INSTITUTE OF BIODIVERSITY AND ECOSYSTEM RESEARCH – BAS

UNION OF SCIENTISTS IN BULGARIA
SECTION BIOLOGY

INTERNATIONAL SEMINAR OF ECOLOGY – 2025

"Modern Ecology – Theoretical and Applied View"

September 25th – 26th, 2025, Sofia, Bulgaria
Hybrid event

Program & Abstracts

INTERNATIONAL SEMINAR OF ECOLOGY – 2025
"Modern Ecology – Theoretical and Applied View"

The Seminar of Ecology is organized by the Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences and the Section "Biology" – Union of Scientists in Bulgaria, Sofia.

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INTERNATIONAL SEMINAR OF ECOLOGY – 2025
"Modern Ecology – Theoretical and Applied View"

This book includes the program and abstracts submitted and accepted for presentation
at the International Seminar of Ecology – 2025,
"Modern Ecology – Theoretical and Applied View",
25 – 26 September 2025,
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THEMATIC SESSION I

BIODIVERSITY AND CONSERVATION BIOLOGY

PL01_01

ALIEN SPECIES IN THE BULGARIAN FLORA: A REVIEW

Vladimir Vladimirov

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Aim: Invasive alien species are recognized as one of the main threats to biodiversity worldwide. Therefore, in recent years, research on alien species, their distribution and impact has been increasing. Based on the accumulated scientific information, attempts are made to discover patterns and predict the invasive potential of the alien species. The aim of this study is to make a review of the alien species in the Bulgarian flora and to outline basic facts and patterns relevant to their control and minimisation of their negative impact.
Material and methods: The analysis is based on data from field research and a review of the main literary sources on alien species in the Bulgarian flora, published over the last 20 years.
Main results: In the last 20 years, alien species in the Bulgarian flora have been the subject of targeted surveys. As a result, the taxonomic composition, geographic origin and distribution of taxa, as well as the most vulnerable habitats and ecosystems, have been largely clarified. The pathways of introduction of alien species and their impact on local biodiversity, the economy and human health have been studied to a significantly lesser extent. Along with these main issues, the presentation will also discuss the main gaps in knowledge and directions for future research, as well as the weaknesses in organizing a national system for monitoring alien species and taking preventive measures or measures to limit the spread and negative impact of invasive plants.

KEYWORDS: Alien species, Bulgarian flora, invasive species, neophytes, vascular plants

Acknowledgements: This analysis has been carried out within the project '*The railway network as a pathway for the introduction and spread of alien plant species in Bulgaria*' funded by the Bulgarian National Science Fund (Contract КП-06-М81/4 of 04.12.2024).

PL01_02

INVASIVE ALIEN SPECIES AND CLIMATE CHANGE – INCREASING THREATS TO BIODIVERSITY: CASE STUDY ON ANIMAL SPECIES IN BULGARIA

Teodora Trichkova[1], Rumen Tomov[2], Milcho Todorov[1], Yordan Koshev[1], Simeon Lukanov[1]
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Aim: Invasive alien species (IAS) and climate change (CC) have been recognised as major and increasing threats to biodiversity and ecosystem services in Europe and worldwide. The aims of this review are: 1) to present information about invasive alien animal species in Bulgaria, including species lists, pathways of introduction and spread, and impact; 2) to emphasize on the effects of CC related to IAS; and 3) to highlight the necessity of applying comprehensive management measures.
Material and methods: The review is based on published and original data. It covers animal IAS of Union concern, but also animal IAS of concern to Bulgaria, belonging to all taxonomic and ecological groups. Categories of the Convention of Biological Diversity are used related to the pathways of introduction and spread of IAS, while categories according to the Environmental Impact Classification for Alien Taxa (EICAT) are used regarding the impact of IAS.
Main results: There are 11 animal IAS of Union concern and additional 20 animal IAS of Danube River Basin concern recorded in Bulgaria. Other IAS with potential negative impact on biodiversity have also been reported. The impact can be expressed by competition, predation, hybridisation, disease transmission, bio-fouling and other mechanisms. Human-caused CC can accelerate the introduction, establishment and spread, as well as the impact of animal IAS.
Conclusion: Management measures and strategies towards IAS should consider the effects of CC in Bulgaria.

KEYWORDS: Invasive alien animals, effects of climate change, impact, management

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***Online* PL01_03**

MARINE BIODIVERSITY AND ITS PROMINENT ROLE IN NOVEL DRUG DISCOVERY

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Aim: The ocean, a vast reservoir of biodiversity, represents an unparalleled source of novel bioactive compounds with immense potential for pharmaceutical development. Marine Pharmacognosy, the study of natural products derived from marine organisms, is a rapidly evolving field that seeks to discover and characterize these compounds for their therapeutic applications. Marine organisms have evolved in diverse and extreme environments (*e.g.* high pressure, salinity, low light). This chemical novelty has led to the development of unique metabolic pathways and the production of secondary metabolites with novel chemical structures, often distinct from those found in terrestrial organisms.
Material and methods: Advances in -omics technologies, including genomics, transcriptomics, and metabolomics, are revolutionizing marine pharmacognosy research. These tools enable the rapid identification of biosynthetic pathways, the characterization of complex metabolic profiles, and the targeted discovery of novel compounds. Furthermore, innovative extraction and purification techniques, coupled with high-throughput screening and bioassay-guided fractionation, are accelerating the process of drug discovery from marine sources.
Main results: Marine organisms are prolific producers of unique secondary metabolites with diverse bioactivities, such as anticancer, antimicrobial, anti-inflammatory, and neuroprotective properties. Marine natural products often serve as "lead compounds" for drug development. Their unique structures can be optimized through chemical synthesis to improve efficacy, reduce toxicity, and enhance pharmacokinetic properties.
Conclusion: The unparalleled biodiversity of marine ecosystems represents an extraordinary reservoir of novel chemical entities. Therefore, marine natural products are concluded as a vast and largely untapped resource with immense potential for addressing human health needs and providing solutions for various industries. The present talk will

showcase cutting-edge research on the isolation, structural elucidation, and biological evaluation of marine natural products.

KEYWORDS: Marine, biodiversity, marine organisms, drug discovery, marine pharmacognosy

***Online* PL01_04**

MOLECULAR ECOLOGY IN PRACTICE

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Aim: Use of molecular markers for inference about species affiliation or diversity dynamics on ecological processes or events related to Iberian wolf monitoring, bird insect predation and hosted insect microbial diversity.
Material and methods: Environmental sample matrices were DNA extracted using different DNA extraction methods. PCR-based techniques were used for barcoding analysis and species identification, monitoring and/or detection. DNA sequencing was performed using Sanger and NGS approaches (Illumina platform).
Main results: Results on molecular markers-based studies will be presented as case studies for three different ecological niches: i) Analysis on C*anis* species affiliation and Iberian wolf monitoring in Northern Portugal; ii) molecular detection of bird insect predation and iii) microbiome of an insect mycangia structure. Technological issues will be discussed for each strategy applied. Data showed how inference from molecular markers explains real life on nature.
Conclusion: Molecular markers are an effective tool to infer ecossystem diversity and population dynamics, even revealing hidden communities.

KEYWORDS: molecular marker, barcoding, population genetics

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Online **PL01_05**

DYNAMICS OF THE ADAPTATION PROCESS IN YEAST CELLS: A RESEARCH BY STATISTICAL MODELING

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Aim: Analyze of the process of adaptation of auxotrophic yeast cells.
Material and methods: Statistical modeling is used to describe the dynamics of cell adaptation and to study the variability of its genes and proteins.
Main results: Analysis of the emergence of adapted cells on nutrient media revealed a sequence of foci of quasi-equilibrium states of pseudo-wild-type cells (PWTC). The sequence of foci forms two groups associated with the cytosolic and nuclear-mitochondrial pathways of reactive oxygen species (ROS). The dispersion of foci expands over time. The frequency of PWTC in the foci of the cytosolic group decreases, and in the nuclear-mitochondrial one it increases.
Conclusion: We assume that changes in the intracellular environment lead to structural changes in the transcribed gene and mRNA underlying the sequence of PWTC foci. Changes in the environment also restructure the tertiary structure of proteins, which creates an expanding funnel of diversity of pseudo-equilibrium states of PWTC and reduces their number. Cell metabolism changes the intracellular environment. A continuous chain of adaptations is associated with the mutual influence of the intracellular environment, the structure of genes and proteins. Stress reduces the frequency and diversity of pseudo-wild cells in the cytosolic group and increases their frequency and stability in the nuclear-mitochondrial group. We show that the process of cellular adaptation is based on the laws of physics.

KEYWORDS: intracellular adaptation, auxotrophic cells, statistical approach, quasi-equilibrium states, changes in the structure of genes and proteins, energy landscape, stress effects

Acknowledgements: no financial support

PL01_06

SYSTEMATICS, PHYLOGENY, BIOGEOGRAPHY, FLOWER EVOLUTION AND POLLINATION BIOLOGY OF THE MEGA GENUS *SALVIA* L. (SAGE) IN LAMIACEAE (MINT FAMILY)

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Aim: The mega genus *Salvia* L., with approximately 1000 species, represents the largest genus in the mint family (Lamiaceae). Notably characterized by a unique staminal lever mechanism aiding in efficient pollination primarily by bees and birds. The aim of this study is to investigate the systematics, phylogeny, biogeography, flower evolution, and pollination biology of *Salvia*.

Material and methods: Approximately 500 specimens from around the globe were used for molecular phylogeny and dating, and biogeographic studies. Additionally, approximately 250 specimens were used for floral traits evolution studies. Although studies on the pollination ecology of the genus *Salvia* are limited, we have conducted studies on about ten sympatric species in three populations.

Main results: Phylogenetic analyses, including Sanger and Next Generation Sequencing approaches, strongly support *Salvia* as a monophyletic group when broadly defined

to include several smaller genera (*Dorystoechas*, *Meriandra*, *Perovskia*, *Rosmarinus*, *Zhumeria*). Molecular dating suggests a gradual increase in speciation rates over the past 32 million years, accelerating significantly within the last 15 million years, correlating with dispersal from its origin in Southwest Asia and the eastern Mediterranean region. Extensive pollination studies involving morphometric analyses and observations of floral visitors revealed distinct patterns of generalization and specialization among *Salvia* species. Ecological isolation mechanisms, such as mechanical differences in flower size, phenological separation in flowering times, and behavioral preferences of pollinators, significantly contribute to reproductive isolation and diversification. Pollinator networks indicate that while many *Salvia* species are ecological generalists, specific taxa exhibit extreme specialization.
Conclusion: This extensive study highlights *Salvia* sensu lato is a monophyletic genus and originated in Southwest Asia and the Mediterranean region. Staminal lever mechanism is a key innovation for the genus. Floral traits and ecological interactions, particularly pollinator specialization and generalization, play a critical role in the evolutionary diversification and reproductive isolation of the mega genus *Salvia*.

KEYWORDS: *Salvia*, phylogeny, speciation, staminal lever mechanism

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PL01_07

GENETIC AND CHEMICAL DIVERSITY OF *NEPETA* SPECIES IN THE CENTRAL BALKANS

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Aim: The aim of the present study was to determine sources of genetic and chemical diversity in *Nepeta nuda* L., *N. cataria* L., and Serbian stenoendemic *N. rtanjensis* Diklić

& Milojević at the inter- and intra-population levels towards prioritizing genotypes/ chemotypes for future conservation.
Material and methods: Leaf samples were collected from Central Balkans populations. Chemical diversity was assessed from both methanol extracts and essential oils through untargeted and targeted metabolomics using LC/MS and GC/MS instruments. Genetic diversity was evaluated based on two genomic (SSRs) and seven genic (EST-SSRs) microsatellite markers.
Main results: *N. rtanjensis* and *N. cataria* were recognized as high-productive species in words of nepetalactone-type iridoids, while *N. nuda* produced significantly lower amounts of these compounds. Genetical and chemical diversity of *N. nuda* primarily resides within populations, implying that metabolomic profiles may be less influenced by geographic distance and variable environmental conditions. Conversely, the analyzed populations of *N. rtanjensis* and *N. cataria* were small, containing nearly clonal individuals, regardless the geographical distance among the populations.
Conclusion: The results of the present study point to the urgency for the adequate conservation measures in the case of *N. rtanjensis* and *N. cataria*, which display a disturbingly low level of genetic variations across the Central Balkans. Protective measures should rely on novel molecular markers and analysis of more populations as well as on re-examining the growth performance, reproductive strategies, and phytochemical characteristics in variable environments.

KEYWORDS: *Nepeta cataria*, *Nepeta nuda*, *Nepeta rtanjensis*, microsatellites, chemical markers

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***Online* PL01_08**

VARIABILITY OF BACTERIA IN THE MOUNTAINS: INFLUENCE OF RADIATION BACKGROUND AND HIGH-ALTITUDE LANDSCAPE

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Aim: To analyze the factors of the mountainous landscape of Armenia that can lead to genetic changes and speciation of tularemia bacteria.
Material and methods: tularemia epizootics, rodent density, tularemia threshold in the populations of microbe carriers; PCR method, statistical analysis.
Main results: The analysis revealed the influence of high-mountain landscape on the outbreaks and infection rate of epizootics, i.e. the threshold of tularemia.
Conclusion: The main factors of variability and speciation of tularemia bacteria are the radiation background and high altitude landscape, as well as epigenetic mechanisms induced by low doses of radiation.

KEYWORDS: tularemia bacteria, epizootics, variability and speciation, gamma background of rocks, high altitude landscape, epigenetics, statistical analysis

Acknowledgements: no financial support

L01_01

EPOICOCLADIUS FLAVENS (MALLOCH, 1915) (DIPTERA: CHIRONOMIDAE): A NEW RECORD FROM REPUBLIC OF NORTH MACEDONIA

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Aim: The aim of the study is to report the novel finding of the phoretic midge *Epoicocladius flavens* (Malloch, 1915) on its mayfly host *Ephemera danica* Müller, 1764 (Ephemeroptera: Ephemeridae) for the entomofauna of North Macedonia.
Material and methods: The sampling was conducted during spring 2022 at the upstream region of Golema reka River watershed, Republic of North Macedonia. The species were collected with hydrobiological hand net (mesh size 500 μm), applying kick&sweep multihabitat procedure.
Results: The chironomid species *Epoicocladius flavens* was found in upper stretch of Golema reka River (North Macedonia) on its host the mayfly species *Ephemera danica*. This finding contributes to the enrichment of the entomofauna of Republic of North Macedonia, but also adds new data on the distribution of the midge *E. flavens* from Balkan Peninsula. According to the published data the species is distributed in Europe, North America and Asia. Usually found in greater numbers, in our case only one midge of *E. flavens* was noted on a single specimen of *E. danica*.
Conclusion: This study contributes to the knowledge about the diversity and distribution of the Chironomidae family in The Republic of North Macedonia.

