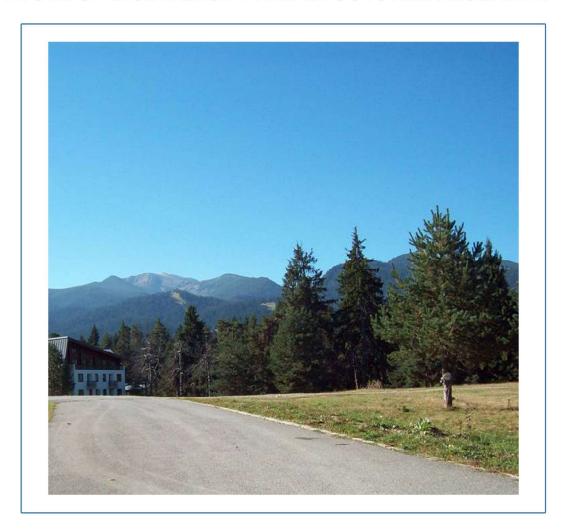
UNION OF SCIENTISTS IN BULGARIA SECTION BIOLOGY

INSTITUTE OF BIODIVERSITY AND ECOSYSTEM RESEARCH - BAS



INTERNATIONAL SEMINAR OF ECOLOGY - 2020 DEDICATED TO THE 10TH ANNIVERSARY OF IBER-BAS

On-line SEMINAR 23-24 April 2020



Програма/Program Абстракти/Abstracts



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CONTENTS:

| PROGRAM | 1 |
|--|----------|
| ABSTRACTS | |
| SESSION I: BIOTIC AND ABIOTIC IMPACT ON THE LIVING NATURE AND MECHANISMS OF ADAPTATION • Lectures | 10 |
| POSTER PRESENTATIONS | 17 |
| SESSION II: LANDSCAPE ECOLOGY • Lectures | 22 |
| SESSION III: ECOLOGY AND EDUCATION • Lectures | 25 |
| SESSION IV: ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE | 2.0 |
| LecturesPOSTER PRESENTATIONS | 26 27 |
| SESSION V: OTHER RELATED TOPICS | |
| POSTER PRESENTATIONS | 28 |
| SESSION VI: BIODIVERSITY AND CONSERVATION BIOLOGY • Lectures | 29 |
| Dectures POSTER PRESENTATIONS | 31 |
| INDEX | 40 |
| TECHNICAL SUPPORT | 44 |

INTERNATIONAL SEMINAR OF ECOLOGY- 2020



DEDICATED TO THE 10TH ANNIVERSARY OF IBER-BAS



ONLINE SEMINAR 23 - 24 April 2020 Sofia, Bulgaria

SCIENTIFIC PROGRAM 23 April 2020

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

THEMATIC SESSION I BIOTIC AND ABIOTIC IMPACT ON THE LIVING NATURE AND MECHANISMS OF ADAPTATION

Chairperson: Assoc. Prof. Michaela Beltcheva, PhD Technical support: Iliyana Demirova and Galia Georgieva

Plenary presentation

9²⁰-9⁴⁰ COMPLEX CHARACTERISTICS OF HIGH LIGHT INDUCIBLE (HIi) PROTEIN OF CHLOROPHYLL PROTEIN COMPLEXES IN CYANOBACTERIA. Nadezhda Yurina, Lubov Sharapova PL01 01

Oral presentations

9⁴⁰-9⁵⁰ HOW CHLORELLA SPECIES RESPOND TO UV-B INDUCED STRESS. <u>Daniela Miteva</u>, Teodora Todorova, Stephka Chankova L01 01

9⁵⁰-10⁰⁰ ISOLATION AND CHARACTERIZATION OF ELECTROGENIC IRON-REDUCING BACTERIA OF THE GENUS GEOBACTER AND POSSIBILITIES OF THEIR APPLICATION IN

MICROBIAL FUEL CELLS. <u>Iskra Yankova</u>, Ralitsa Ilieva, Boyanka Angelova, Mihail Iliev L01 02

10⁰⁰-10¹⁰ STRESS INDUCED DAMAGES IN PSAMOPHILIC BIVALVE SPECIES: A PILOT STUDY ON *DONAX TRUNCULUS* L. FROM BLACK SEA HABITATS IN THE UPPER SUBTIDAL ZONE (BULGARIA). *Lachezar P. Yakimov*, Elina R. Tsvetanova, Almira P. Georgieva, Nesho H. Chipev & Albena V. Alexandrova L01_03

10¹⁰-10³⁰ Discussion

10³⁰-10⁴⁰ Session break

 $10^{40}-10^{50}$ Chairperson: Assoc. prof. Kalina Danova, PhD Technical support: Tsvetan Tsvetanov and Petya Parvanova

10⁵⁰-11⁰⁰ SET OF TESTS FOR CHLORPYRIFOS TOXICITY SCREENING. <u>Teodora Todorova</u>, Petya Parvanova, Hilal Çavuş, Mariya Yovkova, Maria Dimitrova, Samia Mostafa M. Mohafrash, Abdel-Tawab H. Mossa, Krasimir Boyadzhiev, Martin Dimitrov and Stephka Chankova L01_04

11⁰⁰-11¹⁰ EFFECTS OF SALT STRESS ON THE PHOTOSYNTHESIS OF MAIZE AND SORGHUM. <u>Martin Stefanov</u>, Georgi Rashkov, Ekaterina Yotsova, Preslava Borisova, Anelia Dobrikova, Emilia Apostolova L01 05

11¹⁰-11²⁰ RELATIONSHIPS BETWEEN SOIL MICROBIAL ACTIVITY, ABIOTIC FACTORS AND BACTERIAL COMMUNITY STRUCTURE ALONG THE HEAVY METAL CONTAMINATION GRADIENT. Daniel Palov, Michaella Aleksova, Radina Nikolova, Nikolai Dinev, Silvena Boteva, Anelia Kenarova, Roumen Dimitrov, Galina Radeva L01_06

11²⁰-11³⁰ OBSERVED REDUCTION BY A FACTOR OF 10 IN THE WHOLE-BODY TOTAL β-ACTIVITY OF SMALL MAMMALS FROM ALPINE ECOSYSTEMS IN RILA MOUNTAIN, BULGARIA. <u>Peter Ostoich</u>, Michaela Beltcheva, Iliana Aleksieva, J. A. Heredia-Rojas, Elena Geleva, Ivanka Ravnachka, Chisto Angelov L01_07

11³⁰ – 11⁴⁰ UTILIZING THE INDIGENOUS BIOSYNTHETIC CAPACITY OF ARTEMISIA ALBA TURRA THROUGH OPTIMIZATION OF TISSUE CULTURE CONDITIONS. <u>Kalina Danova</u>, Antoaneta Trendafilova, Václav Motyka, Petre Dobrev, Viktoria Ivanova, Milka Todorova L01_08

11⁴⁰-11⁵⁰ INFLUENCE OF SALT STRESS ON GROWTH AND ESSENTIAL OIL PRODUCTION OF CHERVIL PLANTS. <u>M. S. Hussein</u>, S. F. Hendawy, A. E. El-Gohary L01 09

11⁵⁰-12¹⁰ Discussion

12¹⁰-13¹⁰ Lunch break

THEMATIC SESSION II LANDSCAPE ECOLOGY

13¹⁰ – 13²⁰ Chairperson: Assoc. prof. Galina Radeva, PhD Technical support: Teodora Todorova and Mariya Yovkova

Plenary presentation

13²⁰-13⁴⁰ ECOGEOCHEMICAL ASPECTS OF LANDSCAPES. Zornitza Cholakova PL02_01

Oral presentations

13⁴⁰-13⁵⁰ CARBON SEQUESTRATION – A RESEARCH SUBJECT OF A PRESENT IMPORTANCE. <u>Borislav Grigorov</u>, Assen Assenov L02 01

13⁵⁰-14⁰⁰ COMPARATIVE ANALYSIS OF ANTHROPOGENIC TRANSFORMATIONS OF LANDSCAPES IN THE LANDS OF THE SETTLEMENTS OF BELOZEM (PLOVDIV PROVINCE) AND OPALCHENETS (STARA ZAGORA PROVINCE) WITH REMOTE SENSING. *Ilia Tamburadzhiev* L02 02

14⁰⁰-14¹⁰ ASSESSMENT OF FLOOD REGULATION CAPACITY OF DIFFERENT LAND COVER TYPES IN KRUMOVITSA RIVER BASIN (EASTERN RHODOPES). <u>Petko Bozhkov</u>, Borislav Grigorov, Assen Assenov L02 03

14¹⁰-14²⁰ DEVELOPMENT OF ACCURATE CHEMICAL THERMODYNAMIC DATABASE FOR GEOCHEMICAL STORAGE OF NUCLEAR WASTE. PART I. MODELS FOR PREDICTING SOLUTION PROPERTIES AND SOLID-LIQUID EQUILIBRIUM IN BINARY NITRATE SYSTEMS OF THE TYPE 1-1. Stanislav Donchev, Christomir Christov L02_04

14²⁰-14⁴⁰ Discussion 14⁴⁰- 14⁵⁰ Session break

THEMATIC SESSION III ECOLOGY AND EDUCATION

Chairperson: Assoc. prof. Marina Stanilova, PhD Technical support: Ivan Yanchev and Maria Dimitrova

Oral presentations

14⁵⁰-15⁰⁰ SENSITIVITY ANALYSIS OF WATER QUALITY INDEXES – A CASE STUDY OF TOPOLNITSA AND LUDA YANA RIVERS. <u>Marian Varbanov</u>, Kristina Gartsiyanova, Atanas Kitev, Stefan Gentchev, Stela Georgieva L03_01

THEMATIC SESSION IV ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE

Plenary presentation

15⁰⁰-15²⁰ ORGANIC CULTIVATION OF NEW MEDICINAL AND AROMATIC PLANTS AS A SOURCE FOR PHARMACEUTICAL INDUSTRIES. Saber Hendawy PL04 01

Oral presentation

15²⁰-15³⁰ RESPONSE OF AMARANTHS PLANTS TO LIQUID BARITE AND HUMIC ACID APPLICATIONS. <u>A. E. El-Gohary</u>, S.F. Hendawy, M. S. Hussein L04 01

15³⁰-15⁴⁰ Discussion

15⁴⁰- 17⁰⁰ Poster session and Discussion

Chairpersons: Assoc. Prof. Galina Radeva, Assoc. Prof. Marina Stanilova, Assoc. Prof. Kalina Danova

Technical support: Teodora Todorova and Petya Parvanova

POSTER SESSION THEMATIC SESSION I

BIOTIC AND ABIOTIC IMPACT ON THE LIVING NATURE AND MECHANISMS OF ADAPTATION

P01_01 THE RESISTANCE AND RESILIENCE OF SOIL ENZYMES AFTER THE APPLICATION OF FUNGICIDE AZOXYSTROBIN TO LOAMY SAND SOIL. Silvena Boteva, <u>Anelia Kenarova</u>, Stela Dimitrova, Hristo Chanev, Michaella Aleksova, Galina Radeva

P01_02 THE EFFECT OF SEASONALITY OF CLIMATE CONDITIONS ON THE STRUCTURE AND FUNCTIONAL PERFORMANCE OF THE PHOTOSYNTHETIC APPARATUS OF THE MEDICINAL PLANT PETASITES HYBRIDUS. Borislava Borisova, Svetlana Momchilova, Dimitrina Koleva, Albena Ivanova, Albena Momchilova, Liliana Maslenkova

P01_03 SESQUITERPENE PATTERNS OF THE LEAVES AND ROOTS IN LOCAL POPULATIONS OF MEDICINAL PLANT PETASITES HYBRIDUS (L.). <u>Veselina Uzunova</u>, Rumiana Tzoneva, Albena Momchilova, Liliana Maslenkova

