UNION OF SCIENTISTS IN BULGARIA
SECTION BIOLOGY

INSTITUTE OF BIODIVERSITY AND ECOSYSTEM RESEARCH - BAS

INTERNATIONAL SEMINAR OF ECOLOGY - 2021

CURRENT TRENDS OF ECOLOGY

29-30 September 2021
Online

UNION OF SCIENTISTS IN BULGARIA

Program & Abstracs

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CURRENT TRENDS OF ECOLOGY

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INTERNATIONAL SEMINAR OF ECOLOGY- 2021

CURRENT TRENDS OF ECOLOGY

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

Many thanks for the financial support of Bulgap EOOD and Pensoft Publishers EOOD.

Publisher: Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

Publication year 2021, ISBN 978-954-9746-48-8

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Technical support: Martin Dimitrov and Teodora Todorova

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THEMATIC SESSION I

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

PL01_01

Ecocide. Global consequences.

Roumiana Metcheva, Peter Ostoich, Michaela Beltcheva

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

The problem of environmental pollution is becoming increasingly important on a global scale. Man has oversaturated the environment of his habitat with harmful and most often toxic waste. It is difficult to describe all the toxic substances, as a separate book can be written for each group. The term "ecocide" has been introduced, which reflects large-scale destruction of the natural environment. We will focus only on three classes of pollutants that are of particular concern, creating environmental conflicts. These are:

- Pesticides that are becoming less effective as a result of the establishment of resistant strains and species of pests. They create large amounts of waste that bacteria can't handle, accumulate in the organs of some animals, and become toxic.
- Waste from the nuclear industry and radioactive fallout from nuclear explosions. It is especially dangerous that radioactive elements can be concentrated in certain organs.
- Petroleum products - often large quantities end up in the seas and oceans, along with industrial waste of various kinds, impossible to compensate for by nature and they pose a serious threat to ecosystems, many of which have already been destroyed.

At the submolecular level, chemical and physical effects can lead to genetic rearrangements (mutations); destructive ionization in the tissues of every living being, sometimes with completely unexpected consequences for humans.

Keywords: pesticides, radionuclides, petroleum products

PL01_02

Green and nano pesticides: new trends in environmental protection

Abdel-Tawab H. Mossa*

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Extensively uses of synthetic pesticides have adverse effects on human, animals, plants, non-target organisms and ecosystem. Therefore, green and nano pesticides have posted as alternative to chemical pesticides. It can be use in agriculture and in public health to control a variety of pests. Green or natural products such as plant extracts and essential oils (EOs) are a composite of many compounds with different mechanisms and sites of toxic action that enhances their activity. Moreover, nanotechnology such as nanoformulations is important to re-solve the problems of application, persistence and activity of natural compounds such as EOs. For example, nanoemulsions of some EOs were developed by ultrasonic emulsification, characterizations and evaluated for insecticidal and acaricidal activities. The droplet size ranged between 20-120 nm and the time of sonication, ratio of surfactant related to stability and droplets size. In green and nanoformulations, the insecticidal and acaricidal activities were increased by 50-90%. Ecotoxicology using Microtox and acute and sub-chronic toxicity of these formulations were studied on male rats. Stability of green and nanoemulsions and high activity suggest the possibility of developing safe green and nanopesticides.

L01_01

Light and auxin treatments affect morphogenesis and polyphenolics productivity in *Artemisia alba* Turra cell aggregates *in vitro*

Kalina Danova[1,*], Dobrina Pecheva[2]

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Aims: *Artemisia alba* Turra is an essential oil bearing shrub, with an Euro-Mediterranean distribution widespread in the South Eastern parts of Europe. Phytochemical investigations have evidenced the presence of volatile mono- and sesquiterpene derivatives, as well as non-volatile sesquiterpenoids, flavonoids and phenolic acids contributing to the anti-inflammatory, antimicrobial, antioxidant and pro-apoptotic activity of different preparations, obtained from the plant. The current research aims at elucidation of the potential for biotechnological polyphenolic compounds productivity of non-differentiated cell lines of the plant.

Material and Methods: For this purpose non-differentiated cell aggregates of the plant were initiated from either leaf or root explants of the sterile grown plant. The obtained cell aggregates were cultivated in the dark or at 16/8 h photoperiod in liquid media, supplemented with N^6-benzyladenine

benzyl adenine (BA) as auxin. The cytokinin effects of indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) were compared.

Main results: was established that NAA supplementation was superior to IBA, and light treatment - to dark growth conditions in terms of polyphenolic productivity. In addition, NAA supplementation led to better expressed compactness and larger size of the cell aggregates as compared with IBA.

Conclusion: The results of the present experiment indicate that secondary metabolite productivity *in vitro* is a dynamic process closely related to the plant's growth and development and is in close relation to the interactions of the plant with its environmental conditions.

Keywords: *Artemisia alba* Turra cell suspensions, cytokinins, auxins, polyphenolics productivity *in vitro*

Acknowledgements: The authors acknowledge the financial support of grant КП-06-Н39/6, National Scientific Fund, Bulgaria

L01_02

Application of residual sludges from wastewater treatment technologies for construction of biofertilizer

Dobromira Yaneva, Mihaela Belouhova, Yana Topalova

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To stimulate plant development in phytoremediation or in the cultivation of non-food crops in potentially contaminated soils, a biotechnologically created product could be applied.

Aim: to explore the possibility of creation of biofertilizer based on activated sludge combined with bacterial strain with detoxifying and plant growth promoting properties.

Materials and Methods: The presented study is focused on the effect of phenol in the following concentrations: 5 mg/L, 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L, on the metabolic activity of *Brevibacillus laterosporus BT271*. The gradual increased concentration of phenol was used to study the metabolic activity of mineralized activated sludge and *B. laterosporus BT271*.

Results: The CTC/DAPI staining showed high activity of the bacteria even at the highest concentration. The biggest amount of biomass was accumulated at 5 mg/L phenol ($4.44x10^{7}$ cells/mL). At this toxicant concentration, a total dehydrogenase activity of $5.72x10^{-4}$ μgH+/mL/min was found. Studies of the metabolic activity of microorganisms in experiments involving a combination of mineralized activated sludge, *B. laterosporus BT271* and phenol at three concentrations (5mg/L, 250mg/L, 1000mg/L) showed the highest value for dehydrogenase activity in the variant with average phenolic concentration (up to $6.39x10^{-6}$μgH+/mL/min).

Conclusion: The results proved the detoxification potential of *B. laterosporus BT271* when different concentrations of phenol are present. The combination of a mineralized activated sludge and selected highly active biodegrading *B. laterosporus BT271* showed valuable properties of detoxification and metabolic activity and keep these potential up to 1000mg/L phenol.

Keywords: biofertilizer, *Brevibacillus laterosporus BT271*, mineralized activated sludge, phenol.

Acknowledgements: This work has been supported by Project BG05M2OP001-1.002-0019: 'Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy', financed by Operational Program 'Science and education for smart growth', co-financed by the European Union through the European structural and investment funds.

L01_03

Polyphenol constituents of grape pomace from the local Balkan variety Mavrud possess antioxidant and antitumor activity against breast cancer

Iskra Yankova[1], Ralitsa Radoeva[2], Bozhidar Enchev[2], Maria Karsheva[2], Elena Ivanova[2], Inna Sulikovska[1], Ivan Iliev[1]

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Grape pomace is the main by-product of winemaking, a valuable source of polyphenols with antimicrobial, antioxidant and anti-cancer effects. The extracts of grape seeds and marcs are of strategic significance for the development of new therapeutic approaches against certain cancer diseases, including breast cancer.

The **aim of our study** was to investigate the antioxidant and antitumor potential of the polyphenol fraction of grape pomace obtained after the vinification of the local Balkan grape variety Mavrud.

Material and methods: We performed Soxhlet extraction with 50% ethanol solution and determined the total polyphenol content of the extracts by Folin-Ciocalteu colorimetric method. The obtained extracts were characterized by HPLC-DAD and their antioxidant activity was studied with DPPH assay. The cytotoxic effect and antiproliferative effect of compounds were tested on 3T3, MCF-10A, MCF-7 and MDA-MB-231 cell lines by MTT assay.

The **results** showed higher total polyphenol content in the marcs compared to the seed extracts and prevalence of gallic acid, catechin and epigallocatechin. We established a partial correlation between antioxidant activity and total polyphenol content. The studied extracts had a low cytotoxic effect, and the seed extracts showed higher antitumor activity and potential for treatment of luminal breast cancer.

Conclusion: This research provided new data on the biological effects of wine by-products from the local Balkan grape variety Mavrud. Our results have shown high antioxidant potential and opportunities for therapeutic use against luminal breast cancer, prerequisites for application in the pharmaceutical, cosmetics and food industries.

Keywords: grape pomace, breast cancer, polyphenol content, antioxidant activity, cytotoxicity, antiproliferative effect, local Balkan grape variety Mavrud

Acknowledgements: This investigation is partially financed by grant 12079 of the Research division of the University of Chemical Technology and Metallurgy.

L01_04

On the mode of action of *Origanum vulgare* spp. *hirtum* methanolic extract and essential oil on *Chlamydomonas reinhardtii*

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Aim: To evaluate genotoxic, mutagenic, and DNA-damaging potential of methanolic extract (ME) and essential oil (EO), isolated from fresh leaves of *Origanum vulgare* spp. hirtum, on a plant model *Chlamydomonas reinhardtii*.

Material and methods: Aerial parts of *Origanum vulgare* ssp. hirtum from the *ex-situ* collection of IBER, BAS during the flowering were collected. Extraction and isolation procedures as well as GC/MS analysis of EO and ME were performed by standard protocols. The components were identified by comparing their relative retention times with the retention times of authentic standards, and mass spectra with the NIST.

Test system: *Chlamydomonas reinhardtii* strain 137 C+ (WT).

Endpoints: "clonal" assay, a test of “visible” mutations, CFGE.

Statistics: GraphPad Prism program version 6.04 software (San Diego, USA) and One-way analysis of variance ANOVA with multiple comparisons using the Tukey method.

Results: A good correlation was observed between differences among main constituents of EO and ME identified by GC/MS analysis and their mode of action. Our genotoxic and DSBs results demonstrated mild genotoxic and statistically insignificant DNA damaging potential of ME and concentration-dependent well expressed genotoxic and DNA damaging potential of EO. A good relationship between increased DSBs levels and decreased survival might be related to one of the main constituents of EO, suspecting carvacrol. No mutagenic effect for ME and EO was found.

Conclusion: The results presented here could be seen as a good reason to clarify whether a new product for purposes of "green" technologies could be created based on oregano EO or its constituents.

Keywords: cell survival, mutations, DSBs, *Origanum vulgare* spp. *hirtum* methanolic extract, *Origanum vulgare* spp. *hirtum* essential oil, *Chlamydomonas reinhardtii*.

Acknowledgements: This research was funded by The Bulgarian National Science Fund, contract number 16/2 /11.12.2017

L01_05

Polar and nonpolar fraction from *Origanum vulgare* spp. *hirtum* methanolic extract - differences in their bioactivity on *Chlamydomonas reinhardtii* test system

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Aim: To compare the bioactivity of polar and nonpolar fractions of *Origanum vulgare* spp. *hirtum* methanolic extract on *Chlamydomonas reinhardtii*.

Material and methods: The polar and nonpolar fractions are derived from aerial parts of *Origanum vulgare* ssp. *hirtum*, collected during the flowering stage from the *ex situ* collection of IBER-BAS. *Chlamydomonas reinhardtii* 137C+ (WT) was used as a test system. Spot-test, cell survival, test of "visible" mutations and CFGE (for measurement of induced DNA double-strand breaks (DSBs)) were applied.

Results: The polar fraction did not show genotoxic, mutagenic as well as DNA-damaging effect. The situation with the nonpolar faction is quite different. Even at the lowest concentration of 250 ppm, cell survival was decreased by 60% (SF=0.41±0.08). Very strong genotoxic potential of all other concentrations nonpolar faction was found (100% lethality). A weak mutagenic effect was obtained for concentration of 250 ppm nonpolar fraction (MI=4.83±0.004). Well expressed dose – effect measured as statistically significant induced higher levels of DSBs compared to those in the control were calculated after the applying of all concentrations of nonpolar fraction.

Conclusions: The different bioactivity of the two fractions correlate well with their different chemical composition. The polar fraction, rich in sugars, organic acids and flavonoid glycosides, does not show genotoxic and mutagenic potential. The strong genotoxic potential of the nonpolar fraction might be related to carvacrol content (37.08%), which is not present in the composition of the polar fraction. To clarify our presumption further experiments should be done.

Keywords: bioactivity, polar and nonpolar fraction *Origanum vulgare* spp. *hirtum* methanolic extract, *Chlamydomonas reinhardtii*

Acknowledgements: This research was funded by The Bulgarian National Science Fund, contract number 16/2 /11.12.2017

L01_06

On the bioactivity of *Origanum vulgare* essential oil in *Myzus persicae* and potato plants

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Aim: To evaluate *Origanum vulgare* ssp. *hirtum* essential oil (EO) aphicidal activity on *Myzus persicae* and stress response of potato plants.

Material and Methods. A cohort of around 248 potato plants of the variety „Soraya"grown in March-June 2020 in the IBER-BAS experimental field was split into 3 groups: controls - negative Tween20 (0.1%) and positive - Nurelle D® (0.05%); plants uninfected and artificially infested with aphids and consequently treated with 1, 3 and 5µL/ml EO. The aphicidal activity expressed as corrected mortality % = (1- T/K)*100 (where: T – the number of the alive aphids after the treatment; K – the number of the alive aphids in the control) as well as stress response measured as MDA and H_2O_2 contents were evaluated at 2, 24, 72 and 120h post EO application. All results are statistically calculated.