KEYWORDS: Symbiotic species, commensalism, macroinvertebrates, stream, host, Balkan Peninsula

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L01_02

IN SITU AND *EX SITU* CONSERVATION OF THE VULNERABLE SPECIES *ASTRACANTHA THRACICA* FROM ITS ENDANGERED HABITATS IN BULGARIA

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Aim: The xeromorphic shrub *Astracantha thracica* (Griseb.) Podl. (Fabaceae) is a tertiary relict and Balkan endemic, protected by the Biodiversity Act, and included in the Red Data Book of Bulgaria as "Vulnerable" species, while its habitats are rated as "Endangered". The study aimed at *in vitro* propagation of *A. thracica*, acclimatization of the plants, creation of an *ex situ* collection and strengthening of the corresponding natural populations of the species in Bulgaria.
Material and methods: Seeds collected from three populations of the species in Bulgaria were used as starting material for plant propagation. Seed germination was assessed *in vitro*, in soil and in Petri dishes. *Ex vitro* adaptation of *in vitro* seedlings was performed in a phytotron, and then plant acclimatization continued in the unheated greenhouse of IBER. Some plants were used to create an *ex situ* collection in the IBER experimental field, others were returned to the wild to strengthen their populations.
Main results: A protocol for *in vitro* plant propagation was elaborated. Seed germination stimulated by consecutive immersions in boiling and ice water, increasing to 47% under *in vitro* conditions and 41% on wet paper in Petri dishes. Seedlings about 8 cm tall were *ex vitro* adapted in a phytotron, then 30 well-growing plants were further acclimatized in the greenhouse. In the fall of 2018, 8 plants originating from Bakadzhitsite locality, Yambol District, were transferred to the IBER *ex situ* collection, while 4 plants each originating from Lale Bair, Sliven district and Vodentzi, Haskovo District were returned to their natural populations to strengthen them and aid their recovery processes.
Conclusion: Monitoring of the individuals planted in their populations has proven their successful adaptation to the habitats. The plants in the IBER *ex situ* collection have been flowering for several years, with the bushes currently measuring about 50 cm in height and 70 cm in diameter.

KEYWORDS: *ex situ* collection, Fabaceae, *in situ* recovery activities strengthening populations, tertiary relict

Acknowledgements: The research work was partially supported by the Bulgarian Enterprise for management of environmental protection activities (Contract # 11233/ 10 August 2016) and the Project Life08NAT/BG/279 (www.bulplantnet-bg.skay.com) co-funded by the European Commition under the Life+ Programme and by the Ministry of Environment and Water of Bulgaria.

L01_03

IMPACT OF HEAVY METAL POLLUTION ON MICROBIAL FUNCTIONAL DIVERSITY IN SOILS NEAR TOPOLNITSA RIVER, SOUTHERN BULGARIA

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Aim: The study aims to assess the effects of heavy metal pollution on soil microbial metabolic activity and functional diversity, and to evaluate the ecological risk near Topolnitsa River by Biolog Ecoplate system.

Material and methods: Five samples (0-20 cm) polluted with Cu and co-contaminants Zn, Pb, Cd, and As were collected from the area near Topolnitsa River and its tributaries: sediment from Medetska River (S1), adjacent soils of the Topolnitsa River, Medetska River (S2), Zlatishka River (S3), Bunovska River (S4) and cultivated soil around Topolitsa River (S5). The total catabolic activity of bacteria was defined as average well carbon development (AWCD) and community-level physiological profiling (CLPP). The relationships between soil parameters, heavy metal concentration, AWCD, and diversity indices (Shannon (H`), Pielou (E`) and substrate richness (SR)) were analyzed using the Pearson's correlation statistical test (Past 4.13).

Main results: The soils were classified as slightly polluted S1, S2, S3, and heavily polluted S4 and S5. The AWCD values reflected the microbial community's metabolic activity across the different sampling sites, with the relatively high activity in S2 and S5. The most easily utilized carbon groups were as follows: CA (carboxylic acids)>AA (amino

acids)>AM (amines and amides)>POL (polymers)>CH (carbohydrates)>PHE (phenols) (except S3). The Pearson's correlation statistical test showed a strong positive correlation between Zn and As, and between Cd and Pb. A perfect correlation was found between the H` and E`, indicating a close relationship between microbial diversity and community balance. Additionally, soil moisture was positively associated with SR and with P_2O_5.
Conclusion: This study shows that heavy metal pollution influences microbial metabolism and functional diversity in soils near Topolnitsa River. Heavily polluted sites exhibit distinct functional shifts. Correlations between pollutants and microbial indices indicate stress responses. Microbial profiling emerges as a valuable method for ecological risk assessment in industrially impacted ecosystems.

KEYWORDS: heavy metals, soil microbiome, Biolog EcoPlate™, functional diversity

Acknowledgements: This study was financially supported by the Bulgarian Science Fund (Grant KP-06-N76/9/2023).

L01_04

ECOLOGY AND SYNTAXONOMY OF *RUBUS* L. SHRUBLANDS IN BULGARIA

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Aim: The aim of the research is to explore the syntaxonomy and ecology of shrublands, dominated by *Rubus* spp. on the territory of Bulgaria.
Material and methods: During the period 2020–2024 we collected 242 original phytocoenological relevés from communities dominated by *Rubus* spp. following the Braun-Blanquet approach and contributed to the Balkan Vegetation Database (EU-00-013). The plot size was 64 m^2. The hierarchical clustering was performed by the PC-ORD software package applying the Bray-Curtis dissimilarity and the flexible beta clustering algorithm. The species cover values were square root transformed and clusters were standardized to equal size. The diagnostic species were determined by calculating the Phi-coefficient and only the statistically significant values evaluated by Fisher's exact

test (P<0.05) were considered. Detrended Correspondence Analysis was used to reveal the major environmental gradients.
Main results: The Rubus shrublands were classified into 6 plant communities, dominated by *Rubus caesius, R. ulmifolius, R. hirtus, R. idaeus, R. canescens* and *R. grabowskii* (*thyrsanthus*). The most widespread communities were dominated by *Rubus caesius* (204 relevés), followed by *R. idaeus* (19) and *R. grabowskii* (10). The phytocoenoses of *Rubus caesius, R. ulmifolius, R. canescens* and *R. grabowskii* were developed as a successional stage on disturbed habitats, in the vicinity of forests, along roads, riverbeds, springs, within urban territories and hedgerows. The communities of *Rubus idaeus* occured in the mountainous and semi-mountainous regions in the county. All of the phytocoenoses were monodominant, with predominantly underdeveloped or missing herb layers. Litter accumulation and significant shading from the shrub layer were characteristic of them.
Conclusion: This is the first comprehensive research on the species of the *Rubus* genus in Bulgaria which communities are widespread. More analyses are necessary to classify them on the territory of the country to specific syntaxa and accurately place them in the syntaxonomical scheme of the Bulgarian vegetation.

KEYWORDS: shrublands, *Rubus* spp., syntaxonomy, ecology, plant community

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L01_05

DATA ON NESTING AND MORPHOMETRIC CHARACTERISTICS OF EGGS OF *STERNA VITTATA* (ANTARCTIC TERN) ON SOUTH SHETLAND ISLANDS

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Aim: Mapping all established nests in the colony of the Antarctic Tern (*Sterna vittata*). All available eggs were photographed and measured during both visits to the colony.
Material and methods: This study looks at 22 nests of the Antarctic Tern (*Sterna vittata*) in a colony on Livingston Island, Antarctica. The position of each nest in the colony was

recorded using a transect method. Researchers checked what was inside each nest and measured the eggs (length, width, and volume). All eggs were also photographed to compare their color patterns.

Main results: The colony is located on a rocky hill at the foot of Mount Charla, about 130 meters above sea level, near Johnsons Dock Bay. The nests are shallow holes lined with small stones. Out of the 22 nests studied: 12 had no eggs and already had chicks, 3 had two eggs, the rest had one egg each.

The distance between nests was different. On average, it was 3.2 meters for the first group of chicks and 2.8 meters for the second. The eggs were oval-shaped. Their color and markings varied a lot depending on where they were in the colony – from light brown and olive brown to dark brown.

The average egg size was: Length: 4.41 cm, Width: 3.26 cm, Volume: 1.471 cm^3.

Conclusion: The results add to the knowledge of the breeding biology of the Antarctic tern (*Sterna vittata*) on Livingstone Island and provide a basis for future comparisons between different colonies.

KEYWORDS: *Sterna vittata,* Antarctic Tern, eggs, Livingston Island, Antarctica

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L01_06

VEGETATION CLASSES DIVERSITY IN THE PLOVDIV AND RHODOPEAN FOOTHILLS GEOBOTANICAL REGIONS

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Aim: The aim of the research is to uncover the syntaxonomical diversity of the vegetation in the Plovdiv and Rhodopean foothills geobotanical regions at the class level.
Material and methods: We browsed the available phytocoenological literature for the study area and we found 21 publications that include 198 phytocoenological relevés. We excluded 74 of them due to being outdated or collected according to the Dominance approach and used 124 for the analyses. During the period 2020–2025 we sampled all vegetation types of the area intensively and collected 4670 relevés, following the Braun-Blanquet approach. The final dataset consists of 4744 relevés. Each releve includes a full species list, species cover/abundance, GPS coordinates, elevation, slope, exposition, soil moisture. All relevés were stored in the Balkan Vegetation Database (EU-00-013). The species names were standardized according to the Euro+Med PlantBase. We used the EuroVegChecklist Expert System and PC-ORD as catalog windows in the JUICE software to classify the relevés to class level. Diagnostic, constant and dominant species were determined for all classes.
Main results: The vegetation in the study area was classified to 33 classes. The most richests classes are *Papaveretea rhoeadis* (996), *Sisymbrietea* (523), *Phragmito-Magnocaricetea* (493), *Digitario sanguinalis-Eragrostietea minoris* (334), *Festuco-Brometea* (334), *Polygono-Poetea annuae* (215), *Epilobietea angustifolii* (191) and *Artemisietea vulgaris* (173).

Conclusion: As a result of this research, the two geobotanical regions are the most comprehensively studied in the country. The large number of relevés and recorded vegetation classes show the diversity of vegetation type in the region of the Thracian lowland. Even though the region is dominated by agrocoenoses, there are still semi-natural grassland, shrubland, forest and wetland phytocoenoses.

KEYWORDS: Bulgaria, Thracian plain, Rhodopean foothills, vegetation classification

Acknowledgements: The project was funded by the PhD subsidy of the Institute of Biodiversity and Ecosystems Research at the Bulgarian Academy of Sciences.

L01_07

DETERMINATION OF THE EFFECT OF PROLINE CONTENT IN *PINUS BRUTIA* TEN. NEEDLES ON HOST PREFERENCE OF THE PINE PROCESSIONARY MOTH (*THAUMETOPOEA WILKINSONI* TAMS.)

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Aim: *Pinus brutia* Ten., commonly known as Turkish red pine, is the most widely distributed conifer species in Türkiye. Due to its rapid growth and adaptability to large-scale afforestation, it holds significant ecological and economic value. However, one of the major biological threats to both natural and planted stands of *P. brutia* is defoliation caused by the pine processionary moth (*Thaumetopoea wilkinsoni* Tams.). In this study, we investigated changes in proline content in pine samples from both resistant and susceptible clones against *T. wilkinsoni* over one year in a clonal seed orchard near Antalya, Türkiye.
Material and methods: The *P. brutia* clones used in our study are located in a clonal seed orchard established in 1992. The orchard spans 17.8 hectares and contains approximately 1,900 trees representing 30 different clones. The method proposed by Bates et al. (1973) was used to determine the proline content of the trees belonging to the clones.
Main results: Statistically significant differences were observed in the proline content between the resistant and susceptible clones. In particular, differences in proline content between the clones were identified from November to April, which is the period when the pest begins to cause serious damage.

Conclusion: This study serves as an important roadmap for understanding the biology of pest species such as *T. wilkinsoni*, which pose significant threats to forest resources. The results obtained are important as they reveal which parameters of the pine needles the moth monitors while causing damage to *P. brutia*, and which parameters act as feeding barriers for the insect.

KEYWORDS: Forest Pest, Pine processionary Moth, *Pinus brutia*, Prolin, Türkiye

Acknowledgements: This research was financially supported by Turkish Science and Technological Research Council (TÜBİTAK) through Project No: 121Z362.

P01_01

CONTRIBUTION TO THE UNDERSTANDING OF THE YELLOW TIDES PHENOMENON IN THE COASTAL WATERS OF THE SOUTHERN ADRIATIC – BAY OF BOKA KOTORSKA

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Aim: Research on the composition and abundance of zooplankton and phytoplankton was conducted during the yellow tide caused by dinoflagellate bloom in the Bay of Kotor.
Material and methods: This study analyses zooplankton and phytoplankton samples collected from three stations in the Bay of Kotor over two months during the summer of 2016/17. Zooplankton was collected with Nansen nets (100 and 150 µm), and phytoplankton with 5litre Niskin bottles. Salinity and temperature were measured simultaneously with a CTD multisonde (Sea-Bird Electronics Inc., USA).
Main results: The Bay of Kotor is characterised by increased phytoplankton production compared to other bays within Boka Kotorska Bay complex, but the maximum values did not exceed 600,000 cells/L. During the study, elevated temperatures and eutrophication caused an explosive phytoplankton bloom, which turned the water yellow-red. Two species of dinoflagellates dominated: *Prorocentrum scutellum* and *Prorocentrum micans*. In the zooplankton, *Penilia avirostris*, *Paracalanus parvus*, *Centropages kröyeri*,

Oithona nana, and *Monotula subtilis* were dominant. In the surface layer, affected by the bloom, *C. kröyeri* dominated with over 90%. *O. nana* dominated throughout the rest of the water column, all the way down to the bottom.
Conclusion: Intense warming of the Bay of Kotor, prior to heavy rains, led to high surface water temperatures reaching 29 ºC. After the rains and runoff from the land, large quantities of soil with mineralised faecal matter were introduced into the Bay, causing a sudden increase in organic salt deposits, followed by a phytoplankton bloom and disruption of the ecological balance. In the shallow part of the Bay, the entire plankton community degraded due to the abrupt development of *Prorocentrum sp*.

KEYWORDS: zooplankton, phytoplankton, Bay of Kotor, Adriatic

Acknowledgements: This work was supported in part by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia through grants 451-03-137/2025-03/200123 and 451-03-136/2025-03/200287.

***Online* P01_02**

BIODIVERSITY OF LACTIC ACID BACTERIA AND YEASTS ISOLATED FROM NONCONVENTIONAL SOURCES

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In recent decades, alternative sources for isolation of lactic acid bacteria (LAB) and yeasts with physiological and biochemical characteristics of practical interest have been actively explored. Alternative sources, as a potential reservoir of new microbial strains, support the fermentation processes and organoleptic qualities of foods, innovative probiotic additives production, and cost-effective microbial transformations of renewable raw materials into biofuels and biochemicals.
Aim: This study aimed to isolate and identify LAB and yeasts from unconventional non-fermented plant sources (fresh fruits) and study their distribution and co-existence profiles.
Material and methods: LAB and yeast strains were isolated by classical techniques for the isolation of microbial pure cultures. They were identified by MALDI-TOF mass-spectrometry, and conventional species-specific PCR was used to confirm their taxonomic profiles.

Main results: a total of 30 LAB and 12 yeast strains were isolated from grape, apple (*Malus spp*.), plum (*Prunus spp*.), and pear (*Pyrus communis*). Their identification pattern showed that they belonged mainly to the lactobacilli group and ascomycetes yeasts. The LAB taxonomic profile revealed the predominance of *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* in grape, apple, and pear. The yeast strains were represented by *Pichia kudriavzevii*, *Pichia kluyveri*, and *Saccharomyces cerevisiae* – mainly in the grape's matrix. The most frequently observed co-occurrence patterns were between *L. plantarum* and *P. kluyveri* and *L. brevis* and *P. klyuiveri*.
Conclusion: The biodiversity profiles of the studied lactobacilli and yeasts could be used for planning a plant protection strategy based on the natural defense mechanisms, whose representative is the balanced plant microbiome.

