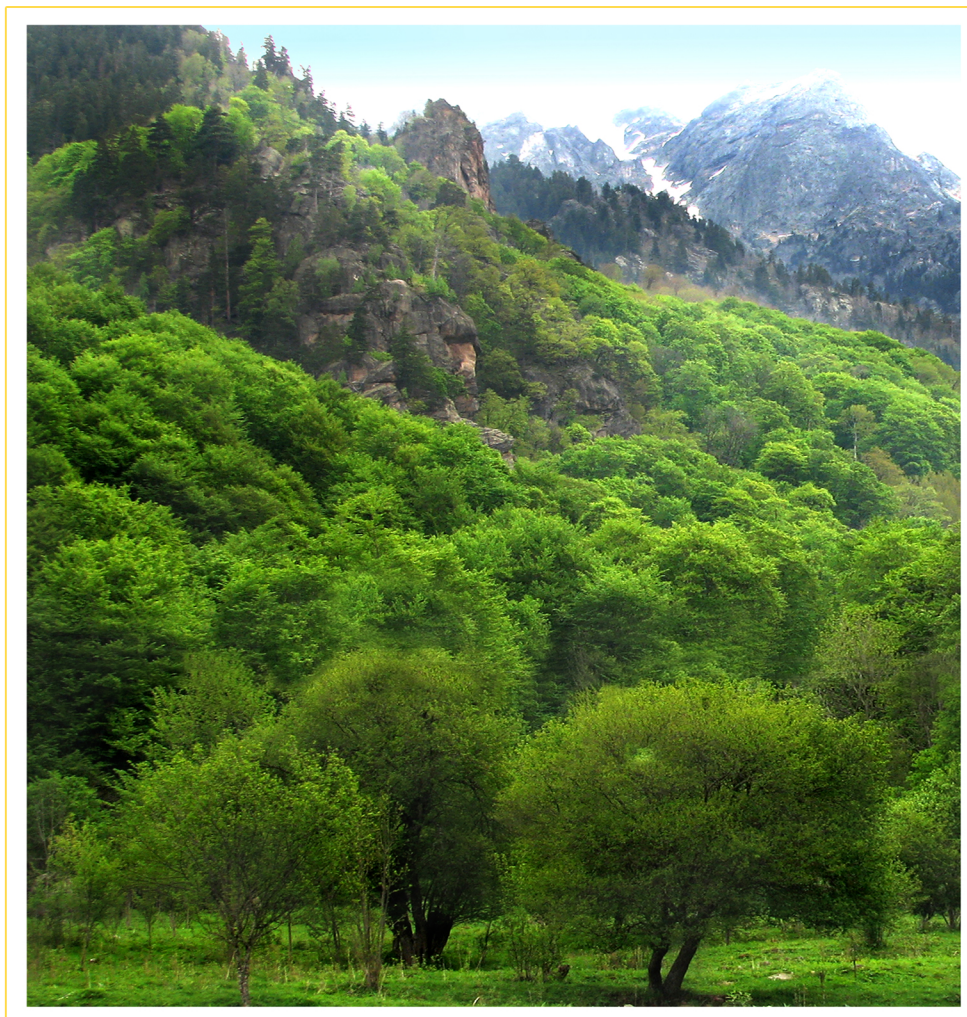


**UNION OF SCIENTISTS IN BULGARIA
SECTION BIOLOGY
INSTITUTE OF BIODIVERSITY AND ECOSYSTEM RESEARCH – BAS**



**“SEMINAR OF ECOLOGY-2014”
with INTERNATIONAL PARTICIPATION**

DEDICATED TO 70 YEARS USB

24-25 April 2014



**Програма/Program
Абстракти/Abstracts**



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This seminar was organized by Section Biology of the Union of Scientists in Bulgaria and the Institute of Biodiversity and Ecosystem Research, BAS, Sofia.

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**“SEMINAR OF ECOLOGY - 2014”
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24-25 April 2014

PROGRAM

24 APRIL 2014

8⁰⁰ – 9⁰⁰ Registration (IBER - BAS)

9⁰⁰ – 9³⁰ Opening ceremony

Family photo

THEMATIC SESSION I

ANTHROPHOGENIC IMPACT ON LIVING NATURE

Chaired by: Yordan Uzunov and Galina Satchanska

Oral presentations

9³⁰-9⁴⁰ - ENZYME PROPERTIES OF *HELIX ASPERSA* MAXIMA HEMOCYANIN *Yuliana Raynova and Krassimira Idakieva – L1_01*

9⁴⁰-9⁵⁰ - INCREASED MUTABILITY IN *TARAXACUM OFFICINALE* GENOME CORRESPONDS WITH THE DEGREE OF HEAVY METAL POLLUTION *Borislava Boteva, Rumiana Djingova, George Miloshev - L1_02*

9⁵⁰-10⁰⁰ - GENOTOXICITY BIOMONITORING OF HEAVY METAL POLLUTION *IN SITU* USING THE COMET ASSAY AND THE MICRONUCLEUS TEST IN NATIVE RODENT *Vesela Mitkovska, Tsenka Chassovnikarova, Hristo Dimitrov - L1_03*

10⁰⁰-10¹⁰ - WATER BUGS (HETEROPTERA: NEPOMORPHA AND GERROMORPHA) AS BIOINDICATORS IN ECOLOGICAL STUDIES
Desislava Stoianova, Snejana Grozeva - L1_04

10¹⁰-10⁴⁰ Discussion

10⁴⁰-10⁵⁵ Coffee break

Chaired by: Rumyana Mecheva

10⁵⁵-11⁰⁵ - TRAFFIC MORTALITY OF AMPHIBIANS, REPTILES, BIRDS AND MAMMALS ON TWO TYPES OF ROADS BETWEEN PAZARDJIK AND PLOVDIV REGION – SUMMARY OF THE RESULTS *Nevena Kambourova-Ivanova - L1_05*

11⁰⁵-11¹⁵ - ZINC POLLUTION OF SOILS AND WATERS NEAR PB-ZN SMELTER AND ITS TOXICITY IN HUMANS *Galina Satchanska - L1_06*

11¹⁵-11²⁵ - PESTICIDES IN THE ENVIRONMENT - SOURCES OF POLLUTION AND EFFECTS ON ECOSYSTEMS *Irena Bogoeva - L1_07*

11²⁵-11³⁵ - ESTIMATION OF GENOTOXIC EFFECT OF INSECTICIDE MOSPILAN AT GOLDEN FISH *Kasum Letaj, Kemajl Kurteshi - L1_08*

11³⁵-11⁴⁵ – HEAVY METAL CONCENTRATIONS IN VEGETABLES WITH GROWTH STAGE AND PLANT SPECIES VARIATIONS *Snezana Veselinovska - L1_09*

11⁴⁵-11⁵⁵ – BIO-MONITORING OF SOIL SAMPLES FROM THE AREA OF KCM PLOVDIV *Zhana Mitrovska, Anife Mahmud, Daniela Miteva, Radostina Hristova, Nadezhda Yurina, Stephka Chankova - L1_10*