Results: Different aphicidal activity of EO depending on the concentration was registered. Analyzing survival data it should be pointed out the well pronounced aphicidal capacity of both concentrations - 3µL/ml and 5µL/ml. No aphids mortality was identified applying 1µL/ml EO.

No effect of both concentrations 1 and 3µL/ml EO is observed concerning lipid peroxidation and H_2O_2 in uninfested and infested plants. Slight lipid peroxidation in uninfested plants was found only 72 and 120 hour post treatment with 5µL/ml EO.

Conclusion: Based on our preliminary data, *Origanum vulgare* EO at concentrations 3 and 5µL/ml possesses well-expressed aphicidal activity, which is not related to induction of significant stress in plants.

Keyword: *Origanum vulgare* ssp. *hirtum* essential oil, *Myzus persicae*, potato plants, MDA and H_2O_2

Acknowledgements: This study was funded by the project "Biocidal activity of extracts from bulgarian plants – screening and potato crop protection" - Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Grant ДН 16/2, 11.12.2017).

L01_07

Correlation between bacterial abundance, soil properties and heavy metal contamination in the area of non-ferrous metal processing plant, Southern Bulgaria

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Aim: This study aimed to assess the correlation between the bacterial abundance, soil physicochemical properties and heavy metals along the Zn, Pb and Cd contamination gradient, located in the area of non-ferrous processing plant "KCM-2000" in the vicinity of Plovdiv city, Southern Bulgaria.

Material and methods: The physicochemical properties of topsoil samples (0-20cm) were determined by standard protocols. The heavy metals concentration was measured by ELAN 5000 Inductively Coupled Plasma Mass Spectrometer (Perkin -Elmer, Shelton, CT, USA) and bioavailable forms of Zn, Pb, Cd was established after extraction with 0.01M $CaCl_2$. The bacterial abundance was assessed by colony forming unites (CFU) and real-time quantitative polymerase chain reaction (qPCR) with universal primers for 16S rRNA gene. Statistical analyses were performed to visualize the correlation between the bacterial abundance, soil properties and heavy metal content.

Main results: Our results showed that the cultivable heterotrophic bacteria and 16S rRNA gene copies decreased along the soil heavy metal contamination gradient. Statistical analyses showed a significant positive correlation (R2=0.83) between bacterial abundance (16S rRNA gene copies) and the heavy metal contamination in the samples. The soil texture was one of the key factors that affects the accumulation of heavy metals.

Conclusion: Soil bacterial abundance decreases along the heavy metal contamination gradient, but soil properties are the main factors that influence on it in long-term contaminated soils.

Keywords: bacterial abundance, 16S rRNA gene, heavy metals, soil properties

Acknowledgements: This study was financially supported by the National Research Fund of the Bulgarian Ministry of Education and Science (Grant-DN11/4-Dec.2017).

L01_08

Differences in bacterial functional profiles from loamy sand and clay loam textured soils under the impact of fungicide Quadris^{R}

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Aim: Fungicides are chemicals of high importance for crop production, but they may have deleterious effects on agroecosystems affecting negatively soil inhabitants. The study aimed to evaluate the effects of fungicide Quadris^{R} on the metabolic activity and functional profiling of soil bacteria – fungicide non-target organisms inhabiting loamy sand (LS) and clay loam (CL) soils.

Materials and methods: Soil mesocosms amended with increasing concentrations of Quadris^{R} (0 (Az0), 2.90 (Az1), 14.65 (Az2) and 35.00 (Az3) mg kg^{-1}) were used. Changes in soil abiotic and biotic properties were followed along four months after fungicide application. Bacterial metabolic activity and functional profiling were evaluated using the technique of community-level physiological profiling (CLPP) via Biolog EcoPlates^{TM} (Biolog Inc., Hayward, CA, USA).

Results: Quadris^{R} caused changes in soil abiotic and biotic properties, and the changes were related to the type of soil texture. The main soil abiotic metrics influenced by the fungicide application were soil pH and nitrate concentrations. The metabolic activity of soil bacteria was fungicide insignificantly stimulated at Az1 (LS and CL) and Az2 (LS) and significantly inhibited at Az3 (LS and CL). CLPPs shifted under Quadris^{R}, and the main contributors to the CLPPs' differences were the utilizations of amines (LS and CL), carbohydrates (LS) and amino acids (CL). The balanced utilization of Biolog carbon substances was significantly disturbed by Quadris^{R} in LS, but not in CL, and the rate of misbalance linearly related to the applied fungicide concentrations.

Conclusions: Quadris^{R} influenced the functioning of soil non-target bacteria for at least four months. The fungicide caused shifting both in the preference and the rate of carbon substances' use, and the shift was soil type dependent.

Keywords: fungicides, Quadris^{R}, soil texture, bacterial metabolism, Biolog EcoPlate^{TM}, CLPP

Acknowledgements: This work was supported by the National Research Fund of the Bulgarian Ministry of Education and Science (Grant number DN 11/6 - Dec, 2017).

L01_09

Screening of *Amorpha fruticosa* and *Ailanthus altissima* extracts for genotoxicity, mutagenicity, and carcinogenicity

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Aim of the study: To evaluate the potential genotoxic/antigenotoxic, mutagenic/antimutagenic, and carcinogenic/anticarcinogenic effect of *Amorpha fruticosa* (AF) fruit, *Ailanthus altissima* bark hexane (AAEH), and methanol (AAEM) extracts on a model system *Saccharomyces cerevisiae.*

Materials and methods: Plants were identified by Ekaterina Kozuharova and extracted following standard protocols. Three concentrations of each extract were tested - 10, 100, and 1000µg/ml. *In vitro* pro-oxidant/antioxidant activities were evaluated by DPPH and DNA topology assay. The potential genotoxic/antigenotoxic, mutagenic/antimutagenic, and carcinogenic/anticarcinogenic effects were revealed *in vivo* by: Zimmermman's test on *Saccharomyces cerevisiae* diploid strain D7ts1, and Ty1 retrotransposition test on *S. cerevisiae haploid* strain 551. Zeocin was used as a positive control.

Results: Based on the *in vitro* antioxidant activity the extracts could be arranged as follows: AF>AAEM>AAEH. AAEH possessed moderate oxidative potential. No genotoxic and mutagenic capacity was obtained *in vivo* for all the tested extracts. The total aberrants, convertants, and revertants were comparable with the control ones. None of the extracts was able to induce Ty1 retrotransposition. Further, all the extracts possessed well-expressed antigenotoxic, antimutagenic and anticarcinogenic activity. Significant reduction of the total aberrants, reverse point mutations and Ty1 retrotransposition was obtained. Only AF was able to reduce the levels of zeocin-induced mitotic gene conversion.

Conclusion: The three extracts did not possess genotoxic, mutagenic, and carcinogenic effects on *Saccharomyces cerevisiae*. Based on their protective activity, they can be arranged as follows: AF>AAEM>AAEH which corresponds well with their phytochemical composition. Further experiments could provide an in-depth analysis of the exact mode of action.

Acknowledgements: This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № Д01-363/17.12.2020).

L01_10

Cellular susceptibility and oxidative stress response to Menadione of logarithmic, quiescent and nonquiescent *Saccharomyces cerevisiae* cell populations

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Aim: To compare cellular susceptibility and oxidative stress response to Menadione of *S. cerevisiae* logarithmic (log), quiescent (Q) and non-quiescent (NQ) cell populations.

Materials and methods: Three main approaches were used: microbiological - cell survival, molecular – constant field gel electrophoresis for detection of DNA double-strand breaks, and biochemical – measurement of ROS levels, oxidized proteins, lipid peroxidation, glutathione, superoxide dismutase and catalase on *S. cerevisiae* haploid strain BY4741. The dose causing 20% (LD_{20}) and 50% (LD_{50}) lethality were calculated.

Results: The effect of Menadione as a well-known oxidative stress inducer is compared in log, Q and NQ cells. Data reveal that Q cells are the most susceptible to Menadione with LD_{50} corresponding to 32 µM Menadione. Dose-dependent DSB induction is measured only in quiescent cells. No effect on DSBs levels is observed in log and NQ cells. Further, the role of oxidative stress is clarified. Results show significantly higher levels of oxidized proteins, SOD and ROS in Q cells than in log cells after the treatment with 100µM Menadione. The malondialdehyde and catalase levels are comparable with these measured in log and NQ cells.

Conclusion: Our study provides evidence that *Saccharomyces cerevisiae* quiescent cells are the most susceptible to the Menadione action. It might be suggested that the DNA damaging and genotoxic action of Menadione in *Saccharomyces cerevisiae* quiescent cells could be related to ROS production.

Keywords: *Saccharomyces cerevisiae*, quiescence, Menadione, stress response

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

L01_11

Environmental impact assessment of treated wastewater discharge in Upper Iskar sub-catchment

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The upper Iskar sub-catchment is the water source with one of the most important economic and social significance in Bulgaria because of its role in drinking water supply of Sofia. The critical factors with potential high-risk levels for water quality in this hydrosystem are the discharge of Samokov Wastewater Treatment Plant (WWTP), diffuse pollution from agriculture and penetration of untreated sewage from the small villages.

Aim: to assess the effect of treated wastewater discharge on water quality, ecological state and self-purification processes in river sector of Samokov WWTP disposal.

Materials and Methods: The assessment was based on complex use of chemical, microbiological, enzymological indicators and biological quality elements.

Results: The concentration of organics, nutrients and macro-/microcomponents was determined and the results confirmed the expected increase for parameters associated with discharge of urban wastewater. The ecological state according macrozobenthos indicators was "good" in the whole river sector but the local deterioration was registered in a proximal location downstream of the WWTP outfall. The analysis of stream water and bed sediment microbial communities by fluorescent technique and enzymological indicators (total dehydrogenase activity and phosphatase activity index) showed the high metabolic activity and intensive transformation processes.

Conclusion: The importance of studied sub-catchment for the functioning of urban water cycle and quality of drinking water of Sofia enforces the extension of an existing monitoring program with more detail assessment of environment impact of wastewater discharge.

Keywords: treated wastewater discharge, Iskar River, microbial community, self-purification, pollution, WWTP

Acknowledgment: This work was financially supported by Project BG05M2OP001-1.002-0019 'Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy', financed by Operational Program 'Science and education for smart growth', co-financed by the European Union through the European structural and investment funds and by Project 80-10-84/25.03.2021 'Functional profile of sediment microbiome in discharge areas of Wastewater Treatment Plants in Iskar catchment', Scientific Research Fund of Sofia University

L01_12

Sexually-manifested variations in pigmentation of *Boeckella poppei* (Copepoda: Calanoida) from the Livingston Island (Maritime Antarctica)

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Antarctic environments are exposed to high levels of ultraviolet radiation (UVR) that are often detrimental to their biota. Recent studies suggest that the genus *Boeckella* (Copepoda: Calanoida) has high level of plasticity in terms of its reaction to UVR, which enables its wide distribution in various water bodies in Antarctica. *Boeckella poppei* is common in freshwater habitats of all three main biogeographic regions in Antarctica: sub-Antarctic islands, maritime and continental.

Aim: To report for the first time a specific pigmentation in populations of *B. poppei* from freshwater habitats in Maritime Antarctica.

Material and methods: Aquatic invertebrates were collected from ponds near the Bulgarian Antarctic Base (BAB) on the Livingston Island, Maritime Antarctica. We used a hand-held net with mesh size of 60 μm for collecting the specimens. They were brought alive to the laboratory of BAB, where were examined using a stereomicroscope.

Main results: The collected samples contained only one species of aquatic invertebrates, the calanoid *Boeckella poppei*. We recorded, for the first time, a typical orange pigmentation of the specimens. In non-ovigerous females and in males, we observed uniform distribution of pigments in the body, while these pigments were almost entirely concentrated in the ovisacs of mature females.

Conclusion: We could speculate this is a means of progeny protection from the teratogenic influence of the high level of UVR in Antarctic environments but further studies are needed to confirm this hypothesis. Unequivocally, such adaptation would facilitate the expansion of *B. poppei* on the continent through colonisation and survival in shallow freshwater habitats.

Keywords: Copepod pigmentation, ovigerous females, progeny photoprotection, Antarctica

Acknowledgments: This study was funded through the National Program for Polar Studies, Polar Research Funding – 2019, projects # 70.25-178/22.11.2019 and 70.25-177/22.11.2019. The authors are grateful to the National Center of Polar Research, Bulgarian Antarctic Institute and the staff of the 28th Bulgarian Antarctic Expedition for their logistic support.

L01_13

In vitro reconstitution of complexes of stress HliA protein with pigments

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Aims: Cyanobacteria are widespread practically in all ecological niches and provide a major part of primary biomass. Recently, much attention has been paid to cyanobacteria due to their widespread use in the production of biofuels and bioremediation. They adapt well to a variety of conditions, including extreme environments, using the synthesis of unique, poorly understood adaptive proteins and secondary metabolites for survival. Identification and characterization of such proteins are necessary both for studying the adaptation of cells to stress, and for increasing their resistance for practical use. In this paper, for the first time, the isolation and characterization of the recombinant stress high light inducing A (HliA) protein of cyanobacterium *Synechocystis* sp.is described.

Materials and Methods: recombinant HliA protein of *Synechocystis* sp. was isolated by Ni-chelate-chromatography and characterized by circular dichroism spectroscopy.

Results: A plasmid encoding the HliA with 6His gene at the C-terminus (HliA-6His) was obtained. The pure recombinant protein HliA *Synechocystis* sp was isolated by Ni-chelate chromatography. The HliA protein with chlorophyll *a* and carotenoids was reconstituted. Using circular dichroism spectroscopy, it was shown that chlorophyll a and carotenoids interact in vitro with the HliA protein.