KEYWORDS: lactobacilli, yeast, biodiversity, microbiome

Acknowledgments: This work is financially supported by SU Science Fund project No 80-10-84/27.05.2025.

P01_03

ESTABLISHMENT OF A PILOT PLANTATION OF *VALERIANA OFFICINALIS* L. USING *IN VITRO* CLONAL PROPAGATION

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Aim: *Valeriana officinalis* is a valuable medicinal plant species, with increasing market demand, but is under a special protection regime in Bulgaria. The present study aimed to establish a pilot valerian plantation using *in vitro* produced plants, starting from a selected individual.
Material and methods: A specimen of valerian from the *ex situ* collection of IBER was chosen as the mother plant due to its vigorous growth. Leaves, stems, raceme stalks, and leaf buds were disinfected and used as a source of primary explants to initiate *in vitro* cultures on two basic media: MS and B5. Besides, eight MS-based media supplemented with the cytokinins Kin or BAP alone or in combination with the auxins NAA or IBA

were tested during the consecutive sub-cultivations. Leaves and root segments from *in vitro* regenerated plants were used as secondary explants. *In vitro* plants were potted in soil mixture and gradually *ex vitro* adapted in a phytotron, then transferred to an open farm field.

Main results: The best primary explants were raceme stalks, with a regeneration potential of 67%. They formed whole *in vitro* plants, up to an average of 2.7 per explant, on medium MS supplemented with 0.5 mg/l Kin. The regeneration potential of both leaf and root secondary explants was only 8% on basal MS medium but increased up to 100% on media with plant growth regulators. *In vitro* leaves oriented with their adaxial side toward the medium formed roots. *In vitro* root segments were the best explants producing an average of 4 plantlets on medium containing 1 mg/l NAA and 0.5 mg/l Kin for about 6 weeks. A total of 150 plants were *ex vitro* adapted and successfully overwintered and bloomed.

Conclusion: A pilot plantation of *V. officinalis* was created. Over a thousand *in vitro* plants can be obtained from a single donor plant in a year.

KEYWORDS: medicinal plants, *in vitro* cultures, plant growth regulators, *ex vitro* adaptation

P01_04

DNA BARCODING IN THE IDENTIFICATION OF SPECIES WITHIN THE GENUS *DIGITALIS*

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Aim: Understanding the molecular basis of a plant taxon begins with its accurate identification, a task that may present a major challenge. Traditional plant taxonomy relies primarily on morphological characters, but these often lack the precision required for reliable classification. A more accurate approach is the integration of genetic data through DNA barcoding. Combining plastid DNA sequences with nuclear ITS regions is a powerful approach in species identification and plant phylogenetics. Plastid DNA, inherited maternally in most plants, provides information on evolutionary relationships, while nuclear ITS regions provide higher resolution for distinguishing closely related species.

Material and methods: Among the 27 recognized species within the genus *Digitali*, we analyzed five that occur in the Balkan Peninsula: *D. lanata* Ehrh., *D. laevigata* Waldst. & Kit., *D. ferruginea* L., *D. viridiflora* Lindl. and *D. grandiflora* Mill. (syn. *D. ambigua* Murray). Four plastid markers commonly used in molecular plant studies (*mat*K, *trn*L-F, *rbc*L and *psb*A-*trn*H), complemented with nuclear ITS regions, were PCR amplified and subsequently sequenced to facilitate genomic characterization.
Main results: The generated data for the five *Digitalis* species analyzed within the present study were integrated with the publicly available data to reconstruct the phylogenetic relations. The obtained ITS sequences facilitated species determination and aid in explaining phylogenetic relations among the analyzed taxa. In addition, the sequences of all plastid regions were deposited in GenBank, contributing to an improved resolution of the inferred phylogeny.
Conclusion: Integration of plastid and nuclear markers increases the accuracy of phylogenetic relations by enabling direct sequence comparisons with reference databases, thereby ensuring both unambiguous species identification and deeper insights into genetic evolution. In addition, continuous expansion of reference public databases with taxonomically representative genetic datasets can improve the discriminatory resolution within the genus *Digitalis* and thus the reliability of botanical identification of species.

KEYWORDS: DNA barcoding, *Digitalis* spp., ITS, *mat*K, *trn*L-F, *rbc*L, *psb*A-*trn*H

Acknowledgements: This project was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia and the Ministry of Science and Technology of the People's Republic of China, approved through the call for strategic bilateral scientific and technological cooperation: Comparative Analysis of High-Quality Germplasm Resources in China and Serbia: A Study on the Efficacy Components of Traditional Herbal Medicine Using Spatiotemporal Multidimensional Technology as well as by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (NITRA) (grant no. 451-03-66/2024-03/200007).

THEMATIC SESSION II

LONG-TERM ECOSYSTEM RESEARCH

***Online* PL02_01**

ECOLOGY, RELIGION AND ETHICS

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Aim: To link Ecology and Society mainly with Ethics and Religion.
Material and methods: Theoretical Research.
Main results: Ecology is a scientific discipline but has profound societal implications since it depends in many aspects from human activities and actions. Although ecology it is embedded on a bio system controlled by the forces of species evolution the recent appearance of man (homo sapiens) and the agricultural society first and then the industrialisation created impacts on the ecosystems going from species disappearance and pollution effects with its impact on climate cycles. The fact that most religions consider that the natural world was created by God this implies that man activity leading to disruption of bio systems with causes affecting economics and social systems has to be considered in relation to God Himself and puts in question the relationship, "the religion", itself. A new discipline of Theology, Ecotheology, is dealing with the interactions between Ecology and Theology. The result it is the acceptance of a new environmental conscience where man activities concerning the environment are becoming to be seen under the norms of Ethics.
Conclusion: Only with a new behaviour of man in respect of the results of ecology but also of the respect of the intrinsic nature and dignity of all beings may ensures sustainable progress for humanity.

KEYWORDS: Ecology, Ecotheology, Religion, Ethics

PL02_02

METHODOLOGICAL FRAMEWORK FOR INTEGRATED ASSESSMENT OF ECOSYSTEMS AND THEIR SERVICES IN NATURA ZONES IN BULGARIA

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Aim: To present the Framework for Integrated Ecosystem Assessment (FIEA) which is developed under the EU funded SELINA (Science for Evidence-Based and Sustainable Decisions about Natural Capital). The framework builds on the MAES, IPBES and Capitals Coalition schemes by making comparison between them, clustering different aspects into steps and integrating all relevant steps.
Material and methods: FIEA is a comprehensive and systematic approach to the planning, implementation and evaluation of the ecological, economic and social aspects of an ecosystem assessment and explicitly aims at informing and supporting management decisions. The Framework consists of six phases: frame, scope, design, assess, share, and act. Each phase has several steps that guide the user through the process of defining the purpose, selecting the methods and indicators, collecting and analyzing the data, and communicating and applying the results. Depending on the user group and the specific purpose of the assessment, the phases may vary in their level of detail. The Framework is based on existing Frameworks (i.e. the IPBES values assessment, the Capitals Coalition protocol, the "MAES" framework developed in ESMERALDA and the Integrated approach to planning) to suit the specific context and objectives of the respective Integrated Ecosystem Assessment.
Main results: The framework was adapted to the needs of the national scale mapping and assessment of ecosystems and their services in NATURA zones in Bulgaria. An updated typology of ecosystems and a set of indicators for ecosystem condition are proposed.
Conclusion: The framework provides a holistic and integrated view of the ecosystem and its services, and to support the implementation of ecosystem-based planning and management, fostering the incorporation of the value of nature into decision-making in order to move towards transformative societal change.

KEYWORDS: Integrated Ecosystem Assessment, MAES, IPBES, mapping of ecosystem services, decision-making

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L02_01

DEVELOPMENT OF LAND COVER IN CHERNA GORA MOUNTAIN, WESTERN BULGARIA

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This article explores land cover changes in Cherna Gora Mountain, part of the Kraishte region, over the period 1990–2018. Cherna Gora is one of Bulgaria's lesser-known mountains, as it remains underexplored and relatively obscure both scientifically and publicly. The mountain is not part of the NATURA 2000 network and contains no protected areas.
Aim: The aim of the study is to trace the development of land cover in the Cherna Gora Mountain.
Material and methods: This study is based on desk research utilizing CORINE Land Cover (CLC) data, incorporating all available datasets for Bulgaria (CLC 1990, 2000, 2006, 2012, 2018). Each dataset for the respective years reflects the transformation of land cover in Cherna Gora. A detailed analysis was conducted across all parts of the mountain, and the findings are presented in this article. The study also includes five maps generated using a GIS database.
Main results: The results indicate the most significant differences between the CLC 1990 and CLC 2000 datasets, compared to those from 2006, 2012, and 2018. This discrepancy is primarily attributed to the extended time gap. Notably, the CLC 2018 data reveal certain changes in specific land cover classes relative to CLC 2006 and 2012. For instance, while CLC 2000, 2006, and 2012 data indicate an expansion of agricultural land compared to CLC 1990, the CLC 2018 dataset shows a decline. Additionally, a reduction in forest and pasture areas is observed, alongside an increase in natural grasslands (meadows). The primary driver of these land cover changes in Cherna Gora appears to be rural depopulation.
Conclusion: The results show that the development of land cover has changed over the years but has been only slightly influenced by human activity.

KEYWORDS: Cherna gora, Land Cover, changes, GIS

Acknowledgements: none

L02_02

PRELIMINARY STUDY OF DIRECTIVE 92/43/EEC ON GRASSLAND HABITATS IN THE SOKOLNA RESERVE

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Aim: The goal of the study is to reveal the preliminary habitat diversity of Sokolna Reserve.
This article is a preliminary study of habitats in the Sokolna Reserve, located within the Central Balkan National Park. The National Park is one of the most valuable and largest protected areas in Europe – category II according to the International Union for Conservation of Nature (IUCN).
Material and methods: The study is based on information provided by the Ministry of Environment and Water and the mapping of the NATURA 2000 sites of the Central Balkans (BG0000494) and the Central Balkans – Buffer (BG0001493). The data are processed using a GIS software product. This information will be used as a basis for field surveys, mapping, and habitat verification.
Main results: The results of the study show that there is a high abundance of different groups of grassland habitats with typical grassland vegetation. A wide variety of grassland habitats are represented on the territory of the Sokolna Reserve, which is relatively high for the geographical area it covers. The investigation shows promising results that can be used as a basis for other studies in this area. As well as being suitable for the purposes of management of protected areas and zones, for monitoring the condition, and especially the conservation status of habitats.
Conclusion: The results show promise, and the study can be extended to the neighbouring areas.

KEYWORDS: habitats, research, vegetation, species

Acknowledgements: none

L02_03

IMPACTS OF INCREASING SEAWATER TEMPERATURES ON BENTHIC COASTAL ECOSYSTEMS IN THE SW BLACK SEA

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Aim: To investigate the long-term change in temperature of surface marine waters in the SW Black Sea coastal region (Burgas Bay) and study the possible impacts of temperature changes on the biodiversity, structure and functioning of selected coastal benthic ecosystems (reefs with algae and mussels, seagrass beds, soft-bottom communities).
Material and methods: Multiannual variation in temperatures was studied by combining and comparing continuous in-situ data collected with temperature loggers (2009 – 2023) and satellite derived data from satellite-based estimates of SSTs from the CMEMS Black Sea High Resolution L4 Sea Surface Temperature Reprocessed dataset (1982-2024), thus obtaining a continuous dataset with daily SSTs for the whole 1982-2024 period. Annual periods of optimal SSTs for growth and reproduction of important benthic species were calculated based on literature data (*Rapana venosa, Mytilus galloprovincialis, Cystoseira barbata* sensu lato, *Cystoseira crinita* sensu lato). Data on species composition and quantities (cover, biomass) of coastal reefs in the study area were obtained during scuba surveys and sampling (2010 vs 2023 – 24). The impact of marine heat waves (MHW) on sediment metabolism with and without *Zostera noltei* seagrasses were studied in mesocosm lab experiments.
Main results: We observed a continuous increase in average annual, seasonal and monthly SSTs and positive anomalies staring from 2005. Maximum summer SSTs were recorded in July 2010 (29.1^{O}C) and July 2024 (27.85^{O}C) continuing for a period of up to a month. Periods with optimal temperatures for the growth of black mussels decreased from 282-338 in the 1980s to t 239-247 in the 2010s, while those for the *R. venosa* increased from 40-70 to 90-120. This coincided with continuous destruction of mussel beds and decrease in their biomass and spatial distribution. Cystoseira macroalgal canopies remained stable between 2010 and 2024, despite increasing summer SSTs. Lab experimental results showed a change in nutrient and oxygen fluxes in seagrass and bare sands at temperatures above 28^{O}C, with decrease in O_2 penetration depth, increase in NH^{+4} and PO_3^{-4} sediment concentrations, as well as increased sediment stocks and sediment to water fluxes.
Conclusion: Increasing sweater temperatures and MHWs in the last decade in the coastal SW Black Sea have negative impacts on benthic communities' diversity, structure and functional parameters.

KEYWORDS: Black Sea, SST, marine heat wave, mussels, macroalgae, nutrient fluxes, climate change

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L02_04

RELATIONSHIPS BETWEEN PARAMETERS OF OCEAN ACIDIFICATION AND EUTROPHICATION IN THE NORTHERN BULGARIAN COASTAL ZONE

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Aim: To study of relationships between phytoplankton "blooms" and their impact on the elements of the carbonate system and acidification.
Material and methods: Multi-year field data of temperature, salinity, oxygen, pH, alkalinity, Chl-a were collected and analyzed (2019 – 2025; Varna Bay and Shkorpilovtsi Research Base). The *in-situ* data series were related to the information of automatic stations at the monitoring points Varna Bay – Karantina and Shkorpilovtsi (2023 – 2025). Based on these better time resolution data sets, a statistical analysis was conducted between the indicators of the two processes, eutrophication and acidification.
Main results: Well-defined spring and autumn "blooms" typical of the Black Sea were observed. In the last two years, long periods of phytoplankton "blooms" have been noticed during the summer season, and the cold months, such as January.
The typical spring-autumn "blooms" are characterized by a moderate to significant positive correlation between Chl-a and pH values (0,5-0,7), and a weak to moderate negative correlation between Chl-a and alkalinity.
Conclusion: Due to the specificity of its seawater, the Black Sea is less susceptible to acidification. However, the studied interrelationships between the two processes lead to destabilization of the buffer properties of seawater and facilitation of the acidification processes.

KEYWORDS: ocean acidification, pH, alkalinity, Chl-a, eutrophication, Black Sea

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L02_05

THE WHOLE SYSTEM APPROACH OF THE BULGARIAN LONG-TERM ECOSYSTEM RESEARCH NETWORK LTER-BG – TOWARDS A FUTURE OF LINKED DATA FROM THE ENTIRE NETWORK

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Aim: The advances in big data and artificial intelligence require a transformation of the research landscape. The European Long-Term Ecosystem Research Network (eLTER) is forming a Research Infrastructures consortium and Bulgaria became the first country to formally decide joining it. As a member of eLTER, LTER-BG is undergoing a transformation from a loose group of sites to a coherent national research infrastructure. We report the recent advancements on this track.

Material and methods: To harmonise the networks' activities, a strategic planning between sites is a continuous process. It includes:

1. Sharing expertise through network level activities, bilaterally between sites and through international cooperation;
2. Implementing the Standard Observations agreed by eLTER and their protocols;
3. Mobilisation of legacy data; ongoing collection of new data;
4. Semantic linking of data.

