSICA JUNCEA*)

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Abstract: *Screen house experiment was conducted to study the different forms of Cadmium (Cd) and Nickel (Ni) in the soil after harvest of Indian mustard (Brassica juncea L.) as affected by chelating agents and bioinoculants from metal enriched soil. A seven-step sequential extraction procedure was applied to evaluate the association of metals with soil constituents. The different chemical forms of fraction of Cd and Ni extracted were exchangeable + water soluble carbonate, organic matter complexed, Mn-oxide bound, occluded in amorphous and crystalline Fe-oxide and residual mineral fraction. The present study revealed that dominant Cd fraction was organic followed by carbonate, Manganese oxide, exchangeable, crystalline Fe-oxide, amorphous Fe-oxide and residual Cd fraction. Application of chelating agents increased the exchangeable fraction causing an increase in its availability in the soils. It may be ascribed that application of chelating agent caused an increase in the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, Mn-oxide, AFe-oxide and CFe-oxide bound fractions. Highest amount of exchangeable fraction was observed in EDTA treated soil. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd (greater than non-bioinoculants treated soil). Application of chelating agent (EDTA) significantly increased the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, MnOX, AFeOX, bound fractions. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd. Fractionation of soil Cd after the harvest of Brassica juncea crop showed that the application of chelating agent (EDTA) excellently improved the exchangeable pool of Cd.*

Keywords: *Bioinoculant, Cadmium, Chelating agents, Different fractions, Nickel, Indian mustard*

INTRODUCTION

Cadmium (Cd), recognized as one of the most hazardous elements, is not essential for plant growth

(Kabata-Pendias, 2000). World production of Cd increased from 11000 t in 1960 to 20200 t in 1990 (Alloway, 1995). The main source of Cd contamination is industries involved in protective plating on steel and production of various alloys, pigments, stabilizers for plastic, Ni-Cd dry batteries and other miscellaneous items including photovoltaic cells and control rods for nuclear reactors. The other sources of Cd contamination come from mining, ore dressing and smelting of Cadmium sulphide ore, which may contain up to 5 per cent Cd (Alloway, 1995).

Nickel (Ni) is considered an essential micronutrient for plant, humans, and animals. It can exist in trace amount in air, food, drinking water, and soils. Although, Ni plays an important role in the metabolism of humans and animals, its intake in excessive amount or over a prolonged period could pose health risks. Studies have shown that children living in polluted areas have hypertrophy of tonsils, enlarged lymphatic nodes, and enlarged livers. There is also evidence that soluble Ni particulate is linked to acute lung injury. The conservation of a healthy agricultural ecosystem is prerequisite for protecting the food chain from bioaccumulation of hazardous substances e.g., heavy metals. Among the pollutant elements, nickel is of great environmental concern as it is hazardous and highly carcinogenic (De et al., 1997).

Unlike organic compounds, metals cannot be degraded and their cleanup requires their immobilization and toxicity reduction or removal. In recent years, scientists and engineers have started to generate cost effective technologies which includes use of microorganisms/ biomass or live plants for cleaning of polluted areas Qiu et al., 2000),. Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long-term applicability (Richard, 1954, Su and Wong, 2004).

Microbial populations can change complexes of heavy metals to affect their availability to the plant through release of chelating agents, siderophores, acidification, phosphate solubilization and redox changes, and therefore, have potential to enhance phytoremediation processes. Application of farm yard manure (FYM) or compost or biological wastes is a common practice and traditionally followed in Indian agriculture. Integrated approach, involving, plants (hyper accumulators), suitable chemical and biological amendments, and microbial strains should be required for successful remediation of heavy metal contaminated soils. Amongst the commercial crops grown in India, Indian mustard (*Brassica juncea* L.) has been reported to produce higher biomass and accumulate significant amount of heavy metals in their tissues when induced through the addition of chelating agents (Blaylock et al., 1997, Huang and Cunningham, 1996). Additions of chelating agents have been reported to increase Cd and Ni contents in the solution phase (Gray et al., 1999, Huang and Cunningham, 1996). Hence, higher extractability of Cd and Ni due to chelating agents may lead to their increased availability to plants (Mishra, 2004).