P01_04 ANTI-SALMONELLA ACTIVITY OF LACTOBACILLI FROM DIFERENT HABITATS. <u>Lili Dobreva</u>, Miglena Koprinarova, Vasilena Georgieva and Svetla Danova

P01_05 SEASONAL COMPOSITION AND DENSITY OF MARINE LITTER ON ASPARUHOVO BEACH, VARNA, BULGARIA. <u>Marina Panayotova</u>, Radoslava Bekova, Kremena Stefanova, Valentina Todorova, Media Gumus, Violeta Slabakova, Bogdan Prodanov, Svetla Mihova

P01_06 PLASTIC DEGRADATION BY EXTREMOPHILIC MICROBIAL COMMUNITIES. <u>Tsvetelina Paunova-Krasteva</u>, Nikolina Atanasova, Nadya Radchenkova, Ivanka Boyadzhieva, Stoyanka Stoitsova, Margarita Kambourova, Yulia Taktarova, Dina Malakhova, Elena Tsavkelova, Maria Egorova, Maria Dorofeeva, Ivan Bubnov, Irina Kotova, Elizaveta Bonch-Osmolovskaya, Tatyana Sokolova, Alexander Elcheninov

P01_07 CANOPY EFFECTS ON THE DISTRIBUTION OF OPHRYS INSECTIFERA (ORCHIDACEAE) – PRELIMINARY RESULTS FROM HEMISPHERICAL IMAGING ANALYSIS. <u>Andrey Popatanasov</u>, Asen Asenov

THEMATIC SESSION IV ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE

P04_01 FIRST IMPLEMENTATION OF MSFD FRAMEWORK FOR BENTHIC BROAD HABITATS ASSESSMENT IN THE BULGARIAN BLACK SEA. Valentina Todorova, Valentina Doncheva, Ekaterina Trifonova

P04_02 EFFECT OF MIXED CROPPING SYSTEMS ON YIELD AND QUALITY OF LETTUCE (LACTUCA SATIVA L.). Ivanka Mitova, Lilyana Koleva

THEMATIC SESSION V OTHER RELATED TOPICS

P05_01 INVESTIGATION OF RIVER WATERS BY THE METHOD OF WATER SPECTRA. <u>Stefan Todorov</u>, Lidia Popova

24 April 2020

THEMATIC SESSION VI BIODIVERSITY AND CONSERVATION BIOLOGY

9⁰⁰ – 9⁰⁵ Chairperson: Prof. Vlada Peneva, PhD

Technical support: Galia Georgieva and Maria Dimitrova

Plenary presentation

905-925 EGYPTIAN ENCYCLOPEDIA OF WILD MEDICINAL PLANTS AS A MILESTONE FOR CONSERVATION OF GENETIC RESOURCES AND FOLK MEDICINE. Elsayed Omer PL06_01

Oral presentations

9²⁵-9³⁵ MONITORING THE IMPACT OF CLIMATE CHANGE ON THE ALPINE LICHEN AND PLANT DIVERSITY – THE GLORIA-BULGARIA PROJECT. Anna Ganeva, Vladimir Vladimirov, Svetlana Bancheva, Rayna Natcheva, Veselin Shivarov, Malina Delcheva, Elina Yankova & Harald Pauli L06 01

9³⁵–9⁴⁵ MONITORING WETLAND DYNAMICS USING REMOTE SENSING AND GIS. <u>Rumiana Vatseva</u>, Davis Dinkov, Stefan Genchev, Kristina Gartsiyanova L06_02

9⁴⁵-9⁵⁵ COMPARATIVE ANALYSIS OF THE MYRIAPODA (DIPLOPODA, CHILOPODA) FAUNA OF THE SHUMEN PLATEAU AND THE MADARA PLATEAU (NORTHEASTERN BULGARIA). <u>Sevda Hamza</u>, Darina Bachvarova, Aleksandar Doichinov L06 03

9⁵⁵-10¹⁰ Discussion

10¹⁰- 10²⁰ Session break

10²⁰- 11⁴⁰ Poster session and Discussion

Chairpersons: Assoc. Prof. Galina Radeva, Assoc. Prof. Marina Stanilova, Assoc. Prof.

Kalina Danova

Technical support: Tsvetan Tsvetanov and Mariya Yovkova

POSTER SESSION THEMATIC SESSION VI

BIODIVERSITY AND CONSERVATION BIOLOGY

P06_01 INTEGRATIVE INVESTIGATION ON THE ECOLOGY OF THE BLACK SEA MUSSEL MYTILUS GALLOPROVINCIALIS LAM. AND ITS HABITAT. Borislava Pavlova, Sevginar Ibryamova, Nina Arhangelova, Dimitar Dimitrov, Radoslav Ivanov, Nesho Chipev, Nikolay Natchev, Tsveteslava Ignatova-Ivanova

P06_02 ALKANNA TINCTORIA: AN APPROACH TOWARD EX SITU CULTIVATION. Boryanka Traykova, Irena Grigorova, Marina Stanilova, Emil Molle, Elina Yankova-Tsvetkova

P06_03 HIERACIUM PILIFERUM AGG. (ASTERACEAE), THE FIRST RECORD IN THE BULGARIAN FLORA. Vladimir Vladimirov

P06_04 STRUCTURE AND COMPOSITION OF THE AMPHIBIANS AND REPTILES, ACCIDENTALLY FALLING IN PITFALL TRAPS DURING STUDIES OF THE EPIGEAL FAUNA. *Ivo T. Dobrinov, Teodora M. Teofilova, Nikolay D. Kodzhabashev*

P06_05 ASSESSMENT OF MARINE CETACEAN POPULATIONS IN BULGARIAN BLACK SEA IN 2017 ACCORDING TO INDICATORS OF THE EU MARINE STRATEGY FRAMEWORK DIRECTIVE. <u>Marina Panayotova</u>, Radoslava Bekova, Bogdan Prodanov

P06_06 RECENT OBSERVATIONS ON THE SIZE STRUCTURE OF CHAMELEA GALLINA AND DONAX TRUNCULUS IN THE BULGARIAN BLACK SEA AS STATUS INDICATORS OF COMMERCIALLY EXPLOITED SHELLFISH UNDER THE MARINE STRATEGY FRAMEWORK DIRECTIVE (MSFD). Mediya Gumus, Valentina Todorova, Marina Panayotova

P06_07 BIO-ECOLOGICAL CHARACTERISTICS AND SIZE-AGE CLASSIFICATION OF THE ALPINE NEWT (ICHTHYOSAURA ALPESTRIS (LAURENTI, 1768)) DURING THE BREEDING SEASON IN REPRESENTATIVE REGIONS FOR BULGARIA. Teodora M. Teofilova, Pencho G. Pandakov, Nikolay D. Kodzhabashev

P06_08 POPULATION OF THE FLATHEAD GREY MULLET (MUGIL CEPHALUS, LINNAEUS 1758) FROM THE BAY OF BURGAS, BULGARIAN BLACK SEA COAST. <u>Radoslava Bekova</u>, Marina Panayotova, Bogdan Prodanov

P06_09 RELATIONSHIPS BETWEEN SIZE, WEIGHT, AGE AND FECUNDITY OF THE CHELON AURATUS AND CHELON SALIENS (MUGILIDAE) FROM THE BULGARIAN BLACK SEA COAST. Radoslava Bekova, Marina Panayotova, Galerida Raikova-Petrova, Bogdan Prodanov

P06_10 DIETARY ANALYSIS OF WINTERING GROUPS OF LONG-EARED OWLS (ASIO OTUS, LINNAEUS, 1758) IN THE REGION OF SILISTRA (NE BULGARIA). <u>Stela Dipchikova</u>, Teodora M. Teofilova, Nikolay Kodzhabashev

P06_11 MACROPTEROUS GROUND BEETLES (COLEOPTERA: CARABIDAE) PREVAIL IN EUROPEAN OILSEED RAPE FIELDS.

<u>Teodora Teofilova</u>, Anja Schmidt, Tibor Hartel, Daniel Ston, Vlada Peneva, Josef Settele

P06_12 STATUS AND THREATS OF THE POPULATIONS OF ORCHIS PROVINCIALIS IN MT RHODOPES, BULGARIA. Andrey Popatanasov

1140 CLOSING PROCEDURE - Prof. Stephka Chankova, PhD



THEMATIC SESSION I

BIOTIC AND ABIOTIC IMPACT ON THE LIVING NATURE AND MECHANISMS OF ADAPTATION

PL01 01

Complex characteristics of high light inducible (Hli) protein of chlorophyll protein complexes in cyanobacteria

Nadezhda Yurina, Lubov Sharapova

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

Aims: It was shown that stress Hli proteins (Hlips) of cyanobacterium *Synechocystis* sp. are necessary for the survival of cells and adaptation in conditions of intense light. Nonetheless the complete picture of Hlips functioning and distribution is not fully understood and data for other cyanobacteria is missing. In the current study Hlips of multicellular *Arthrospira platensis* were investigated.

Materials and Methods: The A. platensis and Synechocystis sp. cells were exposed to light stress (500 μ mol photons /m²·s, 1 h). Then two dimensional electrophoresis and mass spectrometry were used to determine the association of Hli proteins with chlorophyll protein complexes of thylakoid membranes.

Results: According to the NCBI database, there are three Hli genes in the *A. platensis* genome that encode proteins of 47, 64, and 69 aa long. Our data demonstrated that MALDI-TOF mass spectrometry analysis revealed presence of Hli 47 aa long only. This data allows us to conclude that the identified Hli protein is associated with photosystem II and is a homologue of HliC *Synechocystis* sp.

Conclusions: Bioinformatics analysis of the amino acid sequence of the identified Hli protein of *A. platensis* revealed a high degree of homology with the amino acid sequences of proteins of a number of other multicellular cyanobacteria and a lesser degree with the Hli amino acid sequence of singlecelled cyanobacteria.

Keywords: high light inducible proteins, stress proteins, abiotic stress

Acknowledgements: this work was partially supported by the Russian Foundation for Basic Research (Grant No. 19-04-00798).

How Chlorella species respond to UV-B induced stress

Daniela Miteva, Teodora Todorova, Stephka Chankova

Institute of Biodiversity and Ecosystem Reseasch, Bulgarian Academy of Sciences, Sofia, Bulgaria

Different mechanisms of adaptation to environmental stress have been developed by organisms during the evolution. We hypothesize that algal species isolated from habitats with extreme environmental conditions would have more efficient cellular defence mechanisms to various types of stress.

Unicellular green algae are a robust model because of the following reasons: Photosynthetic eukaryotic organisms with typical for plants cell structure and genome organization; Cell - organism with short life cycle - the response of a single cell is equivalent to the response of an individual organism;

The aim of this study is to evaluate stress response of *Chlorella* species, isolated from different habitats to UV-B irradiation. *Chlorella vulgaris* (Antarctic) is isolated from soil in island Livingston, Antarctic, *Chlorella vulgaris* 8/1 (Thermophilic) is isolated from thermal springs in region of Rupite, Bulgaria and *Chlorella kesslery* (Mesophilic) is from the Trebon collection. *Chlorella* species are cultivated on TAP medium under standard conditions $23^{\circ}\text{C} \pm 0.3$ and 5000-5500lx in a growth sharphar Phytotran CC 40. Cell symposium in the end of the symposium of the

in a growth chamber Phytotron GC 40. Cell suspensions in the end of the exponential/ beginning of the stationary phase are used.

The cells are irredicted in PLY 254 (Life Technology LIV cross linker) as a source of LIV P.

The cells are irradiated in BLX-254 (Life Technology, UV cross linker) as a source of UV-B irradiation (λ = 312nm).