Main results: LTER-BG is developing a number of national structures: a coordination council planning the development directions, mobile laboratory including drone services, sharing of experts between sites, server infrastructure for data collection. Internationally, LTER-BG is active on setting priorities in standard observations and develops some of the protocols in hydrosphere observations. We participate in multiple scientific exchanges with European sites, including a data mobilization pilot for legacy data where Srebarna and Mesta were the only SEE sites to participate.
We present the advances in semantically linking these heterogeneous data sources to both the earlier ecosystem integrity indicator system of eLTER that groups indicators and parameters in structural and functional groups, and the currently adopted indicator structure by sphere (athmosphere, geosphere, hydrosphere, biosphere, and sociosphere).
Conclusion: The future directions of eLTER include transition to automated data processing pipelines that link FAIR data to Machine Learning and Artificial Intelligence to allow building digital twins. Bulgaria's contribution to this process is in the flexible semantic linking of different conceptual systems, legacy and current.

KEYWORDS: Standard Observations, Whole System approach, Semantic linking

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P02_01

LONG-TERM INSIGHTS FROM ZOOPLANKTON LTER-BG RESEARCH: A CASE STUDY FROM THE BULGARIAN BLACK SEA COAST

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Aim: This study aims to utilize long-term ecological research (LTER) to enhance understanding of marine ecosystem dynamics, with a focus on zooplankton as sensitive

indicators of environmental variability and their crucial role in connecting primary producers to higher trophic levels.

Material and methods: This study presents a comprehensive analysis of almost 20-year dataset (2000 – 2023) of zooplankton communities collected along the Bulgarian Black Sea coast at two areas (c. Galata with Varna Bay and c. Kaliakra) recognized as macrosite Black Sea in LTER – BG. The dataset, collected through consistent methodologies across coastal stations, includes high-resolution records of species composition, abundance, and biomass.

Main results: Our analysis reveals significant long-term trends, including shifts in biodiversity, density and biomass, comparing both areas. Correlations with environmental parameters – such as rising sea surface temperatures, salinity fluctuations, nutrient enrichment, and oxygen availability – indicate that both climate variability and human pressures (e.g., eutrophication, overfishing) have played substantial roles in reshaping community dynamics. Notable shifts are evident after 2010, aligning with climatic dynamics in the Black Sea region. These shifts suggest a reorganization of zooplankton assemblages with potential cascading effects on fisheries productivity and biogeochemical cycles. This long-term case study emphasizes the critical need for sustained monitoring efforts to detect subtle and cumulative ecosystem changes.

Conclusion: By integrating zooplankton LTER data with regional oceanographic and climate datasets, we provide a robust framework for understanding ecological resilience and vulnerability in semi-enclosed seas. The findings contribute to the broader LTER mission by supporting adaptive ecosystem management and offering a scientific basis for forecasting future ecological scenarios in the Black Sea and beyond.

KEYWORDS: Marine plankton fauna, Ecosystem monitoring, biodiversity, indicators

Acknowledgements: The study is supported by the Projects: Upgrading of distributed scientific infrastructure – Bulgarian Network for Long-Term Ecosystem Research (LTER-BG), (agreements with Ministry of Education and Science DO1-320/30.11.2023).

Online **P02_02**

EFFECT OF AERATION ON ENERGY SPECTRA OF WATER

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Aim: Water is one of the most important factors in nature, and in human life and activity. This is due not only to its huge reserves, prevalence and accessibility, but also a number of unique physical and chemical properties. Being an important component of living organisms, biological, physical and chemical processes take place in it. The aim of this work is to investigate in laboratory conditions the influence of aeration process on water and how it affects its energy spectrum, to check the presence of 'memory' under such influence.
Material and methods: In nature, aeration occurs naturally during turbulent motion in the air environment, in falling rainwater, in waterfalls, sea wave crests, surf, etc. This process is important, for example, for some aquatic organisms that require more oxygen-saturated water. It can also be useful for the development of some plants. In this article, we study the energy spectra (obtained by the method of Prof. A. Antovov) of water samples enriched with air, and we trace the residual effect of aeration on these spectra over time, noting a kind of "memory" of water, observed under other influences and by other authors.
Main results: Sets of pairs, an aerated sample and a non-aerated control, were investigated. Measurements were taken immediately after aeration, after 6 hours, 30 hours and 54 hours. The corresponding relationships were plotted graphically and their mean energies were calculated. The results of the mean energies of the sample and control in this case decreased 'with a fraction of a hyperbola'.
Conclusion: The results show that aeration significantly changes the spectrum of deionized water. A residual effect (presence of 'memory') is observed for more than 30 h but less than 54 h.

KEYWORDS: deionized water, energy spectrum, aeration

THEMATIC SESSION III

ENVIRONMENTAL STRESS FACTORS – IMPACT AND PROTECTION

PL03_01

THE CONTRIBUTION OF ANTIOXIDANT AND CHAPERONE SYSTEMS TO THE FORMATION OF AN ADAPTIVE RESPONSE INDUCED BY OXIDATIVE STRESS IN MODEL ORGANISMS: A REVIEW

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The aim: To clarify the contribution of antioxidant and chaperone systems to the formation of oxidative stress-induced adaptive response (AR) in model organisms. Here, we want to test the hypothesis that both antioxidant and chaperone systems could play a role in the formation of AR induced by oxidative stress.
Material and methods: A comprehensive research on the available literature was conducted across multiple academic databases, including PubMed, ScienceDirect, Web of Science, etc. The Research was confined to experimental studies published in English and Russian. Our results were compared to the information gained.
Main results: In addition to literature data, our results with paraquat on the *Chlamydomonas reinhardtii* test system illustrate the partial contribution of the antioxidant system and revealed dependence on the strain's genotype. On the other hand, 1.5-fold higher ROS levels were detected after low-dose treatment in *Saccharomyces cerevisiae*. Research on other model systems reveals the increased production of antioxidant enzymes when pretreatment with low-dose stressors is applied, resulting in resistance to higher doses of the same or different stressors.
Concerning the Hsp70 family of proteins, it was found that the modulation of the activity level of Cu/Zn-SOD and the levels of the cytoplasmic HSP70 and chloroplast HSP70B chaperones in higher plants are coordinated, indicating the interaction of these

two cellular defense systems under heat stress. The chaperone overexpression has been detected after pretreatment with some types of stressors. Similar overexpression of other small HSPs had also been found to lead to an induced increase in glutathione (GSH). This could also serve as an example for the relationship between the two systems in terms of the formation of an adaptive response.
Conclusion: The future perspectives in such studies should be directed to the evaluation of the potential relationship among DNA repair, antioxidant and chaperone systems in the formation of AR.

KEYWORDS: adaptive response, antioxidant system, heat-shock proteins

Acknowledgements: This work was performed under the project KP-06-N71/13 "Contribution and relationship of DNA double-strand breaks' repair, chaperone, and antioxidant systems for the formation of an adaptive response", funded by the Bulgarian National Science Fund – Ministry of Education and Science.

PL03_02

SPREAD OF ANTIBIOTIC RESISTANCE GENES AMONG THE MICROFLORA OF ANTARCTIC ANIMALS

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Aim: Studying the spread among Antarctic animals' fecal microbiotas of antibiotic resistance genetic determinants (ARGD), determining resistance to antibiotics used only in human medicine.
Material and methods: DNA isolation; PCR with primers targeting different ARGD; agarose gel electrophoresis.
Main results: 22 Southern sea elephant (*Mirounga leonina*) samples, 35 gentoo penguin (*Pygoscelis papua*) and 3 krill samples were collected and analyzed for the presence of 29 types of ARGD. Among the sea elephants, 59% were positive for vancomycin A ARGD associated with different enterococcal species, 31% for aminoglycoside ARGD, associated mainly with *E. coli*, 27% for carbapenems ARGD, associated with *Pseudomonas* and *Acinetobacter* species, and finally 23% for colistin ARGD, associated with *E. coli* and *Klebsiella*. Concerning the gentoo penguin, 42% of the samples were positive for the presence of IMP-type metallo-ß-lactamase ARGD, associated

with *Klebsiella* and *Pseudomonas* species, 17% methicillin ARGD, associated with staphylococcal species, and 14% for colistin ARGD. All three krill samples were positive for vancomycin B ARGD, associated with members of the genus Enterococcus. Single samples were also positive for most of the other ARGD we investigated; however, even more disturbingly, many of the samples were positive for two different ARGD, and some were also positive for three or more ARGD.

Conclusion: Until recently, due to its remoteness, accessibility difficulties, lack of economic activities, and minimal human presence, Antarctica was considered a pristine and largely untouched natural environment, largely unaltered by human influence. Unfortunately, experimental data acquired during the 30th and the 31st Bulgarian Antarctic Expeditions revealed substantial evidence that this could be a largely overoptimistic statement, especially in the case of the spread of ARGD determining resistance to antibiotics used only in human medicine, but not in agriculture and/or aquaculture.

Acknowledgements: "Spatial and stratificational characterization of the microflora of the surface waters up to 50 m in depth in the South Bay in Livingston Island by NGS-based metagenomics and isolation of bacteria with potential for the biotechnologies, the industry, and other domains", National Center for Polar Studies, Grant № 70-25-11/15.01.2024.

PL03_03

ENVIRONMENTAL TOXICITY: THE ROLE OF CLIMATE CHANGE

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Aim: This lecture explores how climate change alters pesticide behavior and toxicity, affecting ecosystems and human health. It examines how rising temperatures, shifting rainfall, and extreme weather influence pesticide fate and impact, emphasizing the urgent need for adaptive risk assessments and regulatory strategies in our rapidly changing environment.

Material and methods: This lecture integrates environmental science, toxicology, and climate research to examine how climate change alters pesticide behavior and toxicity. Drawing on literature reviews, models, and case studies, it highlights increased contamination under extreme weather. Laboratory and field data reveal organismal

responses to combined stressors. The lecture also critiques current regulations and proposes adaptive strategies for risk assessment and policy.

Main results: Climate change significantly influencing the environmental behavior and toxicity of pesticides. Elevated temperatures increase pesticide volatilization and degradation, often producing more toxic byproducts such as Oxon derivatives from organophosphates. Shifting precipitation patterns lead to greater surface runoff and leaching, raising pesticide concentrations in aquatic systems, especially during storm events. Organisms under climate-induced stress, like heat or drought, show heightened sensitivity to pesticide exposure, as seen in pollinators exposed to neonicotinoids. These findings highlight the urgent need for adaptive pesticide risk assessments and updated regulatory frameworks that account for climate variability and regional environmental conditions.

Conclusion: Climate change is reshaping pesticide behavior and toxicity, increasing risks to ecosystems and human health. Higher temperatures and altered rainfall boost pesticide mobility and harmful byproducts. Organisms under climate stress are more vulnerable. These changes demand urgent updates to risk assessments and regulations to protect environmental and public health.

KEYWORDS: Climate change, Pesticide toxicity, Environmental fate, Adaptive risk assessment, one health

PL03_04

HOW MUCH OF HYDROLOGICAL STRESS STRESSES FRESHWATER ECOSYSTEMS AND HOW?

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Natural hydrological regimes are often modified by a wide array of natural or human-impacted factors, consequently resulting into habitat deterioration, fragmentation of physical environment, shore instability, decline of food resources or even aid degradation of ecosystem integrity. As a consequence of the anticipated increase of frequency and magnitude of extreme climatic events, freshwater ecosystems, already damaged by the overuse of water, stand to be impacted even further by global changes. This explicates the increased research effort on the responses of inland freshwater systems to hydrological pressures and the potential consequences for the integrity of whole ecosystem from

the second half of the 20th century and yet more so over the last decades, the output of which efforts I aim to briefly discuss during my talk. I will also present some case studies and examples of tools we employ in order to better comprehend the relationships among hydrological stress, aquatic habitats and invertebrate biota. We will see that hydrological stress affects various levels of community organisation in aquatic (and adjacent terrestrial) ecosystems.

KEYWORDS: freshwater systems, mesocosms, observational studies, water-level fluctuations, ecosystem response

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***Online* PL03_05**

UNRAVELING THE TOXICITY OF ENVIRONMENTAL POLLUTANTS AND THEIR DEGRADATION BY NON-THERMAL PLASMA: INSIGHTS FROM ORGANISMS LACKING AN ENDOCRINE SYSTEM

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Environmental contamination with endocrine-disrupting chemicals such as bisphenol A (BPA) poses a significant threat to ecosystems and human health. While most research

focuses on organisms with endocrine systems, little is known about the effects of such pollutants – and especially their degradation products – on organisms lacking endocrine regulation. In this lecture, we present findings on the toxicity of BPA and its degradation products, generated by non-thermal plasma (NTP), in different model organisms that include two yeast species (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe)*, nematode *Caenorhabditis elegans*, and plants *Pisum sativum* and *Hordeum vulgare*.
We demonstrate the efficiency of NTP in BPA degradation and assess the biological activity of the resulting by-products through phenotypic and molecular responses in the tested organisms. Our analysis includes viability assays, oxidative stress markers, morphological alterations, and gene expression profiling related to stress responses. These results highlight that even organisms without classical endocrine systems can exhibit sensitivity to both BPA and its degradation intermediates. This underscores the importance of evaluating the ecological safety of advanced remediation technologies such as NTP, not only in terms of pollutant removal efficiency but also with respect to the biological impact of the resulting compounds.

KEYWORDS: bisphenol A, non-thermal plasma, toxicity, degradation

Acknowledgements: This work was supported by the Slovak Research and Development Agency (APVV-22-0247), and the Slovak Grant Agency for Science VEGA (1/0443/25).

***Online* PL03_06**

INTEGRATED APPROACHES FOR RISK ASSESSMENT OF ENVIROMENTAL POLLUTANTS WITH A FOCUS ON GENOTOXICITY

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Aim: The impact of emerging chemical pollutants on the status and functioning of ecosystems is widely recognized. In Europe, the REACH Regulation has registered over 100,000 chemical substances that are released daily into the environment. This challenge underlines the urgent need for innovative, fast and reliable monitoring methods. Within this framework, the inclusion of Effect-Based Methods (EBMs) in monitoring programs has been emphasized.

Material and methods: EBMs are *in vivo* and *in vitro* bioassays that can be employed as an integrated approach to environment quality monitoring. EBMs allow for the evaluation of direct effects on organisms, generating data at the individual level that can be extrapolated to the population level. In this context, the assessment of genome stability through genotoxicity biomarkers can enhance conventional physicochemical methods for environmental risk assessment by the analysis of DNA damage in organisms exposed to pollution sources. Since DNA is a common molecular target across living organisms, genotoxicity data may provide comprehensive insights relevant for risk assessment. Several EBMs are availabe for the detection of genotoxic effects, which result from damage to DNA or cellular structures responsible for maintaining genome stability. To implement EBMs for research and policy uses, the present study involved a network of institutions with the aim to integrate chemical, toxicology and genotoxicology methods for the monitoring of surface water bodies. Different eco-genotoxicological assays were applied to detect genotoxic activity of water samples from Tiber river, concurrently characterized by chemical analysis.
Main results: The EBMs revealed widespread chemical pollution and ecotoxicological effects across three sampling stations, with genotoxic effects confirmed using different tests and organisms. The chemical analyses confirmed the presence of emerging contaminants, such as pharmaceuticals, pesticides, personal care products, PFAS.
Conclusion: These findings support the integrated use of EBMs for future studies to monitor chemical contamination and adverse effects on the ecosystem.