Conclusions: The binding of pigments to the HliA protein indicates the protective role of this protein Apparently, Hli proteins are involved in the coordinated delivery of pigments in the biogenesis of photosynthetic complexes, reducing the risk of accumulation of phototoxic unbound chlorophyll molecules. The results are important for understanding photoprotection processes in both cyanobacteria and algae and higher plants.

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).

P01_01

Assessment of PAHs content in wedge clams (*Donax trunculus* Linnaeus, 1758) from the Bulgarian Black Sea Coast

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Aim: The aim of the study was to investigate the presence of polycyclic aromatic hydrocarbons (PAHs) in *D. trunculus*, collected from different localities along the Bulgarian Black Sea coast.

Material and methods: Wedge clam samples were obtained from clam catchers who collect them by manual dredging in sublittoral sandy localities. Concentrations of 13 PAHs in the sampled clam individuals were measured by gas chromatography system with mass spectrometry detection.

Main results: The wedge clams from different localities showed variations in their content of PAH compounds. Phenanthrene and fluorene were the most abundant compounds in all studied samples. The concentrations of benzo(a)pyrene (BaP) in wedge clams did not exceed the limit set in EC Regulation although it was detected in 20% of analyzed samples. The total PAHs content (sum of 13 PAHs compounds) varied in the range from 5.59 to 50.50 ng/g wet weight and was comparable with data from other European studies. The results showed that low molecular weight (LMW) PAHs (2 and 3 aromatic rings) were predominant accounting for 84% of total PAH levels, while high molecular weight (HMW) PAHs (4-, 5- and 6- rings) constituted 16% at average.

Conclusion: The ratio LMW/HMW PAHs higher than one, suggested presence of pollution predominantly of petrogenic origin. Principal component analysis (PCA) clearly separated Sozopol samples from those from Arkutino and Kranevo (PC axis 1) by the PAHs content. Along the second PC axis wedge clams from the southern localities were separated from the northern ones in their PAHs content.

Keywords: Bulgarian Black Sea, *Donax trunculus* L, polycyclic aromatic hydrocarbons

Acknowledgements: This work was supported by grant КП-06-Н31/6 of National Science Fund, Bulgaria.

P01_02

Heavy metal stress response of microalgal strains *Arthronema africanum* and *Coelastrella sp.* BGV

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The aim of the study was to compare the stress response of two microalgal strains - *Arthronema africanum* (Cyanoprokaryota) and *Coelastrella sp.* BGV (Chlorophyta), after heavy metals' treatment.

Material and Methods: In the course of the experiment, the changes of algal growth, pigment and protein content were analyzed, after adding 50 µM and 100µM Cu, Cd and Pb to the medium. The variations in the activity of superoxide dismutase and catalase, and the stress markers malondialdehyde and total peroxides were studied. The accumulation of heavy metals in the algal biomass was also determined.

Main Results: Cd and Pb significantly inhibited the growth and protein content of *Arthronema africanum* and *Coelastrella sp.* BGV, while the effect of Cu remained less pronounced. In both strains, a decrease of chlorophyll content was observed, while carotenoids markedly increased, especially in *Coelastrella sp.* BGV. The addition of 100µM Cu and 100µM Pb to the medium caused an enhancement of total peroxides' and malondialdehyde levels in *Coelastrella sp.* BGV. Those changes were accompanied by a strong increase in the activities of superoxide dismutase and catalase. The antioxidant enzymes of *Arthronema africanum* responded differently to the heavy metal stress - their activity was most strongly affected by the cadmium ions.

Conclusion: The strains that were studied showed a high absorption capacity for metal ions, especially for Cd, which was absorbed to a much greater extent than Pb and Cu. For that reason, we assumed that both microalgal strains and, in particular, *Coelastrella sp.* BGV, could be successfully used for treatment of contaminated waters.

Keywords: heavy metals, microalgae, *Arthronema africanum*, *Coelastrella sp.* BGV, pigments, superoxide dismutase, catalase

Acknowledgements: That study was supported by grant № 80-10-76/25.03.2021, Sofia University

P01_03

Plant-feeding nematode assemblages from oil-bearing rose and lavender plantations in the Rose Valley: effects of plant species and management type

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Bulgaria has a long-standing traditions in cultivation of aromatic and medicinal plants. The oil-bearing rose and lavender are the two major aromatic plants growing in the Rose Valley region. In the frame of the National Research Program *"Healthy Foods for a Strong Bio-Economy and Quality of Life"*, selected functional groups of soil nematodes are studied as bioindicators for assessing plant and soil health in rose and lavender perennial agrosystems.

Aim: To study the effects of plant species and management type on plan-feeding nematode assemblages from oil-bearing rose and lavender plantations grown by conventional versus organic farming systems.

Materials and methods: In total 20 rose and lavender fields located in the Rose Valley were studied. Ten plots from each plant crop (0.5 ha), managed by conventional and organic farming were selected and sampled in 2019-2020. From each site two multiple core soil samples consisted of 20 sub-samples taken around the plant roots and at a depth of up to 30 cm were collected. Nematodes were isolated from the soil by applying two standard methods, fixed in a 4% formalin/1% glycerol solution, dehydrated and mounted on permanent slides.

Main results: The overall generic richness of plant-feeding nematode assemblages was high (25 genera). The nematodes belonging to this group have diverse morphological and life-history traits and were assigned to 5 functional sub-groups: sedentary parasites (1), migratory endoparasites (3), semi-endoparasites (2), ectoparasites (12) and epidermal/root hair feeders (8). Among plant-parasitic nematode genera several important pests known to cause severe damages on crops were found (e.g. *Geocenamus, Meloidogyne, Pratylenchus, Pratylenchoides and Xiphinema).* No distinct nematode assemblages related to plant and management type were distinguished.

Conclusion: A comprehensive study of plant-feeding nematode assemblages is an important step for diagnostics of plant pests, assessment of *plant health status and* application of proper management practices.

Keywords: biosecurity, plant-parasitic nematodes, conventional agriculture, organic agriculture, *Rosa damascena*, *Lavandula officinalis*

Acknowledgments: The study is supported by the Bulgarian Ministry of Education and Science; National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" DCM # 577/17.08.2018" http://www.nnp-food.au-plovdiv.bg/

P01_04

State of antioxidant defense system in wedge clams from Bulgarian Black Sea as a measure of resistance to environmental impacts

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Aim: The study aimed to assess the changes of oxidative stress (OS) parameters in soft tissues of *Donax trunculus* (Linnaeus, 1758) sampled from different sites along Bulgarian Black Sea coast in response to environmental pressure.

Material and methods: The wedge clams were sampled from different localities by dredging with a fishing dredge operated by boat. The soft tissues of adult clams *D. trunculus* (23-35 mm) were homogenized in 100 mM K-PO_4 buffer, pH 7.4 and centrifuged for 10 min at 3000 g to obtain a post-nuclear fraction, where the glutathione (GSH) concentrations were measured. A part of this fraction was re-centrifuged for 20 min at 12 000 g and the supernatants were used for measurement activities of antioxidant enzymes.

Main results: Concentration of GSH and activities of antioxidant enzymes varied by seasons and among localities. Higher activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were registered in wedge clams collected in autumn compared to those collected in spring. In the autumn wedge clams showed higher activity of the major phase II detoxification enzyme glutathione-S-transferase (GST), which was observed at localities with intensive summer tourism such as Slunchev Bryag, Primorsko, Arkutino, suggesting an activation of the detoxification processes, presumably in response to negative environmental impact after the touristic summer season.

Conclusion: The presence of elevated enzyme activities suggested activation of the antioxidant protection system of the wedge clams in response to environmental pressure indicating their ability to cope with induced oxidative stress and thus maintain the good ecological state of the Black Sea infralittoral sandy habitats.

Keywords: antioxidant enzymes, Bulgarian Black Sea, *Donax trunculus*, glutathione

Acknowledgements: This work was supported by grant КП-06-Н31/6 of National Science Fund, Bulgaria.

P01_05

Ecology friendly high efficient extraction of industrial hemp

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The industrial hemp is the only plant that produces chemicals known as cannabinoids, but the most important for human biology are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). They themselves are odorless and the specific aroma of the plant is due to the essential oil, which consists of

monoterpenes and sesquiterpenes. Moreover, it can be a source of flavonoids and polyunsaturated fatty acids. For decades, hemp and hemp-derived products have been classified as a federally illegal substance, but in recent years they were removed from the restricted status and legalized them as an agricultural commodity.

The nature of the liquefied 1,1,1,2- tetrafluoroethane (R134a) and the treatment conditions (low pressure, easy operation, low energy and investment costs) make it a suitable extractant for the target substances, particularly for cannabinoids and terpenes. It is generally recognized as safe (GRAS) and consumer friendly.

Aim: To evaluate the efficiency of the cannabis subcritical extraction with respect to the yield and chemical composition of the product.

Materials and Methods: Dried inflorescences of five hemp cultivars were purchased from the local growers in the USA. The patented installation with 20L volume extractor and the food grade R134a as solvent were used. HPLC/UV was performed to quantify the cannabinoids (identified as THC, CBD, their acid forms THCA, CBDA and cannabinol) and GC/MS was used to determine the essential oil compounds.

Results: The efficiency of the extraction with respect to total cannabinoids ranged from 64% to 95% and of terpene compounds from 90% to 97%.

Conclusion: The extraction of industrial hemp with 1,1,1,2- tetrafluoroethane at subcritical conditions can be highly efficient method for producing cannabis extractives - both cannabinoids and terpenes.

Keywords: *Cannabis sativa* L., subcritical extraction, cannabinoids, terpenes

P01_06

Investigation on the impact of the environment on the microflora of the white mussel *Donax trunculus* from the region of Arkutin (Bulgarian Black Sea aquatory)

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Aim: *Donax trunculus*, the abrupt wedge shell or wedge clam, is a bivalve species in the family *Donacidae*. In many countries it is consumed by humans as in the last years the source of this mussel is the south Black sea aquatory.

Materials and methods: The samples for this study were collected from southern Black Sea Bulgarian coast in the period May 2020 to December 2020. The study of different types of microorganisms was performed by using the microbial identification system model: MicroLog M® BIO45101 BiologInc and software product GEN III.

Results: For the whole period we have isolated and determined the following types of microorganisms - *Enterococcus hirae* was isolated in May and August. The genus *Escherichia* was represented by the species *Escherichia vulneris*, which occurs in July, August and

September with the warming of the water and the increasing of the number of tourists. The species *Citrobacter farmeri* was discovered only in early September. The species *Acinetobacter gyllenbergii* and *A. johnsonii* were isolated only in October. The species *Pseudomonas viridilivida* and *P. alcaligenes* were isolated only in December.

Conclusion: Our results demonstarate an increase of the quantity of the coliforms in the region of Arkutino in July the quantity of the fecal coliforms is 190 times the norms prescribed in the Ordinance No. 4 from 20.10.2000 for the quality of fisheries water and the breeding of shellfish (the amount of fecal coliforms in the inter-shell content should be less than 300 NVB).

Keywords: *Donax trunculus*, Black Sea, microbial identification, pathogenic

Acknowledgements The contributors express their gratitude for the funding by fund scientific research Bulgaria programme grant H31/6 KP-06-H31/6/10.12.19, the project by Shumen University project 08-67/25.01.2021 Department of Biology.

P01_07

Determination of the bactericidal properties of antimicrobial substances isolated from lactic acid bacteria related to the Black Sea mussel *Mytilus galloprovincialis* Lam.

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Aim: The present study reports on the determination of the bactericidal properties of antimicrobial substances from lactic acid bacteria (ICD) isolated from the Black sea mussel *Mytilus galloprovincialis* Lam.

Materials and methods: The samples were collected in the period of August 2018 until March 2021. The BIOLOG system was used for microbiological determination.

Results: From the mussel *M. galloprovincialis* Lam. three species of ICD were isolated - *Sporolactobacillus kofuensis, L. sakei*, *Streptococcus gallolyticus ss gallolyticus* and *L. brevis*. The assay for antibacterial activity of the tested strains was performed by the diffusion method. The activity of the strains was determined against test cultures (*Escherichia coli* 3398, *Staphylococus aureus* 745, *Bacillus subtilis* 6633, *Salmonella typhimurium* 3591, *Listeria monocytogen* 863 *Enterobacter aerogenes* 3691, *Aspergillus niger, Penicillium claviforme, Saccharomyces cerevisae, Candida albicans 8673* and *Candida glabrata 72*). Before the analysis for antimicrobial activity, the ICD were cultured in media with different concentrations of sugars - 2, 5 and 10%. To monitor of the stability of the synthesized proteins/peptides, the neutralized supernatants of ICD strains were subject to heat treatment (10 minutes on a boiling water bath). In order to prove the protein nature of the active

substance, enzymatic hydrolysis of neutralized supernatants with proteinases - trypsin and proteinase K was performed. The results showed that 4 strains cultured on glucose and oligosaccharides completely lost their activity in all studied variants.

Conclusion: Therefore, some carbohydrates (glucose) and oligosaccharides induce the synthesis of biologically active molecules, which can probably be attributed to peptides/proteins.

Keywords: lactic acid bacteria, *Mytilus galloprovincialis* Lam, bacteriocins, Black Sea, antimicrobial activity

Acknowledgements The contributors express their gratitude for the funding by fund scientific research Bulgaria programme grant KP-06-H21/7/18 Dec 2018, the project by Shumen University project 08-67/25.01.2021 Department of Biology.