FYM, vermicompost, chelating agents and bioinoculants (N_2 fixer and phosphorus solubilizing bacteria) and their interaction leading to changes in different fractions of Cd and Ni in contaminated soil is to be studied for proper understanding of their role in chemically enhanced phytoextraction. Gray et al. (1999) showed that there was a wide range in the concentrations of Cd associated with individual soil fractions. The greatest concentration of Cd is associated with the organic and residual fractions, while the lowest concentration of Cd is in the exchangeable form. Fractionation of Cd in some soils indicated that a substantial portion of Cd added to the soil with P fertilizer applications ends up in the residual fraction of the soils. It is suggesting that a substantial proportion of fertilizer applied Cd in these soils has reverted to forms likely to be unavailable for plant uptake. It is reported that biosorption process for removal of the heavy metal Cd^{2+} from contaminated soil due to its economy, commercial applications and because it acts without destroying soil structure (phytoremediation). Kozáková and Kandrac (1998) studied the binding forms of selected heavy metal Cu, Pb, Cd and Ni in carbonate type soil. The present study thus was conducted to investigate an effect of chelation agents and bioinoculants on different fractions (forms) of Cadmium and Nickel in the soil after harvest of Indian mustard crop.

MATERIALS AND METHODS

i) Collection and Processing of Soil Samples:

The bulk surface sample (0-15) of a sandy loam soil was collected from the experimental area of the

Department of Soil Science, CCS Haryana Agricultural University, Hisar, Haryana. It was dried, ground to pass through a 2 mm stainless steel sieve and mixed thoroughly. The processed soil sample was used for laboratory and screen house studies. Its physico-chemical properties are given in Table (1).

Table (1): Physico-chemical characteristics of experimental soil

Properties	Contents
(A) Sand (%)	61.3
(B) Silt (%)	19.0
(C) Clay (%)	18.6
Textural class	Sandy loam
pH (1:2)	8.1
$EC_{1:2}$ (dS m^{-1})	0.4
Organic	0.36
CEC [Cmol (P^+) kg^{-1}]	12.7
$CaCO_3$ (%)	0.33
Available nutrients (kg ha^{-1})	
(A) Nitrigen	190.4
(B) P_2O_5	17.7
K_2O	200.6
DTPA extractable metal (mg kg^{-1})	
Fe	38.70
Mn	9.78
Zn	0.48
Cu	0.36
Cd	0.25
Ni	0.92
Pb	1.01
Total Metals (mg kg^{-1})	
Cd	3.15
Ni	11.25

ii) Collection and Processing of Farmyard manure:

The bulk sample of well decomposed farmyard manure (FYM) was taken from the manure pit of Dairy Farm, CCS Haryana Agricultural University, Hisar. The chemical composition of FYM used is given in Table (2).

Table 2: Chemical composition of experimental FYM

Properties	Content
Nitrogen (%)	1.23
Phosphorus (%)	0.61
Potassium (%)	1.90
Organic carbon (%)	29.34
C/N Ratio	24.88
Total metal (mg/kg)	
Cd	0.31
Ni	4.29

iii) Collection and Processing of Vermicompost

The bulk sample of a well decomposed Vermicompost (VC) from department of Agronomy, CCS Haryana Agricultural University, Hisar. The chemical composition of Vermicompost used is given in Table (3).

iv) Screen house Studies:

Screen house experiments were conducted to study the phytoremediation of Cadmium (cd) and nickel (Ni) by mustard (*Brassica juncea* L.) as affected by chelating agents and bioinoculants from metal enriched soil. The experimental details given below:

Table 3: Chemical composition of the experimental vermicompost

Properties	Content
Nitrogen (%)	1.75
Phosphorus (%)	0.68
Potassium (%)	0.93
Organic carbon (%)	16.18
C/N Ratio	11.40
Total metal (mg/kg)	
Cd	0.21
Ni	34

v) Imposition of Treatments:

The processed soil sample was equally divided into two halves and one half was artificially spiked with Cd using $CdCl_2$ as a source of Cd and remaining half soil was artificially spiked with Ni using $NiCl_2$ as a source of Ni. These two bulk soil samples were spread evenly on polythene sheets placed over the raised platform of screen house. Pre- calculated amount of $CdCl_2$ (i.e., 100 mg Cd/250 ml distilled water) and $NiCl_2$ (i.e., 100 mg Ni/250 ml distilled water) were dissolved in distilled water. The solution so prepared were sprinkled over the uniformly spread soils at the rate of 250 ml solution per kg of soil. After sprinkling this solution, the soil samples were covered with plastic sheet for 48 hours to minimize evaporation for proper equilibration. Thereafter the covers were taken off the soils were allowed to dry to workable moisture content. Each soil sample was thus thoroughly mixed, respread uniformly over the plastic sheet and moistened to near field capacity moisture content using distilled water. This cycle was repeated thrice for proper equilibration and uniform enrichment of both soils with added Cd and Ni respectively. The Cd and Ni enriched bulk soil samples were then air dried and divided in eight equal lots. One lot was kept as such. Second lot was treated with well decomposed dry farm yard manure (FYM) @ 2% by weight. The third lot was treated with well decomposed dry vermicompost (VC) @ 2% by weight and fourth lot was kept for Ethylenediaminetetraacetic acid (EDTA) treatment @ 2mmol/kg (0.4 mmol daily for 5 days in 5 split doses) at 40 days after sowing (DAS). The remaining four lots were also treated with above said treatments. In addition to those treatments, last four lots were also treated with N_2-fixer (i.e., *Azotobacter sp.*) and phosphorus solubilizing bacteria (i.e., *Pseudomonas sp.*) as seed treatment at the time of sowing.

FYM and vermicompost @ 2% by weight will mixed uniformly in Cd-spiked and Ni-spiked soils one week before sowing.

A. Experiment No. 1: ***Effect of chelating agents and bioinoculants on different fractions of Cd by mustard (Brassica juncea L.)***

1. Bulk spiked soil with 100 mg Cd/kg soil
2. FYM / vermicompost / chelating agents treatment combinations:
   A. Control (Non-spiked soil)
   B. Cd-spiked soil (Cd_{100})
   C. Cd-spiked soil (Cd_{100}) + FYM @ 2%
   D. Cd-spiked soil (Cd_{100}) + vermicompost @ 2%
   E. Cd-spiked soil (Cd_{100}) + EDTA @ 2 mmol/kg soil at 40 DAS (Day after sowing)
3. Bioinoculants treatment combinations:
   A. Control + PSB + N2 fixer
   B. Cd-spiked soil + PSB + N_2 fixer

C. Cd-spiked soil + PSB + N_2 fixer + FYM @ 2%
D. Cd-spiked soil + PSB + N_2 fixer + vermicompost @ 2%
E. Cd-spiked soil + PSB + N_2 fixer + EDTA @ 2 mmol/kg soil at 40 DAS

4. Crop: mustard (Brassica juncea L.) variety: Laxmi
5. Number of treatments: 5+5 = 10
6. Replication: 3
7. Number of pots: 10x3 = 30
8. Experimental design: CRD
9. Harvesting stage: Two weeks after chelating agent application

B. **Experiment No. 2:** ***Effect of chelating agents and bioinoculants on different fractions of Ni by mustard (Brassica juncea L.)***

1. Bulk spiked soil with 100 mg Ni/kg soil
2. FYM / vermicompost / chelating agents treatment combinations:
   A. Control (Non-spiked soil)
   B. Ni-spiked soil (Ni100)
   C. Ni-spiked soil (Ni100) + FYM @ 2%
   D. Ni-spiked soil (Ni100) + Vermicompost @ 2%
   E. Ni-spiked soil (Ni100) + EDTA @ 2 mmol kg-1 soil at 40 DAS (Day after sowing)
3. Bioinoculants treatment combinations:
   A. Control + PSB + N_2 fixer
   B. Ni-spiked soil + PSB + N_2 fixer
   C. Ni-spiked soil + PSB + N_2 fixer + FYM @ 2%
   D. Ni-spiked soil + PSB + N_2 fixer + Vermicompost @ 2%
   E. Ni-spiked soil + PSB + N_2 fixer +EDTA @ 2 mmol kg-1 soil at 40 DAS
4. Crop: mustard (Brassica juncea L.) Variety: Laxmi
5. Number of treatments: 5 + 5 = 10
6. Replication: 3
7. Number of pots: 10x3= 30
8. Experimental design: CRD
9. Harvesting stage: Two weeks after chelating agent application

Five kg capacity earthen pots lined with polythene were used to grow the crop. The polythene lining was provided mainly to avoid leaching of soil solution and interaction of metals with the earthen pots. All, 60 pots were filled with 5 kg of thoroughly mixed air-dried soil. In each pot basal doses of N, P, K, Fe, Mn, Zn and Cu @ 50, 50, 60, 10, 5, 5 and 5 mg/kg soil through Urea, KH_2PO_4, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, and $CuSO_4.H_2O$ respectively, in solution form @ 5oo ml solution in each pot separately. After 24 hours, the nutrient treated soil from each pot was separately taken out, mixed thoroughly and refilled.