11⁵⁵-12³⁰ Discussion

12³⁰-13³⁰ Lunch

THEMATIC SESSION II

MECHANISMS OF ADAPTATION OF LIVING SYSTEMS

Chaired by: Stephka Chankova and Margarita Pesheva

Oral presentations

13³⁰-13⁴⁰ - INTERDISCIPLINARY INTERACTION FOR THE BIOTECHNOLOGICAL DEVELOPMENT OF BALKAN MEDICINAL PLANT SPECIES *Milka Todorova, Antoaneta Trendafilova, Sashka Krumova, Krasimira Idakieva, Viktorya Genova, Yuliana Markovska, Yuliana Raynova, Lujba Evstatieva, Evelyn Wolfram, Kalina Danova – L2_01*

13⁴⁰-13⁵⁰ - ANTIMUTAGENIC AND ANTICARCINOGENIC EFFECT OF AMYGDALIN IN SACCHAROMYCES CEREVISIAE *Atanaska Todorova, Ivan Iliiev, Margarita Pesheva - L2_02*

13⁵⁰-14⁰⁰ - STRESS RESPONSE OF *CHLAMYDOMONAS REINHARDTII* STRAINS TO ZEOCIN DEPENDS ON THE GENOTYPE *Maria Dimitrova, Daniela Miteva, Zhana Mitrovska and Stephka Chankova - L2_03*

14⁰⁰-14¹⁰ - PROTECTIVE ACTIVITY OF DIFFERENT EXTRACTS OF *CLINOPODIUM VULGARE* L. *Teodora Todorova, Daniela Miteva, Ventsislav Bardarov, Krum Bardarov, Atanas Atanassov, Stephka Chankova - L2_04*

14¹⁰-14²⁰ – ANTIGENOTOXIC EFFECT OF SALVIA EXTRACT AGAINST OXIDATIVE DNA DAMAGE INDUCED BY ZEOCIN *Svetla Gateva, Gabriele Jovtchev, Alexander Stankov - L2_05*

14²⁰-14³⁰ – COMPARISON OF BIOCHEMICAL AND MOLECULAR RESPONSES OF *PHASEOLUS VULGARIS* CULTIVAR AND THREE MUTANT LINES TO DROUGHT STRESS *Tsveta Angelova, Petya Parvanova, Zhana Mitrovska, Daniela Miteva, Diana Svetleva, Darya Mokerova, Nadezhda Yurina, Stephka Chankova - L2_06*

14³⁰-15⁰⁰ Discussion

15⁰⁰-15¹⁵ Coffee break

THEMATIC SESSION III

ECOSYSTEM RESEARCH AND SERVICES

Chaired by: Nesho Chipev and Svetla Bratanova-Doncheva

Oral presentation

15¹⁵-15³⁵ - ASSESSMENT OF ECOSYSTEM FUNCTIONS AND SERVICES AT LANDSCAPE LEVEL OF SREBARNA LTER SITE *Svetla Bratanova-Doncheva, Nevena Kamburova-Ivanova, Nesho Chipev – L3_01 (Plenary lecture)*

15³⁵-15⁴⁵ - VALUE OF CULTURAL ECOSYSTEM SERVICES PROVIDED BY ECO-TRAIL "CANYON FALLS" NEAR SMOLYAN *Assen Assenov, Bilyana Borisova, Petar Dimitrov - L3_02*

15⁴⁵-15⁵⁵ - AIR QUALITY IN REPUBLIC OF MACEDONIA DEPENDING ON PRESENCE OF SULFUR DIOXIDE *Biserka Dimiskovska, Dragi Dojcinovski, Vesela Radovic - L3_03*

THEMATIC SESSION IV

LANDSCAPE ECOLOGY

Chaired by: Nesho Chipev and Svetla Bratanova-Doncheva

Oral presentations

15⁵⁵-16⁰⁵ - ALGORITHM FOR LANDSCAPE ECOLOGICAL RESEARCH IN MOUNTAIN AREAS *Bilyana Borisova, Assen Assenov, Petar Dimitrov – L4_01*

16⁰⁵-16³⁵ Discussion

16³⁵-17⁰⁵ Coffee break and poster session

17⁰⁵-18³⁵ Poster presentation and discussion

Chaired by: Nevena Kamburova-Ivanova and Petya Parvanova

POSTER SESSION I

ANTHROPOGENIC IMPACT ON LIVING NATURE

- P 1_01** ASSESSMENT OF THE SENSITIVITY OF *CHLORELLA VULGARIS* TO HERBICIDES WITH DIFFERENT CHEMICAL STRUCTURES
Georgi Rashkov, Anelia Dobrikova, Irina Pouneva, Amarendra Misra, Emilia Apostolova
- P 1_02** DEVELOPMENT AND APPLICATION OF A COMPLEX APPROACH TO ASSESS CELL TOXICITY OF METAL COMPOUNDS
Lora Dyakova, Tanya Zhivkova, Milena Georgieva, George Miloshev, Reni Kalfin, Gabriela Marinescu, Daniela-Cristina Culita, Luminita Patron, Radostina Alexandrova
- P 1_03** INFLUENCE OF LOW LIGHT AND HIGH LIGHT INTENSITIES ON ELECTROKINETIC POTENTIAL OF THYLAKOID MEMBRANES FROM *HORDEUM VULGARE* L.
Virginia Doltchinkova, Nadejda Radovanova, Katya Georgieva
- P 1_04** INFLUENCE OF SONIFICATION AND LECTIN ON THYLAKOID MEMBRANES FROM *HORDEUM VULGARE* L.
Nadejda Radovanova, Virginia Doltchinkova
- P 1_05** SPECTRAL REFLECTANCE SIGNATURES IN SOIL SALINITY STUDIES
Rumiana Kancheva, Denitsa Borisova, Georgi Georgiev
- P 1_06** MACROPHYTE-BASED ECOLOGICAL STATUS ASSESSMENT OF SUB-MEDITERRANEAN RIVERS IN BULGARIA
Yordanka Hristeva, Gana Gecheva, Karin Pall, Lilyana Yurukova

- P 1_07** **OZONE MONITORING IN THE RHODOPES** *Mariana Doncheva-Boneva, Georgi Kadinov*
- P 1_08** **TAXONOMIC COMPOSITION OF THE FUNCTIONAL FEEDING GROUPS OF BENTHIC FAUNA AT SOME BULGARIAN RIVERS** *Maria Kerakova, Emilia Varadinova, Radka Fikova, Yordan Uzunov*
- P 1_09** **PHOTOSYNTHESIS AND GROWTH RESPONSES OF FIVE PAULOWNIA LINES TO SALT STRESS** *Katya Ivanova, Nikolina Tzvetkova, Teodora Georgieva, Yuliana Markovska*
- P 1_10** **TRACE ELEMENTS IN SOILS AND HERBS IN THE VICINITY OF SMELTER “KCM 2000” - PLOVDIV (BULGARIA)** *Stilyana Slavova, Goran Yankov, Iliana Velcheva, Slaveya Petrova*
- P 1_11** **HYPOGLYCAEMIC EFFECTS OF GLYPHOSATE BASED HERBICIDE ON COMMON CARP (*CYPRINUS CARPIO* L.) AND BIGHEAD CARP (*ARISTICHTHIS NOBILLIS*, RICH.) LIVER** *Stela Stoyanova, Vesela Yancheva, Iliana Velcheva, Pepa Atanasova, Elenka Georgieva*
- P 1_12** **EFFECTS OF METAL-CONTAMINATED WATERS OF TOPOLNITSA RESERVOIR ON GILLS OF EUROPEAN PERCH (*PERCA FLUVIATILIS*, L.)** *Vesela Yancheva, Stela Stoyanova, Iliana Velcheva, Elenka Georgieva*
- P 1_13** **CFGE A PERSPECTIVE METHOD FOR DSBs DNA DETECTION IN HUMAN LYMPHOCYTES *IN VITRO*** *Svetla Gateva, Olga Angelova, Stephka Chankova*
- P 1_14** **POTENTIAL THREATS FOR AIR POLLUTION IN WESTERN RHODOPES: A REVIEW** *Nikolina Gribacheva, Lilyana Yurukova, Gana Gecheva*