P01_08

Seasonal changes in the pro/antioxidant status of *Mytilus galloprovincialis* (Lamarck, 1819) from Bulgarian Black Sea coast

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The changes in pro/antioxidant status of marine organisms as a response to environmental pressure are being increasingly used in environmental assessment and monitoring, providing valuable information on ecological state of habitats and ecosystems.

Aim: to assess seasonal variations in pro/antioxidant status of mussels *M. galloprovincialis* sampled from different localities.

Materials and Methods: Mussels growing on mediolittoral rocks were gathered from 9 sites of the southern and 7 sites of the northern Bulgarian Black Sea coastal area in June and September. Lipid peroxidation (LPO), glutathione levels (GSH), activities of the antioxidant enzymes catalase, superoxide dismutase (SOD), glutathion peroxidase, glutathion reductase (GR) and glutathione-S-transferase (GST) were measured spectrophotometrically in tissue homogenates.

Results: Increased LPO, along with reduced concentration of GSH, was present in mussels, gathered in autumn, compared to those in spring from the northern sites, with exception of the mussel farms and Port Sozopol. Significantly increased GST activity was also measured in all autumn mussel samples compared to the spring samples, thus indicating activation of detoxification processes. These findings confirmed presence of increased oxidative stress in mussels from northern sites. Activities of SOD and GR were reduced in all autumn samples from the southern sites, in contrast to the northern samples. Fluctuations in antioxidant enzymes activity in mussels reflect responses to environmental pressure.

Conclusion: The observed decrease in antioxidant enzyme activity indicated that mussels may have difficulty overcoming oxidative stress induced by anthropogenic pressure on the marine environment which in turn may have negative consequences on coastal ecosystem health in the long term.

Keywords: antioxidant enzymes, Black Sea, *Mytilus galloprovincialis*, seasonal changes

Acknowledgements: This study was supported by grant КП-06-Н21/7 of National Science Fund, Bulgaria.

P01_09

Cell cultures as model systems for biorisk assessment

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Introduction: Cell cultures are widely used as model systems in biomedical research and biotechnology as well as in biorisk assessment.

Aim: The purpose of this study was to summarize the main characteristics, advantages and disadvantages of cell cultures used in investigations in the fields of molecular/cellular toxicology and ecotoxicology.

Materials and methods: The cytotoxicity/cytocompatibility of a wide range of agents such as metal [Zn, Cu, Co, Ni, Fe, Mn, Mg, etc] complexes with different ligands, drugs (antibiotics, antitumor drugs, non-steroidal anti-inflammatory agents, statins, disulfiram), alkaloids, nanoparticles, wound dressings, materials for bone implants, etc. has been evaluated. The experiments were performed using cytotoxicity assays, cytological/immunocytochemical, biochemical and molecular biological/genetic methods and techniques with different cell targets (molecules, organelles) and mechanisms of action and a broad spectrum of cell cultures (human and animal permanent cell lines and primary cell cultures, 2D and 3D cell cultures, tumor and non-tumor cell cultures).

Results: The obtained results outline the advantages and limitations of different types of cell cultures and their role in determining the cytotoxic/genotoxic effect of compounds and materials with different chemical composition, structure, chemical and physico-chemical properties.

Conclusion: Cell cultures are appropriate model systems for the needs of biomedicine, ecotoxicology and environmental safety studies. Their choice should be consistent with the purpose of the study, the type of the examined compounds/materials and the experimental design.

Keywords: cell cultures, cytotoxicity/genotoxicity, molecular/cellular toxicology, ecotoxicity, environmental safety

Acknowledgement: This study is supported by National Science Fund, Bulgarian Ministry of Education and Science (Grant № КП-06-КОСТ16/2020); Subsidy for PhD students from the Bulgarian Academy of Sciences; COST Action 18132; Bilateral project between Bulgarian Academy of Sciences and Romanian Academy, Bilateral project between Bulgarian Academy of Sciences and Lithuanian Academy of Sciences.

P01_10

Influence of proline and methyl jasmonate on *in vitro* seed germination and seedling development of *Chelidonium majus*

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Aims: The aim of the study was to evaluate the influence of proline and metyl jasmonate on the seed germination ability and seedling development of *Chelidonium majus* under different stress conditions.

Materials and methods: The seeds were sown on water agar supplemented with different types and concentrations of heavy metals (Pb^{2+}, Cd^{2+}, Zn^{2+}), NaCl and Polyethylen glycol 6000 (PEG 6000). They were previously immersed for 24 hours in distilled water (control), 30 mM proline or 1 mg/L metyl jasmonate (MJ).

Main results: Proline and MJ did not influence significantly the seed germination and seedling development on water agar supplemented with the HM and PEG 6000. However, both proline and MJ increased the percentage of germinated seeds when NaCl was applied in higher concentrations.

Conclusion: The influence of proline and MJ on seed germination and seedling development depends on the type of the stress factor applied.

Keywords: heavy metals, NaCl, PEG 6000, proline, methyl jasmonate

Acknowledgements: This research was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Project КП-06-М26/4 from 01.12.2018).

P01_11

Natural zeolites as detoxifiers and modifiers of the biological effects of lead and cadmium in small rodents: a review

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Aim: The aim of the present investigation is to analyze the literature about the toxicity of Cd and Pb in small rodent's organism and the role of natural zeolites as modifiers of the biological effects.

Material and methods: An array of ecotoxicological, morpho-physiological, hematological, genetic and biochemical methods as most representative are under discussion as a basic point for further exploration of biological effects in laboratory mice.

Results: The review of existing results demonstrated that there is abundant data on the sorption of lead and cadmium by modified natural zeolites in water and soils. Nevertheless, there is insufficient data on the ion exchange capacity and biological effects of this sorbent in living organisms, especially regarding Cd detoxification.

Conclusion: Based on the current review it is possible to conclude that the investigations in this field will elucidate the potential of the use of zeolites as successful detoxifiers against heavy metals and other toxic elements in living organisms.

Keywords: natural zeolites; small mammals; lead; cadmium.

Acknowledgements: This work was supported by the National Science Fund of the Republic of Bulgaria under project КП-06-Н44/3 "Crystal-chemical and structural characteristics of modified natural clinoptilolite and correlation between its sorption properties, ion exchange capacity for heavy metals and biological response *in vivo* and *in vitro*".

P01_12

Fish Fauna alterations affecting ecological status in a heavily modified Bulgarian River

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Vacha River is located in south Bulgaria and is the main right tributary of the Maritsa River. It is 104 km long and is formed by the confluence of the rivers Chairdere and Buynovska. It flows through three big dams: Tsankov Kamak, Vacha and Krichim, as well as Kadievo small hydropower plant (HPP) and other small weirs. Its artificially flooded area is about 40% of the river length, contributing serious habitat and temperature alterations; also hydropeaking and bio-corridor fragmentation. Other pressures are low as the catchment is mainly mountainous (belonging to national river types R3 and R5) without high urbanization rates.

Aims: The aim of the study was to establish the dam impact on the fish community in Vacha River during 2019.

Material and methods: For the purpose it was performed multi-habitat fish sampling according CEN 14011 and two fish indices calculated, according to Bulgarian Ordinance H4 – Trout River Index and Bulgarian Fish Index. Hydromorphological main features of the nine sampling sites were also registered.

Main results and conclusion: Unlike the relevant River Management Plan reporting good ecological status of the river and dams regarding biological parameters, we registered good condition for the undisturbed/controlling site only, as well as another one affected by the dams. In most cases Trout River Index indicates higher status than the Bulgarian Fish Index, because hydromorphological and hydrologic changes lead to a non-typical fish community with essential trout domination, especially closer to the estuary.

Keywords: WFD, Balkans, fish ecology, ecologic assessment, aquatic management, river fragmentation.

Acknowledgements: This study was supported by doctoral grant.

P01_13

Radiation status of soils from the region of the Eastern Rhodopes (Southern Bulgaria)

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Aims: Establishment of local reference values in the natural radiation background based on undisturbed soils and plants from unexplored regions of the Eastern Rhodopes. Assessment of the impact of increased anthropogenic activity as a potential factor for radiation pollution.

Material and methods: Sampling of virgin soils (0-5 cm layer) was performed to monitor the content of radionuclides in the surface soil layer and samples up to 20 cm layer to characterize the processes in depth.

The specific activity of natural *radionuclides* ^{238}U, ^{226}Ra, ^{232}Th, ^{40}K and technogenic ^{137}Cs in the studied samples was determined by gamma spectrometric analysis with Multichannel analyzer DSA 1000, production of CANBERRA and HPGe-detector. To study potential pollution, soil samples from areas affected by human activity in the liquidated lead-zinc mines "Madzharovo", the gold mine "Adatepe" Krumovgrad, *LZC* "Kardzhali", "Neochim" Dimitrovgrad, deposits for extraction of gneiss, marble quarries, etc. were analyzed to study possible pollution.

Main results: The results of studies of natural radionuclides in soil samples do not indicate significant difference from the data reported in the literature. ^{210}Pb was detected in three of the analyzed plant samples. ^{137}Cs activity was found in some of the soils, mostly as a result of the Chernobyl accident. No extra pollution was detected.

Conclusion: The establishment of the content of radionuclides in anthropogenically affected areas of the Eastern Rhodopes allows for an in-depth assessment of the radioecological situation and tracking the trends in its change. The obtained results do not show deviations from the average values for our latitudes, cited in the literature.

Due to the systematic use of unregulated drinking water sources, a recommendation is given for assessment of the radiation status of groundwater and surface water from the studied areas.

Keywords: *Natural and technogenic* radionuclides, radiation pollution, *virgin soils*, anthropogenic activity.

P01_14

Trace metal accumulation in tissues of wedge clams from sandy habitats of the Bulgarian Black Sea

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Aim: The present study aimed at assessing the content of trace metals in tissues of wedge clams (*Donax trunculus* Linnaeus, 1758) and the distribution of metal contamination of sandy habitats of Bulgarian Black Sea coastal reas.

Material and methods: Wedge clams were sampled by dredging from sublittoral sandy habitats at different regions along the Bulgarian Black Sea coastal area. Soft tissues of individual clams were digested with nitric acid followed by OES-ICP analytical determination.

Main results: The content of trace metals in the wedge clams varied among the studied regions. High metal content was present in wedge clams from several localities: Sveti Vlas, Shkorpilovtsi, Sunny Beach, Ahtopol and Kranevo. Highest values of Pb (2.51 mg/kg) and Cd (0.32 mg/kg) were found in samples from Sveti Vlas; of Cu (34.12 mg/kg), Fe (269.52 mg/kg) and Ni (0.32 mg/kg) - in wedge clams from Shkorpilovtsi. The maximum content of Cr (0.58 mg/kg) was present in samples from Sunny Beach, together with very high values of Fe. The highest content of Zn (18.04 mg/kg) and high values of Cr and Fe were measured in wedge clams from Irakli.

Conclusion: Accumulation of metals in marine organisms can cause oxidative stress and negative impact on their survival and reproduction. In addition to the role of *D. trunculus* in maintaining functionality of marine ecosystems, it became a commercially exploited shellfish in the Bulgarian Black Sea with increasing economic importance. In this context it is important that the values of Cu measured in the soft tissue of wedge clams collected for commercial use from Ahtopol and Shkorpilovtsi exceeded the limits of content (30 mg/kg) for food. The content of the other trace metals studied was within the acceptable limits for food sources for human consumption.

Keywords: Black Sea, *Donax trunculus*, trace metals

Acknowledgment: This study was supported by grant КП-06-Н31/6 of National Science Fund, Bulgaria.

P01_15

Quiescent yeast cells for assessing Zeocin toxicity

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Aim: Studying the mechanisms underlying adaptation to environmental changes is a major challenge for modern biology. However, the lack of in-depth knowledge on the toxicological effects of many harmful substances complicates risk assessment analyzes. Therefore, cell populations of logarithmic, quiescent (Q) and non-quiescent (NQ) cells of *S. cerevisiae* were used as a model to examine the cytotoxic effect of Zeocin.

Materials and methods: Q and NQ cells of *Saccharomyces cerevisiae* were isolated and levels of ROS, carbonylated proteins, lipid peroxidation and glutathione were analyzed after treatment with IC_{50}, IC_{100} and suprainhibitory doses of Zeocin.

Results: Zeocin is often used as antitumor drug and is known to generate oxidative changes in the cell. Studying its effect on the intracellular redox homeostasis showed that quiescent cells are less susceptible to it, showing minor intracellular damages – only 50% increase in ROS, oxidized proteins, and lipids. It was also revealed that the spontaneous DSBs depended on the growth phase with NQ cells being the most sensitive - 1.5-fold higher DSBs in comparison to logarithmic and Q cells. High Zeocin concentrations trigger the overexpression of glutathione in the cell as well.

Conclusion: The comparative analysis of different yeast cell populations – logarithmic, Q and NQ – revealed that the cellular physiological state is critical factor determining the resistance to environmental stress with Q cells being the most robust.

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

Key words: yeast, quiescence, Zeocin, stress response

P01_16

Acute and chronic assessment of pesticide toxicity on histochemical and biochemical changes in the liver of common carp (*Cyprinus carpio* 1758, Linnaeus)

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Aim: The main aim of the present study was to assess the short (96 h) and long (30 days) exposure of two commonly applied pesticides, both in agriculture and households – Chlorpyrifos (CPF) and Cypermethrin (CYP) on the common carp, which is a bioindicators fish species in aquatic toxicology.

Materials and Methods: We applied decreasing concentrations of CPF and CYP, based on the EU legislation in laboratory conditions. Therefore, histochemical (PAS reaction and SUDAN staining), as well as biochemical (ASAT, ALAT and LDH) biomarkers in the liver were investigated.