Indian mustard (*Brassica juncea* L.), variety Laxmi, was taken as the test crop. Before sowing, half of the total seeds were inoculated with microbial inoculants (*Azotobacter sp.* and *Pseudomonas sp.*). Twenty inoculated seeds of this crop were sown in each pot on 22nd Oct. 2013.

Thinning was done to 5 plants per pot on 10th day of seeding and plants were allowed to grow up to 8 weeks from the date of sowing (i.e., up to blooming stage). The pots were irrigated deionized water as and when required.

In the present study, EDTA, FYM and vermicompost were used as chelating agents. EDTA (Disodium salts) was applied at the rate of 2 mmol per kg soil (0.4mmol daily for 5 days in 5 split doses) in twelve pots filled with the Cd and Ni treated soil in 40th day of sowing.

Composite soil samples from each pot were taken before sowing and after harvesting of plants with stainless steel tube auger. These soil samples were air dried, ground, sieved and stored in polyethylene bags for their chemical analysis.

Analytical method

Soil Analysis:

The following methods were used for soil analysis:

(i) Mechanical analysis was done as per international pipette method (Piper, 1996).

(ii) pH and electrical conductivity were determined on 1:2 soil water suspension with the help of glass electrode pH meter and conductivity meter bridge, respectively.

(iii) Organic carbon was estimated by Walkley and Black's rapid titration method.

(iv) Cation Exchange Capacity was determined by Ammonium acetate method as outlined in USDA Handbook No. 60 (Richard, 1954).

(v) $CaCO_3$: was determined by rapid titration method of (Puri, 1949).

(vi) DTPA-exchangeable polyvalent transition heavy metals were extracted by using the procedure of [18] and estimated on AAS.

(vii) Total Cd and Ni was determined using by aqua regia method [7], (Conventional aqua regia digestion was performed in 250 mL glass beakers covered with watch glass. A well-mixed sample of 0.5000 g was digested in 12 mL of aqua regia on a hot plate for 3 hours at 110ºC. After evaporation to near dryness, the sample was diluted with 20 mL of 2% (v/v with H2O) nitric acid and transferred in to a 100 mL volumetric flask after filtering through Whatman No.42 paper and diluted to 100 mL with DDW) digest by atomic absorption spectrophotometer (AAS) (GBC-932 plus).

Sequential extraction of soil samples:

To study the effect of different chelating agents and bioinoculants on fractional distribution of Cd in Cd spiked and Ni in Ni spiked soil.

A seven-step sequential extraction procedure given in Table No.4 was applied to evaluate the association of metals with soil constituents. The different chemical forms of fraction of Cd and Ni extracted were exchangeable + water soluble carbonate, organic matter complexed, Mn-oxide bound, occluded in amorphous and crystalline Fe-oxide and residual mineral fraction.