The cell response of *Chlorella* species is examined on the basis of spot - test, micro-colonies assay, growth rate. The results show that doses higher than 250 J/m² UV-B are effective. The result of sector of photo-reactivation determine that *Chlorella* species are not defective in photo-reactivation.

Based on the complex of used methods, it is established that according their resistance to UV-B induced stress, the different species can be arranged in the following order: *Chlorella vulgaris* > *Chlorella vulgaris* 8/1 > *Chlorella kesslery*.

L01 02

Isolation and characterization of electrogenic iron-reducing bacteria of the genus *Geobacter* and possibilities of their application in microbial fuel cells

Iskra Yankova, Ralitsa Ilieva, Boyanka Angelova, Mihail Iliev

Department of General and Industrial Microbiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

Geobacter species are anaerobic Gram-negative microorganisms of class Deltaproteobacteria, which inhabit soil and water sediments, rice fields and wetlands. They have a crucial role in biogeochemical reduction of iron, manganese and other heavy metals. Therefore they are involved in bioremediation, bioelectricity production and various environmental friendly activities. However, cultivation in a laboratory is still a major problem.

The **aim of our study** is to establish the taxonomic characteristics of iron-reducing bacteria isolated from Bulgarian sources and to test their potential for use in microbial fuel cells (MCF).

Materials and methods: Sludgy or soil samples were collected from different locations in Sofia district and a quantitative and qualitative assessment of microbial communities was carried out. Isolation

procedure was optimized in four steps using insoluble and soluble Fe³⁺ compounds and anaerobic cultivation methods. One- and two-chamber MFC were constructed. Molecular identification of six of isolates includes amplification of 16S rDNA gene and phylogenetic analysis.

Results: The optimized isolation procedure allowed successful isolation of Geobacter species and the proposed cultivation scheme significantly increased their growth rate under laboratory conditions. Sequence analysis revealed the presence of *Geobacter metallireducens* and *Geobacter pelophilus*. The microbial fuel cell inoculated with G. metallireducens SubG2 isolate has the highest efficiency (360My/m^2) compared to other isolates.

Conclusion: This study provided new taxonomic and physiological-biochemical data on the Geobacter species from the territory of Bulgaria. Substantial progress has been made in optimizing the cultivation methods and bioelectricity production of MFC, contributing to the development of bioremediation and applied microbiology.

Keywords: *Geobacter*, iron-reducing bacteria, bioremediation, bioelectricity, microbial fuel cell, isolation

Acknowledgements: The investigation is a part of a graduate thesis developed in collaboration with scientific teams from the University of Mining and Geology "St. Ivan Rilski", Sofia. The Bulgarian National Science Fund under project T02/2 supported this study.

Stress induced damages in psamophilic bivalve species: a pilot study on *Donax trunculus* L. from Black Sea habitats in the upper subtidal zone (Bulgaria)

L01 03

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Abstract: Environmental pollution and climate changes impact the quality status of marine environments, which in turn affects the people's wellbeing. In this context, the biomarkers of oxidative stress (OS) in sentinel species are being intensively developed and applied for assessment and management of the ecosystems' health. The present study aims to assess the oxidative damage indicated by the levels of lipid peroxidation (LPO), protein oxidation (PO) and glutathione (GSH) in Donax trunculus L. from different sites along the Bulgarian Black Sea coast. Wedge clams (2-3 cm shell length) were sampled manually from their habitats or obtained from commercial providers. The soft tissues of 10 specimens from each sample were excised and analyzed separately. The obtained results indicated significant variations in OS biomarkers. The increased concentrations of LPO and PO along with GSH decrease indicated tissue damages and point to stress and threat to ecosystem health. The oxidative modifications of membrane lipids and proteins lead to changes in membrane permeability and transport disruption, ultimately leading to cell death. Glutathione not only interacts with reactive oxygen and nitrogen species and is a cofactor of glutathione peroxidases, but is also crucial for eliminating various other toxicants from cells through the glutathione-S-transferases In conclusion D. trunculus proves to be a suitable bio-indicator of the state of the sandy upper subtidal zone environment in the Black Sea. These regional data could be used for further studies handling the combined effects of anthropogenic pollution and climatic changes on marine ecosystems.

Keywords: Black Sea, Donax trunculus, lipid peroxidation, protein oxidation, glutathione

Acknowledgements: This work was supported by grant № KΠ-06-H31/6 of National Science Fund, Bulgaria. We thank ELECTA Ltd. for assistance in providing material from wedge clams.

L01 04

Set of tests for chlorpyrifos toxicity screening

<u>Teodora Todorova¹</u>, Petya Parvanova¹, Hilal Çavuş², Mariya Yovkova¹, Maria Dimitrova¹, Samia Mostafa M. Mohafrash³, Abdel-Tawab H. Mossa³, Krasimir Boyadzhiev^{1,4}, Martin Dimitrov^{1,4} and Stephka Chankova¹

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Aim: To evaluate the reliability of the proposed by us set of test systems/endpoints for chlorpyrifos toxicity screening.

Two multicellular organisms - aphids *Myzus persicae* and rats, and two unicellular - *Chlamydomonas reinhardtii* – as a model for plant cell and *Saccharomyces cerevisiae* – as a model for animal cell, were used. The potential toxicity was evaluated based on several endpoints: aphids' mortality; mitotic index; chromosomal aberrations and micronuclei on rats; spot-test, clonal assay, induction of superoxide anions, Zimmermann's test, test of "visible" mutant colonies, constant field gel electrophoresis on microalgae and yeast. Chlorpyrifos (CPF) was tested as follows: 5 sec exposure time with concentrations in the range: 5 – 10000 ppm on aphids; 30 min with concentrations in the range 6.5 – 100 ppm on *Chlamydomonas reinhardtii* and 100 – 10000 ppm on *Saccharomyces cerevisiae*.

Dose dependent mortality of aphids was found. The lethal dose causing 50 % mortality (LD_{50}) was calculated to be 31.5 ppm. Further, a 2-fold reduction in the mitotic index, about 7-fold increase in chromosomal aberrations, and about 4-fold increase in the total number of micronucleated polychromatic erythrocytes (MMPSE) were measured in rats after the treatment with concentration corresponding to 1/25 of LD_{50} (25.6 ppm).

Based on the survival fraction, *Chlamydomonas reinhardtii* was found to be more susceptible than *Saccharomyces*. The LD_{50} were calculated - 36.56 ppm for *Chlamydomonas reinhardtii* and 66.05 ppm for *Saccharomyces cerevisiae* strain D7ts1.

Interestingly, the LD_{50} calculated for yeast corresponded well with 7.5-fold increase in ROS levels and 2-fold increase in DSB levels. On the other side, treatment of *Chlamydomonas* with different concentrations CPF did not result in DSB increase.

Data concerning the mutagenic potential of CPF on *Chlamydomonas reinhardtii* revealed the induction mainly of low-size and pigment mutations, which could be considered as a result for impaired cell division and gene point mutations in the nuclear and chloroplast DNA, respectively. Based on data presented a potential phytotoxic effect could be suggested. In *Saccharomyces cerevisiae*, reverse point mutations were induced after the treatment with 100 ppm. Dose-dependent increase was also obtained for the mitotic gene conversion.

Based on this, aphids and *Chlamydomonas reinhardtii* are found to be very susceptible to CPF action suggesting aphicidal and herbicidal effect. Very pronounced clastogenic effect was found in rats. High doses of CPF possess well-expressed prooxidative, DNA-damaging and mutagenic effect on yeast. Therefore, it could be concluded that the application of the proposed set of test systems/endpoints could provide concise information concerning the genotoxicity of different pesticides.

Acknowledgements: This work was supported by a grant from the National Science Fund, Ministry of Education and Science, Project No. DH16/2; "Ecological and genetic assessment of the environmental - management and strategies for overcoming the risk"—Bulgarian Academy of Sciences;

"Preparation and characterization of eco-friendly green and nanopesticides for spider mites and aphids control" Bilateral agreement between Bulgarian Academy of Sciences, Academy of Scientific Research and Technology and National Research Center, Egypt.

L01 05

Effects of salt stress on the photosynthesis of maize and sorghum

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Aim: In the present study, the effects of salt stress on the photosynthetic processes in sorghum (*Sorghum bicolor* L. *Albanu concep*) and maize (*Zea mays* L., *Mayflower*) are compared.

Materials and methods: The plants were grown in $\frac{1}{2}$ Hoagland solutions with different NaCl concentrations (0 – 250 mM NaCl) for 6 days. PAM chlorophyll fluorescence, P_{700} photo-oxidation and pigment analysis were used for characterization of the salinity effects on the studied plants.

Results: The treatment of plants with high concentrations of NaCl led to stronger inhibition of the maximum quantum yield of photosystem II (Fv/Fm), the photochemical quenching (q_P), the rate of photosynthesis (R_{Fd}) and the effective quantum yield of photochemical energy conversion (Φ_{PSII}) in sorghum in comparison to maize. All these changes corresponded to decrease in the pigment composition and changes in regulated and non-regulated heat dissipation (Φ_{NPQ} , Φ_{NO}). The analysis of the P700 photo-oxidation revealed that the PSI photochemistry was inhibited and the cyclic electron flow was stimulated at high NaCl concentrations (in both studied plants. The reasons for different effects of salt stress on the maize and sorghum are described.

Conclusion: Sorghum is more sensitive to salt stress than maize.

Keywords: maize, sorghum, salt stress, NaCl, chlorophyll fluorescence, pigments.

Acknowledgements: The studies for sorghum were supported by the Bulgarian Science Research Fund, KΠ-06-K36/9 and for maize by the Bulgarian Academy of Sciences, National Research Programme "Young scientists and postdoctoral students" 2018-2020.

L01 06

Relationships between soil microbial activity, abiotic factors and bacterial community structure along the heavy metal contamination gradient

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Abstract: As a result of contamination heavy metal concentration and their bioavailability forms change the structure and function of microbial communities. It is well known that changes in microbial community structure and activity depend also on soil variables. The aim of this study was to analyse the activity of soil dehydrogenase complex (Dha) and its interaction with the soil physicochemical properties, bacterial abundance and diversity. For this purpose the top soil from three sites along Cu gradient (53-860 mg kg⁻¹) and co-contaminants Zn and Pb, located in the region of Zlatitsa-Pirdop

valley, Western Bulgaria was studied. Our results showed that the activity of Dha varied from $\mu g/g/h$ (uncontaminated site) to 17.8 $\mu g/g/h$ in the most contaminated with HMs site. The principal component analysis showed that Dha correlates significantly positive with total bacterial abundance (16S rRNA gene copies) and NO₃, as well as positively but insignificantly with bacterial diversity, expressed through RFLP profiles, and PO₄.

There was no correlation between Dha, humidity and clay as well as weak negative correlations between Dha and the total content of HMs and their bioavailable forms. Dha correlates much more negatively with other physicochemical properties (TOC, sand, NH₄, Pb-bioavailable) and biodiversity at phylum and class levels.

Keywords: heavy metals, soil properties, dehydrogenase enzymes, biodiversity, bacterial abundance

Acknowledgements: This study was financially supported by the National Research Fund of the Bulgarian Ministry of Education and Science (grant DN 11/4 - Dec, 2017). M. Aleksova was supported by the National Program "Young scientists and Postdoctoral candidates" of Ministry of Education and Science, 2018

L01 07

Observed reduction by a factor of 10 in the whole-body total β-activity of small mammals from alpine ecosystems in Rila Mountain, Bulgaria

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Recent years have seen a decrease in the overall radionuclide emission activity in Europe, attributable mainly to the Chernobyl accident in 1986. Within the framework of a cooperation between IBER-BAS and INRNE-BAS, whole-body total β-activity was measured in three small mammal species, inhabiting two localities in Rila Mountain, Bulgaria. Near Peak Moussala (2925 m a.s.l.) - the snow vole (*Chionomys nivalis*) and wood mouse (*Apodemus sp.*) were investigated. In the locality of Beli Iskar artificial Dam (1400 m a.s.l.) data was recorded for *Ap. sp.* and bank vole (*Myodes glareolus*). At both localities, results show values within the range 230-424 Bq/kg, with reference values >480 Bq/kg indicating significant contamination. The data observed has been compared to previous results, obtained within the framework of the French-Bulgarian "Moussala OM2" project during the 1990s. In addition, the results have been compared with the hematological indices of the animals analyzed during the same time periods. The comparisons show ~10-fold reduction of total β-activity in the bodies of small rodents from these localities. The results are consistent with radioecological models for the atmospheric deposition, decay and overall body burden decrease of radionuclides in the years after 1986.

