

INSTITUTE OF BIODIVERSITY AND ECOSYSTEM RESEARCH - BAS



**UNION OF SCIENTISTS IN BULGARIA
SECTION BIOLOGY**



INTERNATIONAL SEMINAR OF ECOLOGY- 2022

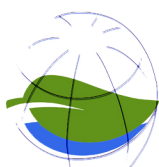
Actual problems of Ecology

Dedicated to

The International Year of Basic Sciences for Sustainable Development 2022

September 29th - 30th, 2022

Online



Program & Abstracts





INTERNATIONAL SEMINAR OF ECOLOGY- 2022 ACTUAL PROBLEMS OF ECOLOGY

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INTERNATIONAL SEMINAR OF ECOLOGY- 2022 **ACTUAL PROBLEMS OF ECOLOGY**

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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- 2022

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INTERNATIONAL SEMINAR OF ECOLOGY-2022
ACTUAL PROBLEMS OF ECOLOGY



Dedicated to
The International Year of Basic Sciences for Sustainable Development 2022

ONLINE SEMINAR
29 - 30 September 2022
Sofia, Bulgaria

SCIENTIFIC PROGRAM
29 September 2022

9⁰⁰ – 9²⁰ Opening Ceremony - *Prof. Stephka Chankova, PhD*

THEMATIC SESSION I

**BIOTIC AND ABIOTIC IMPACT ON THE LIVING
NATURE. ECOLOGICAL RISK. BIOREMEDIATION.**

Chairperson: Prof. Stephka Chankova, PhD and Assoc. Prof. Michaela Beltcheva, PhD

Technical support: Teodora Todorova and Radka Fikova

Plenary presentation

9²⁰-9⁵⁰ Does overproduction of chaperone proteins favor the repair of DNA injuries induced by oxidative stress? *Stephka Chankova, Nadezhda Yurina, Lubov Sharapova, Teodora Todorova, Petya Parvanova* **PL01_01**

9⁵⁰-10²⁰ Modelling of secondary metabolite production by morphogenesis modification *in vitro*. *Kalina Danova, Antoaneta Trendafilova, Kalina Alipieva, Jasmina Petreska Stanoeva, Viktoria Ivanova, Ina Aneva, Marina Stefova, Milka Todorova* **PL01_02**



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Oral presentations

10²⁰-10³⁰ Multi-biomarker approach for PBDEs effects in Common carp (*Cyprinus carpio* Linnaeus, 1785). *Stela Stoyanova, Iliana Velcheva, Vesela Yancheva, Borislava Todorova, Eleonora Petkova, Dobrinka Todorova-Bambaldokova, Elenka Georgieva* **L01_01**

10³⁰-10⁴⁰ Toxicological effects of commonly applied pesticides in Common carp (*Cyprinus carpio* Linnaeus, 1785). *Elenka Georgieva, Iliana Velcheva, Vesela Yancheva, Borislava Todorova, Eleonora Petkova, Dobrinka Todorova-Bambaldokova, Stela Stoyanova* **L01_02**

10⁴⁰-10⁵⁰ Bacterial and fungal abundance in different cooper contaminated soils. *Michaella Petkova, Nadezhda Nankova, Viktoriya Kancheva, Silvena Boteva, Anelia Kenarova, Galina Radeva* **L01_03**

10⁵⁰-11⁰⁰ Genotype differences towards lead chloride toxicity. *Teodora Todorova, Petya Parvanova, Maria Dimitrova, Kiril Zahariev, Krassimir Boyadzhiev, Martin Dimitrov and Stephka Chankova* **L01_04**

11⁰⁰-11¹⁰ The geochemical anomalies of heavy metals in the river sediments of the Dragovishtitsa River as an indicator for cross-border pollution. *Dimitar Zhelev* **L01_05**

11¹⁰-12⁰⁰ Discussion

12⁰⁰-13⁰⁰ Lunch break



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THEMATIC SESSION II ECOLOGICAL AGRICULTURE. ECOLOGICAL EDUCATION.

Chairperson: Assoc. prof. Ventsislava Petrova, PhD and Assoc. prof. Ganka Chaneva, PhD

Technical support: Petya Parvanova and Tsvetan Tsvetanov

Plenary presentation

**13⁰⁰ -13³⁰ Evaluation of phytonutritional characteristics of edible flowers as new functional foods. Laura Pistelli, Ilaria Marchioni, Barbara Ruffoni
PL02_01**

**13³⁰ -14⁰⁰ Green nanoemulsion pesticides: efficiency, application, and safety.
Abdel-Tawab H. Mossa PL02_02**

**14⁰⁰ -14³⁰ Place of ecological knowledge in biology and health education curriculum in the upper secondary level of school education in Bulgaria.
Kamelia Yotovska, Asya Asenova, Victoria Necheva PL02_03**

Oral presentation

**14³⁰ -14⁴⁰ *In vitro* micropropagation of *Tanacetum cinerariifolium* and establishment of an *ex situ* collection of selected clones. Vladimir Ilinkin,
Boryanka Traykova, Marina Stanilova L02_01**

14⁴⁰ -15⁰⁰ Discussion

15⁰⁰ -15¹⁰ Session break

15¹⁰ -17⁰⁰ Poster session and Discussion

Chairpersons: Assoc. prof. Yanka Vidinova, PhD; Assoc. prof. Galina Radeva, PhD and Assoc. Prof. Kalina Danova, PhD


Technical support: Galia Georgieva and Petya Parvanova




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POSTER SESSION THEMATIC SESSION I

P01_01  **Influence of physical factors on logarithmic, quiescent and non-quiescent *S. cerevisiae* cells.** *Polya Marinovska, Emilia Pisareva, Anna Tomova, Ventsislava Petrova*


P01_02 **Effects of foliar application of nitric oxide on sorghum under salt stress.** *Martin Stefanov, George Rashkov, Anelia Dobrikova, Preslava Borisova, Ekaterina Yotsova, Emilia Apostolova*

P01_03  **Comparative study on antioxidative potential of micropropagated and naturally growing plants *Nepeta nuda* ssp. *nuda*.** *Zornitsa Karcheva, Zhaneta Georgieva, Alexander Tomov, Detelina Petrova, Miroslava Zhiponova, Ganka Chaneva*

P01_04 **Evaluation of abundance of microplastics in Bulgarian coastal waters.** *Stanislava K. Georgieva, Zlatina V. Peteva, Mona D. Stancheva*

P01_05 **Recent knowledge about the occurrence of marine biotoxins on Bulgarian coast in 2021.** *Zlatina V. Peteva, Stanislava K. Georgieva, Mona D. Stancheva.*

P01_06 **Alterations in membrane stability after *in vitro* exposure of human erythrocytes to 2.41 GHz electromagnetic field.** *Boyana Angelova, Gabriela Atanasova, Nikolay Atanasov, Momchil Paunov, Maria Gurmanova, Margarita Kouzmanova*


P01_07  **Does the *Drosera rotundifolia* well-being depends on gibberellin and auxin?** *Desislava Mantovska, Zhaneta Georgieva, Zornitsa Karcheva, Mariya Rogova, Veneta Kapchina-Toteva, Aneliya Raycheva, Zhenya Yordanova, Ganka Chaneva, Miroslava Zhiponova*




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P01_08 Hematological profiles of mice sub-chronically exposed to lead and cadmium intoxication via drinking water. *Peter Ostoich, Iliana Alexieva, Michaela Beltcheva.*

P01_09  **Microalgal response against arsenite toxicity.** *Viktoria Vladimirova, Zornica Karcheva, Zhaneta Georgieva, Alexander Tomov, Detelina Petrova, Miroslava Zhiponova, Ganka Chaneva*

THEMATIC SESSION II

P02_01  **Study of the plant growth-promoting capacity of *Pseudomonas putida* 1046 in a model plant system.** *Gloria Georgieva, Trayana Nedeva, Marina Badalova, Veronika Deleva, Valentin Savov*

P02_02  **Challenges of aquaponic plant cultivation.** *Alexander Tomov, Detelina Petrova, Zhaneta Georgieva, Zornitsa Karcheva, Ganka Chaneva, Miroslava Zhiponova.*

P02_03  **Comparative study of metal concentration determination in albumen of hen eggs originating from industrial poultry farms, backyard and free-range hens using ICP-OES technique.** *Plamen M. Kirov, Methody Karadjov, Hristo K. Hristov, Radostina Alexandrova.*

P02_04 Water investigation of some lakes of Rila Mountain. *Stefan T. Todorov, Lidia T. Popova*



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30 September 2022

THEMATIC SESSION III

BIODIVERSITY. CONSERVATION BIOLOGY.

Chairperson: Prof. Valko Biserkov, PhD and Assoc. Prof. Dessislava Dimitrova, PhD

Technical support: Teodora Todorova and Tsvetan Tsvetanov

Plenary presentation

9³⁰-10⁰⁰ Biodiversity conservation and Biocultural heritage:

Bulgaria. *Dessislava Dimitrova, Yulia Bosseva, Teodora Ivanova* PL03_01

10⁰⁰-10³⁰ Ecological renovation as the next step in nature conservation in a changing world. *Valko Biserkov* PL03_02

Oral presentations

10³⁰-10⁴⁰  Phytocenological investigation of Hungarian oak (*Quercus frainetto* Ten.) forests in Maleshevska Mountain. *Iliana Todorova, Mariana Lyubenova* L03_01

10⁴⁰-10⁵⁰ Reasons for the conservation of the local honey bee *A. m. rodopica* and factors for the increasing honey bee colony losses in Bulgaria. *Evgeniya N. Ivanova and Plamen P. Petrov* L03_02

10⁵⁰-11⁰⁰  Orchid biodiversity of Golo Burdo Mountain, Bulgaria – current status and threats. *Andrey Popatanasov, Asen Asenov* L03_03

11⁰⁰-11²⁰ Discussion

11²⁰-11³⁰ Session break



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THEMATIC SESSION IV ECOSYSTEM RESEARCH AND SERVICES. LANDSCAPE ECOLOGY.