KEYWORDS: surface water, genotoxicity, Effect-Based Methods

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INTERNATIONAL SEMINAR OF ECOLOGY – 2025
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PL03_07

MISMATCH AND RECOMBINATION REPAIRS – POSSIBLE PLAYERS IN THE FORMATION OF AN ADAPTIVE RESPONSE: A REVIEW

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Aim: Here we focus our attention on an evolutionary developed, non-specific phenomenon labeled the "Adaptive Response (AR)". AR has been described as a phenomenon that could be induced by various physical and chemical agents when cells have been primed with a low dose of these or other agents and after an inter-treatment time specific for each tested organism, a very high test dose has been given.

Material and methods: The magnitude of AR could be measured by various endpoints – increased cell survival, reduced mutation rates or levels of double-strand breaks (DSBs), or decrease of other damages.

Main results: We discuss the main model organisms, reply to the question of why unicellular green algae and yeast are promising model organisms for studying AR, and the importance of this knowledge for both the environment and human health. We also present information gathered in recent years regarding the possible major players involved in AR formation – three DNA repair systems – DSBs, Mismatch (MMR), and Recombination (rec) repair.

The fundamental role of accelerated DSBs rejoining for the formation of AR was documented by us previously. This process was elucidated to depend on several factors as genotype resistance, the level of priming dose, the inter-treatment and recovery time. The triggering effect of the priming dose was defined as a low level of cell lethality (LD_{20}) or around 2.5-fold increased levels of DSBs compared to those in untreated control cells. Currently, little is known about the contribution of the two repair pathways for the formation of AR – MMR and *rec*. According present state of knowledge, the MMR mechanism is also believed to interact directly with NER and homologous recombination.

Conclusion: This finding indicated that the various DNA repair pathways are not separated, but well interlinked.

KEYWORDS: Adaptive response, mismatch repair, recombinational repair

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PL03_08

SUSTAINABLE MANAGEMENT AND RESTORATION OF THE FERTILITY OF DAMAGED AND CONTAMINATED LANDS AND SOILS

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Aim: This paper is related to a description of the existing methods for technical and biological reclamation with the aim of restoring the fertility of disturbed lands and soils by using humus soil material. New, innovative technologies are recommended to smoothly restore soil fertility by using waste products.
Material and methods: The two stages of reclamation – technical and biological – are presented and the advantages and disadvantages of the various methods of their implementation are examined.Various technological solutions have been justified to restore the fertility of damaged terrain by using reclamation substrates.
Main results: Technological developments for the re-cultivation of landfills by using sludge from waste water treatment plants (WWTPs), which compensate for the shortage of humus materials, have been considered. The characteristics of the sediments reveal that they are an organic mass rich in macro and trace elements, and can be used as a fertilizer.
Conclusion: The conducted long-term laboratory, vegetative and field studies have shown that re-cultivation activities are accompanied by various technical, practical and financial difficulties and problems, namely:

1. The use of humus material in the technical construction of re cultivated terrains leads to a relatively fast recovery of soil fertility but is connected with spending of significant financial resources.
2. The biological stage of re-cultivation is very long, accompanied by planting of a large number of cultures.
3. The technically constructed re-cultivated areas are characterized by different unfavourable features – heavy mechanical composition, low natural fertility, heavy metal content, toxic levels of media reaction (pH), etc.

KEYWORDS: methods of technical and biological re-cultivation, damaged lands, soils, sludge from waste water treatment plants

PL03_09

ENVIRONMENTAL ASPECTS OF TESTICULAR DYSGENESIS SYNDROME (TDS) – PHENOTYPE, ORIGIN AND CELLULAR MECHANISMS

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Aim: It is known that human is exposed to various environmental chemicals known as endocrine disruptors considered as serious risk factors for general health. Male reproductive disorders manifested at birth (cryptorchidism, hypospadias) or in young adulthood (low sperm count, testicular germ cell cancer) are remarkably common, and increasing in incidence. They comprise testicular dysgenesis syndrome (TDS) with a common origin in fetal life related to subtle deficiencies in fetal androgen (testosterone) production. The aim the lecture is to provide recent knowledge on the cellular and molecular mechanism of TDS.

Material and methods: For understanding the phenotype ad mechanisms involved in TDS an experimental model for induction of TDS was developed in rat by gestational exposure to anti-androgenic compound dibutyl phthalate (DBP).

Main results: In fetal testis TDS has a distinct morphology of interstitial cells expressing tubular Sertoli cell markers (AMH, GATA-4). Postnatally, dysgenetic areas are manifested by ectopic expression in seminiferous tubules of 3βHSD, a marker enzyme for steroidogenic interstitial Leydig cell (LCs). COUP-TFII (chicken ovalbumin upstream promoter transcription factor-II) was identified as a cellular biomarker for impaired development of fetal LCs after in utero exposure to DBP. Abnormal persistence of COUP-TFII in fetal LCs is responsible for their dysfunction (T production) within MPW (masculinization programing window) being important in determining the risk and severity of down-stream TDS disorders in later life. Molecular mechanism of action of DPB involved downregulation of key genes for steroidogenesis (StAR, Cyp11a, Cytp17a, 3βHSD) in LCs by epigenetic change (increased methylation/H3K27me3), resulting in compensated adult LC failure manifested by low testosterone and elevated luteinizing hormone levels.

Conclusion: Androgen action during MPW is important for later correct development of male reproductive organs and their final size. Phthalate exposure during fetal life can seriously affect pubertal development and reproductive capability of adult men.

KEYWORDS: endocrine disruptors, phthalates, testis, TDS, Leydig cells, male infertility

Online **L03_01**

COMPARATIVE STUDY OF MICROPLASTIC BIOACCUMULATION IN MOLLUSK SPECIES FROM SOZOPOL BAY (BLACK SEA, BULGARIA): ABUNDANCE, TYPE, SIZE, AND SHAPE

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Plastic pollution is an escalating concern in marine ecosystems, with microplastics (MPs) raising particular risks due to their potential to cause physical and chemical harm to organisms. These impacts can impair vital physiological functions and may contribute to ecosystem disruption. The MPs' presence in commercially important seafood species raises concerns about human exposure through consumption. Understanding the extent and nature of MPs contamination in bivalves is essential for identifying reliable bioindicators for ecological risk assessment, guiding monitoring and mitigation strategies.

Aim: Comparative analysis of accumulation, composition, and characteristics of MPs in *Magallana gigas* (Thunberg, 1793) and *Mytilus galloprovincialis* Lamarck, 1819, from Sozopol Bay (Black Sea), as potential bioindicators of MPs pollution, health, and ecological risks.

Material and methods: *M. galloprovincialis* and *M. gigas* specimens from Sozopol Bay were collected by scuba diving. The presence of MPS in their soft tissues was analyzed using the Agilent 8700 LDIR Chemical Imaging System.

Main results: MPs were detected in 86% of the analyzed individuals, with a significantly higher load in oysters than in mussels (4.53 vs. 0.15 MPs/gram soft tissue). Most particles were rounded or irregular fragments. Polyethylene was the dominant polymer in *M. galloprovincialis* (50%), while rubber prevailed in *M. gigas* (37%). Most of the MPs (61.78%) were in the 20–50 μm size range.

Conclusion: Understanding the extent and nature of MPs accumulation in bivalves not only underscores their role as sentinels of environmental health but also provides critical insight for identifying pollution sources. The results proved substantial MPs' contamination in Sozopol Bay, likely driven by human coastal activities, including tourism. The analysis indicated that *M. galloprovincialis* is a more suitable bioindicator

for MPs pollution and ecological risk assessment, which can be used to inform monitoring and targeted actions to mitigate MPs' inputs and support efforts to preserve coastal marine ecosystems.

KEYWORDS: Black Sea, bivalves, microplastics, ecological risk

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L03_02

IDENTIFICATION AND QUANTIFICATION OF MICROPLASTICS IN BIOTA USING NILE RED DYE

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Aim: This work presents the development of a low-cost, user-friendly, and rapid method for microplastic identification in biological samples using Nile Red dye staining.
Material and methods: A two-step digestion was tested and adapted for processing biological samples to reduce false-positive results after Nile Red staining. Nile red dye in acetone and blue light was used for the fluorescence. A CNC-based device with a microscope camera was constructed to capture images of the filters for rapid microplastic identification. The fluorescent particle analysis was performed by using an adapted for the purpose of this study tool.
Main results: The digestion method, followed by capture and identification using the developed device, was tested on 27 *Mytilus galloprovincialis* spacimens from two locations and 40 *Rapana venosa* spacimens from seven locations along the Bulgarian Black Sea coast. Microplastics were detected in *M. galloprovincialis* at both sampling sites, while no microplastics were found in *R. venosa* specimens at three of the seven locations. The average microplastic concentration was 2 ± 3 MPs/g wet weight (WW) in *M. galloprovincialis* and 1 ± 7 MPs/g WW in *R. venosa*. The device successfully identified particles as small as 30 μm, with particles between 30–100 μm comprising 75% of all detected microplastics. The device and software are capable of capturing and analyzing a single sample in 15 minutes.
Conclusion: The two-step digestion method effectively reduces organic material in the

sample, minimizing the risk of false-positive results. The device is easy to build and use, requiring no special skills. It is non-destructive, preserving samples for further analysis. It detects particles as small as 30 µm and offers a fast, low-cost screening solution suitable for research and non-profit organizations working on microplastic monitoring.

KEYWORDS: Microplastics, Nile red staining, KOH and H_2O_2 digestion, Biota

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L03_03

SMALL MAMMALS AS MODEL ORGANISMS FOR STUDYING DNA DAMAGE INDUCED BY IONIZING RADIATION AND RADIOMIMETIC COMPOUNDS

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Ionizing radiation (IR) and radio-mimetic compounds (RMCs) cause DNA damage, such as single-strand breaks, double-strand breaks, and base modifications, leading to genomic instability, mutagenic and carcinogenic effects. Understanding DNA damage induction, repair mechanisms, and consequences is essential for risk assessment, radiation protection, and targeted therapies. Small mammals are valuable models due to their physiological and genetic similarities to humans, enabling controlled research and relevant translational insights.

Aim: The aim is to comprehensively review and synthesize the current knowledge derived from studies utilizing small mammals, investigating DNA damage induction, detection, and repair, as well as the long-term biological consequences following exposure to IR and various RMCs.

Material and methods: A systematic literature search was conducted across major scientific databases, focusing on the biological effects of genotoxic agents such as UV radiation, IR (e.g., alpha, beta, and gamma rays), and RMCs (e.g., zeocin from

the bleomycin family). Commonly evaluated endpoints include DNA damage (e.g., γ-H2AX, chromosome aberration analysis, micronucleus test) and apoptosis.
Main results: Small mammals are crucial for understanding dose-response relationships of DNA damage (single-strand breaks, double-strand breaks, base damage), evaluating DNA repair mechanisms, and identifying genetic and environmental factors influencing radiosensitivity. Findings highlight their importance in validating exposure and effect biomarkers, assessing radioprotective and radiosensitizing agents, and examining tissue-specific responses and bystander effects in a relevant framework. These studies also provide insights into long-term health effects, such as cancer incidence from genotoxic exposure.
Conclusion: Small mammals remain indispensable *in vivo* radiobiology and toxicology research tools. Their physiological relevance, genetic manipulability, and well-characterized responses to genotoxic stress make them invaluable for advancing our understanding of DNA damage and repair mechanisms and developing strategies to mitigate the adverse effects of radiation and RMCs.

KEYWORDS: small mammals, DNA damage, ionizing radiation, radiomimetic compounds

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L03_04

SHORT-TERM POLYSTYRENE MICROPLASTIC EXPOSURE INDUCES ORGAN-AND SEX-SPECIFIC ANTIOXIDANT RESPONSES IN MICE

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Plastic pollution has become a serious global problem, affecting all living organisms, including humans. Microplastics (MPs) have the potential to penetrate cell membranes

and have been detected in various organs. Their ability to induce damage to organisms draws increasing attention. Polystyrene (PS) is a widely used plastic and a significant environmental pollutant. Laboratory experiments with PS-MPs exposure have been shown to cause liver and brain disorders and affect fetal development. The underlying pathological mechanisms involve inflammation and oxidative stress.

Aim: This pilot study aimed to investigate the PS-MPs' effects on the antioxidant defense system of various organs following short-term exposure in an experimental animal model.

Materials and methods: Male and female SWISS albino mice were randomly divided into (1) Control, and (2) PS-MPs-exposed groups. The control group had ad libitum access to purified water, while the PS-MPs group received 1 μm PS-MPs suspended in purified water at a dose of 0.1 mg/day for 14 days. At the end of the treatment period, animals were euthanized, and the liver, kidneys, lungs, heart, and spleen were dissected. The activities of the organs'antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were measured spectrophotometrically.

Main results: The results showed that 14-day exposure to PS-MPs led to sex- and organ-specific alterations in antioxidant enzyme activities. Comparing to the controls, in male mice, a significant decrease in SOD and GPx activities and increase in CAT and GST were observed in lungs, elevated SOD and GST activities in the liver, whereas in female mice SOD activity in liver was increased.

Conclusion: These findings suggest that even short-term exposure to PS-MPs can disrupt oxidative balance in vital organs, with sex- and tissue-specific vulnerabilities, potentially contributing to the development of disorders.

KEYWORDS: antioxidant enzymes, mice, oxidative stress, polystyrene microplastics

Acknowledgements: This work was supported by Grant № KP-06-H81/2, National Science Fund.

L03_05

CAN ACTIVATED AND MODIFIED CLINOPTILOLITE REDUCE THE GENOTOXIC AND DNA DAMAGING EFFECTS OF TWO HEAVY METALS IN *CHLAMYDOMONAS REINHARDTII* AS A PLANT MODEL?

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The aim: to clarify whether modified and activated clinoptilolite can reduce both genotoxic and DNA-damaging effects of two heavy metals – $PbCl_2$ and $CdCl_2x2.5H_2O$ on the plant model *Chlamydomonas reinhardtii*.
Material and methods: Cell suspensions of *Chlamydomonas reinhardtii* strain 137C at the end of exponential and the beginning of stationary phase, treated for 2 hours with:

1. $PbCl_2$– 8mg/L (LD_{20}), and $CdCl_2x2.5H_2O$ – 20mg/L (LD_{20}), 50mg/L (LD_{50}), and 70mg/L (LD_{80}).
2. Mixture of both heavy metals: $PbCl_2$ (LD_{20}) + LD_{20} $CdCl_2x2.5H_2O$; $PbCl_2$ (LD_{20}) + LD_{50} $CdCl_2x2.5H_2O$; $PbCl_2$ (LD_{20}) + LD_{80} $CdCl_2x2.5H_2O$.
3. 1 and 2 preliminary incubated with 0.005g/ml clinoptilolite for 24 hours.