MECHANISMS OF ADAPTATION OF LIVING SYSTEMS

- P 2_01** **EFFECT OF THE SALINITY OF THE SOIL ON THE FUNCTIONS OF THE PHOTOSYNTHETIC APPARATUS OF TWO LINES OF *PAULOWNIA*** *Martin Stefanov, Georgi Rashkov, Katya Ivanova, Yuliana Markovska, Emilia Apostolova*
- P 2_02** ***IN VITRO* DETERMINATION OF ANTIOXIDANT CAPACITY OF PEA (*PISUM SATIVUM* L. CV. RAN) PLANTS** *Boryana Mihaylova, Ivan Goshev, Lyubomira Atanasova*

ECOSYSTEM RESEARCH AND SERVICES

- P 3_01** ASSESSMENT OF OAK DENDOCHRONOLOGICAL SERIES FOR EUSTRESS IDENTIFICATION IN PROTECTED ZONE *Mariyana Lyubenova, Nadejda Georgieva, Velichka Lyubenova*
- P 3_02** LACTIC ACID CONTENT IN COMMERCIAL AND HOMEMADE YOGURT *Alexander Tomov, Anna Doycheva and Galina Satchanska*
- P 3_03** SIZE-AGE AND DIET COMPOSITION OF SOME OF THE MOST IMPORTANT COMMERCIAL FISH SPECIES IN THE BLACK SEA BASIN *Ioana Georgieva, Georgi Daskalov*
- P 3_04** ON THE APPLICATION OF LIQUID CRYSTALS IN ECOLOGY AND THEIR ROLE AS BUILDING BLOCKS FOR BIODIVERSITY *Stefan Todorov, Lidia Todorova*

25 APRIL 2014

THEMATIC SESSION V

BIODIVERSITY AND CONSERVATION BIOLOGY

Chaired by: Vlada Peneva and Svetlana Bancheva

Oral presentations

9³⁰-9⁴⁰ - POPULATION STATE AND CONSERVATION OF THE BULGARIAN ENDEMIC *VERBASCUM TZAR-BORISII* (SCROPHULARIACEAE)

Svetlana Bancheva, Malina Delcheva – L5_01

9⁴⁰-9⁵⁰ – ECO-BIOLOGICAL ASSESSMENT OF SOME FOREST TREES IN “GOVEDARCI” AND “JASENKOVO” EXPERIMENTAL STATIONS

Elena Nedkova - L5_02

9⁵⁰-10⁰⁰ - CONTRIBUTION TO THE RESEARCH ON MYRIAPODS (CHILOPODA, DIPLOPODA) IN THE MADARA PLATEAU, SHUMEN REGION, NORTH-EASTERN BULGARIA *Aleksandar Doichinov, Darina Bachvarova - L5_03*

10⁰⁰-10¹⁰ - MICROBIOLOGICAL ANALYSIS OF WATER OF RIVER LUMËBARDHI (KOSOVO) DURING WINTER SEASON *Idriz Vehapi, Kemajl Kurteshi, Kasum Letaj - L5_04*

10¹⁰-10²⁰ - ALGOCENOSIS OF RIVER VALBONË (ALBANIA), DURING WINTER SEASON 2012 *Kemajl Kurteshi, Ramë Kortoqi, Fatbardh Gashi - L5_05*

10²⁰-10³⁰ - GENETIC POLYMORPHISMS OF BULGARIAN AND PORTUGUESE COMMON BEAN (*PHASEOLUS VULGARIS*) GERMPLASM *Petya Parvanova, Fernanda Simões, Maria Manuela Veloso, Diogo Mendonça, Joana Guimarães, Tzvetelina Stoilova, Diana Svetleva, José Matos and Stephka Chankova - L5_06*

10³⁰-10⁴⁰ - COMPARISON OF VASCULAR FLORAS OF TREE LIMESTONE TERRAINS IN BULGARIA - ZEMENSKA MOUNTAIN, GOLO BARDO AND BESAPARSKI RIDOVE *Asen Asenov - L5_07*

10⁴⁰-11¹⁰ Discussion

11¹⁰-11²⁵ Coffee break

THEMATIC SESSION VI ECOLOGICAL AGRICULTURE

Chaired by: Marina Stanilova

Oral presentations

11²⁵-11³⁵ – IMPLEMENTATION OF GOOD AGRICULTURAL PRACTICES AND THE BENEFITS FOR AGRICULTURAL PRODUCERS
Kliment Petrov – L6_01

11³⁵-11⁴⁵ - INDUCTION OF RESISTANCE OF PEPPER TO POTATO VIRUS Y BY ACTIVATION OF NATURAL DEFENSE MECHANISMS OF THE HOST PLANT *Nikolay Petrov – L6_02*

11⁴⁵-11⁵⁵ - MONITORING OF FUNGICIDE USE AGAINST THE POWDERY MILDEW AND APPLE SCAB IN NONCOMMERCIAL ORCHARDS IN SOFIA'S FIELD *Antoniy Stoev, Vanyo Aleksandrov – L6_03*

11⁵⁵-12²⁵ Discussion

12²⁵-13³⁰ Lunch

THEMATIC SESSION VII INVASIVE SPECIES

Chaired by: Plamen Kalushkov and Mariyana Lubenova

Oral presentations

13³⁰-13⁴⁰ - IMPORTANT INVASIVE INSECT SPECIES IN BULGARIAN FAUNA *Plamen Kalushkov – L7_01*

THEMATIC SESSION VIII

ECOLOGY AND EDUCATION

Chaired by: Plamen Kalushkov and Mariyana Lubenova

Oral presentations

13⁴⁰-13⁵⁰ - "THE HUMAN – AN INSEPARABLE PART FROM NATURE"
ENVIRONMENTAL EDUCATION MODEL *Galina Treneva, Polina Doncheva –*
L8_01

13⁵⁰-14⁰⁰ - PROJECT- BASED LEARNING ON "SOIL EROSION
PROTECTION" *Mirena Dimitrova –* L8_02

14⁰⁰-14³⁰ Discussion

14³⁰-15⁰⁰ Coffee break and poster session

15⁰⁰-16³⁰ Poster presentation and discussion

Chaired by: Radka Fikova and Kalina Danova

POSTER SESSION II

BIODIVERSITY AND CONSERVATION BIOLOGY

- P 5_01 BULGARIAN COLLECTIONS AND FIRST MOLECULAR DATA
OF *ZEUS OLYMPIUS* (ASCOMYCOTA, FUNGI) *Dimitar Stoykov,*
Boris Assyov, Rossen Alexov, Krasimir Grazdilov
- P 5_02 DETERMINATION OF THE ALKALOID GLAUCINE IN FOUR
BULGARIAN POPULATIONS OF *GLAUCIUM FLAVUM*
(PAPAVERACEAE) *Iva Doycheva, Stefan Philipov, Marina Stanilova*
- P 5_03 ESSENTIAL OIL COMPOSITION OF *IN SITU* AND *IN VITRO*
CULTIVATED *ACHILLEA THRACICA* VELEN. PLANTS
Mariya Rogova, Nia Petrova, Zhenia Yordanova, Milena Dimitrova, Veneta
Kapchina-Toteva
- P 5_04 FIRST RECORD OF *POLYDESMUS SCHAESSBURGENSIS*
VERHOEFF, 1989 (DIPLOPODA, POLYDESMIDA,
POLYDESMIDAE) FROM BULGARIA *Darina Bachvarova,*
Aleksandar Doichinov