Keywords: radioecology, β -activity, terrestrial vertebrates, Rila Mountain

Acknowledgements: The project was supported financially by grant DN04/1/13. 12. 2016, Bulgarian National Science Fund (BNSF)

L01 08

Utilizing the indigenous biosynthetic capacity of *Artemisia alba* Turra through optimization of tissue culture conditions

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The present work gives an overview of the broad research performed by our team on elucidation of the effect of plant morphogenesis on secondary metabolites productivity of *Artemisia alba* Turra *in vitro* cultures. The plant was selected due to the marked variability of essential oil production in its natural conditions.

The experimental models included modification of culture conditions such as application of plant growth regulators and utilizing either agar solidified or liquid media. As a result, differentiated and non-differentiated *in vitro* culture lines were developed. The *in vitro* lines, which were selected based on the morphotypes obtained, consisted of root suppressed and normally root developing differentiated shoot cultures, as well as non-differentiated cell aggregates.

The obtained lines were characterized by selective stimulation of biogenesis of target secondary metabolites characteristic for the species – such as terpenoids and polyphenolics. The observed dependencies were also related to fluctuations in endogenous cytokinin levels of the samples.

Rooting was identified as a decisive factor for monoterpenoid biogenesis and production of bioactive endogenous cytokinins in the differentiated shoots. On the other hand, the degree of dedifferentiation and aggregate size seemed to determine the levels of polyphenolics produced in the non-differentiated cell aggregates grown in liquid media.

The established flexibility of plant secondary metabolism in relation to morphogenesis might serve as a useful tool for obtaining plant material with desired secondary metabolite profile through modeling of plant growth, development and morphogenesis and without the use of genetic modifications *in vitro*.

Keywords: *Artemisia alba* Turra *in vitro*, essential oils, phenolic and flavonoid compounds, endogenous cytokinins.

Acknowledgements: The authors are thankful to grant KΠ-06-H39/6 of the National Scientific Fund, Bulgaria.

L01 09

Influence of salt stress on growth and essential oil production of chervil plants

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Salinization of soils or waters is one of the most serious environmental problems in agriculture. It is necessary to determine the suitable environmental factors under which medicinal and aromatic plants give higher yields and better quality. A pot experiment was conducted during two years (2017-2018) at the greenhouse of National Research Center (NRC), Dokki, Giza, Egypt to investigate the effect of salt stress on plant growth, essential oil yield and its constituents of Chervil. Plants were irrigated with different levels of NaCl (0, 1.7, 3.1 and 4.7 dSm). The plants were harvested after 90 days from transplanting, then air dried and stored in the laboratory for analyzes. Following parameters were

recorded for each sample: fresh and dry weight (g plant-1). In addition the aerial parts of harvested plants which were air-dried in the shade were subjected to hydro distillation to obtain the essential oil. The GC-MS analysis of the essential oil samples was carried out in the second season using gas chromatography-mass spectrometry instrument. This work demonstrated that chervil plants are susceptible to salt stress and that such stress will affect plant growth and essential oil yield and composition.

P01 01

The resistance and resilience of soil enzymes after the application of fungicide azoxystrobin to loamy sand soil

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Fungicide use in crop protection effectively eliminates fungal pathogens of plants. However, fungicides may dissipate to various elements of the environment and cause irreversible changes. The aim of the study was to evaluate changes in soil environment and soil enzyme activities (beta-glucosidase, urease, acid and alkaline phosphatases and arylsulphatase) in response to contamination with azoxystrobin (Az), applied under the trade form Quadris[®]. The study was carried out in the laboratory for 120 days on mesocosms of loamy sand soil (pH of 5.87), amended with increasing Az doses from 0 (control, Az0), through 0.28 (Az1) and 14.46 (Az2) to 28.93 mg kg⁻¹ (Az3). Soil amendment with Az caused changes in soil properties, mainly pH and bioavailable concentrations of nitrate and ammonium nitrogen. Immediately (day 1) after Az application, more resistant to the fungicide were urease and arylsulphatase (Az1-Az3), and alkaline phosphatase (Az1). The resistance of soil microbial enzymes increased during the mesocosms' incubation, and two months after Az amendment of soil mesocosms the highest increase in enzyme resistance was calculated for beta-glucosidase and alkaline phosphatase by 18% (Az1) – 83% (Az3) and by 9% (Az1) – 185% (Az3), respectively. The calculated resilience of soil enzymes on day 120 manifested the highest capacity of urease and acid phosphatase to recover after Az stress, compared to the other studied enzymes. Pearson correlation analysis demonstrated significant linear relationships between enzyme resistance/resilience, and soil Az residues, pH and bioavailable forms of nitrogen and phosphate.

Keywords: fungicide azoxystrobin, soil enzymes, enzyme resistance, enzyme resilience, correlation analysis

Acknowledgements: This study was financially supported by the National Research Fund of the Bulgarian Ministry of Education and Science (grant DN 11/6 - Dec, 2017). M. Aleksova was supported by the National Program "Young scientists and Postdoctoral candidates" of Ministry of Education and Science, 2018

P01 02

The effect of seasonality of climate conditions on the structure and functional performance of the photosynthetic apparatus of the medicinal plant *Petasites hybridus*

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The aim of this study was to relate annual variation in photosystem II activity and chloroplast membranes structure of *P. hybridus* (L.) to environmentally induced constraints in order to elucidate the possible relationship between photosynthetic activity and seasonal variation in the production of the main biologically active compounds.

Materials and methods. Leaves of *P hybridus* (L.) were collected in spring, summer and autumn from plants growing at the region of Kokalyane, Sofia Capital Municipality. The pigments were determined spectrophotometrically. Thylakoids were isolated in Tricine pH 7.8 and re-suspended in MES 6.5. The lipid analyses were performed using thin-layer and gas chromatographic techniques. TEM-analysis was performed by JEOL 1200 EX (Japan) electron microscope. PSII functionality was assessed by alterations in thermoluminescence emission and oxygen evolution patterns.

Results. Comparative analyses of chloroplast ultrastructure revealed specific changes in correlation to the climate conditions during the seasons. The observed variations in the TL glow curves and oxygen evolution patterns might be a result of conformational changes of PSII due to some modification of membrane lipid composition. The analysis of lipid classes and their fatty acids composition confirmed the existence of qualitative and quantitative differences.

Conclusion. The results of this study show specific structural and functional characteristics of photosynthetic membranes in *Petasites* plants, reflecting adaptive strategies to environmental conditions in their natural habitat.

Keywords: Petasites hybridus L., chloroplasts, lipid composition, photosynthetic activity

Acknowlegment. This work was supported by grant KΠ-06-H21/12/2018 of National Science Fund, Bulgaria and partially by Contract DO1-154/28/08/2018/ "Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB)"

P01 03

Sesquiterpene patterns of the leaves and roots in local populations of medicinal plant *Petasites hybridus* (L.)

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Biosynthesis of biologically active substances, products of secondary plant metabolism is highly sensitive to various geographic, ecological and phenological factors. Clarification of these relationships is essential to define their impact on the efficacy and therapeutic potential of phytochemical preparations of medicinal plants.

The aim of this study was to investigate the variation in the pattern of the main biologically active compounds of *Petasites hybridus* (Butterbur), classified as sesquiterpene esters of petasin and iso-petasin in natural population from different habitats in Bulgaria.

Materials and methods. Leaves and subterranean plant parts were collected after the flowering period in different florogeographical regions with various climate and altitude. Thin layer chromatography was performed with silica gel 60 F254, HPLC plates (Merck, Germany). HPLC analysis was performed on HPLC system Waters Alliance e2695 Separations Module.

Results. The results from TLC analysis showed UV absorbing spots with blue and green-blue colour in the Rf range from 0.15 to 0.4 thus confirming the petasin chemo type of the investigated Butterbur extracts. HPLC analysis revealed qualitative and quantitative differences of the six main sesquiterpene esters found in leaf and root extracts.

Conclusion. The present results indicate modulations in the profile and accumulation of secondary metabolites in *Petasites hybridus*, reflecting adaptive strategies to specific environmental conditions in their natural habitats.

Keywords: Petasites hybridus, methanol extracts, HPLC, sesquiterpene esters, TLC.

Acknowledgment. This work was supported by grant KΠ-06-H21/12/2018 of National Science Fund, Bulgaria and partially by Contract DO1-154/28/08/2018/ "Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB)"



Anti-Salmonella activity of lactobacilli from different habitats

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Salmonella is a life-threatening pathogen, with over 2500 serotypes and more than half of them belong to Salmonella enterica subsp. enterica. The modern society is looking for new approaches for improving human health and safety. However, limited data exists on the potential of lactobacilli to play a role of bio-protective agents against foodborne pathogens. With this aim, 45 Bulgarian Lactobacillus strains, with human and dairy origin and 2 multibacterial formulas, a prototype of probiotic product, have been assessed for antagonistic activity against Salmonella enterica subsp. enterica serovar Typhimurium WDCM 00031. They belong to different homo and heterofermentative species. In vitro tests were performed in different model systems - milk, soymilk, De Man Rogose (MRS) medium, to simulate real conditions in the food chain. The highest activity was observed for cell-free supernatants of exponential cultures in MRS broth of the strains, isolated from breast milk, followed by lactobacilli from white-brined and green cheese. A strain-specific effect was due to the produced lactic acid, hydrogen peroxide and probably bacteriocin - like substances, produced during the fermentation. Lactobacillus plantarum strains cultivated in skimmed milk and whey protein are able to inhibit Salmonella's growth. However, a limited inhibitory activity was detected for fermented soymilks. A bacteriocinogenic strains L. salivarius reduced the number of living pathogenic cells during the cocultivation in whole milk. The inhibition was significant only when the L. salivarius was inoculated in predominance. In the case of underrepresented LAB number, Salmonella over-growth is observed.

Six lactobacilli, with bactericidal effect, were pre-selected as promising. Further characterization of their active metabolites, however, is needed in order to be proceeded as bioprotective agents.

Keywords: Lactobaccillus spp., Salmonella, antimicrobial activity

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P01 05

Seasonal composition and density of marine litter on Asparuhovo Beach, Varna, Bulgaria

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Marine litter is a growing environmental problem affecting oceans and seas worldwide. Waste created by humans on land or at sea has been discharged into coastal or marine environments. Marine Strategy Framework Directive establishes the basis of integrated marine assessment taking into account the human pressures and their environmental impacts, including marine litter under Descriptor 10. In this study the composition and density of coastline debris were analysed as indicators of marine litter. Two monitoring campaigns for collection and identification of marine litter took place at Asparuhovo beach (Varna) in 2019, covering surveyed area of 8814 m². In the spring campaign, 3138 items of artificial polymer materials, rubber, textile, paper, processed wood, metal and glass with total weight of 19.591 kg were collected. Litter density was estimated at 0.41 items.m⁻² in abundance and at 0.002 kg.m⁻² in mass. In the autumn survey, the number of collected items decreased to 1461 items and the weight – to 4.189 kg. Compared to spring, the results for beach litter density manifested 2.4 fold decrease in abundance (0.17 items.m⁻²) and 4 fold decrease in mass (0.0005 kg.m⁻²). In both surveys, the artificial polymer materials predominated in the abundance – 87% and 86% respectively. Cigarette buts, plastic/polystyrene pieces, industrial packaging, plastic cups and rings were predominant in marine litter composition.