Results: Overall, we found sever alterations in the liver exposed to the tested pesticides and the lesions were more pronounced after 30 days of exposure.

Conclusions: In sum, both tested pesticides impacted the studied biomarkers in the liver of common carp, even at lower than the permissible concentrations set by law. However, the results of the analysis showed relatively higher toxicity of CYP compared to CPF. To avoid danger or risk, a cautious application of these pesticides must be carried out, especially near water bodies.

Keywords: fish, pesticides, contamination

Acknowledgements: The experiment is kindly financed by the Ministry of Education in Bulgaria (project KP-06-M26/3) and by the Department of Scientific Research – Plovdiv University (FP21-BF-008).

P01_17

Study of the microbial status of intestinal tract in different species of Teleost fish from the Black Sea

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The marine fish, like all marine animals, have characteristic interactions with the microorganisms with which they coexist in their environment. The symbiotic intestinal microbiota of fish plays a key role in their food supply, metabolic homeostasis and immune protection.

Aim: This paper presents a study on the microbial status of different fish species and their habitats in the Bulgarian Black Sea area.

Materials and methods: The samples were collected in the period of January 2021 until March 2021. The fish species we used in this study were Black Sea turbot (*Scophthalmus maximus*), strongil (*Neogobius melanostomus*), shore rockling (*Gaidropsarus mediterraneus*) and sardines (*Sardina pilchardus*). The BIOLOG system was used for microbiological determination.

Results and discussion: Strongil in the winter months lives at a depth of less than 60 m and is a predator. The turbot is a predator and is found at a depth of 80 m buried in the sand. The flounder inhabits thin bottoms at a depth of 50 m and feeds on crustaceans, worms and mollusks. The shore rockling feeds on "worms" and crustaceans. The anchovies feed on plankton. 3 species of microorganisms were isolated from the turbot - the species *Enterococcus villorum, Moraxella nonliquefaciens* and *Pseudomonas synxantha*. *Pseudomonas putida* was isolated from strongils, *Streptococcus entericus* from the shore rockling and *Pseudomonas fulva* from *S. pilchardus*.

Conclusion: This initial study showed that the fish gut microbiology may provide reliable information on the condition of the marine ecosystems.

Keywords: microbial status, marine fish, gastral tract

Acknowledgements The contributors express their gratitude for the funding by fund scientific research Bulgaria programme grant KP-06-PH41/2 28 Sep 2020, the project by Shumen University project 08-67/25.01.2021.Department of Biology.

P01_18

Comparative study on the oxidative stress of fish species of economic importance from localities with different ecological condition of the Bulgarian part of Black Sea

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Aim: The present study aims to assess the oxidative stress (OS) in demersal and pelagic fish species of economic importance inhabiting the Bulgarian part of the Black Sea. OS can affect fish growth and reproduction leading to negative ecological and economic consequences.

Material and Methods: The fish were caught during trawl selectivity experiments from localities with different ecological condition of the marine environment. The OS level in fish individuals was assessed by measuring lipid peroxidation (LPO), glutathione concentration (GSH), activities of superoxide dismutase (SOD) and catalase (CAT) in gills and liver.

Results: Goby and mullet caught in the Nessebar Bay showed clear signs of OS with the highest levels of LPO and the lowest GSH concentration in both liver and gills. On the contrary, goby caught near Maslen Nos (a region with good ecological conditions) were least affected by OS with low LPO and relatively high GSH concentrations and high SOD activity. There were no significant differences in the OS indicators in mullet and horse mackerel from the different localities. Sprat caught in Nessebar Bay showed some OS effects demonstrated by lower GSH levels in both liver and gills and relatively lower SOD activity compared to sprat caught from the other localities.

Conclusion: It can be concluded that the demersal fish (goby and mullet) were more strongly affected by OS induced by the marine environment than the pelagic fish (horse mackerel and sprat). Obviously, further studies are needed for the assessment of OS in fish of economic importance in the Bulgarian part of the Black Sea.

Key words: Black Sea, Bulgaria, demersal fish, oxidative stress, pelagic fish

Acknowledgements: This work was supported by grant КП-06-Н41/7 of National Science Fund, Bulgaria

THEMATIC SESSION II

ECOLOGICAL AGRICULTURE. ECOLOGICAL EDUCATION

PL02_01

Current trends in sustainable agriculture

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The impact of agriculture on the environment, human health, energy crises and climatic changes enforce policy-makers and farmers to rethink the recent model of agricultural production. The present report is considering the ways to promote a strong ecologisation of agriculture all over the world.

Designing and implementing such an agricultural model needs to change deeply the management of the farming systems, natural resources, food – chain and scientific approaches to meet environmental and societal demands. In this aspect the link and interrelationship between traditional, organic and new plant breeding technics including GMO and precision farming will be also considered.

L02_01

Pollinators of *Lavandula angustifolia* – an important factor for optimal production of lavender essential oil

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Lavender essential oil is vastly used in pharmacy, perfumery and food industries. It is one of the key essential oils in aromatherapy due to its valuable pharmacological effects. The producers of lavender essential oil are well aware of the fact that the highest quantity is obtained at the end of flowering and it correlates to the fruit set. Additionally, it was demonstrated that the quality is best at the end of the flowering with gradual increase of monoterpenes and decrease of sesquiterpenes during the flower ontogenesis.

Aim: The aim of this preliminary study was to test the ability of spontaneous self-pollination of *Lavandula angustifolia* L. (cultivar Sevtopolis) and to identify the pollen vectors.

Material and Methods: The field experiments were performed in a lavender plantation near Gorna Lipnitza village, North Bulgaria and the *ex situ* collection in the experimental plot of Botanical Garden of Sofia University.

Results: The enclosed test flowers revealed that spontaneous self-pollination does not occur. The pollinators were polylectic insects such as honeybees, several species of bumblebees, several species of butterflies. The wild pollinators predominated over the honeybees at both study sites.

Conclusion: Our observations showed that all pollinators collected actively nectar. The pollen baskets of most bees were full and this indicates that the adhered on the pollinators' body pollen was transferred in them.

Key words: *Lavandula angustifolia*, pollination, *Bombus, Apis mellifera*, Lepidoptera

L02_02

Organic vs conventional farming of oil-bearing rose: effect on yield and essential oil profile

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Aim: A field study was conducted to compare effects of organic (OF) and conventional (CF) farming on oil-bearing rose (*Rosa damascena* Mill. and *Rosa damascena* Mill. x *Rosa gallica* L.) essential oil yield and composition.

Material and Methods: For a two-year period (2019 – 2020) the private farms with conventional and organic production participated in the experiment. They are localized in Kazanlak valley, southern Bulgaria.

Results: Two annual studies showed that conventional farming superior to organic in oil production, average 30 % for damask and 17 % for the second genotype. GC/FID and GC/MS were performed and 24 major and character compounds were discussed. They presented from 79 % to 92 % of all oil content. The main terpene alcohols were geraniol (20.91 – 35.81 %), citronellol (4.66 – 21.00 %) and nerol (6.12 – 13.91 %), but different ratio was observed between them for the genotypes. The main hydrocarbons were distributed between nonadecane (10.06 – 15.08 %), heneicosane (2.71 – 10.14 %), nonadecene (1.11 – 5.99 %), tricosane (0.90 – 3.01 %) and heptadecane (0.98 – 2.69 %). For paraffins there were no difference in profiling.

Conclusion: According to our results obtained the agriculture system does not significantly affect the essential oil composition.

Keywords: *Rosa damascena* Mill., *Rosa gallica* L., oil composition, organic production

Acknowledgements
This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM № 577/17.08.2018.

P02_01

Plant products with acetylcholinesterase inhibitory activity for insect control

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Acetylcholinesterase (AChE) inhibitors are widely used in Alzheimer's treatment, but they are also crucial for the action of organophosphorus insecticides. The latter exert their toxicity by inhibiting the AChE enzyme in insects, leading to their death. Amaryllidaceae alkaloids have been proven to be potent AChE inhibitors.

Aim: In the present study species of Asteraceae, Lamiaceae, Brassicaceae and Amaryllidaceae were evaluated *in vitro* for AChE inhibitory activity.

Material and Methods: Methanolic extracts (12) and essential oils (4) were obtained from the target species. Ellman's colorimetric method, with modifications by López et al., 2002 was used for AChE activity evaluation.

Results: According to the activity level, the tested plant products were divided into three categories. First – plant products with strong activity comparable to that of galanthamine. Second – plant products with medium activity, with IC_{50} value about 1 mg/mL, and the last group with low activity, with IC_{50} value greater than 1 mg/mL. Essential oils of *Origanum vulgare* subsp. *hirtum* Ietswaart., *Satureja pilosa* Vel., *Monarda fistulosa* L., *Thymus longedentatus* (Degen & Urum.) Ronniger, and methanolic extract of *Leucojum aestivum* L. showed the most potent activity, and were referred to as the first group. Carvacrol was identified as the main component of the most active essential oils. In *L. aestivum* extract galanthamine was found as main alkaloid. The obtained results indicate that essential oils and alkaloid-rich plant extracts possess the strongest AChE inhibitory activity.

Conclusion: Our results give us a reason to recommend these plant products to be tested for insecticidal activity in the future.

Keywords: acetylcholinesterase, alkaloids, essential oils, carvacrol

Acknowledgements: This research was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Grant DN 16/2, 11.12.2017).

Reference article: López, S., Bastida, J., Viladomat, F., & Codina, C. (2002). Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and Narcissus extracts. Life Sciences, 71(21), 2521–2529. doi:10.1016/s0024-3205(02)02034-9

P02_02

Evaluation of viral infections levels in intensive and organic poultry farming

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Whereas early organic farming was mainly focused on plant production, in the last decade the number of organically managed poultry farms within the European Union increased tremendously. Similar to organic crop production, organic animal farming is based on the same principles: welfare-friendly, sustainable production, and resource utilization without or with very little adding synthetic substances like antibiotics and antiparasitic treatments. This and as well the access to wild animals makes the free-range poultry production systems predisposed to different viral diseases associated with potentially higher public health risks or reduction in production quality. On the other side, intensive farming amplifies the impact of viral diseases due to high density, low genetic diversity, and elevated immunodeficiency.

The aim of this analytical study is to compare free-range with intensive poultry systems and the occurrence of different viral diseases in these farms in the EU during the last decade.

Material and methods: The research is based on official data from the statistical office of the European Union, as well as official data from the member countries.

The results were similar in each country and demonstrate that free-range production has a higher incidence of viral diseases with high zoonotically potential.

Conclusion: This makes year-round surveillance absolutely necessary as well the necessity of implementation of additional criteria and requirements towards free-range systems.

Keywords: free-range poultry, intensive farming, meat production, farming systems, European Union

THEMATIC SESSION III
BIODIVERSITY. CONSERVATION BIOLOGY.

PL03_01

Nematodes of Extremes: Polar deserts

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Abstract

Nematodes are one of the most successful groups of organisms taking an important place both in terrestrial and aquatic systems due to their great taxonomic and functional diversity and high abundance. They possess several features (in terms of morphology, genetics, behaviour), perform different life strategies and have diversified life cycles. All this allows them to occupy various niches and to dwell in practically all type of habitats. These habitats include the extreme ones such as volcano mud, abyssal, ice, snow, thermal springs, deep underground waters, deserts. Polar (cold) deserts/barrens represent one of the extreme types of habitats on Earth – at the limits of Life. Based on literature sources and studies performed in IBER a short review of the nematode fauna and ecology in Arctic and Antarctic cold deserts is presented including the brief history of research in these regions. Specific adaptations developed by the nematodes for survival in the harsh polar conditions are outlined. Because of their simplicity, polar deserts served as an excellent model habitat - a natural laboratory to study and monitor the impact of the global change on the biotic communities.

Keywords: adaptations, biogeography, fauna, ecology

Acknowledgements: This study is supported by ANIDIV4 project

PL03_02

Recent review of vegetation diversity of Bulgaria

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Aim: The aim of the presented research is to reveal the recent knowledge of vegetation diversity on the territory of Bulgaria.

Materials and Methods: The existing diversity in the climatic, soil and geological conditions on the territory of the country determine a significant variety of vegetation types. During the last 10-15 years, after the fast development of phytocoenological databases, a lot of data about existing vegetation types in Bulgaria was digitized and made easily accessible. Nowadays, such data is predominantly stored in 1 national (Bulgarian National Database) and 2 regional databases (Balkan Vegetation Database, Balkan Dry Grassland Database). Alongside with this, the fast development of different numerical methods for analysis of vegetation data contributed to the more objective

differentiating of the existing vegetation units on European and national scales. The ArcGIS 10.1 software was used for analysis of vegetation data, distribution ranges and mapping.

Results: The vegetation diversity of Bulgaria is still not fully investigated. The established number of vegetation units depends on the investigation methods and the different concepts for their range and division (at least 39 classes, 67 orders, 94 alliances, 218 associations, 48 subassociations and 36 plant communities). Vegetation data was predominantly collected from semi-mountainous and mountainous regions of Bulgaria and from protected areas and NATURA 2000 network. On the other hand, some vegetation types are poorly studied in lowlands and agricultural areas. The best researched habitat types are grasslands, broad leaved forests and wetlands. Yet available data and knowledge about ruderal, shrubland, fringe and chasmophytic vegetation in the country are insufficient.

Conclusion: The vegetation diversity of Bulgaria is characterized by a great diversity. Its investigation needs to continue and to cover all existing vegetation types systematically on the whole territory of the country.

Keywords: Braun-Blanquet approach, syntaxonomy, vegetation units

PL03_03

Cork oak landscape: a sustainable multiuse resource

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Aims: The cork oak (*Quercus suber*) is a tree belonging to the Fagaceae family mostly found in Southern Europe and North Africa. One of its main characteristics is the production of cork, which covers its trunk and branches and regenerates after removal.