- P 5_05** SPATIAL NICHE PARTITION AMONG FIVE SYMPATRIC LIZARDS IN NORTH-WEST BULGARIA *Emiliya Vacheva, Nikolay Tzankov*
- P 5_06** NEW DATA ON AQUATIC OLIGOCHETS (OLIGOCHAETA LIMICOLA) IN THE BURGASKO (VAYA) LAKE *Galia Georgieva, Elena Nenova, Maria Shishinova, Yordan Uzunov*
- P 5_07** DIVERSITY AND CONSERVATION STATUS OF VASCULAR PLANTS OF THE ROCKY HILLS EAST- AND WESTWARD FROM TRIGRAD VILLAGE *Ina Aneva, Peter Zhelev*
- P 5_08** *PSEUDOMONAS CORRUGATA* – CAUSE OF TOMATO PLANTS DIEBACK IN GREENHOUSES IN BULGARIA *Nevena Bogatzevska, Mariya Stoyanova*
- P 5_09** SPECIES COMPOSITION OF EARTHWORMS (LUMBRICIDAE) IN THE BOYANA LAKE SURROUNDINGS (VITOSHA MOUNTAIN) *Ralitsa Tsekova, Mirjana Stojanovic and Tanja Milutinovic*
- P 5_10** NEW DATA ON THE LICHENIZED FUNGI (*ASCOMYCOTA*) IN BALGARKA NATURAL PARK *Dimitar Stoykov*
- P 5_11** TROPHIC STATE VS. ECOLOGICAL STATUS – A SURVEY OF DIFFERENT WATER BODY TYPES IN BULGARIA *Ivan Traykov and Anita Tosheva*
- P 5_12** EX SITU CONSERVATION OF THREATENED AND ENDANGERED PLANTS OF BULGARIAN FLORA IN THE TERRITORY OF UNIVERSITY BOTANIC GARDEN ECOPARK – VARNA *Petya Boycheva*

ECOLOGICAL AGRICULTURE

- P 6_01** WHEAT VARIETIES AND WHEAT LINES AND THEIR REACTION DEGREE OF SENSITIVITY TO TAKE-ALL DISEASE CAUSED BY *OPHIOBOLUS GRAMINIS* (SACC) *Anna Rachova, Radka Kancheva*
- P 6_02** VIRUS DISEASES ON MEDICINAL PLANT *SILYBUM MARIANUM* (L.) GAERTN *Bistra Dikova*
- P 6_03** INFLUENCE OF DOUBLE MICROBIAL ASSOCIATIONS WITH AM-FUNGI AND RHIZOBIUM ON THE GROWTH OF ALFALFA AND RED CLOVER AND ON THE SOIL STRUCTURE *Efrosina Djonova, Galina Petkova, Ira Stancheva*

- P 6_04** **STUDY OF THE RADIUM-226 AND THORIUM-234 ACCUMULATION IN COTTON PLANTS FROM CONTAMINATED SOILS OF BUHOVO AREA** *Donka Staneva, Ivanka Yordanova, Lidia Misheva*
- P 6_05** **EFFECT OF PLANT EXTRACTS TO *CLAVIBACTER MICHIGANENSIS* - CAUSAL AGENT OF BACTERIAL CANKER OF TOMATO** *Mariya Stoyanova, Miroslava Valkova*
- P 6_06** **REDUCTION OF PLANT AND FRUIT DAMAGES CAUSED BY TOMATO MOSAIC VIRUS AND CUCUMBER MOSAIC VIRUS IN TOMATOES THROUGH NATURAL INDUCERS OF RESISTANCE** *Nikolay Petrov*
- P 6_07** **BULGARIAN SORTS OF FIELD BEANS WITH EXCELLENT TASTE** *Dobri Dobrev, Radka Kancheva, Stajka Stratieva*

INVASIVE SPECIES

- P 7_01** **INVASIVE PLANT SPECIES ALONG THE MAJOR RIVERS IN STRANDZHA NATURE PARK** *Dimcho Zahariev*

ECOLOGY AND EDUCATION

- P 8_01** **„I PRACTISE SOME SPORT = I AM HEALTHY AND FIT” (MODEL FOR BIOLOGY AND HEALTH EDUCATION)** *Hristina Samardzhieva, Peter Lazarov*

16³⁰ - Awards and closing procedure of the “SEMINAR OF ECOLOGY-2014” with international participation.

ABSTRACTS
THEMATIC SESSION I
ANTHROPHOGENIC IMPACT ON LIVING NATURE

L1_01

**ENZYME PROPERTIES OF *HELIX ASPERSA* MAXIMA
HEMOCYANIN**

Yuliana Raynova, Krassimira Idakieva

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Aim: Hemocyanins (Hcs) are oligomeric copper-containing proteins that function as oxygen carriers in the hemolymph of several mollusks and arthropods. Hcs are related to phenoloxidases (POs). Both are type-3 copper proteins and show similarities in their structure, sequence and active site architecture. In the present study, we investigated the intrinsic and inducible phenoloxidase (PO) activity of Hc, isolated from snails *Helix aspersa maxima* (HaH).

Material and methods: HaH was purified from the hemolymph of garden snails *Helix aspersa maxima*. The intrinsic and inducible PO activity of HaH was studied using catechol as substrate. Limited proteolysis by subtilisin DY was used to enhance the PO activity of HaH. The subtilisin-treated HaH, immobilized in a chitosan film thin layer on a glass plate, was used as a biosensor for detection of catechol.

Results: The kinetic analysis of the reaction of catalytic oxidation of catechol to quinone, catalyzed by native HaH was carried out ($K_M = 11.8$ mM; $k_{cat}/K_M = 0.093$ mM⁻¹min⁻¹). Upon treatment of HaH with subtilisin DY more than sixty fold increase in the enzymatic efficiency was achieved ($k_{cat}/K_m = 5.9$ mM⁻¹ min⁻¹). The high proteolytically induced *o*-diPO activity of HaH allowed using it for preparation of a biosensor for detection of catechol. The Hc-based biosensor showed a linear response at different concentrations of catechol, ranging from 0.05 mM to 0.9 mM, and a response time of 5 min.

Conclusion: After activation of Hcs, making the active sites more accessible to the molecules of the substrate, they can function as POs. This fact reveals a potential use of PO activity of Hcs for preparation of biosensors.

Keywords: hemocyanin, phenoloxidase activity, biosensor

Acknowledgements: The authors thank the National Science Fund of Bulgaria (research grant DTK 02/78) for the financial support.

L1_02

**INCREASED MUTABILITY IN *TARAXACUM OFFICINALE*
GENOME CORRESPONDS WITH THE DEGREE OF HEAVY
METAL POLLUTION**

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The aim of this study was to investigate genetic alterations in *Taraxacum officinale* (common dandelion) populations in heavy metal polluted environment. Dandelions reproduce asexually

which makes them suitable for studying of point mutations and large genetic rearrangements obtained as a result of stress.