The clinoptilolite was kindly provided by prof. Yana Tzvetanova – Institute of Mineralogy and Crystallography „Acad. Ivan Kostov" – BAS. The genotoxic and DNA-damaging effect was evaluated based on "clonal assay" and constant-field gel electrophoresis, respectively.
Main results: Minor, but statistically significant decrease of harmful effects of heavy metals despite of the experimental scheme was obtained. Increased cell survival $PbCl_2$ (LD_{20}) – 1.25-fold; LD_{20} $CdCl_2x2.5H_2O$ – 1.1-fold; $PbCl_2$ (LD_{20}) + LD_{50} $CdCl_2x2.5H_2O$ – 1.2-fold was calculated. A dose-dependent, statistically significant DSBs increase was measured after single $CdCl_2x2.5H_2O$ treatment. When a mixture of both metals was applied, more than 1.6-fold lower DSB levels were observed compared to single cadmium application. The interaction analysis revealed a statistically proven antagonistic interaction between both heavy metals. Analyzing levels of DSB induced by heavy metals without or incubated modified clinoptilolites, it becomes clear that the modified and activated clinoptilolites reduce the DNA-damaging effect of heavy metals in *Chlamydomonas reinhardtii*.
Conclusion: Our findings demonstrated a minor capacity of modified and activated clinoptilolites used by us to reduce both genotoxic and DNA-damaging action of cadmium. Our results did not correspond to results obtained in other experimental models. Probably, the optimal conditions for activating and modifying clinoptilolites are different for various organisms.

KEYWORDS: cadmium, cell survival, *Chlamydomonas reinhardtii*, clinoptilolites, DNA double-strand breaks, lead

Acknowledgements: This work was supported by a grant from the National Science Fund, Ministry of Education and Science, Project No. KP-06-PN44/3.

L03_06

PARASITIC ARTHROPODS ISOLATED FROM WILD BIRDS NEAR THE ATANASOVSKO LAKE, BULGARIA IN THE PERIOD 2024-2025

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Aim: To make a screening on the different types of parasitic arthropods found in several species of wild migratory birds (*Erithacus rubecula, Hirundo rustica, Fringilla coelebs* and *Phoenicurus phoenicurus, etc.*) captured in the area of Lake Atanasovsko Nature Reserve, Burgas province, Bulgaria.
Material and methods: The samples were obtained from the plumage and skin of wild migratory birds during routine bird ringing in the area of Lake Atanasovsko Nature Reserve.
After obtaining the parasitic arthropods, they were stored at -20°C, and subsequently brought to the parasitological laboratory for isolation and identification.
Main results: Based on morphological features, the arthropods were identified as a few separate species belonging to the large groups of parasitic acari and insects. Different species of parasitic arthropods were established, such as *Ixodes ricinus* (Ixodidae, hard ticks) and *Ornithomya avicularia* (Hippoboscidae, parasitic fly).
Conclusion: more detailed research covering a larger area of the country, as well as regular monitoring on the parasitic infestation of the migratory birds, is advisable, since some of them can transmit various infectious disease which affects both domestic and wild birds and even humans.

KEYWORDS: migratory birds, parasitic arthropods, acari, insects

Acknowledgements: The participation in the conference was funded by the Student Council at the University of Forestry, Sofia, Bulgaria

L03_07

A REVIEW OF LEAD AND CADMIUM ACCUMULATION IN ORGANS IN REPRESENTATIVES OF CERVIDAE FAMILY

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Aim: Venison is gaining popularity, not only among hunters, but also among health-conscious consumers and culinary enthusiasts, due to its health-promoting properties and distinctive flavor. This review aimed to assess the potential health risks associated with deer meat and edible offal consumption through compiling the reported concentrations of lead (Pb) and cadmium (Cd) in the muscle tissue, liver, and kidneys of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and fallow deer (*Dama dama*). Additionally, we aimed to examine possible differences in heavy metal accumulation with respect to age, sex, and habitat location and to compare the findings with the permissible limits established by European regulations.
Material and methods: A review of the existing research published between 2014 and 2024 was conducted. Data on Pb and Cd levels in meat, liver, and kidneys of the three specified *Cervidae* species, as well as deer age, sex, and habitat proximity to industrial sites were summarized.
Main results: The analysis revealed varying levels of Pb and Cd in different organs and deer species. Differences in heavy metal concentrations were observed in relation to both the age of the animals and proximity of their habitat to polluted areas. Some reported concentrations exceeded permissible limits, indicating a potential health hazard in the case of human consumption. The position in the food chain of the Cervidae family representatives makes them susceptible to the accumulation of various environmental pollutants in their organisms, and can be used as bioindicators for monitoring heavy metal contamination.
Conclusion: These findings highlight the major issue of Pb and Cd bioaccumulation in deer's organs, emphasizing the need for effective health risk assessments related

to the consumption of game meat and edible offal. Environmental monitoring and the implementation of appropriate strategies against heavy metal pollution are crucial for preserving both wildlife and human health.

KEYWORDS: Lead, Cadmium, Deer, Bioaccumulation, Health risk assessment

Acknowledgements: The participation in the conference was funded by University of Forestry, Sofia, Bulgaria through the project NIS-B 1406/2025 "Screening of the levels of certain heavy metals in meat and internal organs of roe deer (*Capreolus capreolus*, L., 1758) in regions with high or low risk of pollution".

L03_08

MICROPLASTIC ACCUMULATION IN WILD BIRDS IN EUROPE: A REVIEW

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Aim: This review examines current research on wild birds' exposure to microplastics across terrestrial, freshwater, and marine ecosystems in Europe. The goal is to identify the possible ways of contamination with microplastics, the most common organs/systems of the body where accumulation occurs as well as the types of plastics involved.
Material and methods: The information was collected from internationally recognized scientific databases such as Scopus, ProQuest, Google Scholar, and ResearchGate. This review covers a 25-years' period, from 2000 to 2025.
Main results: Microplastic pollution has emerged as a growing threat to birds inhabiting marine, freshwater, and terrestrial ecosystems. This review synthesizes findings from recent research examining microplastic exposure in birds, with emphasis on the most affected organs and systems of the body and particle types. Plastic ingestion is common across species, especially in seabirds and is influenced by foraging behavior, habitat, and age. Identified plastics include fibers, fragments, films, and pellets, with polymers such as polyethylene and polypropylene most frequently reported. Microplastics have been found not only in the digestive tract, but also in feathers, feces, regurgitates, and even lung tissues, pointing to both ingestion and inhalation as exposure pathways. While some experimental studies report few immediate health effects, others document growth delays, reproductive abnormalities, and potential endocrine disruption.

Conclusion: Our understanding of long-term consequences remains limited. This is due to short study durations, lack of standard protocols, species-specific responses, and challenges in isolating the effects of plastic-associated contaminants. Moreover, interactions with environmental stressors in wild populations further complicate interpretation. Birds may also act as vectors, depositing microplastics in nesting environments through excretion. These findings underscore the importance of birds as indicators of the levels of plastic contamination and highlight the urgent need for long-term, ecologically relevant studies to assess chronic impacts on avian health and population dynamics.

KEYWORDS: Microplastics, Birds, Ingestion, Pollution, Ecosystems, Bioindicators

Acknowledgements: The participation in the conference was funded by the Student Council at the University of Forestry, Sofia, Bulgaria.

L03_09

THE GENOTOXICITY OF ZEOCIN STRONGLY DEPENDS ON THE HOMOLOGOUS RECOMBINATION REPAIR

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Aim: To analyze the role of certain genes related to the genotoxic effects of Zeocin treatment.
Materials and methods: Wide range of concentrations of the radiomimetic Zeocin (0.1 – 300 μg/ml) was tested on 6 strains of *Saccharomyces cerevisiae* – BY4741 (parental strain) and the isogenic ones – Y06913 (deletion in *SOD1* gene, responsible for the synthesis of the cytoplasmic Cu/Zn superoxide dismutase), Y06401 (deletion in *RAD51* gene, responsible for the synthesis of a protein participating in the recombinational repair of double-strand breaks), Y06240 (deletion in *MSH2* gene, responsible for the synthesis of a protein participating in the mismatch repair), Y04718 (deletion in *CTT1*gene, responsible for the synthesis of catalase), Y00403 (deletion in *SSA1* gene, responsible for the synthesis of an ATPase involved in protein folding and NLS-directed nuclear transport; member of the HSP70 family). Cell suspensions in the end of exponential and the beginning of stationary phase were treated with Zeocin at the range of concentrations 0.1 – 300 μg/ml for 1 min as previously determined. The genotoxic effect was evaluated based on two endpoints: cell survival and induction of DNA double-strand breaks (DSBs).

Main results: Treatment with Zeocin resulted in variation in the sensitivity of the strains depending on the genotype. The dose causing 50% lethality was calculated for all the strains. Based on it, they can be arranged as follows: Δ*RAD51* < Δ*CTT1* < Δ*SSA1* ≤ Δ*SOD1* ≤ Δ*MSH2* < BY4741.
Further, the induction of DSBs was tested. Our results indicated that the levels significantly differ when treatment was performed with concentrations up to 10μg/ml. The highest levels were detected in strain Y06401 (1.5-fold higher than the control – untreated cells).
Conclusion: Our results provide confirmatory data that the Zeocin's genotoxicity is mainly due to the direct DSBs induction and strongly depends on the presence of recombinational repair.

KEYWORDS: *Saccharomyces cerevisiae,* Zeocin, genotype, recombinational repair

Acknowledgements: This work was performed under the project KP-06-N71/13 "Contribution and relationship of DNA double-strand breaks' repair, chaperone, and antioxidant systems for the formation of an adaptive response", funded by the Bulgarian National Science Fund – Ministry of Education and Science.

P03_01

THE ANTIOXIDANT CAPACITY STUDY OF TWO EUKARYOTIC MODEL SYSTEMS: *CHLAMYDOMONAS REINHARDTII* AND *SACCHAROMYCES CEREVISIAE*

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Aim: Maintaining cellular homeostasis and low ROS levels is vital for oxidative stress adaptation. While various organisms possess different adaptive strategies, the antioxidant system's role remains unclear. To investigate this, two model organisms, *S. cerevisiae* and *C. reinhardtii*, differing in their genotypes, were used to elucidate the molecular basis of antioxidant capacity and redox homeostasis maintenance in eukaryotic cells.
Material and methods: Wild-type and mutant strains of *C. reinhardtii* (WT 137C, UVS-10, UVS-14) and *S. cerevisiae* (BY4741, Y06913, Y04718, Y06401, Y00403, Y06240),

defective in different types of repair mechanisms or with different tolerance to oxidative stress, were used in this study. The basic levels of SOD, catalase, ROS, carbonylated proteins, lipid peroxidation and glutathione were analyzed.
Main results: *C. reinhardtii* mismatch and recombination repair deficient strains were characterized with increased intracellular ROS levels triggering as a natural adaptive mechanism the upregulation of oxidative stress response regulators, such as glutathione and SOD. In *S. cerevisiae* the knock-out mutations in SOD and catalase-encoding genes, as well as those in the DNA repair mechanisms-related genes, also resulted in excessive ROS accumulation in all tested cells. Contrary to the *C. reinhardtii* in all *S. cerevisiae* mutants the upregulation of SOD enzyme was not always the general protective mechanism. However, all of them were characterized with elevated glutathione levels. These data indicate the important role of glutathione for overcoming oxidative stress in eukaryotes. In both model systems the highest susceptibility to stress was observed in recombination repair-deficient strains.
Conclusion: The comparative analysis of the antioxidant potential in both eukaryotic model organisms, revealed that in *Chlamydomonas reinhardtii* the key role in natural adaptive mechanisms played the SOD and glutathione, while in *Saccharomyces cerevisiae* the main response to the elevated ROS levels involved the prime upregulation of components from the non-enzymatic defence system.

KEYWORDS: *S. cerevisiae*, *C. reinhardtii*, ROS, oxidative stress response, redox balance

Acknowledgment: This work was supported by a grant from the National Science Fund, Ministry of Education and Science, Project No. КП-06-Н71-13/16.07.2024.

P03_02

DESIGN OF A SMART MONITORING AND IRRIGATION SYSTEM TO SUPPORT DROUGHT STRESS STUDIES IN *ARABIDOPSIS THALIANA*

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Aim: Drought is an important environmental stress factor affecting plant growth and development. In this study, we present an *in silico* concept for a smart environmental monitoring and irrigation system tailored to the model plant *Arabidopsis thaliana* to

investigate drought stress responses mediated by ethylene signaling in mutant plants such as *etr1-1*, *ctr1-1*, *ein2-1* and *ein3eil1*.

Material and methods: The proposed system integrates multiple sensors to continuously monitor and record abiotic stress factors including soil and air humidity, temperature, light intensity, air flow and gas concentrations (CO_2 and O_2) and control irrigation. The design allows for future integration with predictive algorithms to derive insights for *A. thaliana* drought response.

Main results: The conceptual model demonstrates how environmental data can be collected using a sensor-based non-invasive monitoring system that enables real-time observation of abiotic factors and provides precise irrigation of the plants. The proposed system has the potential to reduce the need for high-cost and labor-intensive wet-lab experiments in the field of plant physiology and environmental stress research, as the collected data, along with the gene expression and biochemical marker data obtained from plant hormone signaling mutants, can be used in the development of predictive *in silico* simulations.

Conclusion: The smart monitoring and irrigation system concept offers a reproducible and energy-efficient way to study abiotic stress in *A. thaliana*. It provides a foundation for future experimentation and computational modeling in order to better understand ethylene's role in drought tolerance.

KEYWORDS: drought, smart monitoring, irrigation, *Arabidopsis thaliana*, ethylene, environment

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P03_03

MIRNA RESPONSE TO HEAVY METAL POLLUTION IN *HYPNUM CUPRESSIFORME* FROM MOSS WALL INSTALATIONS IN PLOVDIV REGION

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Aim: *Hypnum cupressiforme*, a nonvascular moss species, is used as a biomonitor for abiotic stress, including heavy metal pollution. Heavy metal exposure endangers both the

environment and human health and can modify gene expression in exposed individuals. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression post-transcriptionally and are considered potential biomarkers of environmental stress. This study aims to assess miRNA expression changes in *H. cupressiforme* walls installed in selected locations in Plovdiv region, Bulgaria, in response to heavy metal pollution.
Material and methods: Moss samples were collected from moss walls (MW) installed at two locations in Plovdiv and one location in Kuklen, Bulgaria. Total RNA was extracted from the moss tissues using the RNeasy Plant Mini Kit (QIAGEN), followed by DNase treatment to eliminate genomic DNA contamination. Small RNA library was prepared from non-poluted control collected from Stara Planina, and sequenced by Novogene (UK). miRNAs were identified based on homology with plant miRNAs (miRbase). For selected microRNAs, cDNA synthesis was performed using the ProtoScript® II First Strand cDNA Synthesis Kit (New England BioLabs). Quantitative real-time PCR (qRT-PCR) was carried out using the qPCR Master Mix (Genaxxon bioscience) to evaluate the expression levels of target miRNAs.
Main results: Preliminary study of the heavy metal pollution of the MW classified the Kuklen village as a moderate-risk site. Homology-based analysis identified 26 miRNAs in moss, of which miR533a, miR536a, miR538a, and miR319a were compared between non-polluted control and the Kuklen MW. RT-qPCR analysis confirmed down-regulation of all miRNAs in the Kuklen sample, with miR319a exhibiting the most noticeable change.
Conclusion: These results suggest that miRNAs in *H. cupressiforme* respond to heavy metals and may serve as sensitive biomarkers for environmental monitoring. Further research is needed to identify their target genes and enhancing biomonitoring methods.

KEYWORDS: *Hypnum cupressiforme*, moss walls, miRNA, heavy metals

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P03_04

ISOLATION AND CHARACTERIZATION OF ANTARCTIC FRESHWATER MICROBES FROM LIVINGSTON ISLAND

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Aim: The main objective of this study is to characterize microbial isolates collected from Livingston Island, Antarctica. The investigation focuses on assessing the effects of UV radiation, temperature tolerance, and resistance to selected heavy metals.