Keywords: marine litter, monitoring, Bulgarian Black Sea, MSFD, Descriptor 10

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P01 06

Plastic degradation by extremophilic microbial communities

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This work reports the potential impact of extremophiles in resolving one of the biggest contemporary ecological problem - accumulation of huge amounts of plastic pollutants.

Lye (C1) and water mud (C2) from Atanasovsko ezero, Bulgarian salterns were enriched in a mineral salt medium with four synthetic plastics, Polycaprolactone (PCL), Polyvinyl alcohol (PVA), Polystyrene (PS) and Polypropylene (PP). Comparison of the microbial growth revealed better growth of C1 on PP, PS and PCL than in control. Esterase activity was measured for C1 on PVA and for C2 on PCL and PVA. SEM analysis of the plastics showed most explicit alterations on PCL. While the presence of C1 in the culture medium caused damage of the plastic surface, C2 interacted with it directly by forming a biofilm in surface breaks.

Thermophilic microorganisms in samples from Kamchatka hot springs were enriched in a medium with high- and low-density polyethylene (HDPE and LDPE) in aerobic and anaerobic conditions, at 30 and 55°C. Degradation of polyethylene was monitored by scanning electron microscopy of its surface and the weight losses after incubation. Results of 16S rRNA genes profiling compared with control variants revealed a domination of bacteria of phylum *Dictyoglomy*, family *Defluvitaceae* and uncultured clostridia in anaerobic thermophilic Kamchatka enrichments in the presence of polyethylene. The share of *Bacilli* decreased in compost-originating aerobic mesophilic enrichments after incubation with LDPE, while uncultured *Chitinofagaceae* increased. These results show the ability of thermophilic anaerobes to degrade efficiently two types of polyethylene, as well as the existence of novel polyethylene-degrading mesophilic aerobes.

Keywords: microbial communities, halophiles, Plastic degradation, Esterase activity, SEM

Acknowledgement: This study was supported by the National Fund for Scientific Research, Bilateral Projects 2019 – BULGARIA – RUSSIA (Grant KP-06-Russia-17).

P01 07

Canopy effects on the distribution of *Ophrys insectifera* (Orchidaceae) – preliminary results from hemispherical imaging analysis

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Aim: Ophrys insectifera L. is the northernmost representative of the remarkable Ophrys genera with predominantly Mediterranean distribution. Due to its specific biology and ecological requirements it's among the most threatened vascular plants in Bulgaria. The study aimed to explore and evaluate canopy effects on the species distribution in some of the largest locations in Bulgaria.

Materials and methods: Exploration and investigation of few populations of the orchid *Ophrys insectifera* in some locations in the Southwest mountainous region in Bulgaria was done from 2014 till 2018. For evaluation of the canopy effects on the plants' distribution. were recorded geospatial data and hemispherical photographs were collected and analyzed.

Results and discussion: The results show that more open areas with the index of canopy openness more than 60% are more favorable for the distribution of the species.

It is known that many shade-intolerant plants have optimized their photosynthetic apparatus and its efficiency to the full light spectrum of the solar irradiation therefore the understorey microenvironment with reduced amount of the available light and changed spectrum may have prohibitive and hampering effect on the normal physiology. Howbeit further research is necessary for the determination of the the precise underlying physiological and molecular processes and mechanisms.

Conclusion: The study suggests that shading may have some role as limiter for the distribution of this species.

Keywords: Ophrys insectifera, canopy effects, hemispherical imaging analysis, orchids, Orchidaceae

THEMATIC SESSION II LANDSCAPE ECOLOGY

PL02 01

Ecogeochemical aspects of landscapes

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Landscapes are natural-anthropogenic systems distributed both in the terrestrial and the aquatic environment of the Earth. The current environment state is a result of the millennial development and interaction of these systems. From the point of view of systematic approach, interactions in nature become direct and inverse relationships between individual elements (components), in which flows of energy, substances, chemical elements, etc. are transformed. Some of these elements have a hazardous effect on living organisms. They disrupt the dynamic equilibrium between system elements in landscapes and disrupt the natural interactions between them. The reason for this impact is the increasing technogenic (anthropogenic) pressure. Even limited at a later stage, its chemical footprint remains long in the landscape. Its functional and structural nature is altered irreversibly and its self-recovering, if possible, takes place under different natural conditions and creates new natural-technogenic (anthropogenic) complexes, systems or elements. The study of these changes is important both for the theory itself for the development of natural anthropogenic systems, as well as for the assessment of the environmental impact and risk to the health and life of man and living organisms. Environmental geochemistry places the focus on geochemical landscapes and their impact on the flow of chemical elements that migrate into nature and are distributed among its components. The individual geochemical landscapes and their constituent components - soils, weathered crust, underground water, living organisms - are very closely connected in a more complex system - landscape sphere.

Keywords: ecogeochemistry, landscape, natural-anthropogenic system, environment

L02 01

Carbon sequestration – a research subject of a present importance

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Aim: The current study investigates published data, concerning carbon sequestration on global scale in order to reveal the research focus, regarding this issue.

Materials and Methods: The investigation is based on the use of the search engine of Nature Research Journal in order to acquire information about the studies, concerning carbon sequestration – the latter words were used as key words. Two main periods are examined, covering the years 1845-1999 and 2000-2019. The comparative method is also applied in order to accomplish the aim of the investigation. Results: During the first period - 1845-1999 58 articles, regarding carbon sequestration. were published. The second period saw an unprecedented boom when a total count of 1806 research articles came to being. Some of the most important among them are a part of several scientific journals, including Scientific Reports, Nature Communications, Nature Journal, Nature Geoscience and Nature Climate Change and these numbers should not be underestimated since only Nature Journal has an impact factor

of 43.070 in 2018. This can serve as a proof of the quality of scientific research. The results also show a division by country of several of these scientific works.

Conclusion: The authors of the current investigation stress on the need of such an overview study for the revelation of the subjects that scientists around the world are excited about.

Keywords: carbon sequestration, Nature Research Journal, impact factor

L02 02

Comparative analysis of anthropogenic transformations of landscapes in the lands of the settlements of Belozem (Plovdiv Province) and Opalchenets (Stara Zagora Province) with remote sensing

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In this study, a remote analysis of the factors that play a fundamental role in the formation of contemporary landscapes in part of the Pazardzhik-Plovdiv area - the lands of the villages Belozem (Plovdiv Province) and Opalchenets (Stara Zagora Province), as well as of the landscape structure, determined by the complex interaction of natural and anthropogenic factors. The study area was selected based on the registration of significant differences in the character of the land use in the lands of the two villages. Remote sensing contributes for more profound investigation of the structure and dynamics of anthropogenic landscapes in the study area. Satellite data, as well as landscape metrics analysis of the landscape structure in the study area are used. A multi-temporal analysis was conducted to study the dynamics of landscapes over different periods. Significant differences have been registered in the structure of agricultural landscapes in the study area, which could be explained by the different type of organization of the agricultural process on the territory of Plovdiv and Stara Zagora provinces. The manifestation of versatile by its character anthropogenic activity is a major factor in the formation of specific types of land use, which depend to a large extent on the socio-cultural and economic features of the environment, which in turn influences the formation of the landscape structure. This is precisely what necessitates the study of these transformations and interactions and in a temporal aspect, so that qualitative and quantitative conclusions can be synthesized about the "pattern-process" interconnections in the landscapes.

Keywords: landscapes, landscape pattern, anthropogenic effect, landscape metrics, satellite image, remote sensing

L02_03

Assessment of flood regulation capacity of different land cover types in Krumovitsa River basin (Eastern Rhodopes)

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Floods are common phenomena, which are restricted by the climate, landforms, geological settings, vegetation and land use. Different land cover types have various capacity for flood regulation and flow

retention. The area of interest located in the Eastern Rhodopes, where the winter precipitation maximum often causes floods and material damages.

Aim: The aim of the presented research is to perform an assessment of flood regulation capacity of different land cover types in the basin of Krumovitsa River, one of the main tributaries of Arda River. Therefore, a drainage network and different land cover types are analyzed and discusses.

Materials and Methods: Due to the size of the area of interest (673 km²) and its variety the entire study is based on analysis of drainage network and available CORINE land cover classes from different years. High resolution imagery and digital elevation model is used for the visual interpretation of the derived maps.

Results: The end result of the image classification is a land cover map showing the spatial pattern of each land cover class. The changes in land cover and its spatial pattern in different years are evaluated. The area of each land cover type is calculated both in absolute in relative units (% of the whole study area). An attempt is made to relate the drainage density with different land cover types.

Conclusion: Presented results show relevant information for land cover and flood regulation management. Land cover maps could be used in further evaluation of regulating ecosystem services.

Keywords: ecosystem services, floods, Eastern Rhodopes, land cover change

L02 04

Development of accurate chemical thermodynamic database for geochemical storage of nuclear waste. Part I. Models for predicting solution properties and solid-liquid equilibrium in binary nitrate systems of the type 1-1

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Abstract: In this study we developed very well validated thermodynamic models for solution behaviour and solid-liquid equilibrium in 7 nitrate binary systems of the type 1-1 (HNO₃-H₂O, LiNO₃-H₂O, NaNO3-H2O, KNO3-H2O, RbNO3-H2O, CsNO3-H2O, and NH4NO3-H2O) from low to very high concentration at 298.15 K. The models for nitrate systems described in this study are of high importance, especially in development of strategies and programs for nuclear waste geochemical storage. Models are developed on the basis of Pitzer ion interactions approach. To parameterize models for binary systems we used all available raw experimental osmotic coefficients data (φ) for whole concentration range of solutions, and up to saturation point. Data for supersaturation zone, available for LiNO₃-H₂O system, are also included in parameterization. The recommendations on mean activity coefficients ($\gamma \pm$) given in literature are model-dependent. Therefore, they are not used in parameterization process. However, these data are used to validate the resulting models. The predictions of new developed here models are in excellent agreement with experimental osmotic coefficients data, and with recommendations on activity in binary solutions from low to very high concentration: up to ≈ 29 mol.kg⁻ ¹ in HNO₃-H₂O, and up to 26.8 mol.kg⁻¹ in NH₄NO₃-H₂O. The thermodynamic solubility products (as ln K°sp) of 6 solid phases [LiNO3.3H2O(s), NaNO3(s), KNO3(s), RbNO3(s), CsNO3(s), and NH4NO3(s)] have been determined on the basis of evaluated binary parameters and using experimental m(sat) solubility data.

Keywords: Nuclear waste sequestration; Computer chemical and geochemical modelling; Pitzer approach; Binary nitrate systems HNO₃-H₂O, LiNO₃-H₂O, NaNO₃-H₂O, KNO₃-H₂O, RbNO₃-H₂O, CsNO₃-H₂O, and NH₄NO₃-H₂O; Solution and solute activity; Solid-liquid phase equilibrium.

Acknowledgement: The work was supported by the European Regional Development Fund within the Operational Programme "Science and Education for Smart Growth 2014-2020" under the Project CoE "Universities for Science, Informatics and Technologies in e-Society (UNITe) BG05M2OP001-1.001-0004", as well as by Shumen University Research Program, Project No. RD-08-120/04.02.2019.