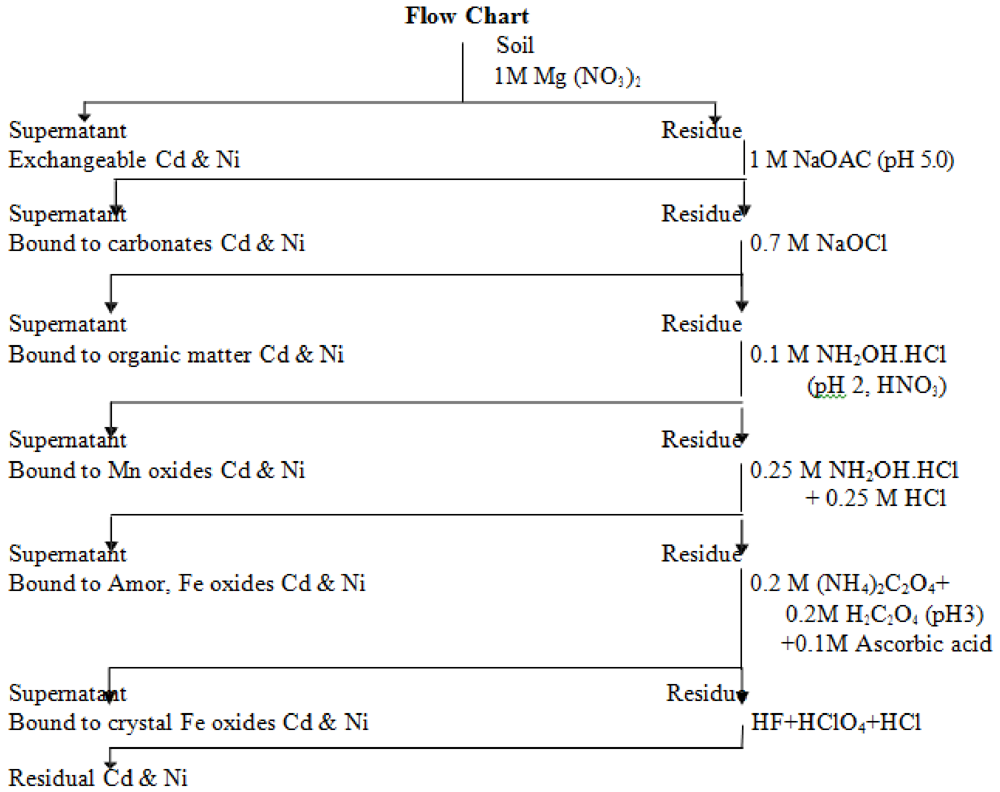

Figure (1): Flow chart of sequential extraction scheme for Cadmium and Nickel

Sequential fractionation technique was used as a measure of availability, mobility and potential bioavailability of Cd and Ni to plants. Several methods of fractionation of heavy metals have been proposed by several workers (Chao and Zhou, 1983, Chao, 197], Gupta and Chen, 197, Shuman, 1998, Tessier et al., 1979). Here, sequential extraction procedure adopted by Singh *et al*. (1988) was used which is described in Table (4) and Figure (1). Reagents used in fractionation scheme were selected from those cited in the literature as being selective for specific chemical form in soil. All procedures were carried out in triplicate. The fractionation scheme generally starts with the weakest, least aggressive ones. Ten gram of each soil samples was taken in centrifuge tube; the extractant was added to it in required ratio. The contents of tube were centrifuged and filtered after fulfilling the essential conditions such as shaking, boiling, digestion etc. The soil residue was washed twice with double distilled water. Then five gram of soil sample was taken in centrifuge tube for determining Mn-oxide form.

Residual metal analysis:
Residual from CFeOX extraction was washed twice with double distilled water. It was subsequently dried and ground to pass through a 2 mm stainless steel sieve. A 0.5 g samples were digested with 10 ml conc. HF and 2-3 ml conc. $HClO_4$ using Teflon beaker, over stand and bath heater. Remaining material taken up in 6N HCl and volume was made to 25 ml. The procedure as described by (Tessier et al., 1979) was adopted and supernatant liquids for all chemical forms were stored in plastic bottles. The amount of Cd and Ni fractions were estimated by Atomic Absorption Spectrophotometer.

Table: 4. Sequential extraction steps for Cd and Ni fractionation

S. No.	Fractions	Reagent/Extractant	Soil (g)	Solution (ml)	Experimental condition	Reference
1	Exchangeable (EX)	1 M Mg $(NO_3)_2$	10	40	Shake 2 h at 2ºC	Shuman (1985)
2	Bound to carbonate (CARB)	1 M NaOAc (pH 5.0 CH_3COOH)	10	40	Shake 5 h at 25ºC	Tessier et al. (1979)
3	Bound to organic Matter (OM)	0.7 M NaOCl (pH 8.5)	10	20	Shake 30 min. in boiling water bath, stir occasionally	Shuman (1983)
4	Bound to Mn oxides (MnOX)	0.1 M $NH_2OH.HCl$ (pH 2, HNO_3)	5a	50	Shake 30 min.	Chao (1972)
5	Bound to Amor. Fe oxides (AFeOX)	0.25 M $NH_2OH.HCl$+0.25 M HCl	5	50	Shake 30 min. at 50ºC in water bath	Chao and Zhou (1983)
6	Bound to Crys. Fe oxides (CFeOX)	0.2 M $(NH_4)_2C_2O_4$+0.2 M $H_2C_2O_4$ (pH 3)+0.1 M Ascorbic acid	5	50	Boiling30 min. in water bath stir occasionally	Shuman (1982)
7	Residual (RES)	10 ml conc. HF + 2 ml $HClO_4$ + 6N conc. HCl in sequence	0.5	25	Sand bath, cool, boil gently and cool	Tessier et al. (1979)