Material and methods: Samples were collected in January 2025 near the Bulgarian Antarctic Base "St. Kliment Ohridski". Sterile aliquots of liquid nutrient media were used during sampling. Pure cultures were obtained using classical microbiological techniques. Isolates were tested for resistance to UV radiation at 254 nm (20, 40, and 60 minutes) using the serial dilution method. Temperature tolerance was evaluated in sterile 24-well plates at 4°C, 18°C, 37°C, and 42°C. MIC values for $ZnSO_4$, $CuSO_4$, and $CdCl_2$ were tested in various millimolar concentrations.

Main results: A total of 19 pure cultures were obtained. All isolates showed abundant growth at 4°C after 24 hours, with no growth at 37°C or 42°C in that time frame. After 48 hours, 7 isolates grew at 37°C, and only NC09 and G08 at 42°C. UV resistance was tested in 9 isolates, along with controls *Pseudomonas aeruginosa* 3732 and *Bacillus subtilis*. The highest survival rates under UV exposure were observed in G03 (22.7%) and R01 (18.2%). MIC testing showed Zn^{2+} inhibited most isolates, with 6 having an MIC of 25 mM and only G08 reaching 100 mM. More isolates grew in the presence of Cu^{2+}; 5 tolerated up to 180 mM. In the presence of Cd^{2+}, 9 isolates grew, with only one showing an MIC of 100 mM. The rest had MICs between 5–10 mM.

Conclusion: All isolates demonstrated abundant growth at 4°C. No strong UV resistance was observed. Zinc had the highest inhibitory effect, with the fewest isolates able to grow in its presence.

KEYWORDS: UV light, temperature range, minimal inhibitory concentration

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***Online* P03_05**

THE TOXICITY OF CLOVE ESSENTIAL OIL AND ITS NANOFORMULATION DEPENDS ON THE MISMATCH REPAIR

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Aim: To evaluate the potential mechanisms of action of clove essential oil and its nanoformulation on a model system *Saccharomyces cerevisiae*.
Material and methods: Clove essential oil and the nanoformulation, kindly provided by prof. Abdel Tawab Mossa, National Research Center, Egypt were tested in the range of concentrations 0.250 – 1%. Four *Saccharomyces cerevisiae* strains were used: BY4741 (parental strain), Y06913 (deletion in *SOD1* gene responsible for the synthesis of the cytoplasmic Cu/Zn superoxide dismutase), Y06401 (deletion in *RAD51* gene, responsible for the synthesis of a protein participating in the recombinational repair of double-strand breaks), Y06240 (deletion in *MSH2* gene, responsible for the synthesis of a protein participating in the mismatch repair). Cell suspensions in the end of exponential and the beginning of stationary phase of growth were treated for 30 min at optimal for cell growth conditions. The genotoxic effect was evaluated based on a cell survival assay.
Main results: The cell survival data concerning the potential toxicity of the clove oil revealed that the strains could be arranged as follows: Y06401, BY4741, Y06913, Y06240. All the tested concentrations revealed strong genotoxic potential. In terms of the effect of nanoemulsion, the cell survival of most of the strains was comparable to the control – untreated cells. The most susceptible strain was found to be Y06240 – the one with the impaired mismatch repair.
Conclusion: The approach provided in the current study allowed us to evaluate the role of three cellular defense systems – the antioxidant, the recombinational repair of DSBs, and the mismatch repair in response to the treatment with clove essential oil and its nanoemulsion.
Our data revealed that the strain with a deletion in the *MSH2* gene is more sensitive to the action of clove essential oil and its nanoemulsion, suggesting that the mismatch repair plays a pivotal role in *Saccharomyces cerevisiae*'s response towards these compounds.

KEYWORDS: *Saccharomyces cerevisiae,* clove essential oil, nanoformulation, mismatch repair

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P03_06

EFFECT OF SUBCHRONIC ORAL EXPOSURE TO POLYSTYRENE MICROPLASTS ON FEMALE RATS

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Aim: The present study aimed to determine the effect of oral exposure to different sizes of polystyrene microplastics (PS-MPs) on the sex-related hormonal profile and ovarian oxidative status of female laboratory rats.
Material and methods: Female rats aged 28 days and body weight of 35-40g were randomly divided into a control group (Co) and two experimental groups: G1-treated with a size of 1 μm and G2-treated with a size of 5μm PS-MPs at a dose of 0.1mg/24h, suspended in the drinking water, for 30 days. At the end of the period, the animals were anesthetized. Blood was obtained by cardiac puncture and centrifuged to separate serum, where hormones: estradiol (E2), testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone(LH) were examined by ELISA method. The ovaries were dissected, homogenated and lipid peroxidation (LPO) and total glutathione (GSH) were measured spectrophotometrically.
Main results: It was observed an increase in the levels of E2 (4.51±0.17ng/dL) and T (3.73±0.68ng/dL) in G1 compared to Co (E2-4.35±0.16ng/dL; T-3.40±0.20ng/dL) and G2 (E2-4.33±0.23ng/dL; T-3.43±0.40ng/dL). In animals from G1 the FSH decreased (2.23±0.77ng/dL) compared to Co (2.60±1.15ng/dL) and G2 (2.70±1.55ng/dL) groups. The LH levels increased in G2 (3.73±0.55ng/dL) compared to the both Co (2.03±1.53ng/dL) and G1 (2.10±1.03ng/dL). Regarding the oxidative profile, it was found that LPO levels significantly increased in G1 compared to the Co and G2 (0.25±0.66 vs 0.16±0.25 ($P \leq 0.01$) and 0.17±0.02 nmoles MDA/mg protein, respectively). The ovarian GSH increased significantly in both experimental groups (G1 – 234.52±54.53ng/mg protein; $P \leq 0.05$; G2 – 304.70±57.75ng/mg protein; $P \leq 0.01$) compared to the Co (157.14±52.13 ng/mg protein).

Conclusion: Subchronic oral exposure to PS-MPs affects the hormonal and oxidative profile in female rats, with the size of the particles ingested being of significant importance. Considering the widespread environmental pollution with microplastics, these results raise the alarm about possible long-term effects on fertility and reproduction, including in humans.

KEYWORDS: polystyrene microplastics, oxidative stress, sex hormones, female rats

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P03_07

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES, SYNTHESIZED WITH MUCUS OF THE GARDEN SNAIL *CORNU ASPERSUM*, AND THE UNDERLYING MECHANISM OF ACTION

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Aim: The aim is to study the antibacterial activity of silver nanoparticles (AgNPs) obtained with *Cornu aspersum* mucus and to reveal the mechanism of their antimicrobial action.
Material and methods: The antibacterial tests were carried out with four $Gram^+$ and four $Gram^-$ bacterial strains, applying the agar well-diffusion method. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were also determined. The intracellular generation of ROS was determined fluorometrically with DCFH-DA. Catalase activity was evaluated by the H_2O_2 decomposition assay. The levels of lipid peroxidation were measured with an MDA colorimetric assay kit. The activity of superoxide dismutase (SOD) was defined spectrophotometrically. The levels of protein carbonylation were determined by derivatization of protein carbonyl groups with DNPH. The cellular concentration of reduced and total glutathione was measured by DTNB colorimetric assay.
Main results: The silver NPs were found to have antibacterial activity against all

eight experimental bacterial strains. The MIC of AgNPs for *S. aureus* was 256 µg/mL, while for the other seven strains – 512 µg/mL. AgNPs caused concentration-dependent increase in intracellular ROS levels in *E. coli* and *S. aureus*. Catalase and SOD activity was markedly increased in both studied bacterial strains after one to six hours of NPs treatment. The levels of protein carbonylation and lipid peroxidation were also elevated. The concentration of reduced glutathione in *E. coli* and *S. aureus* decreased in a time-dependent manner during incubation with NPs.
Conclusion: AgNPs with *Conru aspersum* mucus exhibit strong antibacterial activity against the tested $Gram^+$ and $Gram^-$ bacterial strains. The intracellular generation of ROS and occurrence oxidative stress was identified as a leading mechanism in antibacterial action.

KEYWORDS: nanoparticles, mucus, *Cornu aspersum*, antibacterial, reactive oxygen species

Acknowledgement: This research was carried out with the support of the project KP-06 PN61-8/2022 funded by the Bulgarian National Science Fund.

P03_08

EFFECTS OF THE FOLIAR APPLICATION OF IRON AND MAGNESIUM NANOPARTICLES ON BASIL PLANTS UNDER DROUGHT STRESS

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Aim: The present study was conducted to assess the effects of foliar-sprayed Fe_2O_3 and MgO nanoparticles (NPs) on green basil plants (*Ocimum basilicum* L., cv. *Italiano Classico*) under drought stress.
Materials and methods: The plants were grown in ½ Hoagland solution for about 40 days and then treated with 20% PEG 6000 (medium drought stress) for 3 days, followed by recovery for 5 days. Foliar treatments with Fe_2O_3 or MgO NPs (20 mg/L) were

applied to the plants 3 days before PEG addition. Chlorophyll fluorescence analysis (PAM parameters), leaf pigments, total phenolic content, membrane lipid peroxidation (MDA) and oxidative stress (H_2O_2) were determined to evaluate the effects of these NPs under drought stress. Both types of NPs were synthesized in the Institute of General and Inorganic Chemistry, BAS.

Main results: Drought stress caused a decrease in the chlorophyll content and inhibited the photosynthetic function in basil plants as a result of the increased oxidative stress and membrane damage. Foliar application of both types of NPs mitigated drought-induced impairment of the photosynthetic function and led to more successful plant recovery than the control plants. Data also revealed that treatment with MgO NPs resulted in less reduction in the chlorophyll content under drought stress compared to Fe_2O_3 NPs. At the same time, total phenolic content in leaves increased more strongly after treatment with Fe_2O_3 NPs, accompanied by lower levels of oxidative stress and membrane damage during drought.

Conclusion: This study provides new knowledge into the protective effects of Fe_2O_3 and MgO NPs on basil plants subjected to drought stress, which could be useful for improving plant resistance in water-limited conditions.

KEYWORDS: nanoparticles, drought stress, pigments, total phenolics, oxidative stress, photosynthesis

Acknowledgements: This work was supported by the Bulgarian Science Research Fund, project КП-06-М 76/3.

P03_09

BOTTOM TRAWLING IMPACT ON BENTHIC ECOSYSTEMS IN A MARINE PROTECTED AREA IN THE SW BLACK SEA, BULGARIA

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Aim: To test and verify a methodology, quantifying the degree of impact of bottom trawling on the sea-bottom and benthic ecosystems in the SCI BG001001 Ropotamo Natura 2000 area.

Material and methods: Bottom-trawling is recognized as one of the most prominent anthropogenic pressures on benthic ecosystems of the world oceans. In order to study the degree of trawling impacts on the sea-bottom in an area frequently visited by fishing vessels, we applied a combination of side-scan sonar scans, video cameras and ROVs. The survey vessel scanned the sea-bottom along a 12 km transect line, starting 3 km east of Cape Korakia. The scanning was done with a Klein 4900 side-scan deployed by a winch at 10 m above the sea-bottom, scanning 2.35 km^2 with 10x10 cm resolution. A video drop camera was deployed to visually verify sediment types and macrofauna, and observe the actual trawl impact. Data was analyzed with the SeaView MOGA, and edited by outlining the visible bottom-trawl tracks (QGIS). The total area. In-situ results were compared with previous studies of the degree of trawling impacts based on VMS data analysis.
Main results: A total of 219 trawl marks were identified along the transect, with a length of 23000 m, and area of 51782.38 m^2, representing 0.5% of the surveyed sea-bottom area. Areas with higher trawl-mark density coincided with areas with higher ship traffic densities and higher degree of trawling impact (MSFD D6C2). The actual impact of bottom trawling on the biodiversity, ecological status and functional parameters of these ecosystems requires precise bottom sampling by divers or ROVs. and lab analyses of samples.
Conclusion: Our methodology provided relevant data on the degree of physical disturbance of the sea floor in the study area and could be applied in other coastal and offshore areas of the Bulgarian Black Sea.

KEYWORDS: Black Sea, bottom trawling, trawl marks, side-scan, sea-bottom integrity

Acknowledgements: LTER-BG (Grand agreement ID: 101060072).

P03_10

THE CONTRIBUTION OF THE MODEL ORGANISM *CHLAMYDOMONAS REINHARDTII* IN THE STUDIES OF ADAPTIVE RESPONSE

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Chlamydomonas reinhardtii is robust model organism in studies of "Adaptive response" (AR) phenomenon. AR means increased cell resistance to high dose by giving both –

appropriate priming dose and intertreatment time. Unfortunately, some recent studies misunderstand and classified organisms' adaptation and acclimation to different environmental factors as an AR.

Aim: Here, we aim to analyze the main data known for the contribution of *Chlamydomonas reinhardtii* as plants model organism in this process.

Material and methods: Our own results and selected articles listed in the WoS, Scopus, and PubMed databases, with no publication date restriction. "*Chlamydomonas reinhardtii*" and "Adaptive response" were used as keywords.

Results:

What is known about the factors affecting the magnitude of the AR in *C. reinhardtii*?

- experimental design – inter-treatment and recovery time;
- low priming dose – inducing LD_{20-30};
- low level of initially induced DSB;
- strains genotype – genotype resistance did not abrogate the capability of radio/zeocin resistant strains to develop an AR;
- constitutive levels of HSP70B.

Our strains CW15, 137C, UVS10, UVS14, AK-9-9 and H3 that differ in their genotype resistance were documented to differ in their capability to develop AR.

Does the AR involve induction of new proteins synthesis?

- Protein synthesis inhibitors (cycloheximide and chloramphenicol) prevented the formation of AR in *C. reinhardtii*.

What role have the antioxidant systems?

- Data on paraquat-induced AR to paraquat revealed the partial involvement of the antioxidant system, depending on the strain genotype.

Does the AR operate via up-regulation of DNA repair?

- Up-regulation of the DSBs and acceleration of DSBs rejoining were found as one of the mechanisms responsible for the formation of AR.

Conclusion: The mechanisms of AR include the activation of DNA repair pathways, antioxidant and chaperone systems. Still unanswered question remains the contribution of the MMR and the *rec* repair of DNA to the AR.

KEYWORDS: *Chlamydomonas reinhardtii*, adaptive response

Acknowledgements: This work was performed under the project KP-06-N71/13 "Contribution and relationship of DNA double-strand breaks' repair, chaperone, and antioxidant systems for the formation of an adaptive response", funded by the Bulgarian National Science Fund – Ministry of Education and Science.

THEMATIC SESSION IV

ECOLOGICAL AGRICULTURE AND EDUCATION

***Online* PL04_01**

AGROECOLOGY: GOOD PRACTICES IN MEDITERRANEAN AGRICULTURE

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Aim: Agroecology represents a transdisciplinary, participatory, and system-based approach to sustainable agriculture, offering robust solutions for the complex socio-ecological challenges facing Mediterranean farming systems. The Mediterranean basin is marked by a diversity of agroecosystems, yet commonly suffers from water scarcity, soil erosion, biodiversity decline, and increasing climate variability. We tackle the integration of agroecological principles into Mediterranean agriculture, emphasizing ecologically sound, socially just, and economically viable practices tailored to the region's unique climatic and cultural conditions.

We herein highlight key agroecological practices including polycultures, legume-based rotations, and pasture and forage diversity based on solid and continuous research and science based data. Agroecological aligns the objectives of both ecological resilience and rural development.