Chairperson: Prof. Marina Stanilova, PhD and Assoc. prof. Svetla Bratanova-Doncheva, PhD

Technical support: Galia Georgieva and Ivan Yanchev

Plenary presentation

11³⁰-12⁰⁰ The new challenges in Ecosystem research or how to see the whole without overlooking the details. Svetla Bratanova-Doncheva PL04_01

Oral presentations

12⁰⁰-12¹⁰ Forest habitats of Godech Municipality, Western Bulgaria. Borislav Grigorov, Nikolay Velez, Assen Assenov, Momchil Nazarov, Beloslava Genova, Kiril Vassilev L04_01

12¹⁰-12³⁰ Discussion

12³⁰-13³⁰ Lunch break

13³⁰-15⁰⁰ Poster session and Discussion

Chairpersons: Assoc. prof. Yanka Vidinova, PhD; Assoc. prof. Galina Radeva, PhD and Assoc. Prof. Kalina Danova, PhD

Technical support: Teodora Todorova and Petya Parvanova

POSTER SESSION

THEMATIC SESSION III


P03_01 Social parasitic ants in Bulgaria – new distribution records and overview. Albena Lapeva-Gjonova




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P03_02 Ant-associated *Rickia lenoirii* Santamaria, 2015 (Ascomycota: Laboulbeniales) on the Balkan Peninsula: first records. Albena Lapeva-Gjonova, Sándor Csősz, Ferenc Báthori, Walter Pfliegler

P03_03  How to use the terrestrial orchid *Ludisia discolor* for investigations? Alexander Tomov, Nikolay Krumov, Boris Tsenov, Viktoria Hristova, Georgi Bonchev, Ganka Chaneva & Miroslava Zhiponova

P03_04 Overview of *Xylariales* (Ascomycota) in Bulgaria. Dimitar Stoykov

P03_05  Distribution, ecology and external morphology of the Snake-eyed Lizard *Ophisops elegans* Ménétries, 1832 (Reptilia: Lacertidae) in Bulgaria. Georgi Krastev, Emiliya Vacheva, Borislav Naumov

P03_06 On the flora and vegetation of the area of Aldomirovsko Blato. Gabriela Petrova, Georgi Kunev, Anita Tosheva, Desislava Raykova, Miglena Borisova

P03_07 Boreal relict shrub habitats of Willow-leaf meadow sweet (*Spiraea salicifolia*) and Shrubby cinquefoil (*Potentilla fruticosa*) in Western Rhodope Mts. and their role for the mammals. Kalina Pachedjieva, Rossen Tzonev, Daniela Simeonovska-Nikolova, Venislava Spasova, Gabriela Petrova, Krastio Dimitrov

P03_08  Do brown bears hibernate in captivity? Katerina Zareva-Simeonova, Daniela Simeonovska-Nikolova, Venislava Spasova, Vladimir Todorov

P03_09 New records of hymenolepidid cestodes (Cyclophyllidea: Hymenolepididae) from waterfowl in Bulgaria. Margarita H. Marinova & Gergana P. Vasileva

15⁰⁰ Closing Procedure - Prof. Stephka Chankova, PhD



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Book of Abstracts

THEMATIC SESSION I

BIOTIC AND ABIOTIC IMPACT ON THE LIVING NATURE. ECOLOGICAL RISK. BIOREMEDIATION.

PL01_01

Does overproduction of chaperone proteins favor the repair of DNA injuries induced by oxidative stress?

Stephka Chankova¹, Nadezhda Yurina², Lubov Sharapova², Teodora Todorova¹, Petya Parvanova¹

¹Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

Genotype resistance to oxidative stress, induced by various physical /chemical stimuli has been in the focus of scientists for the last decades, with two main aspects –ecological (the formation of genetic elite of population) and medical (radio- chemotherapy).

Genotype resistance to oxidative stress is regarded as the integration of different morphological, physiological, biochemical, metabolic, and genetic characteristics. Currently, it is believed that the mechanisms involved in the formation of genotype resistance to oxidative stress are inter-correlated and inter-dependent, comprising changes in genes, proteins, enzymes, different metabolic pathways, and/or biological networks. According to the present state of knowledge, various cellular targets, resulting in genotoxic stress, induction of DNA damage, mutations, genomic instability or apoptosis can trigger different signal transduction pathways, activating DNA repair, antioxidant, and chaperone defense systems.

Till now, a lot of experimental data have been accumulated concerning the contribution of DNA repair to the formation of genotype resistance to oxidative stress. At the same time, genotype resistance of organisms is largely determined by the ability of molecular chaperones to maintain conformational homeostasis of proteins (folding – misfolding – refolding, or aggregation – degradation). The role of chaperones in protein homeostasis and cell death, especially in apoptosis, is well discussed in the literature, but much less is known about their function in DNA repair. In this regard, here we addressed the question of whether the overproduction of chaperone proteins contributes to the repair of DNA damage caused by oxidative stress.



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Acknowledgments: This review was elaborated under the project “Ecological and genetic assessment of the environment, management and strategies for risk overcoming” - BAS.

PL01_02

Modelling of secondary metabolite production by morphogenesis modification *in vitro*

Kalina Danova¹, Antoaneta Trendafilova¹, Kalina Alipieva¹, Jasmina Petreska Stanoeva², Viktoria Ivanova¹, Ina Aneva³, Marina Stefova², Milka Todorova¹

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²Institute of Chemistry, Faculty of Natural Science and Mathematics, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia

³Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

Medicinal and aromatic plants are an irreplaceable source of many biologically active compounds, vital for maintenance of human health. Bulgarian flora is exceptionally rich in species, utilized as teas, extracts and standardized pharmaceutical products. Conservation of plant populations and integrity of their natural habitats is a considerable issue in utilization of natural plant resources. Another important point is the achievement of stability in the content of active ingredients, this problem often not solved by the cultivation of the medicinal species in the farm.

Although genetically pre-determined, plant metabolic profile is a dynamic trait, dependent on the complex interrelations of the plant organism with the factors of its environment.

The present work gives an overview of the results obtained on targeting of the production of compounds with terpenoid, phenylethanoid, pherylpropanoid and polyphenolic structure by modification of plant growth, development and morphogenesis in *Sideritis scardica* Griseb., *Artemisia alba* Turra, *Hypericum perforatum* L., *H. richeri* L., *H. tetrapterum* Fries. and *H. calycinum* L. plant cell tissue and organ culture.

Thus, the obtained results are indicative that modification of the parameters of *in vitro* culture can direct secondary metabolites production and is even capable of induction of new compounds not previously identified in the intact field collected plant.

Acknowledgements we are thankful to the National Scientific Fund, Bulgaria, Grant num. KII-06-H39/6.



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L01_01

Multi-biomarker approach for PBDEs effects in Common carp (*Cyprinus carpio* Linnaeus, 1785)

Stela Stoyanova, Iliana Velcheva, Vesela Yancheva, Borislava Todorova, Eleonora Petkova, Dobrinka Todorova-Bambaldokova, Elenka Georgieva

Faculty of Biology, Plovdiv University, Plovdiv, Bulgaria

Polybrominated diphenylethers (PBDEs) are a group of chemicals that have been marketed since the 1960s. PBDEs belong to a class of hydrophobic, poorly degradable organic pollutants, which are persistent and bioaccumulate in the fatty tissues and biomagnify through the food web.

Aim: We aimed in the present experiment to study how PBDEs, which concentration was based on the EU legislation, would affect different biomarkers in Common carp.

Materials and Methods: The test fish species (Common carp) has well-defined characteristics, which make it an excellent bioindicator and also serve as an excellent test organism in ecotoxicological studies because it is relatively resilient to water pollution. Healthy juvenile carps (1 summer) without any visible pathological lesions were treated with different PBDEs concentrations, based on the allowable concentrations in water and biota according to the Water Framework Directive for 96 hours (acute exposure) under laboratory conditions. In terms of the applied biomarkers, histopathological alterations in the gills were studied.

Main results: Overall, the effects of PBDEs on Common carp were studied for the first time and we found different alterations, such as proliferative, degenerative and changes in the circulatory system caused by the acute exposure to PBDEs under laboratory conditions.

Conclusion: Further research in this particular field is needed in order to obtain knowledge on how commercial freshwater fish would react/adapt in polluted with PBDEs waters.

Keywords: pollution, toxicity, fish, biomarkers.

Acknowledgments: The study was conducted with the financial help of project FP 21 BF 008 – “*Ex situ* assessment of xenobiotic-contaminated aquatic ecosystems by applying multi-biomarkers in selected in bioindicator species” – NPD – Plovdiv University.

L01_02

Toxicological effects of commonly applied pesticides in Common carp (*Cyprinus carpio* Linnaeus, 1785)

Elenka Georgieva, Iliana Velcheva, Vesela Yancheva, Borislava Todorova, Eleonora Petkova, Dobrinka Todorova-Bambaldokova, Stela Stoyanova

Faculty of Biology, Plovdiv University, Plovdiv, Bulgaria

Pesticides are a group of man-made chemical compounds, invented to battle food shortages and meet the world's rising human population, which can increase the yield of crops and control pests that damage them, as well as disease-causing vectors.



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Aim: The present study deals with the investigation of short-term (96 h) effects of different concentrations of three commonly applied pesticides – 2,4-D (herbicide), pirimiphos-methyl (insecticide), and propamocarb hydrochloride (fungicide), based on their LC_{50} , on different biochemical and histological biomarkers in the liver of Common carp.

Materials and Methods: Healthy juvenile carps (1 summer) without any visible pathological lesions were purchased from the Institute of Fisheries and Aquaculture in Plovdiv, Bulgaria. Then they were treated with decreasing concentrations of the tested pesticides for 96 hours. The enzymatic activity of antioxidant enzymes CAT, GPx and GR and metabolic enzymes (ASAT, ALAT and LDH), as well as the histopathological alterations in the liver were studied. The obtained results were analyzed with GraphPad Prism 7, USA.

Main results: Overall, the effects of three different pesticides on Common carp were studied for the first time, which allowed us to discuss which pesticide and which concentration had more severe effects. In this regard, we found different alterations, ranging from cellular to physiological caused by the acute exposure to 2,4-D (herbicide), pirimiphos-methyl (insecticide), and propamocarb hydrochloride (fungicide) under laboratory conditions.

Conclusion: Further research in this particular field is needed in order to obtain knowledge on how commercial freshwater fish would react/adapt in polluted with various pesticides waters.