RESULTS AND DISCUSSION

Fractionation studies:

Effect of chelating agents and microbial inoculants on different fractions of Cd in soil after harvest of *Brassica juncea:*
The data pertaining to different fractions of Cd as influenced by bioinoculants and chelating agents are presented in Table 5. A perusal of data in table revealed that the transformation of Cd to different fractions in Cd spiked soil is influenced differentially by different chelating agents and bioinoculants. The fraction wise description is given below:

(i) Exchangeable-Cd (Ex-Cd)

The mean value of exchangeable-Cd varies from 0.23 to 27.02 µg/g soil (Table 5). With application of EDTA a significant increase was observed in exchangeable form. The application of EDTA yields 2.19 and 2.21 times higher Ex-Cd than without EDTA in Cd_{100} and Cd_{100}+M treated soil. The organic amendments significantly decreased the exchangeable fraction of Cd (approx. 2 times lower than EDTA treated soil). Bioinoculants did not play a significant role either in increasing or in decreasing this fraction of Cd.

(ii) Carbonate bound- Cd (Carb-Cd)

It is evident from data presented in Table 5 that carbonate bound-Cd fraction was significantly higher in soil where none of the chelating agent was applied. The mean value of Carb-Cd fraction decreased from 19.51 at Cd_{100}+M to 16.05 µg/g soil in EDTA treated Cd spiked soil. The bioinoculants significantly increased Carb-Cd fraction in soil where chelating agents were not applied.

(iii) Organic bound- Cd (Org-Cd)

Organically bound-Cd fraction was significantly higher in soils where EDTA was not applied than EDTA treated soil (Table 5). The mean content of Org-Cd was 29.95 µg/g in Cd_{100}+M but decreased significantly with the addition of chelating agents. The organically bound-Cd fraction was comparatively higher than either carbonate or manganese oxide bound forms in both i.e., with or without bioinoculants soils. The Org-Cd fraction increased in organically amended soil, approximately 1.34 (without bioinoculants) 1.38 (with bioinoculants) times more than EDTA applied soil.

(iv) Manganese oxide bound-Cd (MnOX-Cd)

Data related to the effect of chelating agents and bioinoculants on the MnOX-Cd fraction are presented in Table 5. It is clear from the data that application of chelating agent caused a significant reduction in the MnOX-Cd fraction as compared to Cd_{100} where chelating agent was not applied. The MnOX-Cd fraction decreased from 15.22 in Cd_{100} to 14.75 µg/g soil in Cd_{100}+EDTA treated soil.

However, FYM and vermicompost proved useful in maintaining this pool as compared to chelating agent. Slightly lower content of MnOX-Cd fraction was observed in bioinoculants treated soil than untreated soil as the bioinoculants release some chelating agents or siderophores which make the micronutrients available for the plants for their growth.

(v) Amorphous Fe-oxide bound Cd (AFeOX-Cd)

The AFeOX-Cd fraction was higher in Cd_{100} but decreased slightly due to addition of FYM, vermicompost and EDTA (Table 5).

The mean content of AFeOX-Cd fraction was 5.44 and 5.08 µg/g in Cd_{100}+FYM and Cd_{100}+VC treatments whereas in Cd100+EDTA treatment, 4.78 µ g/ g observed. In case of bioinoculants treated soil, the values for AFeOX- Cd fraction was higher than the treatments which were not inoculated with bioinoculants.

(vi) Crystalline Fe-oxide bound Cd (CFeOX-Cd)

The mean content of CFeOX-Cd fraction decreased significantly with the addition of chelating agent (EDTA) (Table 5). The CFeOX-Cd fraction (13.95 µ g/g) was higher in Cd100 but decreased slightly due to addition of vermicompost whereas the addition of FYM and EDTA caused a significant reduction in the value of CFeOX-Cd fraction.

The mean value of CFeOX-Cd fraction was 12.92 and 12.31 µg/g soil in FYM and EDTA treatments. In case of bioinoculants treated soil the values for CFeOX-Cd fraction was higher than the treatments which were not inoculated with bioinoculants.

(vii) Residual-Cd (Res-Cd)

Residual fraction showed a decreasing trend with addition of organic amendments and chelating agent (Table 5). Among all, EDTA was more effective in decreasing Res-Cd fraction as compared to others. The mean value of Res-Cd fraction decreased from 7.15 to 4.95 μg/g on the addition of EDTA.