In Portugal cork oak covers 736 775 ha which represents 34% of the world production and 23% of the forest area in the country.

We herein describe the work that has been performed during the past two decades ranging from genetic diversity analysis using microsatellite markers, transcriptome analysis, set up of an F1 generation using controlled pollination and whole genome sequencing with the aim of understanding the genetics of cork oak and identifying genes of interest for high quality cork production.

Material and methods: Genetic studies were based on nucleic acids (both DNA and RNA) extracted from leaves and other tissues of *Q. suber*, analysed using PCR and RT-PCR, NGS and bioinformatics tools. F1 was prepared using frozen pollen collected from selected trees used to pollinate mature female flowers. Mature acorns were removed and seeded in small vases in green-house and 1 m high trees were planted in the soil.

Results: An F1 generation was established in the field which will enable future genotype/Phenotype comparisons. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank (Data Citation 1). Raw read files are available at NCBI Sequence Read Archive (Data Citation 2). A comprehensive assessment of the transcriptome of cork oak (*Quercus suber*) through EST sequencing has been achieved.

Keywords: Cork oak, *Quercus suber*, genome analysis

L03_01

Monitoring pollinator visits to the medicinal plant *Gentiana asclepiadea* with three decades distance

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Constantly piling data point that bumblebees decline. The key factors causing declines in their abundance and diversity are: 1) habitat destruction; 2) loss of floral resources; 3) emerging diseases; 4) increased use of pesticides (particularly neonicotinoids).

Aim: to monitor the bumblebee visits to *Gentiana asclepiadea* as the habitat of this mountain plant species is supposed to be less exposed to the hazards.

Materials and methods: Three study sites were chosen in Mt. Vitosha (SW Bulgaria) where grow natural populations of *G. asclepiadea*. The observations of bumblebee's activity in the flowers of *G. asclepiadea* were conducted during the flowering seasons (August and September) of 1990 - 1994, and later on in 2017 – 2020 in the same study sites. The free pollination fruit set was tested by monitoring 100 *G. asclepiadea* flowers each year for development of fruit capsules. The seed set was tested by counting the matured seeds and non-fertilised ovules of 10 fruit capsules each year.

Results: A slight decline in bumblebees' activity was recorded in 2017 – 2020 in comparison to 1990-1994. This reflected on the fruit set and the seed set.

Conclusion: Our data demonstrate that even in a mountain whit fewer direct hazards for bumblebees the negative effect is still detectable. This corresponds to the research study which provides evidence that insect biomass fell by 76% on German nature reserves between 1989 and 2016 (Hallmann et al. 2017).

Keywords: Bombus, bumblebees decline, pollinators, monitoring

L03_02

Bioconservatory status of some critically endangered orchids in Bulgaria – update

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Aim: Orchids have rather peculiar biology and some of them have relatively narrow environmental requirements which put them among the most threatened and endangered vascular plants in Bulgaria. On the territory of Bulgaria are found 9 species that fulfill the IUCN criteria for critically endangered and 2 for regionally extinct and are included also in the Red Data Book and Biodiversity Act. The present study aimed to explore and evaluate the bioconservatory status of the populations of *Cypripedium calceolus*, *Traunsteinera globosa*, *Orchis spitzelii* and *Epipactis greuteri*.

Materials and methods: The populations of the mentioned critically endangered orchids in Bulgaria were explored and evaluated. The shoots were counted and GPS coordinates were recorded for mapping of their distribution.

Results: Among the current 2 species with regionally extinct status there should be added two more namely *Epipactis geuteri* and *Orchis spitzelii*.For the other critically endangered species *Cypripedium calceolus* and *Traunsteinera globosa* were discovered few new locations.

Conclusion: All of the known locations of these species have rather low number of shoots - less than 50 shoots per place. All these facts show that there is a high stress on the perspectives of the populations' survival.

Keywords: Critically endangered, *Epipactis greuteri*, *Orchis spitzelii*, *Cypripedium calceolus*, *Traunsteinera globosa*, Bioconservation.

L03_03

Mechanism of copulation of *Tortrix viridana* L. (Lepidoptera: Tortricidae): possible relation to sexual selection

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Aims: The fine mechanisms of copulation and functional morphology of the internal copulatory organs in Lepidoptera are poorly studied. Since the internal genitalia of both sexes are in tight contact, their role in sexual selection seems obvious but its mechanism is only tentative. The present study is focused on the internal genitalia of *T. viridana*, a species with relatively simple copulatory structures. The study aims to reveal the mechanisms of copulation and functional morphology of the internal copulatory organs of a model Lepidoptera species and their role in sexual selection.

Material and methods: Various techniques were used in the current study, such as: micro-CT scanning and histology of couples fixed at different stages of copulation ("serial morphology"), SEM, video recording of males poisoned with dichlorvos.

Main results: The preliminary results reveal a relatively long copulation (90 min) that can be divided into certain phases, in which different substances of the ejaculate are ejected and the endophallus achieves different stages of eversion. During the second half of the copulation most of the ejaculate is already transferred to the female bursa, the endophallus is maximally extended and the sclerotised cornuti on its tip perform pulsating movements in a certain area of the ductus bursae of the female, the insertion of ductus seminalis.

Conclusion: It can be assumed that the endophallus with its cornuti is involved in mechanical stimulation of the female, but neither spatially corresponding structure, nor sensory organs were detected in the counterparts of the females.

Keywords: copulation, sexual selection, functional morphology, Lepidoptera

Acknowledgements: This study was supported by National Science Fund of Bulgaria, grant No. КП-06-Н31/4–10.12.2019 “Sexual selection in moths: copulatory mechanisms and functional morphology of the copulatory organs (Insecta: Lepidoptera)”.

L03_04

Species composition and some genetic and population characteristics of the fish fauna in the shallow shelf zone of the South Bay (Livingston Island, Antarctica)

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The aim of the study is to present data on species composition and some population characteristics of the fish fauna in the understudied Antarctic coastal zone (5–25 m) of the Livingston Island.

Material and methods: A total of 165 fish belonging to 6 species were caught near to the Bulgarian Antarctic base in January and February 2020. Species determination was conducted by using combination of both morphological and molecular approaches.

Main results: The greatest species diversity was found in the deep areas (over 15 m depth) where a total of 5 out of 6 species were recorded. The shallower areas were dominated by predatory fish whereas all smaller species were found at bigger depths where they avoid competition and direct threat of large predators.

The exponent *b* values of the length-weight relationship in both species *Notothenia rossii* and *N. coriiceps* were not statistically different from 3, indicating isometric growth. The average values of the Fulton’s, Modified and Relative condition factor in *N. rossii* were 1.32, 1.07 and 1.65, respectively, while the same values in *N. coriiceps* were 1.50, 2.28 and 2.10, respectively, which indicated very good general condition of the fish.

Overall, ten dietary classes were recorded in *N. rossii* and five in *N. coriiceps*, although only two of them (krill and amphipods) formed a total of 84.1% and 79.3% of the species diet, respectively. Both dominating classes formed 54.1% and 30.0% of the food biomass, respectively.

Conclusion: A total of 6 fish species were found in the shallow coastal zone of the South Bay, Livingston Island. The fish fauna was dominated by the predatory species *N. rossii* and *N. coriiceps*. In both species an isometric growth as well as very good general condition was found. Their diet was dominated by krill and amphipods.

Keywords: species composition, length-weight relationship, condition factor, diet, Notothenioidei, Livingston Island

Acknowledgements: The study was supported by Project No. 70-25-172/22.11.2019, funded by the Polar Research Fund and managed by the National Museum of Natural History, Sofia.

P03_01

Comparative determination of antimicrobial activity of the Balkan endemic species *Stachys thracica* Davidov during the process of *ex situ* conservation

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Aim: The aim of the present work is *ex situ* conservation of the endemic species *Stachys thracica* Davidov *(Thracian woundwort)* and comparative study of antimicrobial activity of extracts isolated from *in situ* wild, *in vitro* cultivated and *ex vitro* adapted plants.

Material and methods: *In vitro* shoot culture of *S. thracica* was induced by sterilization of ripe dried seeds with ethanol. *Ex vitro* adaptation was successfully accomplished in phytotron chamber, greenhouse and experimental field. The antimicrobial activity of the methanolic extracts obtained from *in situ*, *in vitro* cultivated and *ex vitro* adapted plants from the three species was tested against three gram-positive bacteria *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis*, seven gram-negative bacteria *Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae, Acinetobacter calcoaceticus, Enterobacter cloacae, Escherichia coli* and the yeast *Candida albicans* by agar disk diffusion method and broth microdilution assay.

Results: *In vitro* shoot culture from *S. thracica* was successfully induced and maintained on hormone-free MS media. *Ex vitro* adaptation was successfully accomplished in phytotron chamber, greenhouse and experimental field with 100 % survival. All three extracts were most active against *Acinetobacter calcoaceticus* with MBC values 8 mg/ml for *in situ* plants and 4 mg/ml for *in vitro* and *ex vitro* adapted plants.

Conclusions: The methanolic extracts from *Stachys thracica* show moderate activity against gram-negative bacteria. A collection of *in vitro* cultivated and *ex vitro* adapted plants was established which is an alternative approach for the preservation of this endemic species.

Keywords: Thracian woundwort, *ex situ*, antimicrobial activity

P03_02

Influence of some environmental factors on the distribution of zooplankton complexes in Mandra Reservoir, in Southeastern Bulgaria.

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Aim: The aim of the present study was to trace the influence of some environmental factors on the distribution of zooplankton communities in the system Reservoir Mandra and the ecotone zones formed at the confluence of rivers Fakiyska, Sredetska, Izvorska and Rusokastrenska.

Materials and Methods: The study was conducted in the period February 2020 - January 2021. Four samplings were performed at seven sites. A total of 48 zooplankton samples were collected by using an Apstein plankton net 55 µm mesh size and via filtering of 100 dm^3 of water through the net and were fixed in 4% formalin. After determining the species composition and abundance, the results were subjected to structural analysis and Canonical correspondence analysis (CCA).

Results: A total of 67 taxa were identified, with about 48% of the Rotifera group, 27% of Cladocera and 19% of the Copepoda and only 6 % from Protozoa. The Shannon -Weaver index for individual species diversity was between 2.37 and 0.62. The positive and negative correlation of zooplankton distribution in CCA shows that the relative abundance of any species depends on specific environmental variables.

Conclusion: Analysis showed that temperature and wind had the strongest impact on the distribution of zooplankton.

Keywords: zooplankton, Mandra Reservoir, structural analysis, CCA

P03_03

New data about the distribution of the alien *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta: Naididae) in Bulgaria

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Aim: The study reports about new localities of non-indigenous tubificid species *Branchiura sowerbyi* Beddard, 1892, registered after 2011 in water bodies in Bulgaria.

Materials and methods: Within a large-scale study on the macrozoobenthos, numerous standing and lotic water bodies in Bulgaria were visited and sampled in the spring and summer of 2020. The 205 samples were taken following the multi-habitat sampling and ISO-standards. Basic

physical and chemical water parameters were measured *in situ*. An analysis of the distribution of the species in relation to some environmental factors is also presented.

Results: The current survey showed that *B. sowerbyi* was identified in samples from 15 lentic and 4 lotic water bodies of 168 analyzed oligochaete samples. New localities were registered – 5 in standing and 2 in running waters. Also, the present data on distribution of *Br. sowerbyi* were associated with those of the previous 8 years period (2012-2019) when the species were found in 96 localities. Thus, for the entire 9-year period, a total of 115 localities of *Br. sowerbyi* were identified.

Conclusions: For the last 55 years *B. sowerbyi* has demonstrated a spreading presence upstream of the Danube River's tributaries from the mouths to upper parts, thus the river is likely a vector for species invasion. Note that the species shows also a lasting trend of occupying new biotopes at higher altitudes. To assess the degree of diffusion and invasion of this alien species, the need for further periodic research on the *Branchiura sowerbyi* distribution on the territory of the entire Balkan Peninsula is outlined.

Keywords: *Branchiura sowerbyi*, alien species, aquatic oligochets, Danube River, inland water bodies, Bulgaria

P03_04

Pteromalid fauna (Hymenoptera: Pteromalidae) in rapeseed (*Brassica napus* L.) fields and surrounding grasslands in Bulgaria – composition and perspectives for biological control

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Parasitoid wasps belonging to the family Pteromalidae are widespread and abundant members of the insect communities in the temperate regions of the World. As many other chalcids do, pteromalids serve as natural enemies of the pests in various crop fields and have importance for biological control of these harmful insects. Here we present the results of a field study in Bulgaria which was focused on the diversity of the Pteromalidae family in ten rapeseed fields and their adjacent grasslands. All samples were collected in equal numbers by sweep netting on the border line or inside the crops as well as along transects through the grass habitats. A total of 101 and 68 individuals were collected from the rapeseeds and grasslands, respectively. The most abundant genus in the crop fields was *Mesopolobus* - 67% of the sampled pteromalids. It was also the most numerous in the semi-natural grass habitats - 31%, followed by *Pteromalus* (16%) and *Systasis* (10%). The most abundant species in the rapeseeds was *Mesopolobus morys* - well known key parasitoid of the cabbage seed weevil, *Ceutorhynchus obstrictus*, in Europe. In the present work we discuss the overall species composition of Pteromalidae obtained from the sampled areas and present our point of view on the perspectives for biological control by keeping the grasslands as natural sources for parasitoids.

Keywords: agriculture, biological control, parasitoids, rape fields, grasslands.

Acknowledgements. The present study was carried out thanks to the Project BiodivERsA-FACCE2014-47 "SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources (STACCATO)".