Keywords: pollution, pesticides, fish, biomarkers

Acknowledgments: The study is conducted with the financial help of project FP 21 BF 008 – “*Ex situ* assessment of xenobiotic-contaminated aquatic ecosystems by applying multi-biomarkers in selected in bioindicator species” – NPD – Plovdiv University.

L01_03

Bacterial and fungal abundance in different copper contaminated soils

Michaella Petkova¹, Nadezhda Nankova², Viktoriya Kancheva³, Silvena Boteva², Anelia Kenarova², Galina Radeva¹

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²Department of Ecology and Environmental Protection, Faculty of Biology, Sofia University “St. Kliment Ohridski”, Sofia, Bulgaria

³N. Poushkarov Institute of Soil Science, Agrotechnologies and Plant Protection, Agricultural Academy, Sofia, Bulgaria

Introduction: Copper (Cu) is one of the most common heavy metals that causes serious risks to humans and the environment. Excessive inputs of this metal in ecosystems can cause negative effects on soil microbial communities by affecting their abundance, diversity, and community structure.

Aim: The aim of this study was to assess bacterial and fungal abundance in different soils contaminated with copper.

Methods: Five soil samples with different Cu concentrations (ranging from 28.05 to 198.9 mg kg^{-1}) were collected from the valley of Topolnitsa River and its tributaries (S1-Medetska River, S2-



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Zlatishka River, S3-Pirdopska River, S4-Bunovska River and S5-Muhovo Village). Soil physicochemical properties were determined using standard protocols.

Total DNA was extracted, using E.Z.N.A soil DNA kit (Omega Bio-tek, USA). Real-time quantitative polymerase chain reaction (qPCR) was performed to assess the fungal and bacterial abundance. Primer sets ITS1/ITS4 and Eub338f/Eub518r were used, targeting the ITS fungal ribosomal DNA (rDNA) and bacterial 16S rRNA gene. Q-PCR was performed using iTaq™ Universal SYBRGreen Supermix (BioRad). Agglomerative hierarchical clustering (Ward Linkage) was conducted to evaluate the distance between sampling sites, soil physicochemical properties, and bacterial and fungal abundances, respectively.

Results: Results showed that the bacterial abundance was not influenced by the Cu concentration, whereas the fungal abundance decreased with the increasing concentration of Cu in soils. Cluster analysis identified two main clusters: cluster I - S5 and cluster II, consisting of two subclusters – uncontaminated soil samples S4 and S2, and contaminated soil samples S3 and S1. Our results showed that the 16S rRNA gene copy number correlated with $\text{NO}_3\text{-N}$, SOC and P_2O_5 , fungal ITS copy number correlated strongly with pH and slightly with $\text{NH}_4\text{-N}$, and Cu concentration correlated with water content. Both Cu contamination and local soil environmental variables change the fungal but not the bacterial abundance.

Keywords: Cu contamination, 16S rRNA gene, ITS rDNA

Acknowledgements: The study was funded by the Bulgarian Ministry of Education and Science – National Scientific Fund (Grand KP-06-M41/3).

L01_04

Genotype differences towards lead chloride toxicity

Teodora Todorova, Petya Parvanova, Maria Dimitrova, Kiril Zahariev, Krassimir Boyadzhiev, Martin Dimitrov and Stephka Chankova

Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

The aim of the study was to evaluate the pro-oxidative, toxic/genotoxic, mutagenic and DNA damaging potential of PbCl_2 on different test systems.

Materials and Methods: *Chlamydomonas reinhardtii* (WT) and *Saccharomyces cerevisiae* (D7ts1) in the end of exponential and the beginning of stationary phase were treated with various PbCl_2 concentrations (0.45 – 4.5mM). Cell survival, photosynthetic pigments, malondialdehyde (MDA), intracellular peroxides (H_2O_2), “visible” mutations, gene conversion, reverse mutation, mitotic crossing-over and DNA double-strand breaks (DSBs) were analyzed. The inhibitory and pro-oxidative potential on *Pisum sativum* cultivar Ran1 after the PbCl_2 treatment in concentrations’ range of 0.45 – 3.6mM was also evaluated.

Results: Genotype differences between *C. reinhardtii* (0.047mM) and *S. cerevisiae* (1.66mM) were observed by two endpoints: concentrations that can induce 50% lethality (LD_{50}) and DSB induction. Contrary, no mutagenic effect was found for both unicellular test models. Slight toxic capacity of lead, measured as inhibition of *Pisum sativum* seed germination as well as around 20%



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root length reduction were revealed after the treatment with concentrations equal or higher than 1,8mM.

The variety of stress response among the test models was demonstrated comparing two biochemical markers – MDA and H₂O₂. Around 2-fold increase in MDA levels without dose dependence was obtained after the treatment with concentrations higher than LD₅₀ of *C. reinhardtii* and *S. cerevisiae*. A dose-dependent increase of H₂O₂ levels was observed for *C. reinhardtii*. Analyzing the kinetics of MDA and H₂O₂ in pea leaves, the most pronounced effect of concentration was shown for 2.7mM. Despite that two experimental designs were applied in our study – short-term on *C. reinhardtii* and long-term in *P. sativum* treatments, a decrease in the photosynthetic pigments was detected.

Conclusion: In this study we confirmed our previous finding that *C. reinhardtii* is more sensitive model object than *S. cerevisiae* and *P. sativum*. The magnitude of stress response towards lead was found to be genotype specific.

Keywords: *Chlamydomonas reinhardtii*; *Saccharomyces cerevisiae*; *Pisum sativum*; lead chloride; pro-oxidative effect; toxicity/genotoxicity; mutagenicity; DNA damaging potential

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

L01_05

The geochemical anomalies of heavy metals in the river sediments of the Dragovishtitsa River as an indicator for cross-border pollution

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Aims: The article depicts the geochemical properties of the river sediments in the area of the Dragovishtitsa River in West Bulgaria. The research is conducted by examining of heavy metals' (Cu, Pb, Zn, Co, Cr, Mn, Ni) content in samples of river sediments in the local landscapes. The research aims to analyze the concentration of heavy metals as an indicator for cross-border contamination caused by mining activities upstream in Serbia.

Material and Methods: The study implements the coefficient of Clarke concentration. The deviation from the background concentrations is a ratio between the element concentration in the collected environmental samples and the Clarke value of the element. The coefficient has a scale from 0 to a particular positive value, corresponding to the level of enrichment of the sample in comparison to the background Clarke value. The values corresponding to the Clarke concentration are equal to 1, the lower values are between 0 and 1 (dispersion), and any value higher than 1 is a case of concentration (enrichment).

Main results: The obtained results display the researched territory as an area with a higher concentration of heavy metals in the sediments. The content of heavy metals in the river sediments of the surveyed area (mg/kg, median value) by chemical elements is Cu (41), Zn (416), Pb (70), Mn



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(1665), Ni (82), Co (18), and Cr (88). That is why it could be defined as unimpacted by human activities and is uninfluenced by natural geochemical anomalies.

Conclusion: There are geochemically anomalies in the researched landscapes. Heavy metals pollute them. This outcome is obtained by the geochemical content of the investigated heavy metals in the river sediments.

Keywords: mining, environmental impact, landscape assessment, contamination, ecogeochemistry

P01_01

Influence of physical factors on logarithmic, quiescent and non-quiescent *S. cerevisiae* cells



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Aim: The influence of environmental conditions on eukaryotic cells, including *Saccharomyces cerevisiae*, has been a subject of numerous scientific studies. One of the most important physical factors affecting eukaryotic microorganisms is temperature, pH, UV, osmotic pressure, mechanical, gravitational force, and ultrasound. Therefore, cell populations of quiescent (Q), non-quiescent (NQ), and logarithmic cells of *S. cerevisiae* were used as a model to examine the deleterious effect of these exogenous stressors.

Materials and Methods: Q, NQ, and logarithmic cells of *S. cerevisiae* were subjected to the different physical stresses and their survival rate was determined as CFU/ml. As a control untreated Q, NQ, and logarithmic cells were used.

Results: Physical parameters refer to the abiotic factors of an ecosystem. They affect living organisms and can have either stimulating or negative effects on cells. Studying their influence on the survival of *S. cerevisiae* yeasts revealed that Q cells are more resilient to various physical impacts from the environment. At the same time logarithmic and NQ cells showed from 10 % to 90 % lower resistance depending on the physical factor.

Conclusion: The complex action of abiotic factors on a living cell determines its biological path, namely entering different phases of the cell cycle, triggering proliferation, or formation of resting forms, which directs its adaptability to changed environmental conditions. Therefore, using *S. cerevisiae* cells as a model for revealing the impact of physical parameters on yeast growth in different physiological states is of fundamental importance.

Keywords: physical factors, yeast, quiescence, non-quiescence, logarithmic growth

Acknowledgment: This work was supported by a grant from the National Science Fund, Ministry of Education and Science, Project No. DH11/10.



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P01_02

Effects of foliar application of nitric oxide on sorghum under salt stress

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Aim: The present study was conducted to investigate the effect of exogenous nitric oxide (NO) on two varieties of sorghum (*Sorghum bicolor* L. Albanus and *Sorghum bicolor* L. Shamal) under salt stress.

Materials and Methods: The plants were grown in 1/2 Hoagland solutions and on 14th day were treated with 150 mM NaCl for 6 days. The co-treatment with different concentrations (0 – 300 µM) SNP (donor of NO) was applied through foliar spray on the plants. Chlorophyll fluorescence (PAM and JIP test), P₇₀₀ photo-oxidation, pigment analysis, determination of the membrane integrity and antioxidant markers were used for characterization of the protective role of NO under salt stress.

Results: The treatment of plants with 150 mM NaCl led to an inhibition of the functions of the photosynthetic apparatus: the maximum quantum yield of primary PSII photochemistry (ϕ_{P_0}), the photochemical quenching (q_P), the electron transport flux from Q_A to Q_B (ET_o/RC) and electron transport flux until PSI (RE_o/RC). These changes corresponded with membrane damage, a decrease of the pigment composition, an increase of the hydrogen peroxide and non-photochemical processes. The analysis of the P₇₀₀ photooxidation revealed that the PSI photochemistry was also inhibited by NaCl. Data revealed that the treatment with NO decreases the negative effect of salt stress on the studied plants. Experimental results also showed different salt-induced changes, as well as different protection by NO in studied varieties of sorghum. The reasons for different sensitivity and protection of studied plants are indicated.