Materials and Methods: *Taraxacum officinale* plants were collected from four sites covering a range of heavy metal polluted fields. These included two industrial areas around “Kremikovtzi” and “Stomana” metal works, one naturally rich in heavy metals area next to Bosnek village and a control site located north of Lokorsko village at the skirts of Stara planina mountain. Genomic DNA was extracted from leaf tissue and was used for amplifying three DNA loci: the *trnT-trnF* region of chloroplast DNA, the intergenic spacer of ribosomal DNA and the microsatellite MSTA64 locus. Additionally, on the first two markers Restriction Fragment Length Polymorphism (RFLP) analysis was performed.

Results: Using the results from RFLP assays and microsatellite polymorphism analyses we classified the examined plants in several genotype groups including some unique genotypes. A good correspondence between the number of unique genotypes and heavy metal soil pollution at the studied sites was observed.

Conclusion: As unique genotypes are considered to be result of mutations, our findings support the hypothesis that heavy metal stress increases mutational rate in *Taraxacum officinale* populations. Moreover, the frequency of mutations follows the degree of heavy metal contamination. Thus, the genetic markers used in this study are suitable for revealing biomonitoring and the degree of soil contamination with heavy metals.

Keywords: *Taraxacum officinale*, genetic alterations, heavy metal pollution

Acknowledgements: This work is supported by grant № DDVU – 02/61

L1_03

GENOTOXICITY BIOMONITORING OF HEAVY METAL POLLUTION *IN SITU* USING THE COMET ASSAY AND THE MICRONUCLEUS TEST IN NATIVE RODENT

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Exposure to heavy metal pollution represents an important concern for possible long-term health effects.

The **aim** of the present report is: 1) to describe the application and verification of the alkaline comet assay in rodent species in order to detect the possible genotoxicity of heavy metal pollution; 2) a comparison of the comet assay results with peripheral blood micronucleus (MN) assay results performed.

Material and methods: The wood mice (*Apodemus sp.*) and Common vole (*Microtus arvalis*) were collected in polluted region near lead-zinc smelting factory-Asenovgrad (Southeastern Bulgaria) and in background region in Strandja National Park (Southwestern Bulgaria).

The results indicate that there was an increase in cells with DNA damage in individuals environmentally exposed to heavy metal pollution, as demonstrated by the alkaline comet assay and micronucleus assay. The alkaline comet assay showed gender differences in the response, whereas such sex differences were not found in the micronucleated cells. The comet assay results suggest that adult females are the principal population affected by pollutants. The increased levels of heavy metals pollution were associated with the elevated levels of damaged cells observed in the wild rodent.

Conclusion: The comparative analysis of results confirms that the *Apodemus* species may be suitable bioindicators for biomonitoring studies using MN test and comet assay.

L1_04

WATER BUGS (HETEROPTERA: NEPOMORPHA AND GERROMORPHA) AS BIOINDICATORS IN ECOLOGICAL STUDIES

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Aim: To review the possible applications of water bugs (Heteroptera: Nepomorpha and Gerromorpha) as bioindicators in ecological studies.

Material and Methods: Papers from “Web of science” and the search engine “Google scholar” have been selected by key words “indicator” and “Heteroptera”.

Results: Only the articles most relevant to the topic of the current study were selected. The studies show that Gerridae and Notonectidae species can be used as heavy metal indicators. Micronectidae and Aphelocheiridae are good indicators of low trophism and high oxygen content of waters. The species of aquatic Heteroptera (Nepomorpha and Gerromorpha) are suitable as indicators for terrestrialisation of floodplain habitats. The positive relationship between gerromorphan richness and the Habitat Integrity Index could be applied in ecological studies.

Conclusion: The observed data point at the suitability of the water bugs as bioindicators for organic and heavy metal pollution, terrestrialisation and habitat integrity in certain conditions.

Keywords: indicator, Heteroptera, bioindicator, pollution

L1_05

TRAFFIC MORTALITY OF AMPHIBIANS, REPTILES, BIRDS AND MAMMALS ON TWO TYPES OF ROADS BETWEEN PAZARDJIK AND PLOVDIV REGION – SUMMARY OF THE RESULTS

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Aim: The aim of the study is to explore the mortality of birds, amphibians, reptiles and mammals caused by traffic on the Trakiya highway in the section: road fork for the village of Kalugerovo – village of Manolsko Konare - 68 km and one control road section (secondary road - between the town of Belovo and the town of Plovdiv) – 58 km.

Material and Methods: To observe the number of killed amphibians, reptiles, birds and mammals on the highway, 11 one-kilometer transects and on the control section 10 transects were chosen. They were visited in 2009, 2011 and 2012-2013 in 72 days overall. During the visits of transects every animal, the species, sex and age (if possible) and also the exact distance from the end of the road at which the individual was found were registered. The choice of transects was accomplished according to a casual principle.

Results: In total, 4056 corpses of amphibians, reptiles, birds and mammals were found. From them 84% were amphibians, 36.3% birds, 21.6% mammals and 17.1% reptiles. The marsh frog (*Pelophylax ridibundus*) accounted for 84% of all killed animals and 98% of the amphibians.

The result shows that in three studied years on the highway the animal mortality is higher than on the first-class road. There is not a significant difference in number of dead vertebrate animals between the three studied years. If we excluded the large number of migrating marsh frogs the value for the animal mortality on the two types of road changed, but the difference is was only 65 individuals. On the highway the most abundant group of killed animals are birds – 49.6% and on first-class road are amphibians – 36.5% (excluding the migrating marsh frog). For the assessment of monthly dynamic of animal mortality in 2012-2013 transects were visited every month. The results show that the traffic is most dangerous for animals in autumn (146 individuals) and least dangerous in winter (21 individuals). The birds are most vulnerable in autumn and winter, the amphibians – in autumn, the reptiles – in summer and mammals – in spring.

Conclusion: The results from this study show that the amphibians and birds are most affected from the traffic on the two types of roads between Pazardjik and Plovdiv.

Key words: Traffic, mortality, amphibians, reptiles, birds, mammals, Trakia highway

Acknowledgements: This study is funded by National Science Fund of Ministry of Education, Science, Youth and Sport, Contract N 02-303/18.12.2008

L1_06

ZINC POLLUTION OF SOILS AND WATERS NEAR Pb-ZN SMELTER AND ITS TOXICITY IN HUMANS

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Aim: The aim of the study was to investigate the zinc pollution of soils and waters in the area of Pb-Zn smelter near the town of Plovdiv as well as to overview the toxic effects of zinc in humans.

Materials and methods: Zinc concentrations in soil and water samples were estimated according the standard protocols using Inductively coupled plasma – Mass spectrometry ICP-MS (Perkin Elmer) and HR-ICP-AES ULTIMA 2 (JobinYvon, France), respectively. Analyzes were conducted in conformity with ISO 11885, after dissolution of the solid phase with HNO₃ (also in the exchangeable phase after extraction with MgCl₂).