P03_05

Spore morphology of the *Aneura pinguis* (Marchantiophyta) species complex

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The aim of this study was to reveal variation in spore morphology within the liverwort species complex *Aneura pinguis*.

Material and methods: *Aneura pinguis* is a paraphyletic taxon, composed of several cryptic species. The exact number of species within the complex remains unknown. They are distinguished solely on molecular basis. Samples of *A. pinguis* s.l. were collected in 2018-2021 from various locations and habitats in Bulgaria. Sporophytes were collected and spores were isolated for the purposes of the present study. Spore morphology was observed via scanning electron microscope (SEM).

Results: In result of the analysis variation in the spore ornamentation was observed. The surface ornamentation is of tuberculate type and differences in tubercle size, density and arrangement were present. Two major sub-types of ornamentation were observed.

Conclusions: Our study indicates variation within the *A. pinguis* species complex. The two spore morphotypes correspond to different habitat types but could not be assigned to morphologically distinguishable units. The systematic implications of spore ornamentation within the complex remain to be clarified when data on DNA variation is obtained.

Keywords: *Aneura pinguis*, species complex, cryptic species, spore morphology, SEM

Acknowledgements: This work was held within the project "Cryptic species in Bulgarian flora - molecular species delimitation in the *Aneura pinguis* complex" funded by Bulgarian National Science Fund.

P03_06

Distribution of predatory nematodes (order Mononchida) associated with riparian vegetation in Bulgaria

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Riparian zones are characterized by high and specific biodiversity. Riverbanks play a significant role of bio corridors, which support the existence and dispersal of many animal and plant species. Order Mononchida are exclusively predatory nematodes inhabiting both terrestrial and aquatic habitats.

Aim: To study the distribution patterns of mononchids in different riparian vegetation in Bulgaria**.**

Materials and methods: Multiple core soil samples (3 per site) were collected from each site (15×15 m sampling plot or along the riverbank) around the roots of the dominant tree and from litter. Nematodes were isolated from 200 g of soil (by decanting and sieving method) and from 10 g of litter (Baermann funnel method), fixed, dehydrated and mounted on permanent slides.

Main results: Sixty-seven samples have been collected from 46 localities (32 rivers) with various vegetation types. The most intensively sampled trees among all 14 plant genera studied were *Fraxinus* (17 samples), *Salix* (15), *Alnus glutinosa* (14) and *Ulmus* (4). More than 90% of all soil and litter samples contained at least one mononchid genus. Seven genera: *Prionchulus*, *Clarkus*, *Coomansus*, *Mononchus* (fam. Mononchidae), *Mylonchulus* (fam. Mylonchulidae), *Miconchus* and *Anatonchus* (fam. Anatonchidae) were recovered. Genus *Mylonchulus* was the most widespread (67 % of all samples) in soil while *Prionchulus* spp. prevailed in litter, occurring in almost half of the collected samples. *Clarkus* was the third common genus, occurring mainly in soil. The highest richness of mononchids (6 genera each) was registered in the rhizosphere of *Fraxinus*, *Salix* and *Ulmus*.

Conclusions: The study revealed that mononchids associated with riparian vegetation, are widespread and form different assemblages in soil and litter.

Keywords: Anatonchidae, litter, soil, Mononchidae, Mylonchulidae, floodplain forests

Acknowledgements: This study is part of the PhD thesis of Stela Dipchikova.

P03_07

Effects of land use type on ground beetle (Coleoptera: Carabidae) assemblages in South-Central Bulgaria

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The expansion of cropland areas and their intensive use are the primary drivers of habitat loss and biodiversity decline. Ground beetles (Coleoptera: Carabidae) are widely used as bioindicators in ecological studies due to their great diversity, wide distribution and presence in various types of habitats.

Aim: In the framework of STACCATO project, the effect of landscape structure on carabid composition, diversity and assemblages was studied in rapeseed fields and adjoining semi-natural grasslands**.**

Materials and methods: Ten sites located in South-Central Bulgaria with various proportions of grasslands and oilseed rape areas were sampled in April – August 2018. Pitfall traps (5 in each site) were set. Statistical analyses were performed by PRIMER7 and PERMANOVA+.

Main results: A total of 6451 specimens were collected. Carabids of the oilseed rape fields had higher overall abundance and number of species and genera (5018 specimens, 109 species and 40 genera), as compared to grasslands (1433, 87 and 33, respectively). The most abundant species in fields were *Harpalus distinguendus* and *Calosoma auropunctatum*. The most abundant in the pastures were *Harpalus flavicornis* and *Microlestes fissuralis*. Distinct assemblages related to habitat type were

most unambiguous during the flowering and ripening. Habitat type, sampling period, and the location are major factors affecting carabid assemblages.

Conclusions: Two carabid assemblages related to land use type were distinguished during the flowering and ripening stage. Due to various preconditions the oilseed rape attracts both grani- and herbivorous species, in some cases in extremely high abundances, while in the pastures carabids are less numerous.

Keywords: ecology, rapeseed, semi-natural grasslands, landscape heterogeneity, ecosystem services, Carabidae

Acknowledgements: The present study was carried out with the financial support of the project STACCATO "SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources" (BiodivERsA-FACCE2014-47).

P03_08

Plant parasitic nematodes from rapeseed fields in South-Central Bulgaria

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Rapeseed is an annual winter crop grown mainly for industrial oil, widely used in crop rotation with wheat, sunflower and maize. Although it is often used as an intermediate crop to suppress some parasitic nematodes, there are number of nematode genera that can be a threat for the yield.

Aim: To study the occurrence and distribution of plant parasitic nematodes (PPNs) in selected rapeseed fields (*Brassia napa* L.) in Plovdiv and Haskovo provinces.

Material and Methods: In total, sixty multiple core samples (each of 10 sub-samples) were collected in 2018 twice – during the flowering (May) and ripening (June) stage of rape at 3 distances from the field margin (10, 60 and 110 m) in 10 fields. Nematodes were isolated from 25 g of soil by the Baermann funnel method, processed and mounted on permanent slides.

Results: Twelve genera were recovered from all sites, the number of genera varying between 4 and 7 per site. Based on type of feeding they belonged to three sub-groups, ectoparasites being the most diverse (9 genera), migratory endoparasites and semi-endoparasites represented by two and one genus, respectively. *Pratylenchus* spp. that are migratory endoparasites feed ectoparasitically on rapeseed roots. The genus *Pratylenchus* occurred in all sites, followed by *Geocenamus* and *Paratylenchus* (9) and *Pratylenchoides* (7). The genus *Bitylenchus* containing economically important species was recorded in two plots. *Geocenamus,* and *Pratylenchus* were also the most abundant genera reaching up to 170 and 146 specimens/25 g of soil, respectively.

Conclusion: PPNs were widespread in studied rapeseed fields. The detected parasitic genera (*Pratylenchus*, *Geocenamus*) which were common in studied fields and present in high numbers could have negative impact on rapeseed.

Keywords: agriculture, distribution, ectoparasitic nematodes

Acknowledgements: The present study was supported by the Project BiodivERsA-FACCE2014-47 "SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources (STACCATO)"

THEMATIC SESSION IV
ECOSYSTEM RESEARCH AND SERVICES. LANDSCAPE ECOLOGY

PL04_01

The ecosystem mirage: is it possible for us to become ecosystem people again

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Abstract

For a long historic period humans evolved and lived as "ecosystem" people who were directly and critically dependent for survival on their surrounding ecosystems which provided them with food, shelter and resources. With the development of technology however, people became less and less dependent on their surrounding ecosystems and began to utilize resources from all over the world, thus becoming more and more "biosphere" people. The price of this transition is the increasing pressure on nature, pollution, biodiversity loss and ecosystem degradation. Ecosystems are the fundamental functional units of the planet which provide essential ecosystem services. That is why we do have the responsibility to study, understand and manage reasonably the ecosystems on Our Planet. Some of the main goals of Ecosystem Management are to protect essential ecological processes such as nutrient cycles, succession, hydrologic processes, etc., to maintain viable populations of native species *in situ*, to manage over long enough time periods in order to sustain the evolutional potential of species and ecosystems. The main task is to integrate ecological, economic, and social goals into a unified management approach. This is an integrated New Science. A logical question arises: are we in Bulgaria ready for this challenge? It seems we are not. Our science is lagging behind both in modern ecosystem research, building capacity and competence, which is also followed by education. Goal oriented reorganization of science and education seems to be urgently needed.

Keywords: ecology science, ecosystem management, ecological education

L04_01

Grassland habitats of Godech Municipality

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The investigated territory is located in the Western parts of Bulgaria.

Aim: The aim of the presented research is to reveal the diversity of grassland habitats in Godech Municipality.

Materials and Methods: Following the Braun-Blanquet approach, 418 relevés were collected during the 2019-2020 field seasons and 3422 field points were verified as well. The latter represent terrain samplings that prove or disprove preliminary habitat data.

Results: The following habitat types (10 in total): 6110, 6210, 6230, 6240, 62A0, 62D0, 6410, 6430, 6510 and 6520 are included in Directive 92/43/EEC.

Conclusion: The successful results may be used as a basis for a territorial expansion of the study in the neighboring municipalities.

Keywords: vegetation, NATURA 2000, GIS

Acknowledgements: This investigation was carried out with the financial help of the NSP "Young scientists and postdoctoral students, 2020", contract № 22-603/09.03.2020 and the National Science Fund (Contract ДКОСТ 01/7/19.10.2018).

6110 – 0.08 km²; 6210 – 80.99 km²; 6230 – 9.53 km²; 6240 – 0.55 km²; 62A0 – 8.62 km²; 62D0 – 1.46 km²; 6410 – 0.37 km²; 6430 – 1.37 km²; 6510 – 15.53 km²; 6520 – 0.43 km²

L04_02

Erosion regulation capacity of different habitats in Vitosha Mountain

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The area of interest located in the Western Srednogorie Region of Bulgaria and is entirely covered by the Vitosha (BG 0000113) site of the ecological network NATURA 2000.

Aim: The aim of the presented research is to perform an assessment of erosion regulation capacity of different habitats and mosaics in Vitosha Mountain. The higher the density in a habitat or a mosaic of habitats, the higher the potential of channelizing overland flow is. Erosion regulation capacity is expected to be lower in areas with dense drainage network.

Materials and Methods: Habitat data is provided by the Ministry of Environment and Water of Bulgaria. Habitats within the mountain extent are covering a total area of 160.5 km². Drainage network is extracted from digital elevation model using GIS software. Values of drainage density per square kilometer within each habitat are calculated.

Results: The spatial pattern of drainage network and its density are presented and discussed. High resolution imagery is used for the visual interpretation of the derived maps. The area of each land habitat is calculated both in absolute in relative units (% of the whole study area). An attempt is made to classify erosion regulation capacity of each habitat with total area exceeding 1 km^2.

Conclusion: Presented results show relevant information for habitat diversity and erosion regulation management. Derived maps could be used in further evaluation of regulating ecosystem services.

Keywords: ecosystem services, drainage network, Vitosha Mountain, habitats

L04_03

Pre-monitoring geochemical research of the river sediments in the area of Ada Tepe gold mining site (Eastern Rhodopes)

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Aims: This work depicts the geochemical properties of the landscapes in the area of the Ada Tepe gold mine before its launching. The research is conducted by examination of heavy metals' (Cu, Pb, Zn, Co, Cr, Mn, Ni) content in samples of river sediments in the local landscapes. The research aims to analyze the concentration of heavy metals before the launch of the gold mining.

Material and Methods: There is a system of coefficients which present the ratio between the content of chemical elements of comparable objects. The most important of them are the clarke of concentration and the clarke of dispersion. Clarke of concentration is the ratio of the element's content in a particular natural component towards its clarke in the lithosphere. If the clarke of concentration coefficient is lower than 1 it is necessary to be calculated a reverse quantity for emphasizing the result – clarke of dispersion.

Main Results: The obtained results display the researched territory as a natural background area. The content of heavy metals in the river sediments of the researched area (mg/kg, median value) by chemical elements is: Cu (15), Zn (72), Pb (17), Mn (461), Ni (35), Co (8), and Cr (60). That is why it could be defined as unimpacted by human activities and it is uninfluenced by natural geochemical anomalies.

Conclusion: The researched landscapes are not polluted by heavy metals before the beginning of the mining. This outcome is obtained by the geochemical content of the investigated heavy metals in the river sediments.

Keywords: gold mining, environmental impact, landscape assessment, pollution, ecogeochemestry

L04_04

Forest habitats of Breznik Municipality

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

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

Materials and Methods: Initial data was collected from the Ministry of Environment and Water and the Forestry Management Plans. Polygons were spatially processed with the application of the software product of ArcGIS 10.1. Terrain studies added more scientific information for analysis.

Results: Forests are covering 149.6 km². Their phytocoenoses are dominated by *Quercus dalechampii* (17.75 km²), *Q. frainetto* (1.2 km²), *Fagus sylvatica* (35.24 km²), *Carpinus betulus* (13.26 km²). There are also plantations with *Pinus nigra* (26.59 km²) and *P. sylvestris* (34.48 km²). Non-native species, such as *Robinia pseudacacia* (1.19 km²) and *Quercus rubra* (0.41 km²), are present as well.

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

Keywords: Braun-Blanquet approach, vegetation, GIS

Acknowledgements: This investigation was carried out with the financial help of the NSP "Young scientists and postdoctoral students, 2021", and the National Science Fund (Contract ДКОСТ 01/7/19.10.2018).

L04_05

Landscape analysis of coastal areas suitable for hygrophilous forests restoration. Case study: Baltata locality, North Bulgarian Black Sea coast.