THEMATIC SESSION III ECOLOGY AND EDUCATION

L03 01

Sensitivity analysis of water quality indexes – a case study of Topolnitsa and Luda Yana rivers

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The study focuses on the analysis and sensitivity assessment of some of the worldwide use of surface water index estimates. To obtain a comprehensive assessment of the water status of the Topolnitsa and Luda Yana rivers in Bulgaria, three different indices were used - WQI (Water Quality Index), Water Pollution Index (WPI) and the Bavarian Surface Quality Index (CJ). The first allows the researcher to select a set of physicochemical indicators and reference values. In the second, the set of indicators and reference values are defined, while in the third, the researcher fully respects the pre-set indicators and reference values. Through their implementation, an attempt is made to evaluate the ability of the indices to record both the transformations of individual quality indicators and the comprehensive assessment of river water quality. The results obtained would assist the researchers in selecting appropriate indices for assessing river water quality.

Keywords: water quality indexes, Topolnitsa, Luda Yana

Acknowledgments: Financial support from Bulgarian National Scientific Fund project KΠ-06-OΠP 03/3 (Profiles of spatial differentiation of the quality of river waters is basins with various anthropogenic impact) of the Ministry of Education and Science, Bulgaria is gratefully acknowledged.

THEMATIC SESSION IV ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE

PL04 01

Organic cultivation of new medicinal and aromatic plants as a source for pharmaceutical industries

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Medicinal and aromatic plants continue to be the subject of novel and straight forward applications as a source of active constituents for pharmaceutical industries.

Fertilizers are an important factor in modern-day agriculture. They are responsible for substantial increases in crop yields, and allow crops to be planted in soil that would otherwise be nutrient deficient. Chemical fertilization is one of the most important tools for higher production of different crops. It is a well-known fact that the extensive and intensive use of chemical fertilizers may result in accumulation of chemicals in the increment interest in chemical free products have drawn the attention to new techniques for cultivation of certain medicinal plants for getting on clean products to be more suitable for foreign markets. Instead of chemical fertilizers, some organic fertilizers could be used to meet the demand of plants of NPK, as well as some micronutrients. Farmyard manure (FYM), compost etc., are used in the fertilization at different rates and different combination to determine the best time and rate of application to improve the quantity and quality of medicinal plants.

The present work is intended to review some of the worldwide performed research to elaborate the benefits of organic cultivation of some novel medicinal and aromatic plants such as *Coleus forskohlii*, *Satureja hortensis L.*, some amaranth as well as other species, relevant to be introduced to the climate of Egypt.

L04 01

Response of Amaranths plants to liquid barite and humic acid applications

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This experiment was conducted at Al-Adlya Farm, El-Sharkia Governorate, Sekem Company. The seeds of Amaranths were sown on 1st February, on a prepared nursery beds, watered regularly using a watering can and checked for seedling emergence. Transplanting of amaranths seedlings into their respective plots in the field took place 45 days after sowing in nursery. After 30 days from sowing, plants were sprayed with Barrite (0, 1 and 2 g/l) and / or Humic acid (0, 1, 2 and 3 g/l). Yield and its components as plant height (cm), fresh weight of plant (g), leaves number and seed yield (g / plant and Kg / Fed). Moreover, fixed oil (percentage and yield) and its constituents were determined. The pronounced effects of the different organic fertilizers were noticed.

P04 01

First Implementation of MSFD framework for benthic broad habitats assessment in the Bulgarian Black Sea

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Benthic habitats are ecosystem elements required to be assessed under MSFD Descriptors 1, 6 -"Biodiversity" and "Seabed integrity". This study represents the first environmental status assessment of the Bulgarian Back Sea benthic broad habitat types accomplished according to the criteria and methodological standards of COM DEC (EU) 2017/848. In 2017 a total of 238 macrozoobenthos samples were collected from the shelf sediments at 107 monitoring points. Adverse effects on habitats condition from eutrophication (D5C8) were assessed using indices (S, H', AMBI, M-AMBIn) and classification systems for macrozoobenthos developed under the WFD. These were also considered to be indicative of adverse effects from physical disturbance (D6C3). The spatial extent of adverse effects was estimated by interpolation of EQR_{M-AMBIn} point data within broad habitat types modelled in EuSeaMap. The interpolation is converted to good or not good status areas corresponding to values above or below good status threshold. These results were aggregated with poor oxygen saturation extent (D5C5) and extent of adverse effects by permanent alteration of hydrographical conditions (D7C2) to produce the combined extent of overall adverse effects (D6C5). Four out of five coastal and both of the shelf assessment areas did not achieve Good Environemtnal Status (GES), meaning that more than 80 % of the broad habitat types present were assessed in not good status. The only coastal area in GES was "Emine-Maslen nos". These assessment results ought to be utilized in the first cycle reporting under MSFD Article 17(2), which was due in 2018 but is not yet submitted.

Keywords: benthic broad habitats, Bulgarian Black Sea, environmental status, MSFD

Acknowledgements: The study was funded under Contract № Д-33-36/28.05.2018 between MOEW and IO-BAS for implementing the legal obligations under the Water Act Art.171, paragraph 2, p. 3 to execute Black Sea monitoring.

P04 02

Effect of mixed cropping systems on yield and quality of lettuce (*Lactuca sativa* L.)

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The production of leafy vegetables is aimed at increasing the yields and improving the quality. The amount of organically grown lettuces is increasing compared to conventional products. Studying the relationship between grown together plant species helps to find suitable technologies to create mixed crops, ensuring sustainable production and environmentally friendly production.

The purpose of this work is to determine the quantitative and qualitative changes in biometric and biochemical parameters of lettuce plants when grown mixed with medicinal and vegetable species.

The experiments were carried out in a growing house. The plants used to create mixed crops were lettuce (*Lactuca sativa* L.), cutivar 'Bohemia', tagetes (*Tagetes* sp. L.), basil (*Ocimum basilicum* L.), calendula (*Calendula officinalis* L.) summer savory (*Satureja hortensis* L.) and arugula (*Eruca sativa* L. Cav.).

The results of the analyses showed that the lettuce plants developed together with calendula had a significantly greater leaf and root mass.

The calendula plants were 66.5% higher than the average height of the lettuces in the experiment, and those developed together with savory and basil resp. 15.3% and 14.5%. The lettuce plants with the best vegetative development also had a high chlorophyll content. The highest value of the indicator Ch-a + Ch-b was measured in the variant of lettuce grown together with arugula. The lettuce in the containers with arugula had the highest solids content (10.68%) and total sugars (9.8%) in their leaves, while the vitamin C content was highest in the lettuce grown together with calendula (8.57 mg/100g fresh weight).

Keywords: mixed farming, Lactuca sativa, container experiment

THEMATIC SESSION V OTHER RELATED TOPICS

P05 01

Investigation of river waters by the method of water spectra

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The aim of the article is to study the water spectra of several rivers. The material consists of water samples taken from: Musalenska Bistritsa, Maritsa, Cherni Iskar, Iskar (Sofia), Iskar (Dolni Lukovit), Beli Iskar and Ledeno Ezero for comparison.

The method of investigation consists in measuring the contact angle of an evaporating drop of a test sample using an optical microscope.

The results obtained are displayed as graphs in the form of differential spectra containing several peaks. From the results obtained it can be concluded that the cases of cleaner waters (mountain rivers and lakes) their correlations and peaks are higher than the peaks and correlations of the more polluted waters (near cities) It is interesting to continue the study on other rivers.

Keywords: water spectra, river waters, water pollution.

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THEMATIC SESSION VI BIODIVERSITY AND CONSERVATION BIOLOGY

PL06 01

Egyptian encyclopedia of wild medicinal plants as a milestone for conservation of genetic resources and folk medicine

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The flora of Egypt includes 2145 species in 755 genera and 129 families of which 61 are endemic and more than 1000 are useful or of potential use for medical purposes. Due to the need of Egypt to Encyclopedia for wild medicinal plants like many other countries in this field, the Academy of Scientific Research and Technology (ASRT) initiated and finically supported a project for preparing, editing and publishing of this encyclopedia. The encyclopedia provides all available information on the wild medicinal plants together for the maximum benefit of the readers. The Encyclopedia (a series of ten volumes) of wild medicinal plants consists of monographs on the selected wild medicinal plants in Egypt. Each monograph follows a standard format with information followed by a reference list. It provides practical and theoretical information on wild medicinal plants of Egypt. Some monographs contain DNA bar-coding of the available plants which had been carried out by a scientific team specialized in genetic engineering and biotechnology. A total of 335 monographs in eight volumes are now available in hardcopy form. It is expected that the ten volumes of this Encyclopedia would encourage the conservation of wild medicinal plants and interest in herbal medicine in general and would supply research workers with a source of scientific information in the chemistry and biological activity of medicinal plants in particular.

L06 01

Monitoring the impact of climate change on the alpine lichen and plant diversity – the GLORIA-Bulgaria project

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Climate change is increasing the risk of extreme weather – increased frequency of heat waves, changes in precipitation patterns, catastrophic events (floods, extreme droughts, hurricanes), which have strong impact on biodiversity. Alpine ecosystems are among the most sensitive environments on Earth to such change, especially warming, since they are determined by low temperatures, higher humidity, and shorter vegetation seasons. An international research network – GLORIA – the Global Observation Research Initiative in Alpine Environments, was established two decades ago in order to collect data and analyse the effects of climate change on alpine biodiversity. Bulgaria is in a process of joining the network by the designation and surveying of one four-summit site in Rila Mountains. The aim of the study is to install permanent monitoring plots and to periodically collect the necessary data. The following hypotheses will be tested: (1) Elevation strongly affects plant community composition

patterns, and (2) Topography causes different microclimatic conditions and, thus, different community compositions within the same elevation levels. The site comprises four summits representing an elevation gradient from 2380 to 2790 m a.s.l. In 2019, data collection was carried out following strictly the internationally adopted methodology of GLORIA. The following major parameters were measured or assessed: soil temperature, species composition of bryophytes, vascular plants and lichenised fungi in the study plots, visual cover estimation of surface types, percentage cover of each species through visual estimation and pointing. The site and the collected baseline data will be presented and discussed with emphasis of the species of conservation concern.

Keywords: alpine biodiversity, biodiversity monitoring, Bulgarian flora, climate change, GLORIA network

Acknowledgements: Financial support of the Bulgarian Science Fund under GLORIA-Bulgaria project, Contract KΠ-06-H21/16 of 19.12.2018, is gratefully acknowledged.

L06 02

Monitoring wetland dynamics using remote sensing and GIS

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Wetlands are especially important ecosystems that perform a range of vital environmental functions and provide numerous beneficial services for people. Due to anthropogenic pressure, these fragile ecosystems are under continuous threat and require documentation of recent historical changes, particularly those known to have anthropomorphic causes. The present study focuses on the monitoring and visualization of spatio-temporal changes occurring in the wetland of the Dragoman Marsh in Bulgaria. The study aims to map land cover and land use change taking place in the marsh area for the period 1965-2015 using remote sensing (RS) and geographic information system (GIS). The Dragoman Marsh is the biggest natural karst wetland in Bulgaria. In the beginning of the 1930's the marsh was subject to drainage and was turned into agricultural land as most of the natural wetlands in Bulgaria. Thus, human activities terminate the life in the marsh for a long time. In the beginning of the 1990's the draining process was stopped and restoration of the ecological condition of the Dragoman Marsh was started. Nowadays, plant and animal species here are revived and the marsh is a successful case of wetland recovery. The Dragoman Marsh is a Ramsar site since 2011, as well as a part of two NATURA 2000 sites. Identification of land cover and land use change was performed by using aerial photos, largescale topographic maps and high-resolution satellite images. The results show the dynamic change in the extent of the marsh area over a period of 50 years. The study also demonstrates that remote sensing and GIS provides efficient data and tools for monitoring recent historical changes in wetland ecosystem and for identifying the past and present scenarios, which will further support decision making.