Results: ICP-MS analyzes of zinc concentrations in the three soil samples showed 293, 1810 and 4 490 mg kg⁻¹ or up to 13 times exceeding the Maximum permission levels. In water samples the zinc concentrations were 1.5, 33 and 58 mg L⁻¹ or up to 58 times exceeding the Maximum permission levels. These results demonstrate strong zinc pollution in both soil and water samples in the area. Intoxication with zinc leads to neuronal death, metal fume fever, nausea, vomiting, epigastric pain, diarrhea and elevated risk of prostate cancer. Zinc is responsible for copper deficiency in the body as well as for the altered lymphocyte function. In high concentrations the metal is involved in the regulation of apoptosis. Additionally, it induces the release of cytochrome-c from mitochondria into the cytosol inhibiting the redox reactions of the oxidative phosphorylation. Zinc injuries also the heart as a component of the toxic cascade occurring after ischemia.

Conclusions: The pollution with zinc in the area of the Pb-Zn smelter, Plovdiv is strong. In the soil samples its concentrations exceeds up to 13 times the MPL and in the water samples – up to 58 times. Zinc pollution represents serious health risk for the population in the area.

Keywords: pollution, zinc, toxicity, human health, Pb-Zn smelter

L1_07

PESTICIDES IN THE ENVIRONMENT - SOURCES OF POLLUTION AND EFFECTS ON ECOSYSTEMS

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Application of plant protection products is essential in the modern agricultural practice, the yield and food production would be reduced significantly, without their usage. But there are also some clear evidences that the widespread treatment with these substances leads to the pollution of environment and cause irreversible damages to ecosystems and their inhabitants. There are many pathways for pesticides to penetrate to the environment and their residues may remain there for a long time after the treatment. From the environment, pesticides can easily enter into the food and drinking water and to cause a lasting negative effect on the people and human health. Although pesticides are used to control the pests, they also harm other living organisms in the environment, which are necessary for the maintenance of the ecological balance. Neonicotinoide pesticides are considered particularly dangerous, because of their high persistence in the environment and more pronounced toxic effects. Pesticides from the group of POPs (persistent organic pollutants) are also very harmful for the inhabitants of the ecosystems, because of their long-term stay in the soil and bioaccumulation. One of the most negative consequences of availability of pesticide residues in the soil is the formation of highly toxic metabolites. Another harmful effect from improper application of these substances is the provoking a resistance in the pests, which requires creation of new, more powerful tools to combat them. As a consequence, an increase in the amount and frequency of pesticide treatment in agricultural practice is necessary after that. Timely measures are of a great importance, aimed to reduce the global pesticide use and also, the application of alternative approaches to protect crops from pests.

Key words: pesticides, pollution, environment, ecosystem, pest

L1_08

ESTIMATION OF GENOTOXIC EFFECT OF INSECTICIDE MOSPILAN AT GOLDEN FISH

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Aim: The aim of this study is to determine the genotoxic effect of the insecticide Mospilan in peripheral fish erythrocytes in golden fish (*Carassius aureatus*)

Material and Methods: Concentrations of the insecticide Mospilan (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylethanimidamide) were as follows: in the first aquarium 5 ml insecticide Mospilan / 40 liters of water, in the second one 12 ml insecticide Mospilan / 40 liters of water, in the third one 10 ml insecticide Mospilan / 40 liters of water, in fourth aquarium 8 ml insecticide Mospilan / 40 liters of water. The fifth aquarium was used as a control, without the supplementation of the insecticide Mospilan, it contained only drinking water. The fish we treated for two days.

Results: We determined a higher number of micronucleus in the peripheral erythrocytes of fish treated with insecticide mospilan (for each treated group with insecticide), compared with the control group of 4 micronucleus /2000 erythrocytes)

Conclusions: Based on an investigation we can conclude that the insecticide Mospilan damages the chromosomes of erythrocytes of fish *Carasius aureus*.

Keywords: erythrocyte, fish, insecticide, Mospilan induced, micronucleus.

L1_09

HEAVY METAL CONCENTRATIONS IN VEGETABLES WITH GROWTH STAGE AND PLANT SPECIES VARIATIONS

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Aim: Vegetables constitute an important part of the human diet since there contain carbohydrates, proteins, as well as vitamins, minerals and heavy metals. Heavy metals are one of a range of important types of contaminants that can be found on the surface and in the tissues of fresh vegetables. A number of elements, such as lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), chromium (Cr), Copper (Cu) and Selenium (Se) (IV) can be harmful to plants and humans even at quite low concentrations. Soil pollution is caused by misuse of the soil, such as poor agricultural practices, disposal of industrial and urban wastes, etc.

Material and methods: The research was conducted in order to investigate the concentration of heavy metals in leafy vegetables such as spinach – *Spinacia oleracea*, garlic - *Allium sativum* and onion - *Allium cepa*. Spinach, garlic and onion seeds were sown on 23rd November 2011; samples for analysis of these plants were taken at different stages - 20, 30, 40 and 50 days after sowing.

Results: The results showed that the concentration of lead, zinc, cadmium, nickel, and cobalt increased with increasing age of the plant. The percentage of increase of heavy metals was higher from 20th to 30th day, compared to that between 30th and 40th day.

The result indicated that there was significant difference ($P < 0.01$) in mean heavy metal content in the three vegetable species. The result showed significantly higher level of Pb concentration in amaranth compared to spinach and red amaranth. Spinach exhibited significantly higher levels of Cd and Cr than the other vegetables.

Conclusions: Heavy metal content in different leafy vegetables varies significantly. The content varies with time of harvesting and stage of maturity of crops. The Cd and Cr contents in leafy vegetables in this study were detected higher while Pb and Ni were within the permissible limits as per the WHO standard but all the metals were within the maximum allowable level. The magnitude of time dependence of plant metal concentration variations differed among crop species and metals. Further research is needed to obtain more specific information about the effect of age of the plants on accumulation and distribution of the heavy metal in the different plant parts, variations in uptake between different plant species, cropping history and fertilization.

Key words: Vegetables, soil, heavy metals, concentrations, plant species, correlation coefficient.

BIO-MONITORING OF SOIL SAMPLES FROM THE AREA OF KCM PLOVDIV

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The aim this study was application of a complex of fast, highly sensitive to low doses of contaminants methods for monitoring of soil samples from the area of KCM Plovdiv.

Material and Methods: The wild type *Chlamydomonas reinhardtii* 137C, cultivated in our lab for a long time, was used. Five soil samples from different plots near to KCM Plovdiv were studied. Measurement of heavy metals contents in soil was done by ICP-AES. Extraction was performed with 0.01M CaCl₂ solution for 48h. The cell suspension was treated for 72 h under continuous light on a shaker. The toxic and genotoxic potential of the soil extracts was observed by several methods: *Spot* – test, the growth rate, colony forming ability ("clonal" assay"). The mutagenic potential of the soil samples was assessed by the "visible" mutants - assay is based on changes in size, morphology and pigmentation of surviving colonies and biomarkers for oxidative stress MDA and HSPS70B. One way Anova test was performed.

Results: Soil samples analyzed by affected in a different way the cell survival, growth and mutation rate of algae culture. A genotoxic effect was obtained for soil samples № 1 and № 5. The lower value of HSPs 70B for soil extract № 3 corresponds to the result of the survival test.

Conclusions: Our study indicates correspondence between the results obtained using different tests.