The mean values of this fraction in FYM and vermicompost was 6.25 and 5.49 μg/g. But in case of bioinoculants treated soil, the mean content of Res-Cd fraction was reported lower than the soils which was not treated with bioinoculants.

Table 5: Post-harvest distribution of Cd (μg/g soil) into different forms as influenced by different chelating agents and bioinoculants in Cd enriched soil

Treatment	**Ex-Cd**	**Cab-Cd**	**OM-Cd**	**MnOX-Cd**	**AFeOX-Cd**	**CFeOX-Cd**	**Res-Cd**
Control	0.23	0.61	0.20	082	0.47	0.59	2.05
Cd_{100}	12.32	19.36	26.65	15.22	6.10	13.95	7.15
Cd_{100}+FYM	13.85	18.22	25.45	15.91	5.44	12.92	6.25
Cd_{100}+VC	12.71	19.33	26.08	16.11	5.08	13.72	5.49
Cd_{100}+EDTA	27.02	16.05	19.37	14.75	4.78	12.31	4.95
Control+M	0.26	0.67	0.25	0.80	0.51	0.62	1.90
Cd_{100}+M	12.21	19.51	29.95	14.60	6.21	14.02	6.89
Cd_{100}+FYM+M	13.90	19.30	26.16	15.12	7.25	13.79	5.35
Cd_{100}+VC+M	12.79	19.11	29.42	15.78	8.13	14.55	5.05
Cd_{100}+EDTA+M	27.10	17.14	21.29	14.40	5.45	12.10	4.18
CD at 5%	1.24	1.30	1.81	1.08	0.43	0.96	0.41

M: Microbial inoculants VC: Vermi Compost

Effect of chelating agents and microbial inoculants on different fractions of Ni in soil after harvest of *Brassica juncea*

The data pertaining the fractions of Ni in soil after the harvest of *Brassica juncea* are presented in Table 6. The fraction wise description is given below:

(i) Exchangeable-Ni (Ex-Ni)

The mean value of exchangeable-Ni varies from 0.26 to 25.5 μ g g^{-1} soil (Table 6). With application of EDTA a significant increase was observed in exchangeable form. The application of EDTA yields 1.41 and 1.38 times higher Ex-Ni than without EDTA in Ni_{100} and Ni_{100}+M treated soil. The organic amendments significantly decreased the exchangeable fraction of Ni (approx. 2 times lower than EDTA treated soil). Bioinoculants did not play a significant role either in increasing or in decreasing this fraction of Ni

(ii) Carbonate bound- Ni (Carb-Ni)

It is evident from data presented in Table 6 that carbonate bound-Ni fraction was significantly higher in soil where none of the chelating agent was applied. The mean value of Carb-Ni fraction decreased from 15.10 at Cd_{100}+M to 13.12 μg g^{-1} soil in EDTA treated Ni spiked soil. The bioinoculants significantly increased Carb-Ni fraction in soil where chelating agents were not applied.

(iii) Organic bound-Ni (Org-Ni)

Organically bound-Ni fraction was significantly higher in soils where EDTA was not applied than EDTA treated soil (Table 6). The mean content of Org-Ni was 8.14 μg/g in Ni_{100}+M but decreased significantly with the addition of chelating agents. The Org-Ni fraction increased in organically amended soil, approximately 1.08 (without bioinoculants)
1.21 (with bioinoculants) times more than EDTA applied soil.

(iv) Manganese oxide bound-Ni (MnOX-Ni)

Data related to the effect of chelating agents and bioinoculants on the MnOX-Ni fraction are presented in Table 6. It is clear from the data that application of chelating agent caused a significant reduction in the MnOX-Ni fraction as compared to Ni_{100} where chelating agent was not applied.

The MnOX-Cd fraction decreased from 18.50 in Ni_{100} to 17.21 μg/g soil in Ni_{100}+EDTA treated soil. Slightly lower content of MnOX-Cd fraction was observed in bioinoculants treated soil than untreated soil as the bioinoculants release some chelating agents or siderophores which make the micronutrients available for the plants for their growth.