Keywords: Wetland, Monitoring, Remote Sensing, GIS, Land Cover, Land Use

L06 03

Comparative Analysis of the Myriapoda (Diplopoda, Chilopoda) fauna of the Shumen Plateau and the Madara Plateau (Northeastern Bulgaria)

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At present, comprehensive inventories of the myriapod fauna of particular geographic regions in the country and the comparative analyzes of the similarity in their species composition are rather few.

In the current paper, the results of a comparative analysis of the taxonomic, ecological and zoogeographic structure of myriapoda communities are presented in the natural habitats of two regions in Northeastern Bulgaria – the Shumen Plateau and the Madara Plateau. The habitats compared in these two plateaux are characterized by similar species composition of the plant formations. Analysis of the structure of myriapoda communities includes estimating species diversity by determining the Shannon-Wiever index (H') and the Berger-Parker index (d). The degree of similarity in the species composition of the communities was analysed using the Czekanowski-Dice-Sörensen index (Qs). The impact of abiotic and biotic environmental factors on species distribution in both areas was assessed by principal component analysis (PCA). The identified differences in species composition are discussed in relation to the role of specific environmental factors on the distribution and abundance of species.

Keywords: biodiversity, Shumen Plateau, Madara Plateau, Diplopoda, Chilopoda, component analysis.

Acknowledgements: This work was realized with the financial support of a research project RD-08-104/30.01.2020, funded by "Konstantin Preslavski" University of Shumen.

P06 01



Integrative investigation on the ecology of the Black sea mussel Mytilus galloprovincialis Lam. and its habitat

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The present work is reporting a preliminary investigation on the ecology of the Black sea mussel (Mytilus galloprovincialis Lam.) and its habitat by monitoring of the physicochemical indicators of the water, the microbiology and the radionucleids in the region of Shkorpilovci and the Kavarna. The samples were collected in the period of August 2018 until October 2019. The BIOLOG system was used for microbiological determination. The physicochemical parameters of the waters - temperature, pH, salinity, dissolved O₂ and electrical conductivity are determined by SENSODIRECT 150 portable sustem. Additionally, the study includes determination of naturally occurring 7Be, 40K, 232Th, 226Ra, 238U and technogenous ¹³⁷Cs, which are omnipresent in seawater, infesting the marine environment via atmospheric deposition, winds, underground fresh water discharges and riverflows. Enterococcus durans and E. haere were isolated from the Shkorpilovci region and Streptococcus gallolyticus ss gallolyticus, E. haere, Lactobacillus brevis and L. sakei ss sakei were found from the

Kavarna. We isolated the lactic acid bacterium *Streptococcus gallolyticus ss gallolyticus*, a species associated with colon-rectal tumors on the one hand and as a producer of bacteriocins on the other. Gamma-spectrometric analysis indicated that technogenous ¹³⁷Cs radionuclide can be detected in sand samples collected from region of the Kavarna. However, this radionuclide was not detected in the shells and tissues of the animals from the Kavarna. The calculated indicators for radiation hazard, external hazard, absorbed dose and annual effective dose are in accordance with the norms quoted in the Bulgarian and world legislation.

Keywords: Bivalvia, *Mytilus galloprovincialis* Lam, radionucleids, Black Sea, monitoring of the water, radiology, marine habitas

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P06 02

Alkanna tinctoria: an approach toward ex situ cultivation

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Alkanna tinctoria (L.) Tausch is a perennial herbaceous medicinal plant species from Boraginaceae family, with limited distribution and small populations. It is included in the Red Data Book of Bulgaria as endangered species, and protected by the Biological Diversity Act. Experimental work for ex situ cultivation is among the recommended conservation measures for A. tinctoria. Seed germination rate under in vivo and in vitro laboratory conditions, as well as in soil, was very low, around 1%. The present study deals with stimulation of seed germination, acceleration of plants' growth using hydroponic technologies, and assessment of the photosynthetic apparatus of adapted plants. Seeds gathered from 4 natural populations were treated by soaking in gibberellic acid (GA₃), and irradiation with red, blue, or infrared light emitting diodes (LED), in order to stimulate their germination. Seedlings were grown on Cutting board hydroponic system for 6 weeks, than potted in soil substrate. Photochemical activity of adapted plants was characterized by Pulse-Amplitude-Modulated (PAM) chlorophyll a fluorescence. Results showed that all of the 3 factors: seed origin, GA₃ and light quality, influenced germination; furthermore, interaction of factors was also observed. Germination rate was enhanced mainly by GA₃. Best results were noticed for seeds originating from Mikrevo population, treated with GA₃ and irradiated with blue light: 40% of 90 seeds germinated. Growth of 82% of the plants was accelerated. There were no significant differences in the maximum quantum yield of primary photochemistry in dark adapted state Fv/Fm between plants obtained from seeds germinated under different light quality.

Keywords: dyer's alkanet, seed germination, hydroponics, fluorescence of PS II

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P06 03

Hieracium piliferum agg. (Asteraceae), the first record in the Bulgarian flora

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During field work in Rila Mts in 2019, in search of suitable summits to study the effects of climate change on alpine plant diversity, un unexpected taxon was recorded. It belongs to Hieracium piliferum agg. from H. sect. Barbata, and it is the first taxon of this section to be recorded in the Bulgarian flora. This locality is rather isolated and represents the south-easternmost locality of a taxon from this section in Europe. The Bulgarian material is morphologically most similar to H. amphigenum. So far the latter species was known from the Alps, the Carpathians and the western parts of the Balkan Peninsula. In the Balkans, the species was recorded from Bosnia and Herzegovina, Montenegro and Kosovo. Search in the Bulgarian herbaria, especially in SOM, revealed the species was collected once in the past in the same area but remained unrecognized from similar taxa. In Rila Mts, the species grows on siliceous bedrock in subalpine and alpine grassland communities on steep slopes exposed to the east. Most of the accompanying species are typical alpine plants such as Dianthus microlepis, Homogyne alpina, Jasione bulgarica, Leontodon rilaensis, Pedicularis verticillata, Pilosella rhodopea, Potentilla ternata, Pulsatilla vernalis, Ranunculus crenatus, Vaccinium myrtillus, V. uliginosum, Veronica bellidioides. The poster presents the distinguishing characters from morphologically similar taxa, the locality and habitat of the species, as well as its chromosome number and genome size. The conservation value of the taxon is discussed as well.

Keywords: Compositae, *Hieracium* sect. *Barbata*, new record, Rila Mts, alpine flora, plant conservation

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L06 04

Structure and composition of the amphibians and reptiles, accidentally falling in pitfall traps during studies of the epigeal fauna

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Pitfall traps are a main method for studying of the epigeal fauna and have a number of advantages over all other methods. In most such studies, specialists separate the fixed biological material that interests them, and the remaining animals of the sample are discarded. One of the "unused" groups of animals are the amphibians and reptiles, which we cannot protect from accidental falling into the traps. Object of our survey are pitfall traps catches from 5 different regions of Bulgaria, realized in the period 2004 – 2014. As a result of the survey 454 specimens of 16 species were processed – 7 species of Anura, 3 species of Caudata and 6 species of Squamata. Biological material collected by this method may be used for registration and mapping of the populations of established species. In addition to somatic proportions, all other biological data which cannot be taken from live specimens may also be recorded on fixed animals. Information on food, parasites, pathogenic alterations, reproductive potential and many others is valuable for the analysis of the condition of the species, and for the determination of the

general status required in taking conservation measures and monitoring. The analysis of the biological material proves the full suitability of the pitfall trapping for establishing the species composition, spatial distribution, seasonal activity, phenology and the size-age structure of the common species. The results showed the indicative capabilities of the particular fauna as well as of some species that are strongly dependent on certain environmental parameters.

Keywords: Amphibia, Reptilia, alternative methods for studying, pitfall traps, biological information, Bulgaria

P06 05

Assessment of Marine Cetacean Populations in Bulgarian Black Sea in 2017 according to indicators of the EU Marine Strategy Framework Directive

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The cetacean fauna in the Black Sea is presented by three species – harbour porpoise, common dolphin and bottlenose dolphin, which require monitoring and protection by EU member states under the Habitats and Marine Strategy Framework Directives. Assessment of cetacean populations state was found on data, collected during a ship-based visual survey by line transect method, carried out during 24.11.2017 – 22.12.2017 in the whole Bulgarian coastal and shelf areas. Threshold values for population abundance (D1C2) and density (D1C4) indicated in the national monitoring program for Descriptor 1 of MSFD were applied and preliminary data for distributional range (D1C4) are reported. The three cetacean species - P.p. relicta, T. t. ponticus and D. d. ponticus were observed during the study with the total number of 123 sightings and 360 observed individuals. The abundance of Bottlenose dolphin was estimated at 1365 animals, density - 0.113. 10⁻⁶ ind.km² and distributional range of 745.81 km². The common dolphin was less abundant – 963 individuals and with lower density - 0.0796.10⁻⁶ ind.km², but widely distributed (992.99 km²). The most abundant was Harbour porpoise with an estimate of 6474 individuals, density of 0.536.10⁻⁶ and a range of 2145.09 km². The integrated assessment of the status within species and to species group of marine mammals in coastal and shelf areas in 2017 according to Descriptor 1 (MSFD) criteria and indicators showed that the three species of cetaceans are in "Not good" status and the good environmental status is not achieved by species group.

Keywords: cetaceans, monitoring, indicators, Bulgarian Black Sea, MSFD, Descriptor 1

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Recent observations on the size structure of *Chamelea gallina* and *Donax trunculus* in the Bulgarian Black Sea as status indicators of commercially exploited shellfish under the Marine Strategy Framework Directive (MSFD)

P06 06

P06 07

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Healthy stock of commercially exploited fish and shellfish are determined by MSFD as one of the marine environmental status descriptors (D3). The clams Donax trunculus and possibly Chamelea gallina have become commercially exploited shellfish in the Bulgarian Black Sea since 2012. Mixed catches due to habitat range partial overlap and lumped landings statistics create uncertainty about the catch's species composition and ratio but personal communication with clam catchers suggests predominant harvest of D. trunculus. Rapidly increasing landings to a maximum of 819 t in 2017 dropped to 506 t as soon as 2019. This study examines the wild population status of *Donax trunculus* in front of Chernomorets beach (Varna) by investigating the size and weight structure, and the condition index as observed in February 2020. The predominant size class is 22 mm (36,5% of the sample), as the smallest and the largest observed specimens were 14.69 mm and 38.81 mm respectively. The b-value of the length - weight relationship was 2.82 (p < 0.0001), which was indicative of negative allometric growth. The good status thresholds of the indicators 95th percentile of the Length (L95) and Height (H95) defined under MSFD D3 Criterion 3 were not reached with values calculated at 28.26 mm and 18.00 mm, respectively. The average condition index was 15.5. Overall deterioration of the population status is possibly associated with harvesting pressure. Year-round monthly surveys are planned to study the annual population dynamic with the objective to devise improved indicators and thresholds for better assessment of the population status.

Keywords: *Donax trunculus*, landings, size structure, length – weight relationship, Bulgarian Black Sea, MSFD

Acknowledgements: The study was funded in parts by MOEW for fulfilment of the monitoring requirements under MSFD, MASRI project of the National roadmap for scientific Infrastructure (2017-2023) of Republic of Bulgaria and BAS for PhD education.