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Introduction. Longoses are endemic forests native to the East Balkans that once used to be common along the river downstream sectors flowing into the sea. Key environmental factors for their existence are the humid subtropical climate with mild winters and the river inundations occurring twice-yearly. On the Bulgarian coast, these hygrophilous forests are excellent flood storage providers, an important ecosystem service that is linked to environmental flood risk management. However, because of spatial reduction and fragmentation, longoses are nowadays red-listed as ***critically endangered*** in Bulgaria. Accordingly, they are subject to preservation and restoration in compliance with the EU Habitats Directive and Annex 1 of the National Biodiversity Act.

Aims of the study are: to assess the spatio-temporal reduction of the longos forests at Baltata Locality, bordering Albena Resort (North Bulgarian coast); to identify the ownership of the cadastral parcels once occupied by these endemic woods; to analyze the present-day primary purpose and land use of the reclaimed terrains; to review the legal framework regulating coastal land conversion in Bulgaria; to propose areas suitable for forest landscape restoration.

Material and methods. Spatial analyses were carried out in GIS by means of comparisons of topographic maps from the late-19th century versus contemporary cadastral data.

Main results. The findings of the study demonstrate a significant reduction and fragmentation of longoses at Baltata Locality due to land reclamation for agriculture, infrastructure and resort construction over the years. This implies for a deteriorated environmental status and impaired flood storage capacity, well correlating with recent extreme fluvial floods in the area.

Conclusions. Landscape restoration of coastal hygrophilous forests at the expense of abandoned land is necessary for proper flood risk management in the light of the integrated coastal

zone management (ICZM) along the north Bulgarian coast.

Keywords: landscape change detection, land reclamation, land conversion, cadastral data, coastal spatial planning, ICZM

L04_06

Urban green spaces in Paris and Sevlievo cities

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Urban living can increase exposure to certain environmental hazards, such as air and noise pollution. Many urban areas face increasing pressure from expanding populations, limited resources and growing impacts of climate change.

The aim of this study is to investigate urban green spaces that offer innovative approaches to increase the quality of urban settings, enhance local resilience and promote sustainable lifestyles, improving both the health and the well-being of urban residents.

Material & Methods: Sevlievo is a small sustainable town, European capital of pumpkin that in 2012 turned its old barracks into a beautiful urban park system. On the other hand, Paris has a rich biodiversity with diverse natural flora and vegetation. Since the 10th century, Paris has been one of the main cities of France: it is located in the heart of a rich agricultural region (like Sevlievo). Manes et al. (2014) and dispersion modeling of PM10 - SELMA-GIS and others.

Main results: The green cover of Sevlievo is 27.49% and the green cover of Paris is only 9.49%. The residents of Sevlievo are 33 099 and 2 161 000 for Paris.

Conclusion: The urban green spaces of Paris and Sevlievo (on the order of every other city in the world) have the following types of value – Ecological, Economic and Social. Increasing the sustainability of cities, requires reducing the degradation of their ecosystems. Here the concept of sustainable development intervenes, meeting the needs of the present without compromising the capabilities of future generations.

Keywords: urban landscape, ecosystem services, human well-being, climate change, sustainability

L04_07

Climate change in the Bulgarian homegardens – rural communities perspectives

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Homegardens, being the closest ground of interaction between people and nature, provide not only subsistence and well-being for their owners', but are also a source of immediate impressions of people about climate changes.

The aim of the current study was to reveal the effect of climate changes as perceived by local communities in rural areas of Bulgaria.

Material and methods: We focused on personal opinions of homegarden owners in 35 rural settlements. Semi-structured interviews were conducted among male and female residents between 35-84 years in the period 2017-2019. Data on perceived climate change and its influence on everyday life were documented.

Main results: Homegardens visited during the study served both for provisional and recreational purposes. Climate changes were perceived as: i) warmer and shorter winter season that allows the growing of new fruits and vegetables in mountain areas; ii) hot and dry summers that result in wild fires and/or need of additional irrigation for vegetable crops; iii) unpredicted and extreme weather causing harvest loss and iv) additional pest and disease outbreaks that decrease the yields. Our respondents, however, did not formulate their observations as climate change because this term was not familiar to them, partly because it is not a frequent topic on national and local media. In some areas unsustainably executed renewable energy projects even contributed to negative responses to climate change mitigation activities.

Conclusion: Urgent measures are required so to alleviate public concerns related to climate change mitigation measures and to gain public awareness and positive engagement.

Keywords: crops, subsistence farming, traditional knowledge

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

L04_08

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

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The main purpose of this study is to develop new thermodynamic models for solution behavior and solid-liquid equilibrium in 10 nitrate binary systems of the type 2-1 ($Mg(NO_3)_2$-H_2O, $Ca(NO_3)_2$-H_2O, $Ba(NO_3)_2$-H_2O, $Sr(NO_3)_2$-H_2O, and $UO_2(NO_3)_2$-H_2O), 3-1 ($Cr(NO_3)_3$-H_2O, $Al(NO_3)_3$-H_2O, $La(NO_3)_3$-H_2O, $Lu(NO_3)_3$-H_2O), and 4-1($Th(NO_3)_4$-H_2O) from low to very high concentration at 298.15 K.

Modeling Technology: To construct models we used different versions of standard molality-based Pitzer approach. To parameterize models we used all available raw experimental osmotic coefficients data (φ) for whole concentration range of solutions, and up to supersaturation zone.

Results: The predictions of developed models are in excellent agreement with φ-data, and with recommendations on activity coefficients ($\gamma_{\pm}$) in binary solutions from low to very high concentration. The Deliquescence Relative Humidity (DRH), and thermodynamic solubility product (as ln K^o_{sp}) of 10 nitrate solid phases have been calculated.

Summary: The models for nitrate systems described in this study are of high importance for the development of strategies and programs for nuclear waste geochemical storage.

Keywords: Nuclear waste sequestration; Chemical modelling; Pitzer approach; DRH and K^o_{sp} of $Mg(NO_3)_2.6H_2O$(s), $Ca(NO_3)_2.4H_2O$(s), $Ca(NO_3)_2.3H_2O$(s), $Ba(NO_3)_2$(s), $Sr(NO_3)_2$(s), $UO_2(NO_3)_2.6H_2O$(s), $Al(NO_3)_3.9H_2O$(s), $La(NO_3)_3$(s), $Lu(NO_3)_3.6H_2O$(s) and $Th(NO_3)_4.6H_2O$(s)

Acknowledgement : The work was supported by the European Regional Development Fund, Project BG05M2OP001-1.001-0004, and by Shumen University Research Program, Project RD-08-131/04.02.2021

L04_09

Development of accurate chemical thermodynamic database for geochemical storage of nuclear waste. Part III: Models for predicting solution properties and solid-liquid equilibrium in cesium binary and mixed systems

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The main purpose of this study is to describe very well validated thermodynamic models for solution behavior and solid-liquid equilibrium in 8 cesium binary ($CsF-H_2O$, $CsCl-H_2O$, $CsBr-H_2O$, $CsI-H_2O$, $CsOH-H_2O$, $CsNO_3-H_2O$, $Cs_2SO_4-H_2O$, and $Cs_2SeO_4-H_2O$) and 13 cesium mixed ternary ($CsCl-MgCl_2-H_2O$, $CsBr-MgBr_2-H_2O$, $CsCl-NiCl_2-H_2O$, $CsBr-NiBr_2-H_2O$, $CsCl-MnCl_2-H_2O$, $CsCl-CoCl_2-H_2O$, $CsCl-CuCl_2-H_2O$, $Cs_2SO_4-CoSO_4-H_2O$, $Cs_2SeO_4-CoSeO_4-H_2O$, $Cs_2SO_4-NiSO_4-H_2O$, $Cs_2SeO_4-NiSeO_4-H_2O$, $Cs_2SO_4-NiSO_4-H_2O$, $Cs_2SeO_4-NiSeO_4-H_2O$) systems at 25°C.

Modeling Technology: The models are developed on the basis of Pitzer ion interactions approach. To parameterize models for binary systems we used all available experimental osmotic coefficients data (φ) for whole concentration range of solutions. To evaluate Pitzer mixing parameters (θ and ψ) solubility data in ternary systems have been used.

Results: Using evaluated binary and mixing parameters important thermodynamic characteristics (thermodynamic solubility product (K^o_{sp}), standard chemical potential (ΔG^o_m)) of 8 cesium simple salts and 16 cesium double salts, precipitating from saturated binary and mixed solutions have been determined.

Summary: The models described in this study are of high importance in development of strategies for nuclear waste geochemical storage.

Keywords: Geochemical nuclear waste sequestration; Computer thermodynamic modeling; cesium binary and mixed systems; Pitzer approach

Acknowledgement: The work was supported by the European Regional Development Fund, Project BG05M2OP001-1.001-0004, and by Shumen University Research Program, Project No. RD-08-131/04.02.2021.

P04_01

Effects of land use on butterfly assemblages in selected rural areas in Bulgaria

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The expansion of cropland areas and their intensive use are the primary drivers of habitat loss and biodiversity decline. Homogenous landscapes dominated by large arable areas are created that can change significantly the biological communities and their ecological functions.

Aim: In the framework of STACCATO project the effect of landscape structure on butterfly assemblages of rapeseed fields and semi-natural grasslands was studied.

Material and methods: Ten sites located in the South-Central Bulgaria with various proportions of grasslands (pastures) and rapeseed areas were sampled in April and May 2018 using the standard protocols for European monitoring schemes. In each site, two 1000 m transects, at the rapeseed field margin and in the adjacent semi-natural grassland (pasture) were walked and all butterflies registered.

Main results: Overall, 42 different species (30 genera) were found: 33 (24) in the rapeseed fields and 38 (28) in the grassland and pasture areas. The assemblages of rapeseed field margins were dominated by one species, *Pieris rapae* (49-85%), whose larvae usually feed on plants of the family Brassicaceae. *Pontia edusa* and *Isoria lathonia* occurred in both habitat types having slightly higher abundance in areas along the rapeseed fields. Species belonging to the genera *Coenonympha*, *Maniola*, *Polyommatus*, *Melitaea*, *Lycaena*, *Colias* and *Plebejus* prevailed significantly in the grassland/pasture areas. The species richness and diversity of grassland assemblages were higher and the similarity lower as compared to those of rapeseed field margins.

Conclusions: Two butterfly assemblages related to land use type and time (sampling month) were distinguished. Species richness and diversity were higher and species composition distinct in two sites located in areas with more heterogeneous landscape structure. The study illustrates the importance of preserving grasslands and low intensity pastures within cropland areas for conserving butterfly assemblages.

Keywords: agriculture, landscape heterogeneity, ecosystem services, Lepidoptera, semi-natural habitats

Acknowledgements: The present study was carried out with the financial support of STACCATO "SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources" project (BiodivERsA-FACCE2014-47).

P04_02

Wild bee assemblages of rapeseed fields and the adjacent grasslands in South-Central Bulgaria

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Agricultural landscape simplification resulting in natural habitat loss can negatively affect wild bees regarding both their species diversity and composition. Arable fields planted with annual crops such as *Brassica napus* L. are an example for landscape simplification.

Aim: We examined wild bee assemblages of semi-natural habitats (grasslands) and rapeseed fields in 10 localities at the territory of five municipalities in South-Central Bulgaria.

Material and Methods: Solitary bees were caught with colored pan traps and with sweep net along 100 m long transects at the margin of flowering *B. napus* fields in April and May 2018 and across adjacent semi-natural grasslands.

Results: A total of 888 individuals representing 111 species from 17 genera we recorded in both types of habitats. The most abundant and diverse genera were *Lasioglossum*, *Halictus* and *Andrena*. One species was particularly abundant and widespread *L. pauperatum* (18% of all individuals, 10 localities), followed by *H. phryganicus* (7%, 9 localities) and *L. pauxillum* (7%, 8 localities). The assemblages were dominated by ground-nesting species (*Andrena*, *Anthophora*, *Halictus* and *Lasioglossum* spp.) whereas above-ground nesting species were rare (e.g. *Ceratina* and *Osmia* spp.). Polylectic species which forage for pollen on a diverse array of plants prevailed. Cuckoo bees (*Bombus vestalis*, *Nomada* and *Sphecodes* spp.), parasitising the nests of other bee species, accounted for 3% of total abundance. A significant difference between wild bee assemblages of semi-natural grasslands and flowering rapeseed fields was detected.

Conclusion: Our results suggest that heterogeneous landscapes with bigger areas of open semi-natural habitats, support more diverse assemblages of spring wild bees by providing flowering plants throughout the foraging period and diverse niches to meet different species' requirements.

Keywords: agriculture, ecosystem services, Hymenoptera, pollination, semi-natural habitats

Acknowledgements: The present study was carried out with the financial support of the project BiodivERsA-FACCE2014-47 "SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources (STACCATO)"

P04_03

Habitat mapping for environmental sustainability of grassland and annual crops by GIS – a Bulgaria case study

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Geographical Information system (GIS) was used in this study for terrestrial mapping according to the STACCATO project protocol around semi-natural grasslands and oilseed rape fields in Bulgaria target sites. Recording system of habitats and the database is performed as outlined, and reclassified the available areas and objects in polygon, linear, and point elements. The permanent land use categories or type of vegetation has been recorded by EUNIS habitat classification or redefined in accordance with the proposed method. Types and physical characteristics of soils were determined in grassland and oilseed rape field sites. Species composition of plants was recorded for semi-natural grasslands adjoining rape.

Keywords: ecological management, oilseed rape, semi-natural grassland

Acknowledgements: The present study was carried out thanks to the financial support of the Project BiodivERsA-FACCE2014-47 “SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources (STACCATO)”

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