Key words: soil samples, oxidative stress, survival fraction, mutants, biomarkers

Acknowledgements: This work was supported by the Bulgarian Ministry of Education, Youth and Sciences, project number "DTK 01/105" and „Ecological and genetic risk: methods and strategies for overcoming“ – BAS

ASSESSMENT OF THE SENSITIVITY OF *CHLORELLA VULGARIS* TO HERBICIDES WITH DIFFERENT CHEMICAL STRUCTURES

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Aim: In this study, the sensitivity of the green alga *Chlorella vulgaris* to two types of herbicides: atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) and DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) was investigated. These herbicides interact with Q_B-binding site of the photosystem II (PSII) and inhibit its activity.

Material and methods: The degree of the PSII inhibition by atrazine and DCMU was evaluated using PAM chlorophyll fluorescence and polarographic oxygen rate electrode.

Results: Results show higher sensitivity of the primary PSII photochemistry and the oxygen evolution to DCMU than to the atrazine, as well as higher sensitivity of the oxygen evolution in comparison to the PSII photochemistry. Moreover, data also reveal an increased sensitivity of the green algae to both herbicides in comparison to the thylakoid membranes of higher plants.

Conclusion: The higher sensitivity of *Chlorella* cells in comparison to the thylakoid membranes of higher plants could be result from the decreased amount of blocked PSII centers and decreased turnover time of oxygen-evolving centers (i.e. increased rate constants of excited S_i states) in algae. This study can be utilized to use *Chlorella* cells as a sensitive receptor in biosensor systems, applying the principle of the polarographic oxygen rate electrode.

Acknowledgements: The work was supported by the Bulgarian Academy of Sciences.

P1_02

DEVELOPMENT AND APPLICATION OF A COMPLEX APPROACH TO ASSESS CELL TOXICITY OF METAL COMPOUNDS

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Introduction: The prevalence and clinical application of bile acids (BAs) as well as the data on participation of BAs in pathogenesis of several liver diseases and gastrointestinal (colon) tumorigenesis provokes the interest in the biological activity of these compounds and their derivatives.

Aim: The aim of our study was to evaluate the putative cytotoxic/cytostatic effects of metal (Cu, Co, Ni) complexes with bile acids *in vitro*.

Materials and methods: Cell cultures (permanent cell lines and/or primary cultures) of various origins (chicken, mouse, bovine and human) were used as model systems in our study. The investigations were performed after 4h–96h of treatment using methods with different molecular/cellular targets and mechanisms of action such as thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake cytotoxicity assay, trypan blue dye exclusion technique, crystal violet staining, double staining with acridine orange and propidium iodide, single cell gel electrophoresis, FACS analysis.

Results: Our results revealed that the compounds exerted affect to varying degrees on the survival and proliferation of the treated cells in a time- and concentration- dependent manner. A positive correlation between the experimental data obtained by the different methods was observed. Applied independently, the ligands express lower cytotoxic/cytostatic activity as compared to the metal complexes.

Conclusion: The above mentioned approach could be used as fast and reliable tool for cell toxicity assessment of different compounds.

Key words: bile acids, metal complexes, cell cultures, cytotoxic/antiproliferative activity

Acknowledgement: Supported by a bilateral project between Bulgarian Academy of Sciences and Romanian Academy and by grant **BG051PO001-3.3.06-0048** from **04.10.2012** of the Operative Programme “Development of Human Resources” of the European Social Fund.

P1_03

INFLUENCE OF LOW LIGHT AND HIGH LIGHT INTENSITIES ON ELECTROKINETIC POTENTIAL OF THYLAKOID MEMBRANES FROM *HORDEUM VULGARE* L.

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Aim: The electrokinetic properties of thylakoid membranes of WT and mutant lines named Chlorina *f2* and Chlorina126 were measured at various illuminations (low light intensity and high light intensity).

Materials and methods: Chloroplasts from barley (Wild type *Hordeum vulgare* L.) and mutant lines Chlorina *f2* and Chlorina126 were isolated. For WT- the main electrokinetic parameters (electrophoretic mobility, zeta potential, surface charge density and lipid peroxidation) were measured by cell electrophoresis. Electrophoretic mobility, zeta potential, surface charge density and lipid peroxidation at various light intensity (1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) were measured for WT, Chlorina *f2* and Chlorina126.

Results: Mutant Chlorina *f2* (LL) is characterized by a low electrokinetic potential as compared to the wild type WT and mutant Chlorina 126.

Conclusion: High intensity illumination of barley plants (Wild type, Chlorina *f2*, Chlorina 126) did not significantly affect their electrophoretic mobility, zeta potential and surface electric charge of their thylakoid membranes.

P1_04

INFLUENCE OF SONIFICATION AND LECTIN ON THYLAKOID MEMBRANES FROM *HORDEUM VULGARE* L.

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Aim: To track changes of electrokinetic’s parameters after the treatment with ultrasound and at presence of lectin (Phytohemagglutinin – P) on isolated chloroplasts of WT barley.

Materials and methods: Chloroplasts from barley (Wild type *Hordeum vulgare*.L) were isolated. The main electrokinetic parameters (electrophoretic mobility, zeta potential, surface charge density and lipid peroxidation) before and after sonification with US = 0.824 kJ or 1.820 kJ in the presence of various concentrations of lectin were measured by cell electrophoresis.

Results: At increasing concentration of lectin in the treatment of thylakoid suspension at dose 1.820 kJ we observed raising of electrophoretic mobility, zeta potential, surface charge density and in 0.824 kJ their lowering.

Conclusion: After the sonification of thylakoid membranes specifically formed aggregates were observed. This was demonstrated by electrophoresis of sonicated chloroplasts. They form aggregates with irregular shape and large size after sonification.

P1_05

SPECTRAL REFLECTANCE SIGNATURES IN SOIL SALINITY STUDIES

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Aim: Soil salinization as a result of natural or human-induced processes is a serious global-scale problem. Numerous studies and efforts in controlling soil salinity have been made. Nearly 60 percent of the salt-affected soils around the world are in irrigated farmlands, and this trend is increasing. Salinization is a major reason for degradation of soil resources and decline of soil fertility. From an ecological and economic point of view it is extremely important to establish both the occurrence and distribution of soil salinization and the intensity of the process. The goal of this study is to examine soil spectral reflectance properties as detectors and quantitative indicators of soil salinity.

Materials and Methods: Remote sensing techniques are widely used in soil surveys for land assessment and monitoring the spatial distribution and temporal change detection of salt-affected soils. In this work, some results are presented from spectrometry studies of saline soils. Ground-based and airborne reflectance measurements in the visible and near infrared ranges of the electromagnetic spectrum were performed over soils with varying degree of salinity. Different data processing methods were applied to relate soil spectral response to salinity.

Results: The specific information carried by the spectral reflectance features was used to identify saline soils and assess the degree of salinization.

Conclusions: The practical application of the research results requires a greater number of *in situ* and remote sensing measurements of soils with different types of salinization, and especially of soils with lower salinity.

Keywords: remote sensing, spectral reflectance, soil salinization

P1_06

MACROPHYTE-BASED ECOLOGICAL STATUS ASSESSMENT OF SUB-MEDITERRANEAN RIVERS IN BULGARIA

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The Water Framework Directive (Directive 2000/60/EC) requires ecological assessment of water bodies with biological communities.

Aim of study: an overview was given on the Sub-Mediterranean Rivers and their ecological assessment based on macrophytes as a biological quality element.

Material and methods: Data for thirteen rivers in South Bulgaria studied in 2009 are presented together with site descriptions (flow velocity, shading, mean depth, substrate type, and altitude) and environmental variables (acidity, electrical conductivity, dissolved oxygen and oxygen saturation, NH₄, NO₃, PO₄, total nitrogen and phosphorus, COD and BOD₅).