Conclusion: The protective effect of NO was better after spraying the plants with smaller concentrations of SNP (25µM -150µM).

Keywords: sorghum, NaCl, nitric oxide, chlorophyll fluorescence, membrane integrity, antioxidant markers

Acknowledgements: This work was supported and by the Bulgarian Science Research Fund, KII-06-H36/9, 2019.



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P01_03



Comparative study on antioxidative potential of micropropagated and naturally growing plants *Nepeta nuda* ssp. *nuda*

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Aim: The objective of the present work was to compare the antioxidative status of catmint (*Nepeta nuda*) - *in vivo* and *in vitro* grown plants. The study aimed to examine changes in the antioxidant enzymes and reducing sugars as markers for the antioxidative potential.

Material and Methods: *Nepeta nuda* subsp. *nuda* was collected from its natural habitat in the Lozen Mountain, Sofia, Bulgaria. Fresh leaves of both, *in vivo* and *in vitro* plants were used to determine the activity of catalase (CAT), guaiacol peroxidase (PER) and superoxide dismutase (SOD). Changes in the reducing sugars' content were also investigated.

Main results: The comparison between *in vivo* and *in vitro* *N. nuda* plants showed that in *in vivo* leaves SOD and CAT activities were approximately three times higher. SOD activity *in vivo* reached 22,5 U/mg protein, as compared to *in vitro* plants - 7,26 U/mg protein. The differences in CAT activity were even greater - 0,78 $\Delta E \text{ min}^{-1} \text{ mg}^{-1}$ protein measured *in vivo*, and 0,23 $\Delta E \text{ min}^{-1} \text{ mg}^{-1}$ protein in the *in vitro* plants. However, in the leaves of *in vivo* grown *N. nuda*, the activity of PER was 1,6 times lower than in leaves *in vitro* propagated of *N. nuda*. Further, *in vivo* catmint plants synthesized 2,5 times more sugars than *in vitro* plants.

Conclusion: The present study showed that naturally growing *N. nuda* possessed higher antioxidant potential than *in vitro* micropropagated plants. Nonetheless, *in vitro* plants still could be used to largescale cultivation as they have the advantage to occupy insignificant growth area.

Keywords: *Nepeta nuda* subsp. *nuda*, *in vitro*, catalase, guaiacol peroxidase, superoxide dismutase

Acknowledgements: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N56/9/12.11.2021



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P01_04

Evaluation of abundance of microplastics in Bulgarian coastal waters

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Plastic pollution in sea waters is ubiquitous, but quantitative estimates on the floating microplastics in Black Sea are still limited. Plastics may adsorb persistent environmental contaminants, thus representing a potential risk for marine organisms.

Aim: The aim of the study was evaluation of the presence and characteristics of microplastic particles (MPs) in waters from the Black Sea coast of Bulgaria.

Materials and Methods: Samples of coastal waters were collected from March 2021 to April 2022 from different station on Black Sea coast, including protected, aquaculture and industrial areas. In order to determine the number of plastic particles in water, 23 samples were collected from surface waters at depth of ca. 1 m close to the Bulgarian shore. Samples were treated with H₂O₂, plastic particles were isolated by density separation and filtered over a membrane filter. Identification analysis of micro particles (< 5 mm) were performed visually by microscopy.

Main results: Results indicated widespread presence of microplastics in coastal waters. Mean MPs concentration was calculated 7.2 ± 4.8 pt/L. The highest concentrations of microplastics were observed in sea waters from industrial region (16.3 pt/L) and from protected area (12.7 pt/L). Comparison of the protected, aquaculture and industrial area showed that there is no significant difference in the abundance of plastic particles – 7.9, 7.8 and 6.3 pt/L resp. The most dominant forms were fibers followed by fragments. The most abundant size class of fragments was 101-500 μ m Ferret diameter.

Conclusion: Further studies are needed in order to fill knowledge gap and to evaluate distribution of plastic particles in the Black Sea and their potential ecological risk.

Keywords: microplastics, sea waters, Black Sea

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P01_05

Recent knowledge about the occurrence of marine biotoxins on Bulgarian coast in 2021

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Marine biotoxins are produced by certain phytoplankton species and use to accumulate in filter feeding marine organisms. Marine biotoxins' occurrence in all aquatic environments and latitudes is variable in time and space. Thus, it is an essentially natural phenomena, but its spread cannot be completely avoided or eliminated. A serious concern appears if these substances accumulate at high levels in seafood.

Aim: The aim of this study was to assess the presence of marine biotoxins in plankton samples from 2021 and to compare the determined levels with previous period.

Materials and Methods: Plankton samples (N=21) were collected in 2021 along the whole Bulgarian coastline (Black Sea). The presence of hydrophilic (domoic acid (DA)) and lipophilic toxins (okadaic acid, dinophysis toxin – 1, dinophysis toxin -2, azaspiracid-1, gonidomin A, pectenotoxin-2 (PTX2) and yessotoxin) was investigated via liquid chromatography – tandem mass spectrometry (LC-MS/MS).

Main results: Results indicated the presence of only DA in three samples and PTX2 in two samples. The positive samples were sporadically distributed along the study period. During 2018-2019 LC-MS/MS analysis confirmed the presence of DA, PTX2, SPX1 and GDA in plankton net samples collected from the same locations reported present study. The matching toxins (DA and PTX2) were at comparable levels in both period of investigation, thus lower than in other European water where harmful algal blooms are registered. There results showed the persistent appearance of some marine biotoxins in Bulgarian waters.

Conclusion: Although concentration levels are low, a constant monitoring is required in order toxic events by seafood consumption to be avoided.

Keywords: domoic acid, pectenotoxin-2, Black Sea, seafood

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P01_06

Alterations in membrane stability after *in vitro* exposure of human erythrocytes to 2.41 GHz electromagnetic field

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The growing use of wireless communication devices has been significantly increasing the level of high frequency electromagnetic fields (EMFs) in the environment, which raises a concern for possible deleterious effects on living organisms. Long lasting exposure to low-intensity EMFs can cause effects on the molecular and cellular level, and a number of morphological and physiological changes.

The aim of this work was to investigate the effects of 2.41 GHz EMF emitted by wireless communication systems on human erythrocytes after *in vitro* irradiation.

Material and Methods: The amount of the hemoglobin released from the cells was measured as an indicator for membrane destabilization. Effects of different exposure times (20 min or 4 hours) and time elapsed after exposure to 2.41 GHz pulsed or continuous EMFs with different intensities, emitted from a textile (0.213–0.238 V/m) or a dipole (5, 20, 40 and 180 V/m) antenna, were investigated.

The obtained results showed that the low intensity EMF had no significant effect on the hemoglobin release from irradiated cells; even a slight tendency for membrane stabilization was noticed 3–4 hours after the end of 20 min exposure to 0.213–0.238 V/m, 2.41 GHz EMF. There was no difference in the effects of continuous and pulsed EMFs. Increased hemoglobin release was observed only during the 4-hour exposure to 180 V/m, 2.41 GHz continuous EMF. In this case, the temperature of the cell suspension had also risen, and we compared these results with the effects of conventional heating. After 1-hour exposure (180 V/m) the released hemoglobin level was a bit higher than the control one but this difference was eliminated one hour after the end of the irradiation.

In conclusion, the *in vitro* exposure of human erythrocytes to 2.41 GHz EMF emitted by wireless communication devices with power density below the exposure reference level for the population does not change the stability of the cell membrane.

Keywords: wearable textile antenna, wireless, temperature effects, hemoglobin release

Acknowledgements: This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, grant number KP-06-H57/11 from 15th November 2021 "Antenna structures for new energy sources in next-generation wireless networks", leader Assoc. Prof. Gabriela Atanasova, South-West University "Neofit Rilski".



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P01_07



Does the *Drosera rotundifolia* well-being depends on gibberellin and auxin?

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Aim: The scope of the present research is to study the influence of phytoeffectors - gibberellic acid (GA), indole-3-acetic acid (IAA), and paclobutrazol (PAC) on the growth and antioxidant status of *in vitro* cultivated carnivorous plant *D. rotundifolia*.

Material and Methods: The *in vitro* culture of *D. rotundifolia* L. was induced from sterilised shoot explants, cultivated on standard MS medium. After 3 months of cultivation, growth regulators were added: 3 μ M GA, 6 μ M IAA, and 2 μ M PAC. The accumulation of intracellular ROS was detected using 2,7-dichlorofluorescein diacetate (DCF-DA). In order to study the non-enzymatic antioxidant capacity, the total phenol and flavonoid content, total antioxidant activity and DPPH radical scavenging activity were determined.

Results: The growth and the antioxidant status of *D. rotundifolia* were reduced upon cultivation with all used growth regulators, as the effect was most pronounced for PAC. The accumulation of ROS was induced by GA in epidermis and trichomes, and it ceased in presence of IAA and PAC. The content of polyphenols and flavonoids was significantly reduced in all variants and the antioxidant activity was fluctuating close to the control.

Conclusions: The present work revealed that *D. rotundifolia* growth and phenolic content were strongly affected by the level of the phytohormone GA, while the effect of IAA was milder. Notably, GA enhanced ROS accumulation but upon IAA the level of ROS was reduced. Our results suggest important role of gibberellins and auxins in *D. rotundifolia* development.

Keywords: *Drosera rotundifolia*, *in vitro*, IAA, PAC, GA



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P01_08

Hematological profiles of mice sub-chronically exposed to lead and cadmium intoxication via drinking water

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Aim: The aim of the present investigation is to determine the effects of sub-chronic exposure to lead and cadmium nitrate, individually or combined, on the main hematological parameters of white laboratory mice.

Material and Methods: Laboratory mice were exposed to $\text{Pb}(\text{NO}_3)_2$ at a concentration of 0.00250M and $\text{Cd}(\text{NO}_3)_2$ at a concentration of 0.00125M, individually or combined, in drinking water in controlled ecotoxicological experiments for a period of 45 days. Their growth performance, as well as their liver, kidney, and spleen indices were recorded at 15-day intervals. Twelve key hematological parameters were scored via a Mindray BC-30Vet semi-automatic hematology analyzer.