(v) Amorphous Fe-oxide bound Ni (AFeOX-Ni)

The AFeOX-Cd fraction was higher in Ni_{100} but decreased slightly due to addition of FYM, vermicompost and EDTA (Table 6). The mean content of AFeOX-Ni fraction was 20.82 and 19.71 μg/g in Cd_{100}+FYM and Cd_{100}+VC treatments whereas in Cd100+EDTA treatment, 19.33 μg/g observed. The Amorphous Fe-oxide bound Ni fraction was comparatively higher than either exchangeable or manganese oxide bound forms in both i.e., with or without bioinoculants soils. In case of bioinoculants treated soil, the values for AFeOX-Cd fraction were higher than the treatments which were not inoculated with bioinoculants.

(vi) Crystalline Fe-oxide bound Ni (CFeOX-Ni)

The mean content of CFeOX-Ni fraction decreased significantly with the addition of chelating agent (EDTA) (Table 6). The CFeOX-Ni fraction (16.60 $\mu g\ g^{-1}$) was higher in Ni_{100} but decreased slightly due to addition of vermicompost whereas the addition of FYM and EDTA caused a significant reduction in the value of CFeOX-Ni fraction. The mean value of CFeOX-Cd fraction was 15.62 and 14.91 μg/g soil in FYM and EDTA treatments. In case of bioinoculants treated soil, the values for CFeOX-Cd fraction was higher than the treatments which were not inoculated with bioinoculants.

(vii) Residual-Ni (Res-Ni)

Residual fraction showed a decreasing trend with addition of organic amendments and chelating agent (Table 6). Among all, EDTA was more effective in decreasing Res-Ni fraction as compared to others. The mean value of Res-Ni fraction decreased from 6.40 to 4.90 μ g/g on the addition of EDTA. The mean values of this fraction in FYM and vermicompost was 5.77 and 5.12 μg/g. But in case of bioinoculants treated soil, the mean content of Res-Ni fraction was reported lower than the soils which was not treated with bioinoculants.

Table (6): post-harvest distribution of Ni (μg/g soil) into different forms as influenced by different chelating agents and bioinoculants in Ni enriched soil

Treatment	Ex-Ni	Carb- Ni	OM-Ni	MnOX- Ni	AFeOX-Ni	CFeOX-Ni	Res-Ni
Control	0.26	0.90	0.48	0.91	1.81	0.72	4.51
Ni_{100}	18.30	14.91	7.51	18.50	22.78	16.60	6.40
Ni_{100}+FYM	19.67	13.49	6.39	18.78	20.82	15.62	5.77
Ni_{100}+VC	18.87	14.70	6.75	19.05	19.71	16.33	5.12
Ni_{100}+EDTA	25.95	13.12	6.24	17.40	19.33	14.91	4.90
Control+M	0.35	0.88	0.41	0.80	1.72	0.77	4.35
Ni_{100}+M	18.76	15.10	8.14	17.80	22.95	16.85	6.00
Ni_{100}+FYM+M	19.80	13.60	7.18	18.36	21.08	15.75	5.50
Ni_{100}+VC+M	18.90	14.52	7.33	19.30	21.27	15.20	4.94
Ni_{100}+EDTA+M	26.12	13.80	6.03	17.11	19.00	15.35	4.44
CD at 5%	1.48	0.96	0.49	1.25	1.48	1.11	0.68

M: Microbial inoculants VC: Vermi Compost

CONCLUSIONS

It is evident that dominant Ni fraction was amorphous iron-oxide followed by exchangeable-Ni, crystalline iron-oxide, manganese oxide, carbonate, organic and residual Ni fraction. Application of chelating agents increased the exchangeable fraction causing an increase in its availability in the soils. It may be ascribed to the fact that EDTA targeted Ni mainly to amorphous and exchangeable fractions whereas other fractions were less effectively solubilized. It has been suggested that levels of Nickel in Fe-Mn oxide fraction depend on how much Mn oxide is absorbed in soil because Ni^{2+} can substitute for surface manganese in mixed valence Mn oxides. Similar results were confirmed by (Ahmed, 2000).

Application of chelating agent (EDTA) significantly increased the exchangeable fraction of Cd with a consequent decrease in carbonate, organic, MnOX, AFeOX, bound fractions. Application of FYM and vermicompost increased organic and carbonate fraction of Cd but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost increased organically bound fraction of Cd.

Application of chelating agents increased exchangeable fraction with a consequent decrease in organic fraction. Highest amount of exchangeable fraction was observed in EDTA treated soil. Application of FYM and vermicompost increased AFeOX fraction of Ni but decreased other fractions. In bioinoculants treated soil, addition of FYM and vermicompost also increased AFeOX fraction of Ni.

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INVITATION LETTER

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