Material and methods: The on-going Agroecology Partnership, funded by Horizon Europe, unites the European Commission and 72 partners across 26 Member States, Associated Countries, and Third Countries and it will support an agriculture sector that is fit to meet the targets and challenges of climate change, biodiversity loss, food security and sovereignty, and the environment, while ensuring a profitable and attractive activity for farmers. The Partnership in particular pools the resources of the EC and the states involved to fund high-level research generating appropriate knowledge and technologies aligned with the core themes described below and in the Strategic Research and Innovation Agenda.

Main results: Recently activities such as the Summer School on **Agroecology: Conservation Agriculture in Mediterranean Systems** have shown to be a good initiative to exchange knowledge, gather and share experiences and close the gap between farmers, researchers, consultants, students, technicians and policy makers.
Conclusion: In conclusion, agroecology offers a scientifically grounded and socially inclusive pathway to address the interconnected crises of climate change, land degradation, loss of biodiversity and food insecurity in the Mediterranean region. Advancing its adoption calls for an enabling policy environment, investment in agroecological research, and the strengthening of knowledge exchange networks among farmers, "technicians", researchers, and civil society actors.

KEYWORDS: Agroecology, Mediterranean agriculture, Sustainability

Acknowledgements: Project: 101132349 – AGROECOLOGY – HORIZON-CL6-2023-FARM2FORK-01.

PL04_02

ECOLOGICAL EDUCATION TODAY – CONTEMPORARY ASPECTS, TRENDS AND CHALLENGES

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Aim: To outline the main dimensions of contemporary ecological education, their determinants, terminological development, educational-political accents, practical manifestations and prospects.
Material and methods: Study of documents and articles reflecting global, European and national policies related to the contemporary development of ecological education; analysis of competence complexes in the context of ecological education; analysis of current educational practices in school and out-of-school environments.
Main results: Ecological education in a national and international context is developing both terminologically-theoretically, as well as educationally-politically, and practically-applied within the framework of activities in formal and non-formal education. In recent years, there has been a dynamic in the terminology that underlies the concept of ecological education. The term environmental education is increasingly being used, as well as education for sustainable development, especially in an educational-

political context – in the global and European policies, including through the concept of green competences. There is also a dynamic development in the concept of ecological education, which is expanding with new thematic fields and interpretations, for example, nature-based solutions integrated into school education. The practice of school and out-of-school ecological education is also developing by enriching thematic areas related to human rights, climate change, social dimensions of food production, sustainable use of resources, active citizenship, etc.

Conclusion: Ecological education is developing dynamically, reflecting trends in the development of society, politics, science, social attitudes regarding a healthy lifestyle, biodiversity conservation, care for nature and learning from nature, etc. These trends should be well known in order to educate people as active and responsible citizens concerned about the life on the planet.

KEYWORDS: ecological education, environmental education, education for sustainable development, green competences

PL04_03

SUSTAINABLE DEVELOPMENT – A GLOBAL PARADIGM FOR THE XXI CENTURY

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Aim: When creating the concept of sustainable development, as a new global paradigm it is for the XXI century, logical to start from sensitizing student to the environment, developing self-confidence, initiative for independence and knowledge, as well as developing communication skills and environmental management skills.

The aim of this research is to determine, through an analysis of curricula, how many and in what way secondary education programs support education for sustainable development, and to identify segments where the principles, goals, outcomes, and content related to sustainable development can be incorporated.

Material and methods: The research was conducted through an analysis of secondary education curricula in the Republic of North Macedonia. Secondary education in the country is provided by 99 public schools and 13 private schools. According to the curricula implemented in public schools, 16 schools offer general secondary education,

40 provide vocational education, 34 implements both general and vocational education programs, 4 are for students with special educational needs, and 5 are art schools.
Results: The research, based on an analysis of all curricula, concluded that it is essential to establish a link between learning objectives in the curricula and the Sustainable Development Goals adopted by the UN General Assembly on September 25, 2015. The Sustainable Development Goals are integrated, indivisible, and encompass the three dimensions of sustainable development: economic, social, and environmental.
Conclusion: Based on the analysis, it can be concluded that most secondary education programs include goals, objectives, and content that are directly or indirectly related to sustainable development. Such goals and content are more prevalent in general education subjects compared to vocational subjects, which may be due to their representation in the overall curriculum structure.

KEYWORDS: sustainable development, sustainable development goals, secondary education programs, analysis of curricula, environmental education

L04_01

ALIEN INSECTS IN SOFIA MUNICIPALITY: A REALITY CHECK ON SPECIES COMPOSITION AND SPREAD

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As the capital of Bulgaria, Sofia functions as a major center of activity for both residents and tourists. The intense movement of people and goods, the abundance of ornamental vegetation, and the availability of recreational and tourist areas in both urban and suburban zones create favorable conditions for the introduction and establishment of invasive alien species (IAS).
The aim of the study was to determine the species diversity of invasive alien insect species and their distribution within the territory of Sofia Municipality.
Materials and methods: The analysis was conducted based on both published and unpublished data, as well as field surveys carried out between 2022 and 2024. The study encompassed 33 biodiversity-rich areas and roadside plantings across the territory of Sofia Municipality.
Results: A total of 32 IAS were confirmed as established within the municipality. Of these, 16 species were recorded in 32 of the surveyed sites. All species were associated

with existing vegetation, and their distribution patterns were strongly influenced by the type and availability of host plants. Urban parks were identified as hot spots of IAS diversity.

Four species associated with *Robinia pseudoacacia* L. – *Obolodiplosis robiniae* (Haldeman), its parasitoid *Platygaster robiniae* Buhl & Duso, *Parectopa robiniella* Clemens, and *Phyllonorycter robiniella* Clemens were detected at low population densities in 24 sites. *Coptodisca lucifluella* (Clemens), found on *Juglans regia* L., was observed in 14 areas. A notable decline in *Metcalfa pruinosa* (Say) populations was documented, likely due to the presence of its parasitoid *Neodryinus typhlocybae* (Ashmead).

Two species exhibited localized outbreaks, but only within urban environments: *Corythucha arcuata* (Say) on *Quercus* spp. in six sites, and *Cameraria ohridella* Deschka & Dimić on *Aesculus hippocastanum* L. in nine sites.

Conclusion: The distribution of these insect species on city streets was found to be closely linked to the composition of urban vegetation.

KEYWORDS: Alien, insects, distribution, capital, Bulgaria

Acknowledgements: This study was conducted in the framework of the National Science Programme 'Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters', approved by Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science of Bulgaria (Agreement № DO-230/06-12-2018); Agreement COA22-ДС96-1288/04.07.2022 Development of 'Strategy for protection of biodiversity of Sofia Municipality for 2030 and Action plan for protection of biodiversity of Sofia Municipality for 2030' and Project NIS-B-1280 "Assessment of the level of study of alien economically important pests of agricultural crops in Bulgaria" funded by the Scientific sector of University of Forestry.

L04_02

TAXONOMIC STUDY OF THE SUBFAM. CARDUOIDEAE (ASTERACEAE) IN BULGARIA

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Aim: The aim of the study is to make a critical inventory of the biodiversity in the subfam. Carduoideae (Asteraceae) in the Bulgarian flora in order to create the scientific basis for its conservation and sustainable use.
Material and methods: The study is based on materials collected from natural populations of all taxa from all floristic regions of Bulgaria, as well as on herbarium specimens from all Bulgarian and numerous foreign herbaria. For taxonomically difficult genera, biosystematic studies (chromosome number, ploidy level, genome size), embryological and metabolomic studies by standard methods were also conducted.
Main results: As a result of the large-scale comparative study of the subfamily Carduoideae, it was established that 148 species and 19 subspecies, belonging to 21 genera, occur on the territory of Bulgaria, as follows: Carlina (5 species и 1 subspecies), Xeranthemum (3), Echinops (6 species и 3 subspecies), Arctium (4), Saussurea (1), Jurinea (11), Carduus (14 species 2 subspecies), Cirsium (17 species и 1 subspecies), Picnomon (1), Ptilostemon (1), Tyrimnus (1), Onopordum (4), Silybum (1), Serratula (1), Klasea (2), Crupina (2), Centaurea (51 species и 11 subspecies), Cyanus (17 species и 1 subspecies), Psephellus (2), Rhaponticoides (1), Carthamus (3). As a result of a critical review of the taxonomic schemes of the target genera and species, 15 new species and 7 subspecies have been identified for the Bulgarian flora, compared to the previous most up-to-date information. Taxonomic descriptions, citation blocks, as well as information on the ecology, biology, distribution and intrageneric variability of each taxon, following the model of the multi-volume edition "Flora of Bulgaria" have been prepared.
Conclusion: The taxonomic diversity of Carduoideae (Asteraceae) in the Bulgarian flora is impressive. The data from the study provide a solid basis for its conservation and sustainable use.

KEYWORDS: Asteraceae, Carduoideae, taxonomic diversity

L04_03

WHAT ARE WE DOING!

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(thesis work for my bachelor degree under the guidance of Chief Assistant Dr. Boyana Pavlova and Associate Prof. Nikolay Aleksiev)
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Aim: The topic is environmental protection. The project's goal is to draw attention to this very important issue in the times we live in. It is much easier for a person to learn habits from an early age that would support the global need to take timely measures.
Material and methods: Digital illustrations and Photographs.
Main results: The project consists of a case and five books inside it.
Each book is dedicated to one of the five most polluting and non-biodegradable materials in nature. Plastic, glass, aluminum, textiles, and e-waste. In its third phase of content the readers can understand how much time it actually takes for the different materials to break down in nature (shown in numbers). The illustrations are showing different type of objects made from the material one of the 5 books its about and if they are recyclable or not.
Conclusion: The book is intended for the adolescent age group, as this is when young people begin to understand more clearly abstract concepts such as conservation, the environment, harmful emissions, climate change, and the consequences of their own actions, and also begin to form themselves as separate individuals with self-awareness. The choice of the project topic is related to the still limited information available in both Bulgaria and elsewhere in the world.

KEYWORDS: polluting materials, book, environment, climate change, pollution, knowledge, educating the new generations

Acknowledgements: I would like to thank my university teachers for their help during the making of this project.

P04_01

PRELIMINARY SURVEY OF THE BIODIVERSITY OF OLD APPLE AND PEAR VARIETIES IN PODGORIE AREA, BELASITSA NATURE PARK

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Aim: The study aims to map old apple and pear varieties and identify the relevant stakeholders in Podgorie region, Belasitsa Nature Park.
Material and methods: We performed stakeholder analysis and locally assisted mapping of fruit varieties in Podgorie, a geographical area at the northern foothills of Belasitsa Mt. which encompass the villages Belasitsa, Kolarovo, Samuilovo, Kamena, Javornica, Kljuch, Skrat and Gabrene, Petrich municipality on the border with Greece and North Macedonia.
Main results: Apple, pear, cherry and plum varieties have been cultivated in Podgorie for decades. Nowadays, most of the old orchards are destroyed due to aging of the population, youth migration, changing of consumers' demand for modern fruit varieties with larger and sweeter fruits, etc. The old pear and apple varieties can be found either in home gardens or as single trees in the fields that once were cultivated. So far, we have established 10 different old varieties of apples (6) and pears (4). Although our respondents claim that the old varieties are more resistant to pests and diseases, they replace them with modern ones to respond to consumers' demands. Disintegrated irrigation system in the area and low subsidies for fruit orchards further demotivate the locals to grow fruit trees.
Conclusion: Old fruit varieties can be an integral part of the local identity and successfully included in the image of Podgorie as a destination for sustainable tourism. Further knowledge and cooperation with local stakeholders are needed to restore old pear and apple orchards in the Podgorie area.

KEYWORDS: apple and pear old varieties, Podgorie, Belasitsa Nature Park

Acknowledgements: (The research is funded with the financial contribution of the European Union through the BESTbelt project "tbd_BN_24_41 Protection of native fruit varieties in Maleshevo and Belasitsa regions" and National Roadmap for Research Infrastructure (2020-2027), LTER-BG-3–ДО1-320/30.11.2023).

P04_02

BIOINOCULANTS OF N_2-FIXING BACTERIA WITH POTENTIAL TO IMPROVE SOIL AND PLANT HEALTH

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The use of plant growth-promoting rhizosphere bacteria (PGPR) in agriculture is progressively increasing as they offer an effective tool to reduce the application of chemical fertilizers, pesticides, and other harmful substances. Among the PGPR, the research of N_2-fixing bacteria as microbial inoculants and their effects on plants have greatly improved crop production.

Aim: This study aimed to assess the N_2-fix potential and plant growth-promoting (PGP) impact of *Azotobacter, Nocardia*, and *Micrococcus* bacterial bioinoculants through test crops' model systems.

Material and methods: The bioinoculants' N_2-fix capacity was determined by the bromthymolblau method. The API 50CH и API 20NE polymicrotests were used for their biochemical profiles' generation. The foliar spray and watering techniques were applied to evaluate the bioinoculants' overall effect on sunflowers (*Helianthus annuus* L.). A seed germination method was used to study the N_2-fix and PGP effect on corn (*Zea mays* L.) seeds.

Main results: The *Azotobacter*, *Nocardia*, and *Micrococcus* model strains possess good N_2-fix (8.46 – 32.77 μg/ml N) capacity and biochemical profiles that reveal their PRP potential – production of esterase (C4), α-galactosidase, α-glucuronidase acid phosphatase enzymes. The model plant systems showed indicator values that indicate positive effects on the crops' physiology and agronomical traits when treated with the bioinoculants at various regimens.

Conclusion: The demonstrated N_2-fix capacity and PGP impact of *Azotobacter*, *Nocardia*, and *Micrococcus* bioinoculants on both tested crops reveal prospects for their application as biofertilizers promoting the plants' growth and development.

KEYWORDS: N_2-fix capacity, PGP, bioinoculant, crops

Acknowledgments: This work is financially supported by SU Science Fund project No 80-10-20/21.05.2025.

P04_03

PARASITISM OF *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE) ON KALE (*BRASSICA OLERACEA* L. VAR. ACEPHALA) INTERCROPPED WITH REPELLENT AND ATTRACTANT COMPANION PLANTS IN URBAN ENVIRONMENT

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Aim: Intercropping has emerged as a promising agroecological practice for enhancing pest control and reducing reliance on chemical inputs in urban farming. This study evaluates the potential of intercropping to influence the interactions between *Plutella xylostella* (diamondback moth) and its parasitoid complex through habitat manipulation.
Material and methods: An open-field experiment was conducted in Sofia, the capital city of Bulgaria, in area suitable for urban farming. Kale "Nero di Toscana" (Brassica oleracea convar. acephala var. palmifolia) was intercropped with different companion plants – *Lobularia maritima, Borago officinalis, Fagopyrum esculentum, Tropaeolum majus, Calendula officinalis, Tagetes patula, Anethum graveolens, Coriandrum sativum, Petroselinum crispum, Ocimum basilicum* and *Allium porrum*. These plants were chosen for their pest-repellent properties or ability to attract beneficial insects. Their role in the host plant-pest-natural enemy relationship is discussed.
Main results: One family of butterflies was reported as economically important pests on kale plants – Plutellidae. The parasitoid complex connected to it is described and the relationships between the companion plants is discussed.
Conclusion: Parasitism rates of *Plutella xylostella* larvae ranged from 50% to 80%, suggesting that intercropping can serve as a viable, sustainable pest management strategy for small-scale urban vegetable production.

KEYWORDS: biological control, intercropping, parasitoids, *Plutella xylostella,* urban farming

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