Results: It was determined that mice exposed to $\text{Cd}(\text{NO}_3)_2$ in drinking water have reduced growth performance. Mouse erythropoiesis was found to be significantly damaged in a very uniform pattern in mice, exposed to Pb and Pb+Cd, leading to the onset of anemia. Cd-exposed mice also developed damage to erythropoiesis, but with much greater individual variation. Decreases in WBC counts were recorded in all toxicant-exposed groups, indicating decreasing immune function and overall damage to the hematopoietic system.

Conclusion: The current study elucidates hematopoietic damage resulting from sub-chronic exposure to lead nitrate and cadmium nitrate in mice. As part of current and ongoing research on low-dose exposure to toxic metals, this work can serve as a baseline for future studies.

Keywords: lead (Pb), cadmium (Cd), hematology, sub-chronic exposure

Acknowledgements: This work was supported by the National Science Fund of the Republic of Bulgaria under project KP-06-N44/3 "Crystal-chemical and structural characteristics of modified natural clinoptilolite and correlation between its sorption properties, ion exchange capacity for heavy metals and biological response *in vivo* and *in vitro*".



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P01_09



Microalgal response against arsenite toxicity

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Aim: The aim of the present study was to investigate the physiological response of two green microalgal strains, *Coelastrella* sp. BGV and *Scenedesmus* sp. BGP, and a cyanoprokaryote, *Arthronema africanum*, towards arsenite toxicity.

Material and Methods: In the course of the experiment, the changes of algal growth, pigment and protein content were analyzed, after adding 1 mM, 5 mM, 10 mM and 20 mM NaAsO₂ to the nutrition medium. The activity of antioxidative enzymes, superoxide dismutase and catalase, were studied.

Main results: The growth of green microalgae was more affected by As than the cyanoprokaryote. The lowest dry weight (65% under control) and protein content (25% under control) were measured in the biomass of *Scenedesmus* sp., at 20 mM As. The addition of 5, 10 and 20 mM arsenite led to a sharp decrease in the chlorophyll content in all three strains, but mainly in *Scenedesmus* sp. (52% below the control). Carotenoids reacted differently to As toxicity - they increased strongly in green algae and decreased in *A. africanum*. A significant increase of phycobiliproteins was also measured when *A. africanum* was exposed to 1 mM NaAsO₂. Changes in the activity of antioxidant enzymes were species-dependent, with the highest activity being recorded in the cells of *A. africanum* - SOD activity was 2-fold enhanced, and CAT activity increased even more - 5-fold over the control value.

Conclusion: Both green microalgal strains were found to be more sensitive than the cyanoprokaryote despite the high concentrations of arsenite they endured. *Arthronema africanum*, as many other species of cyanoprokaryotes, tolerated high concentrations of arsenic dissolved in water, which makes it good candidate for the remediation of As-contaminated water bodies.

Keywords: arsenite, *Coelastrella* sp. BGV, *Scenedesmus* sp. BGP, *Arthronema africanum*, growth, pigments, superoxide dismutase, catalase

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THEMATIC SESSION II

ECOLOGICAL AGRICULTURE. ECOLOGICAL EDUCATION.

PL02_01

Evaluation of phytonutritional characteristics of edible flowers as new functional foods

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Since long time edible flowers (EF) are consumed as food, mainly as vegetables such as broccoli and cauliflowers. Nowadays, new natural food products have been discovered, and edible flowers of new plant varieties (from several families) have been tested. The phytonutritional characteristics have been determined, so EF are recognized as functional foods. Several flowers, mainly belonging to the Lamiaceae family, have been studied and the main antioxidant compounds have been detected to improve their use as novel food, and their VOCs (volatile organic compounds) have been identified. EF are rich in secondary metabolites with antioxidant properties, as polyphenols, flavonoids, anthocyanins, and vitamins as ascorbic acid. They contain also soluble sugars and minerals. They are recognized by specific aromatic profile, that contributes to the fragrance and sensorial aspect. The main critical points for their use are the shelf-life and their proper storage. EF can be consumed either fresh or dried, and their consumption is mainly limited due to the seasonality and perishability of the flowers. Several post-harvest conditions have been tested: freezing, chilling, hot-drying. The freeze-drying method seems to be the best effective solution to maintain unaltered the metabolites for longer period. However, the different flower morphology and their different metabolites content lead to conclude that each species have peculiar characteristics to be investigated in order to establish the best storage condition.

Keywords: nutraceutical properties, antioxidant compounds, aromatic profile, flowers storage, sensory analysis, Lamiaceae family

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PL02_02

Green nanoemulsion pesticides: efficiency, application, and safety

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Abstract

A new trend is starting for using nanotechnology in pest control in both agriculture and public health sectors. This technology proved new nanoformulation such as nanoemulsions, nan capsulation, and new nano pesticides. In this respect, plant extracts and natural oils are recognized in new nanoformulations as green-nano-pesticides as an alternative to synthetic pesticides in agriculture, food protection, and public health sectors for sustainable pest control programs. The promising effects and activity of nanoformulation are because those materials at the nanoscale have properties different from the bulk materials that enhanced properties when they are used in similar applications with similar doses. Currently, application of nanoemulsion in in food and agrochemical industries and pest control is increased. These nanoemulsions have droplet size diameter between 20- 200 nm. Uses of new green nano-pesticides lead to a decrease in the adverse toxic health effects on humans and the ecosystem. The structure and composition of nanoemulsions such as active ingredients, surfactants, and other helping materials are showed the toxicity profiles of nanoemulsions. The biological activity of nanoemulsion is correlated to droplet size or charge and nanoemulsion toxicity. Moreover, control release nanoformulations and encapsulation can decrease losses of these substances and increase chili-life time.

Keywords: nanoemulsion, pests, application, safety.

PL02_03

Place of ecological knowledge in biology and health education curriculum in the upper secondary level of school education in Bulgaria

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With the entry into force of Ordinance 7 / 11.08.2016 in the Bulgarian schools is introduced profiling education in various subjects in 11th and 12th grade. The profiled education is a set of three or four subjects studied in extended course outlines and with an increased number of hours. Two of these subjects are determined at the national level and state matriculation exams are held on them. The new program is valid from the 2020/21 school year and in May 2022 the first matriculation exams have been held. In the present study we have focused on the analysis of the curriculum and the



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teaching of ecology in the profiling education in biology. Our goal is to establish to what extent the ecological knowledge is present in the new curriculum, which is crucial for preparation of students as future active and responsible citizens. Along with the analysis, the study used surveys and narrative interviews of students and teachers regarding their opinion on the place of ecology in the profile subject "Biology and Health Education". From the study we found that despite the importance of environmental knowledge, in the curriculum it is presented with an insufficient number of hours. Although the curriculum allows to compensate for the missing classes by studying ecology in the elective modules, due to a number of circumstances, teachers avoid teaching ecology in these modules. With this study we try to draw attention to the scientific community, which can take an active part in the pedagogical process by conducting various forms of education to promote ecology in the profiling education in 11 and 12 grade.

Keywords: ecology, knowledge, biology and health education, profiling education

L02_01

***In vitro* micropropagation of *Tanacetum cinerariifolium* and establishment of an *ex situ* collection of selected clones**

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Aim: The study deals with rapid *in vitro* multiplication of *Tanacetum cinerariifolium* (Trevir.) Sch. Bip. (Asteraceae), *ex vitro* adaptation of selected clones, and establishment of an *ex situ* collection as a first step towards restoration of commercial plantations of the species in Bulgaria. Pyrethrum is valuable for the bioinsecticides accumulated mainly in its capitula.

Material and Methods: Seeds were surface sterilized and *in vitro* germinated on MS nutrient medium. One-seed derived clones were propagated by consecutive subcultivations on MS free of plant growth regulators (PGRs) and media supplemented with: 1) kinetin and indole-3-butyric acid in different concentrations or 2) antibiotic and CaCO₃. The propagation effectiveness was evaluated as a number of new plants obtained per initial shoot. Clones were selected on the base of the propagation rate. Plants were *ex vitro* adapted in a phytotron, and transferred to the *ex situ* collection in 2019. Clones were compared by the number of capitula per plant.

Main results: Seed germination rate was low in all tested media. Considerable losses of *in vitro* multiplied shoots were noticed due to endophytic contaminations, especially on media supplemented with PGRs; necrosis was also observed. To overcome these problems, the basal MS medium was modified, including antibiotic and increasing Ca-ions, followed by optimizing their concentrations. In the best variant (200 mg/l Medaxone + 75 mg/l Ca⁺) no more infected plants were observed, and the percentage of necrotic plants decreased threefold, which resulted in formation of 38.06±10.11 new plants per initial shoot for a period of 7 months. All 16 plants from four clones



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transferred to the *ex situ* collection bloomed twice in the first year (June and September). An average of 328 capitula per plant formed in the second year for the best clone.

Conclusion: The results are promising towards establishment of a pilot plantation of pyrethrum.

Keywords: Pyrethrum, *Chrysanthemum cinerariaefolium*, Bocc bioinsecticides, *in vitro* cultivation.

Acknowledgements: This research work was financially supported by the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" at the Bulgarian Ministry of Education and Science (DCM № 577/17.08.2018)

P02_01



Study of the plant growth-promoting capacity of *Pseudomonas putida* 1046 in a model plant system

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Nowadays, the need for environmentally friendly fertilizers (biofertilizers) is constantly increasing. Plant Growth Promoting Rhizobacteria (PGPR) are an important part of the rhizosphere since they support the better development of the plants' root system. In the rhizosphere, the PGPR fix nitrogen, dissolve phosphorus, synthesize phytohormones and iron-binding proteins (siderophores), and help the plant to survive biotic and abiotic stresses. All these characteristics make them suitable for application as a microbial fertilizer.

Many species, belonging to g. *Pseudomonas* possess plant growth-promoting (PGP) characteristics. They are suitable for use as biocontrol agents since they grow well and quickly in the rhizosphere. These bacteria take part in plant-microbial symbiosis by promoting the plants' growth and development and by protecting them from their natural enemies.

Aims: This study aimed to check and prove the PGP capacity of bacteria belonging to *Pseudomonas putida* species.