Bio-ecological characteristics and size-age classification of the Alpine newt (*Ichthyosaura alpestris* (Laurenti, 1768)) during the breeding season in representative regions for Bulgaria

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The Alpine newt (*Ichthyosaura alpestris* (Laurenti, 1768)) in Bulgaria is a glacial relict and has a limited distribution in mountainous regions. The object of the study are 773 specimens caught in three different

regions – the Rila Mts. (on the border with the Western Rhodope Mts.) – 104 Specimens, Western Stara Planina Mts. – 107 specimens and Osogovo Mts. – 562 specimens. The animals' gender is determined and total length and body length are measured. Minimum and maximum sizes for both sexes are established and size-age structure of the species is defined for each of the three regions. The results contributed to the development of a hypothetical size-age scale, which might be applied when express data on the state of the Alpine newt breeding groups are needed. Some patterns in the time limits of the entering and exiting from the breeding ponds are established, as well as peculiarities of the duration of the metamorphosis and terrestrial activity. The collected and analysed information is the result of long-term studies in the two educational and experimental forest stations of the University of Forestry, where the species is annually monitored. We observed a persistent trend of reduction of the Alpine newt populations and its disappearance in certain long-standing breeding ponds as a result of established pesticide and organic wastewater contamination and drainage of a quarry microlake where it occurred. The methodology used to analyse the condition of the species is experimental and is in a process of practical development and implementation for the purpose of express evaluations.

Keywords: Alpine newt, size classes, size-age structure, Rila Mts., Petrohan Pass, Osogovo Mts.

P06_08

Population of the Flathead grey mullet (*Mugil cephalus*, Linnaeus 1758) from the Bay of Burgas, Bulgarian Black Sea coast

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The study is aimed to investigate the dynamics of age distribution, size structure, seasonal and annual growth rates of *Mugil cephalus* from the Bay of Burgas. The species inhabits inshore marine waters, as well as estuaries, lagoons and rivers and has high economical importance. Only four age groups were determined for the recent period (2015-2018), compared to the previous period (2010 - 2013) when the flathead grey mullet population has eight age groups. For the period 2010-2013, the size classes ranged from 3.1 cm to 49 cm. For the period 2015-2018 the maximum total length registered was 32 cm. The equation describing the relationship between the length of the body and the radius of the scale is L=2.9034S-5.2894, r²=0.9104. The von Bertalanffy's equation for length growth of the flathead mullet is L_t=199.41[1-e^{-0.03(t+0.11)}], r²=0.994 and for the weight growth is W_t=3138[1-e^{-0.17239(t-0.3720)}]^{3.0654}, r²=0.9276. The equation describing the length – weight relationship of the *Mugil cephalus* is W=0.0122*L^{3.0961}, r²=0.991. Values of Fulton's condition index for the age groups from 1 to 4 varied between 1.54 and 1.82. Comparison between the results obtained for the periods 2010-2013 and 2015-2018 shows the deterioration of the population of the flathead grey mullet.

Keywords: population parameters, von Bertalanffy's equation, Fulton's condition index, Black Sea coast, *Mugil cephalus*

Acknowledgments. The study was realized with the financial support of National Scientific Fund-MES through the project entitled "The influence of environmental condition of Varna and Burgas bays on population-biological parameters of mullets species (*Mugil cephalus, Liza aurata* and *Liza saliens*)", №DM11/2 (15 Dec 2017).

P06 09

Relationships between Size, Weight, Age and Fecundity of the Chelon auratus and Chelon saliens (Mugilidae) from the Bulgarian Black Sea coast

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The study is aimed to demonstrate the relationships between the fecundity, length, weight and age of Chelon auratus and Chelon saliens from the Bulgarian Black Sea coast. Absolute fecundity of Ch. auratus varied from 327126 oocytes for age 3 (L-16.0 cm, EW-55 g) to a maximum of 4103879 for age 9 (L-32.4 cm, EW-321g). For Ch. Saliens, absolute fecundity varied from 162890 oocytes for age 2 (L-16.9 cm, EW-52 g) to a maximum of 892441 oocytes for age 6 (L-33.7 cm, EW-315 g). The mean estimated fecundity was 213642 eggs for Ch. auratus and 54882 eggs for Ch. saliens. Relationship between absolute fecundity-weight, fecundity-length was best fitted by the following equations: Ch. auratus- F=9476+10241*W, $r^2=0.921$ and F=-2384206+152333*L, $r^2=0.906$; Ch. F=215473+1698.7*W, $r^2=0.916$ and F=-457777+36837*L, $r^2=0.924$. The relationship between fecundity andage was best described by the exponential equation: Ch. auratus- F=106354e^{1.5475}, r^2 =0.969 and *Ch. saliens*- F=103041e^{1.1264}, r^2 =0.994. The average weighted relative fecundity was calculated as 492 for Ch. auratus and 272 for Ch. saliens. A more accurate indicator to describe the relative fecundity is the coefficient b from the equation F=a+b*W. For Ch. auratus the coefficient b was 10241, and for *Ch. saliens* was 1698.

Keywords: absolute fecundity, relative fecundity, Black Sea, Chelon auratus, Chelon saliens

Acknowledgments. The study was realized with the financial support of National Scientific Fund-MES through the project entitled "The influence of environmental condition of Varna and Burgas bays on population-biological parameters of mullets species (Mugil cephalus, Liza aurata and Liza saliens)", №DM11/2 (15 Dec 2017).

P06 10



Dietary analysis of wintering groups of long-eared owls (Asio otus, Linnaeus, 1758) in the region of Silistra (NE Bulgaria)

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The object of the study is the diet of two wintering groups of long-eared owls (Asio otus, Linnaeus, 1758) from the town of Silistra. During the period 18 – 21 April 2014, 511 pellets and other skeletal residues are collected separately in the two localities. A total of 1538 specimens are established, of which 1500 skeletal parts of small mammals, 36 of birds and two pairs of Coleopteran elytra. The established average number of victims in one pellet is 2.27 individuals. A total of 23 species of small mammals are identified: 5 species of Insectivora, 3 bat species (Chiroptera) and 22/23 species of Rodentia. Significant in the diet of the owls are Microtus ssp. (59% of the total biomass), Apodemus ssp. (18%) and Mus ssp. (12%). Bird preys consist a negligible part of the diet, both in numbers (36 ind./2%), and as a part of the total biomass (4%). The differences in the prey species composition between the two wintering groups are due to differences in the landscape structure, as well as the degree of synanthropisation. All identified small mammal species, except synanotropic mice and rats, can be considered new to the research area. For the first time after almost 50 years, information about *Apodemus uralensis* is given. Some protected and conservationally significant species are established (Chiroptera: *Nyctalus noctula, Plecotus austriacus, Barbastella barbastellus*; Rodentia: *Muscardinus avellanarius, Mesocricetus newtoni*). They show the exceptional possibilities of this method to establish rare species that are difficult to prove with other methods of research.

Keywords: Asio otus pellets, owls diet, Danube River coast

P06 11

Macropterous ground beetles (Coleoptera: Carabidae) prevail in European oilseed rape fields

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During a research conducted in oilseed rape (*Brassica napus* L.) fields in four European countries (Bulgaria, Germany, Romania and Switzerland), species composition and ecological structure of the ground beetle (Coleoptera: Carabidae) fauna associated with the rape were studied. Field work was carried out in 2017 (2018 in Bulgaria). Pitfall traps (5 in each site) were set in each sampling site in each country. Captured beetles belonged to 179 species and 51 genera. The most diverse were genera *Harpalus* and *Amara* (21 species each), followed by the genera *Carabus* (15 species), *Pterostichus* (10 species), *Microlestes* and *Poecilus* (9 species each), and *Brachinus* and *Ophonus* (8 species each). In Bulgaria were found 107 species, in Germany – 68 species, in Romania – 71 species, in Switzerland – 45 species. Thirteen species were common in all countries. Macropterous species represented 65% (116 species) of all collected carabids (in all countries). Pteridimorphic species were 20% of all (36 species), and brachypterous were only 12% (21 species). For 6 species (3%) there were no data about their wing morphology. The results were similar in each country. Macropterous species were 73% (78 species) in Bulgaria, 60% (41 species) in Germany, 68% (48 species) in Romania, and 69% (31 species) in Switzerland. The prevalence of the macropterous carabids reflects their higher mobility and adaptiveness.

Keywords: carabids, agrocoenoses, ecology, Europe, wing morphology.

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Status and threats of the populations of *Orchis provincialis* in Mt Rhodopes, Bulgaria

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Aim: Orchis provincialis Balbis is among the most threatened vascular plants in Bulgaria due to its peculiar biology, environmental requirements and various threats of anthropogenic origin. The study aimed to explore and evaluate status of the species in some of its largest populations in Bulgaria.

Materials and methods: Exploration and investigation of few populations of the orchid *Orchis provincialis* in some locations in the Mt Rhodopes (East) region in Bulgaria was done from 2014 till 2018. For evaluation of the status of the plants' populations were recorded geospatial data and some morphometric parameters.

Results and discussion: The results show that the population above Kirkovo village is in relatively steady state. However the largest known population in Bulgaria – the one near Lozengradtsi village is in more threatened state. Among the key factors for this condition probably are the climate changes which promote excessive development of understorey vegetation. The excessive development, growth and spread of the understorey vegetation reduces the light availability for the threatened species and restricts the access of the pollinators which as consequence leads to reduced shoot count and lower fruiting success. Taking into account the relatively low lifespan of this orchid all this situation puts a high stress upon the population survivability and renewal.

Conclusion: The study suggests that some more active interventions and actions may be are necessary to secure the future presence of this species in the Bulgarian flora.

Keywords: Orchis provincialis, bioconservation, threats, orchids, Orchidaceae

Index

| Name | Code | E-mail | Student/ PhD student | Page |
|-------------------------------------|-----------------------------------|---|----------------------------|----------------|
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| Bonch- Osmolovskaya Elizaveta | P01_06 | | | 20 |
| Borisova Borislava | P01_02 | | | 18 |
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| Dimitrova Stela | P01_01 | | 17 | |
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| Dobreva Lili | P01_04 | lili.ivailova@gmail.com | 19 | |
| Dobrikova Anelia | L01_05 | aneli@bio21.bas.bg | 14 | |
| Dobrinov Ivo | P06_04 | | 33 | |
| Doichinov Aleksandar | L06_03 | doichinov_shu@abv.bg | 31 | |
| Donchev Stanislav | L02_04 | st.donchev@shu.bg | 24 | |
| Doncheva Valentina | P04_01 | valentinadoncheva@gmail.com | 27 | |
| Dorofeeva Maria | P01_06 | | 20 | |
| Egorova Maria | P01_06 | | 20 | |
| Elcheninov Alexander | P01_06 | | 20 | |
| El-Gohary A. E. | L04_01 | gohary52001@gmail.com | 26 | |
| Ganeva Anna | L06_01 | annaganeva8@gmail.com | 29 | |
| Gartsiyanova Kristina | L03_01; L06_02 | krisimar1979@gmail.com | 25; | 30 |
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| Georgieva Almira | L01_03 | | 12 | |
| Georgieva Stela | L03_01 | | 25 | |
| Georgieva Vasilena | P01_04 | | 19 | |
| Grigorov Borislav | L02_01; L02_03 | borislav.g.grigorov@gmail.com | 22; | 23 |
| Grigorova Irena | P06_02 | djelepova@yahoo.com | 32 | |
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| Hamza Sevda | L06_03 | | 31 | |
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| Kitev Atanas | L03_01 | atanaskitev@abv.bg | 25 |
| Kodzhabashev | P06_04; P06_07; | | 33; 35; 37 |
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| Koleva Lilyana | P04 02 | liljanamarkova@abv.bg | 27 |
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| Kotova Irina | _ | | |
| | P01_06 | | 20 |
| Malakhova Dina | P01_06 | | 20 |
| Maslenkova Liliana | P01_02; P01_03 | lili.maslenkova@gmail.com | 18 |
| Mihova Svetla | P01_05 | | 20 |
| Miteva Daniela | L01_01 | daniela.miteva@abv.bg | 11 |
| Mitova Ivanka | P04_02 | lilikol@web.de | 27 |
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| Molle Emil | P06_02 | | 32 |
| Momchilova Albena | P01_02; P01_03 | albena_momchilovaabv.bg | 18 |
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| Natchev Nikolay | P06_01 | | 31 |
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Technical support

April 23rd

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