Materials and Methods: A seed germination method was used to study the PGP effect of *Pseudomonas putida* 1046 strain on corn (*Zea mays*) seeds through monitoring of the growth indicators germination capacity and growth parameters of the plant root system.

Main results: The test plants subjected to inoculation with bacterial suspension (1×10^6 CFU) for 120 hours showed growth indicators values that indicate a positive effect of the studied strain on the plant physiology. The results obtained demonstrate that the *Pseudomonas putida* 1046 strain enhances the process of corn germination, increases the number of lateral roots, and enlarges the length of the plant root.

Conclusion: The studied PGP capacity of the *Pseudomonas putida* 1046 strain showed positive preliminary results in promoting the growth of the economically important *Zea mays* plant system.



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Keywords: rhizosphere microorganisms, PGP, biofertilizer, *Zea mays*, *Pseudomonas putida*

P02_02



Challenges of aquaponic plant cultivation

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Aim: The aim of the present study was to monitor the fluctuations of nitrate, nitrite, ammonia and phosphate in the media of hydroponic and aquaponic systems, along with some stress markers in the aquaponically and hydroponically grown plants.

Materials and Methods: Plant species *Lactuca sativa* L., *Pisum sativum* L. and *Zea mays* L. were cultivated for 14 days in three experimental systems: hydroponics 1 (tap water); hydroponics 2 (Knop nutrient medium) and aquaponics. A substrate bed type system was used for aquaponics and goldfish (*Carassius auratus* L.) were selected for aquaculture. The amounts of ammonium, nitrate, nitrite and phosphate ions in the three cultivation media were determined. Malondialdehyde (MDA) content and endogenous hydrogen peroxides in the leaves of experimental plants were also assayed.

Main results: The presence of residual ammonium ions, although low, was observed in the pea and maize hydroponic systems. Further, the highest levels of nitrate and nitrite (traces) were found in maize hydroponic media. The enhanced phosphate concentrations were measured in maize aquaponic medium ($3.6 \mu\text{g ml}^{-1}$). In general, the largest amount of nutrition elements was found in *Z. mays* medium, regardless of the cultivation method, which may be a result from the increased root secretions. We found the highest concentrations of malondialdehyde in the pea leaves ($7.2 \mu\text{M/g}$, aquaponics). In maize and lettuce, the amount of MDA increased in the hydroponic system 1. Elevated levels of total peroxides have been reported in aquaponically grown pea ($1.4 \text{ mM g}^{-1} \text{ DW}$). In maize and lettuce, the highest levels of peroxides were observed in the hydroponic system 1 ($0.53 \text{ mM g}^{-1} \text{ DW}$ and $0.83 \text{ mM g}^{-1} \text{ DW}$).

Conclusion: The results showed that the aquaponics is less suitable for pea *P. sativum*. In maize, however, that method proved to be the most favorable for growth, while lettuce developed equally well under both, hydroponic and the aquaponic cultivation.

Keywords: aquaponics, hydroponics, *Lactuca sativa* L., *Pisum sativum* L., *Zea mays* L.

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P02_03



Comparative study of metal concentration determination in albumen of hen eggs originating from industrial poultry farms, backyard and free-range hens using ICP-OES technique

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Because of its role in human nutrition, there have been multiple types of research focused on the relationship between feed ingredients and metal content in the egg white. **The aim** of the present study is to determine the metal concentration in hen eggs and, in particular, to compare the metal concentration in egg albumen originating from industrial poultry farms with that of backyard and free-range hens.

Materials and Methods: All samples were collected in Romania from five separate counties and 10 different farms, over a period of two weeks, and, as a result, a total of 50 samples were collected, 10 for each housing system. The measurements of the metals were taken by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), with a wide range of elements reported. For the essential ones, we have Cr, Cu, Fe, Mn, and Zn. Al, Cd, Ni, and Pb for the heavy ones, and in addition, we measured B, Ba, Sr, Ca, and Mg.

Results: The present study revealed that the metals in eggs from free-range hens are richer in essential elements with mean concentrations as follows: 1.528 for Fe, 3.278 for Zn, 0.058 for Mn, 1.362 for Cu, 1.551 for Al, and 0.273 mg/kg for Pb. Poultry farm eggs contain a higher concentration of 0.052 for Cd, and B with 2.244 mg/kg.

We concluded that the egg quality is closely connected with the housing and feed composition. Furthermore, the results demonstrate that eggs from backyard housing are no better than those from free-range hens in terms of essential metal composition. The heavy and non-essential metal contents present in the albumen in all of the examined eggs were much lower than the maximum allowed concentration, and therefore, egg consumption does not pose any risk to human health.

Keywords: food safety, albumen, egg-white, farming systems, ICP-OES, poultry housing, poultry nutrition



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P02_04

Water investigation of some lakes of Rila Mountain

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Aim: The aim of this work is to investigate lake water samples by energy spectra.

Materials and Methods: Samples were taken from different locations in the Rila Mountains area, remote from urban development. A snow (snow water) sample from Musala Peak area was also investigated for comparison. The method used is based on the presence of hydrogen bonds between water molecules. By studying the evaporation of the droplet of the sample studied and comparing the energy spectra obtained with the spectra obtained for the deionised water sample, the presence or absence of impurities (contaminants) can be inferred.

Results: Typical peaks (minima and maxima) in the region of 0.10-0.105 eV were observed in the differential spectra of all investigated samples of lake and snow water. Correlation coefficients between differential spectra were obtained.

Conclusions: The method showed its sensitivity to the complex influence of various chemical and physical factors affecting even pure samples of mountain water.

Keywords: mountain ecosystem, lake water, snow, energy spectra of water.



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THEMATIC SESSION III

BIODIVERSITY. CONSERVATION BIOLOGY.

PL03_01

Biodiversity conservation and Biocultural heritage: Bulgaria

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The complex and not always favourable human-nature relations have led to a substantial loss of habitat and species diversity and have deteriorated our well-being in the last decades. However, pursuing global or even limited local sustainability poses numerous challenges that require considerable change of economy, technology and also human lifestyle. Biocultural studies were initially focused on the integration of data related to cultural, behavioural and social sciences with biological and environmental data in search of new ways for improvement of human wellbeing. The population aging, changes in land use and growing urbanization of the Global North poses a growing need to preserve the biological cultural heritage, especially in countries like Bulgaria where these processes have reached a critical level in rural areas. Biocultural heritage encompasses indigenous and local community knowledge, innovations, and practices, which are developed within, and linked to, the social-ecological context. According to UNESCO (2008) the definition of biocultural heritage includes “living organisms or habitats whose present features are due to cultural action in time and place”. A variety of factors (climatic, biological, agronomical and socio-economic) put pressure on biocultural diversity *in situ*. However, due to political and in many cases limited involvement of experts in biological and other sciences, many objects of biocultural heritage are not only threatened but also understudied. Ethnobotanical and food studies examples are presented so to illustrate the current state and urgency of interventions for safeguarding Bulgarian biocultural heritage. Challenges and opportunities for grassroots citizen science and interdisciplinary scientific collaborations are discussed.

Acknowledgements: This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science of Bulgaria (Agreement № D01-230/06.12.2018).



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PL03_02

Ecological renovation as the next step in nature conservation in a changing world

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Abstract

The collapse in biodiversity poses the question about the adequacy and sufficiency of conventional conservation actions for restoring ecosystems to their previous state. Until recently approaches like assisted migration, prioritizing conservation objects taking climate change into account, lowering the attention towards unviable populations, cost/benefit analysis of ecosystem replacement were considered unacceptable and controversial.

In the context of climate change, Prober et al. (2019) suggest the term “ecological renovation”, which admits ecological management of active, deep change of ecosystems through renovation to a state with high biodiversity and buffer capacity, which better corresponds to the new conditions. Cross et al. (2012) argue that using a wider spectrum of adaptation strategies is needed to achieve the policy goals.

Climate change, habitat degradation, pollution, over-exploitation, invasive species and disease are the main negative factors for biodiversity. When applying ecological renewal to local populations/ecosystems, we must consider their place on the gradient from core to periphery, which could exacerbate the negative factors. Local populations/ecosystems at or near the boundary could be the first to become unviable, have their support become unjustifiable and lose their priority.

Soon climate changes will make the need for ecological renewal by drastic actions more and more frequent. Currently there is a sharp deficit of data and conservation research to support informed executive decisions. We are critically pressed for time to provide the scientific basis for adequate responses to deepening and accelerating environment changes. Therefore, for concrete local ecosystems the best base for the management of their renovation is provided by data about buffer capacity of the source ecosystem, the degree of change in conditions, the degree of damage and the characteristics of species to be used in the formation of the new ecosystem.

Keywords: biodiversity decline, climate change, conservation biology, ecological renovation, ecosystems.



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L03_01



Phytocenological investigation of Hungarian oak (*Quercus frainetto* Ten.) forests in Maleshevska Mountain

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The aim of the study is to present a characteristic of monodominant communities of Hungarian oak in Maleshevska Mountain and forest Reserve "Sokolata", which belong to the association *Digitali viridiflorae-Quercetum frainetti Gamisans et Herbard 1980* by the floristic classification of Gogushev (2009).

Material and Methods: Phytocenological descriptions were made in 2018 – 2020 (March – September) to evaluate some structural indicators – species composition, abundance estimation, forest inventory. The biological type, the life forms (after Raunkier) and the floral elements (after Assyov et al.) for the vascular plants have been determined.

Results of floristic analysis in the investigated phytocoenosis showed 63 species of vascular plants, which refers to 22 families and 52 genera. The families *Rosaceae*, *Fabaceae*, *Lamiaceae* and *Poaceae* were presented with the greatest number of species. Most of them were perennial herbaceous plants - 77.8 %. The life forms were definitely dominated by hemicryptophytes – 68.3 %. Species distributed in the investigated area relate to 17 floristic elements. The greatest proportion of them are Euro-Asiatic (15.9 %), European (14.3 %), Euro-Mediterranean (12.57 %), sub-Mediterranean (12.57 %) and Euro – Siberian (11.1 %).

Conclusion: This distribution is typical for broad leaved forest communities and for the biological spectrum of Bulgarian flora. The high participation of Euro-Mediterranean and sub-Mediterranean floristic elements talks about Mediterranean climatic influence in this part of the country. The results were compared with other studies for Hungarian oak communities in Bulgaria.

Keywords: Hungarian oak (*Quercus frainetto* Ten.), Maleshevska Mountain, floristic composition, forest inventory



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L03_02

Reasons for the conservation of the local honey bee *A. m. rodopica* and factors for the increasing honey bee colony losses in Bulgaria

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The uncontrolled introduction of foreign genes into the adapted local populations, the stress from the changing environment and its pollution, new pathogens and the global climate changes are the main risk factors for the genetic richness among honey bee populations.

The local Bulgarian bee *A. m. rodopica* is characterized by high levels of queen fertility, productivity, overwintering and hygienic behavior, and low levels of defensive and swarming behavior. At present, different genetic markers could be used for its discrimination.

Aim: The current study aims to reveal causes for the conservation of the local honey bee and study the factors for the increasing honey bee colony losses in Bulgaria.

Material and Methods: In order to study the honey bee colony losses dimension, a standardized international COLOSS questionnaire has been used. The pesticides presence has investigated by chemical chromatographic analysis.

Main results: In the past five years the condition of Bulgarian beekeeping is under great danger because of uncontrolled imports of honey bee queens with foreign origin and the large-scale use of pesticides, related to the reported high mortality of bee colonies in Bulgaria.

For the period 2017 – 2022 the colony losses in Bulgaria increase from 2.04% to over 30%. At the same time, the residues of a total of 27 different pesticides in the samples of bees and the colony food stocks, is found. This is a signal for an objective danger for high mortality and severe weakening of bee colonies in some regions of Bulgaria.

Conclusion: The presented data reveal the need to develop measures for conservation of the national genetic resource of *A. m. rodopica* and conducting activities for detailed studies of the risk factors for the health and viability of honey bees, including the various agrochemicals and their mechanism of action in nature.

Keywords: *Apis mellifera*, conservation, pesticides, honey bee colony losses

Acknowledgments: This study is supported by the National Research Fund through the contract KP-06-H5112/2021 "Complex assessment of genetic and environmental factors related to the losses of honey bees (*Apis mellifera* L.) in Bulgaria".



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L03_03



Orchid biodiversity of Golo Burdo Mountain, Bulgaria – current status and threats

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Aims: the study explores the biodiversity of the species from *Orchidaceae* family in the Golo Burdo Mountain, Bulgaria.

Material and Methods: The mountain is relatively dry and well defined massive and was visited multiple times during the last decade in order to explore the distribution, habitat preference and the status of the *Orchidaceae* species growing in this region.

Main results: While most of the reported previously orchid species was confirmed and their distribution mapped, some of them could not be verified. However new orchid species were found and documented and the chorological data of *Orchidaceae* got enriched.

Conclusion: Although in the small Golo Burdo mountain is found significant portion of the reported for Bulgaria orchid taxa, this is not a reason for complacency. Most of the species are rather rare and vulnerable, and in unprotected territories.

Keywords: orchids, *Orchidaceae*, bioconservation, Golo Burdo Mt.

P03_01

Social parasitic ants in Bulgaria – new distribution records and overview

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Aim: The study makes additions and review on the diversity, type of parasite-host relations and host range of social parasitic ant species in Bulgaria.

Material and Methods: The new data on the distribution of the species were obtained as results of several entomological studies in the country through the use of pitfall traps and manual collection. The review is based on new and published results.

Main results: This study reports new localities of the slave-makers *Myrmoxenus gordiagini* Ruzsky, 1902 and *M. kraussei* (Emery, 1915), and the inquiline *Anergates atratulus* (Schenck, 1852) in Bulgaria. A total of 42 social parasitic ants in Bulgaria have been identified so far. They belong to three of the six subfamilies in Bulgaria - Formicinae (25 species), Myrmicinae (15 species) and Dolichoderinae (2 species). 14 of the genera contain species with social parasitic lifestyle. Most belong to *Lasius* (13) and *Formica* (9), which are mainly temporary parasites, followed by dulotic ant species from *Strongylognathus* (6) and *Myrmoxenus* (3) genera. There are two temporary parasitic species of *Bothriomyrmex*, and the following genera *Myrmica*, *Harpagoxenus*, *Formicoxenus*,



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Chalepoxenus, *Anergates*, *Plagiolepis*, *Camponotus* and *Polyergus* are represented by one species. Parasite-host relationships are most often refer to 4 types: xenobiosis, temporary parasitism, slavery and inquilism (the most advanced), presented by 1, 25, 12 and 4 species, respectively.

Conclusion: Although the social parasitic ants in Bulgaria remain insufficiently studied, they represent 22% of the regional ant fauna.

Keywords: ants, Bulgaria, xenobiosis, temporary parasitism, slavery, inquilism

Acknowledgements: This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers No 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement No Д01--279/03.12.2021).

P03_02

Ant-associated *Rickia lenoirii* Santamaria, 2015 (Ascomycota: Laboulbeniales) on the Balkan Peninsula: first records

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Aim: The study presents first records of the ectoparasitic fungus *Rickia lenoirii* Santamaria, 2015 (Ascomycota: Laboulbeniales) on *Messor* ants from the Balkan Peninsula.

Material and Methods: New data on the distribution and host range of *Rickia lenoirii* were obtained as results of routine myrmecological studies in Bulgaria, Albania and Greece through the use of ant hosts collection by hand. The ant specimens were stored in 96% (or more) ethanol and detection of the fungus and morphometry were made by microscopic examination. Images by Scanning electron microscope (SEM) were also taken.

Main results: This study reports first occurrence data and host associations of the fungus *Rickia lenoirii* (Laboulbeniales) on the Balkan Peninsula. It was found in 9 localities in Bulgaria, 2 in Albania and 1 in Greece. We confirmed the use of the two known ant hosts so far – *Messor structor* (Latreille, 1798) and *M. wasmanni* Krausse, 1910, and added two more – *Messor hellenius* Agosti & Collingwood, 1987 and *M. mcarthuri* Steiner et al., 2018.

Conclusion: *Rickia lenoirii* is no less common than the other *Rickia* species on *Myrmica* ants (*R. wasmanni* Cavarra), but it is limited in distribution according to the range of *Messor* ant species, which are most diverse in the Mediterranean region.

Keywords: Balkan Peninsula, insect parasitic fungi, *Messor*, *Rickia*

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P03_03



How to use the terrestrial orchid *Ludisia discolor* for investigations?

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Ludisia discolor (Ker-Gawl.) A. Rich. is a terrestrial orchid which originates from the southern parts of Asia but is often grown as an ornamental plant in many countries outside its habitat. The plant is known as a jewel-orchid due to its characteristic leaf coloring giving the high value of the plant in floriculture. Beside its decorative features this orchid and closely related species were claimed to have medicinal properties.

Aims: We aimed to apply *in silico* the DNA barcoding approach for choosing an optimal marker for a taxonomic determination. Further, we introduced the plant in *in vitro* conditions to establish experimental system for its detailed study.

Material and Methods: The DNA barcoding approach serves for species identification and establishment of phylogenetic relationships. We used publicly available *in silico* data for *L. discolor* from the Barcode of Life Data System (BOLD) data base. The resulting list of sequenced *L. discolor* marker genes included information about two chloroplast (*matK*, *rbcL*) and one nuclear (*ITS*) DNA barcoding markers. Orchid stem segments were subjected to sterilization and regeneration of *in vitro* plants. Leaves from *in vivo* and *in vitro* regenerated plants were compared anatomically to confirm successful regeneration.

Main results: The phylogenetic tree based on the *matK* marker distinguished *Ludisia* as a sister genus of *Anoectochilus*. In comparison with *rbcL* and *ITS*, *matK* showed highest resolution. Combinations of the markers did not provide precise phylogenetic information. Anatomical analysis confirmed structural similarity in the histological organization of leaves in *in vivo* and *in vitro* *L. discolor* plants, which is an important condition of the high regeneration potential of the species during *in vitro* cultivation and *ex vitro* adaptation.

Conclusion: The established experimental system would allow to test phytoeffectors for enhanced *L. discolor* productivity.

Keywords: DNA barcoding, *Ludisia discolor*, leaf anatomy, micropropagation

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P03_04

Overview of *Xylariales* (Ascomycota) in Bulgaria

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Aim: The aim of this work is to present a preliminary list of the xylarialean fungi, known up to date in Bulgaria.

Material and Methods: Various publications on Bulgarian mycota were searched for records of fungi from order *Xylariales*. Information on species with known asexual morphs is included, according to published sources. The nomenclature and abbreviations of author names in the resultant list are given according to Index Fungorum and MycoBank databases.

Main results: Sixty-four taxa from Bulgaria are arranged alphabetically in the list, including information on their synonyms, known distribution by floristic regions, and most typical plant substrata. Two species, *Anthostoma decipiens* - found on fallen dead oak branch and *Pseudomassaria chondrospora* - on overwintered petioles of *Tilia platyphyllos*, are reported for the first time from Bulgaria. *Hypoxylon howeanum* is confirmed for the country with new host tree in the Eastern Forebalkan. Digital macro- and microphotographs of 14 species are presented in colour figures.

Conclusion: Sixty-four accepted xylarialean fungi, including two new species - *Anthostoma decipiens* and *Pseudomassaria chondrospora*, one variety and one subspecies, were reported in Bulgaria. *Hypoxylon howeanum* is confirmed on dry twigs. This first preliminary list of *Xylariales* in Bulgaria includes the information about the known diversity, published synonym names, regional distribution and typical plant-hosts published so far from our country. We hope the overview will stimulate more intense future research on this taxonomically difficult fungal group.

Keywords: ascomycetes, Bulgarian mycota, xylarialean fungi

Acknowledgements: The study is held within the framework of 'Phylogeny, distribution and sustainable use of fungi' project.

P03_05



Distribution, ecology and external morphology of the Snake-eyed Lizard *Ophisops elegans* Ménétries, 1832 (Reptilia: Lacertidae) in Bulgaria

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Aims: *Ophisops elegans* is one of the least studied lizard species in Europe. It occurs in the Eastern Mediterranean, Iran, part of the Caucasus region, Asia Minor, and the southeastern part of the Balkan Peninsula. The present work aims to explore the distribution, ecology and morphology of *O. elegans* in Bulgaria, where the northwestern most part of the species range is located.



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Material and Methods: In general, the study area covers the eastern part of the Eastern Rhodopes Mts. (the only region where *O. elegans* is known from Bulgaria), but most of the field trips were conducted in the river valley of Byala Reka, Ivaylovgrad District. Data have been collected sporadically in a period of 7 years and include a total of 137 individual registrations of *O. elegans*.

Main results: The presence of *O. elegans* in Bulgaria was confirmed in each of the four squares (10x10 km UTM grid) for which the species is known according to the literature data, moreover several new localities were identified, which fall into three other UTM-squares. Division of the found individuals per habitat type was as follows (as a percentage of all observed individuals): 1) stony terrains with junipers and other shrubs – 64.23%; 2) open terrains with sparse vegetation – 14.60%; 3) outskirts of thin oak forests – 12.41%; 4) rocky slopes over streams and roads – 8.76%. Statistically significant "sexual size dimorphism" (SSD) was found on 10 of the indices used, describing the body proportions.

Conclusion: In Bulgaria, *O. elegans* is more widespread than previously thought. It seems that here, the species prefers (as a habitat) mainly stony or sandy slopes with shrubby vegetation. SSD is expressed in relatively longer limbs and a more massive head in males, while in females the torso is relatively longer (as the distance between the fore and hind limbs).

Keywords: lizards, mapping, ecology, sexual size dimorphism

P03_06

On the flora and vegetation of the area of Aldomirovsko Blato

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Aim: The aim of our presentation is to report the results on the study of the local flora, focused on species of conservation significance, and the current state of marsh vegetation within the protected area "Aldomirovsko Blato". In addition, the plant communities of surrounding area, dominated of mostly mesophyllous, herbaceous species were also studied.

Methods: Fieldwork were carried out in the period of May-October, in vegetation season of 2022. The data were collected by direct observations, transect methods, and the standard Braun-Blanquet's sampling method. The numbers of the species of conservation significance were estimated by direct count, or by evaluation of their relative abundance.

Results: We present a species list of all vascular plants observed within the study area, noted at the field or within the vegetation plots. We analyze chorological spectrum, life forms and Elenberg's Indicator Values (EIV) for each of the species of the list. The current state of the local populations of the two taxa of conservation significance, *Fritillaria meleagroides* (CR) and *Viola pumila* (EN) were evaluated. We sampled vegetation plots in the most representative spots of aquatic, hygrophytic and mesophytic communities and propose a synoptic scheme in agreement with the most recent classification system.

Conclusions: The population of *V. pumila* shows significant increase in its relative abundance, whereas the population of *F. meleagroides* remains stable comparing with previous studies. Our data



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confirmed changes in floristic composition and shifts in structure of the vegetation units comparing with earlier researches within the study area. These changes are mostly related to desiccation, unregulated fires and ploughing.

Keywords: protected area, flora, threatened species, wetland vegetation, classification

Acknowledgements: The research is carried out under the project: “Study of the flora and vegetation in the Aldomirovsko Blato Protected Area” – Contract №80-10-178/27.05.2022

P03_07

Boreal relict shrub habitats of Willow-leaf meadow sweet (*Spiraea salicifolia*) and Shrubby cinquefoil (*Potentilla fruticosa*) in Western Rhodope Mts. and their role for the mammals

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Several types of relict shrub habitats occur in Bulgaria with limited and local distribution. They are located in remote refugial areas in the country, more often are included in protected areas, and are distinguished by specific ecological features.

Aim: The present study focuses on two shrub habitats included in the Red Data Book of the Republic of Bulgaria, Vol. III – Rhodope thickets of Willow-leaf meadow sweet (*Spiraea salicifolia*) and Rhodope thickets of Shrubby cinquefoil (*Potentilla fruticosa*) in the region of Western Rhodope Mts. In the scope of our scientific research are set the following questions: what is the floristic composition and ecological structure of both habitats, what is their representativeness at a European level and what is their conservation significance in the context of the European Red List of Habitats, as well as what mammals are closely related to these habitats.

Materials and Methods: The plant communities were characterized according to standard phytocoenological and floristics methods. The presence and relative abundance of mammals were studied by standard methods as Sherman live traps and camera traps.

Results: The habitats represent F9.2 Salix fen scrub and F2.3 Subalpine deciduous scrub from the European Red list of habitats. Both habitats are composed by dense and closed acidophilous communities. The floristic composition is rich in Boreal, subBoreal and European elements. The results show that they play an important role for the populations of *Martes martes*, which is a threatened species in Bulgaria (category “Endangered”), and *Capreolus capreolus* with large mammals. Among the small mammals with the highest relative abundance is the bank vole (*Myodes glareolus*), which is typical of the region.

Conclusion: Therefore, as relict natural communities, the studied habitats perform important ecological functions and could be buffers in the conditions of climatic and other anthropogenic changes.



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Keywords: Relic shrub habitats, *Potentilla fruticosa*, *Spiraea salicifolia*, flora, mammals, Rhodope Mts.

Acknowledgements: This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers No 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement No Д01-279/03.12.2021).

P03_08



Do brown bears hibernate in captivity?

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Around fifty brown bears (*Ursus arctos*) live in captivity in Bulgaria in licensed and unlicensed zoos and parks. The brown bear is an endangered species in Bulgaria and is included in Annexes II and III of the Biological Diversity Act. The critical period for survival and reproduction for the brown bear is hibernation, when any anxiety can be fatal. Given that, knowledge of the conditions in captivity during the period of preparation and stay in the den or winter cage is extremely important. Moreover, global climate change and associated regional climate variability is impacting the phenology of many species and this should be taken in consideration in the husbandry procedures of the species.

The **aim** of the present study is to evaluate the parameters of the environment and husbandry procedures related to the period of hibernation in captivity.

Material and Methods: Information was collected through interviews with responsible staff of zoos and centers which rear brown bears in Bulgaria.

The **results** showed that less than 50% of the bears kept in zoos have adequate hibernation conditions and have fallen asleep in recent winters, while in Belitsa Bear Park all bears have such conditions and almost all hibernates. The age and individual history of the animals seems also to affect whether bears will hibernate and for how long.

Conclusion: The obtained results allow us to identify the problems which prevent bears from hibernation in captivity and to propose measures to improve the husbandry procedures for this species of conservation importance.

Keywords: hibernation, zoo, husbandry, *ex situ*

Acknowledgements: The authors are grateful for the financial support of grant № КП-06-H51/8-11.11.2021 by the Bulgarian National Science Fund.



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P03_09

New records of hymenolepidid cestodes (Cyclophyllidea: Hymenolepididae) from waterfowl in Bulgaria

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Aim of the study: To present detailed morphological re-descriptions of two cestode species of the family Hymenolepididae parasitising anseriform birds in Bulgaria; to analyse their previous records and to summarise data on their host range and geographical distribution.

Materials and Methods: The study was based on examination of collection specimens preserved in the Helminthological Collection of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences. The cestode specimens have been isolated from the small intestines of 6 specimens of *Aythya nyroca* (Güldenstädt) and 2 specimens of *Cygnus cygnus* (L.), collected in 3 localities in Bulgaria.

Results: Two hymenolepidid species have been recorded for the first time in Bulgaria, i.e. *Fimbriarioides intermedia* (Fuhrmann, 1913) from *A. nyroca* and *Wardoides nyrocae* (Yamaguti, 1935) from *C. cygnus*. The present study contributed to the knowledge of the morphological variability and the taxonomy of these species, adding new data on the structure of their genital organs and the osmoregulatory system. The analysis of the previous records of *F. intermedia* and *W. nyrocae* revealed them as euryxenous species; their hosts include a wide range of waterfowl birds from the subfamilies Anatinae, Anserinae, Aythyinae, Tadorninae and Merginae.

Conclusion: The study presented new geographical records of two hymenolepidid parasites of waterfowl in Bulgaria. New data on their morphology, host range and geographical distribution were presented. The present finding of *Wardoides nyrocae* is the first record of the genus *Wardoides* in Bulgaria.

Keywords: Cestoda, *Fimbriarioides intermedia*, *Wardoides nyrocae*, Anseriformes, Bulgaria

Acknowledgements: The work was supported by the project 5E/2015 of the Trakia University funded by the National Science Fund.



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THEMATIC SESSION IV

ECOSYSTEM RESEARCH AND SERVICES. LANDSCAPE ECOLOGY.

PL04_01

The new challenges in Ecosystem research or how to see the whole without overlooking the details

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Abstract

Basic concepts such as ecosystem research and monitoring and the relationship between them, ecosystem integrity and ecosystem services are examined. The Whole System approach developed by the Long-Term Ecosystem Research Network is presented in detail. Scientific questions are asked and answers are sought related to the whole and the detail, with system and components in a hierarchy, universal and individual, global and local, multidisciplinary and transdisciplinary. Where we are?

L04_01

Forest habitats of Godech Municipality, Western Bulgaria

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The territory of research is situated in Western Bulgaria in a close proximity to the country's border with the Republic of Serbia.

Aim: The current study aims to uncover the forest habitat diversity of Godech Municipality.

Materials and Methods: Initial data was collected from the Ministry of Environment and Water and the Forestry Management Plans. Polygons were spatially processed with the application of the software product of ArcGIS 10.6. Terrain studies added more scientific information for analysis. 418 relevés were collected in 2010-2020 and 3422 field points were verified, as well, following the Braun-Blanquet approach.



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Results: Forests are covering 144.1 km². Their phytocoenoses are dominated by *Fagus sylvatica* (59.22 km²), *Quercus cerris* (14.85 km²), *Carpinus betulus* (4.94 km²), *Quercus dalechampii* (2.39 km²), *Q. frainetto* (2.99 km²). There are also plantations with *Pinus nigra* (20.87 km²), *P. sylvestris* (16.06 km²) and *Picea abies* (11.65 km²).

Conclusion: The results of the study provide the necessary basis for a more in-depth research of the territory of Godech Municipality.

Keywords: Braun-Blanquet approach, vegetation, GIS

Acknowledgements: This investigation was carried out with the financial help of the NSP “Young scientists and postdoctoral students, 2020”.



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