Results: Two river sites were characterized by absence of macrophytes, while additional two rivers were dried out. At the remaining nine sites 40 taxa were identified, among them 7 bryophytes and 1 charophyte. The 32 vascular plants registered, included 11 hydrophytes and 21 helophytes. The most frequently distributed hydrophyte was *Potamogeton natans* L. (registered at 5 sites, relative abundance 56%), while the most common species of the group of helophytes was *Sparganium erectum* L. (registered at 6 sites; relative abundance 67%).

Conclusion: The assessment of the river sites according to the adopted “Reference Index” resulted predominantly in high and good ecological status.

Acknowledgments. The research was a part of the project: “Developing classification system towards ecological status and potential assessment of the defined surface water types (rivers and lakes) on Bulgarian territory (in compliance with System B)” executed by Consortium for Biomonitoring, financed by Operational Programme Environment 2007-2013.

Keywords: macrophytes, sub-Mediterranean rivers, WFD, ecological status

P1_07

OZONE MONITORING IN THE RHODOPES

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The aim of the present study was to determine the changes in the levels of tropospheric ozone in the Rhodopes for the period 2009 to 2013. For the achievement of this goal a profound analysis of the data was performed and as a result of the potential risk for the forest ecosystems in this region was assessed.

Material and methods: The study was carried out on the territory of the Rhodopes and data obtained from Yundola air quality monitoring station and Rojen air background control station were used. Both stations are part of the national monitoring systems for air quality in forest ecosystems. Yundola monitoring station is located in western Rhodopes at 1600 m altitude on the border of a forest-vegetation area far from local sources of pollution. The region is representative for the coniferous forests. The main tree species are spruce (*Picea abies*) and fir (*Abies alba*). Rojen air background control station is located in the southern part of the Rhodopes at 1750 m altitude, far from local pollution sources. The main tree species is spruce (*Picea abies*).

The data collecting procedure was ensured by the “National Automated System for Environmental Monitoring (NASEM)”, with sampling duration of 24 hours.

Results: The ozone concentrations measured at Yundola monitoring station exceeded the target value for the protection of vegetation in terms of cumulative exposure over a threshold ozone concentration of 40 ppb (AOT40) 1.6 to 2.9 times. In the same time there was a trend of a steady increase of ozone levels. The situation at Rojen air background control station did not differ significantly. The ozone background levels were 2.5 times above the target value, a decrease in the ozone levels was observed only in 2010 where the concentration was 1.1 above the threshold.

Conclusion: The high ozone concentrations measured in Rhodopes could be a precondition for emergence of ozone injuries on leaves.

Key words: tropospheric ozone, target value, ozone AOT40

P1_08

TAXONOMIC COMPOSITION OF THE FUNCTIONAL FEEDING GROUPS OF BENTHIC FAUNA AT SOME BULGARIAN RIVERS

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Aim: The study is aiming at describing the taxonomic composition and abundance of functional feeding groups of the benthic macro invertebrate communities at specific sites, differently influenced type, located in four Bulgarian Rivers, which are belonging to three water catchments.

Material and Methods: The trophic structure of benthic communities was studied in rivers Mesta, Tundza, Vit and Veleka during three seasons in 2011 and 2012. The benthos was collected fusing an adapted version of multihabitat sampling method. In parallel, some physical and chemical parameters of the aquatic environment were measured. After primary and secondary processing of the samples, species/taxa established were referenced to six functional feeding groups (shredders, scrapers, collectors, filterers, deposit feeders and predators).

Results: The highest taxonomic diversity was found in groups sensitive to anthropogenic impacts - scrapers, followed by predators and shredders, while the least richness was registered in ones of deposit feeders, filterers and collectors. Changes in taxonomic composition within each functional group varied depending on seasonality as it is determined by the speed and stages of individual development, as well as the strength, duration and type of anthropogenic impacts. In unaffected sites, environmental parameters influenced changes within each functional group. Density of the trophic groups altered proportionally to the degree and nature of anthropogenic impact pressures. Dominant role in the transformation of species composition played external impact on the ecosystem, while shredders and scrapers were characterized with the highest number of taxa.

Conclusion: Taxonomic composition of functional feeding groups was shown as a dynamic characteristic which responded appropriately to external influences on the water ecosystems. Life cycles, seasonality and environmental factors played a structurally important role for the quantitative and qualitative characteristics of the trophic structure of riverine benthic macroinvertebrate communities.

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Keywords: macroinvertebrate, trophic structure, functional feeding groups, rivers

P1_09

PHOTOSYNTHESIS AND GROWTH RESPONSES OF FIVE PAULOWNIA LINES TO SALT STRESS

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Aim: *Paulownia* species are high-yielding trees that can be used for the production of energy, paper pulp and wooden building materials. The species is highly suitable to revalidate agricultural deserted areas, to reclaim mining areas, or to restore contaminated sites. The effect of salt stress on some growth parameters and photosynthesis in five *Paulownia* lines (*P. tomentosa x fortunei* – TF01, *P. elongata x fortunei* – EF02, *P. elongata x fortunei x elongata* - T2, *P. elongata x elongata* - T4, *P. elongata x kawakamii* - EK), grown in hydroponic at three levels of salinity, 50 mmol/l, 100 mmol/l, 200 mmol/l NaCl solution was analyzed.

Material and Methods: Seeds and *in vivo* explants from the species *P. tomentosa* and *P. elongata* and their hybrids with *P. fortunei* and *P. kawakamii* were used for developing of *in vitro* multiplication protocol, licenced by Bio Tree Ltd., Bulgaria. *In vitro* propagated plants were transferred to nutrient solution for 48 days and then were treated with NaCl solutions for 10 days. Leaf area was calculated using software program SigmaScan Pro 5. Net photosynthetic rate, stomatal conductivity and transpiration rate were measured by photosynthetic portable system LI-COR 6400.

Results: The ratio total leaf area/ leaf number decreased in the order: EK>T4>EF02>T2>TF01 for control variants of all lines. With increasing salinity levels this parameter increased for T2>T4 and decreased gradually for the other three lines. Net photosynthetic rate, and namely stomatal conductivity and transpiration rate declined strongly, but water use efficiency of five lines was enhanced.

Conclusions: Our results suggest that under salinity conditions TF01 line was characterized with highest rate of photosynthesis and lowest water use efficiency, while for T4 tendency was opposite.

Key words: salinity, gas exchange, growth, *Paulownia*

P1_10

TRACE ELEMENTS IN SOILS AND HERBS IN THE VICINITY OF SMELTER “KCM 2000”-PLOVDIV (BULGARIA)

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Aim: Area of smelter "KCM 2000"-Plovdiv, is one of the "hot spots" in Bulgaria in terms of contamination with trace elements. For the purposes of this study four test sites were chosen at various distances from the smelter, according to the wind rose. In each one both soil and herb samples were collected simultaneously. Concentrations of As, Cd, Cu, Hg, Ni and Pb in all samples were analyzed by ICP-MS. Coefficient of soil technogenic pollution and the coefficient of bioaccumulation of the studied trace elements in herbs were calculated. Our results showed that the soils in the vicinity of “KCM” are priority contaminated with Pb, Cd, Ni and As.

Plantago lanceolata and *Carex divisa* were found as accumulators of Pb, Cd and Cu, and *Papaver rhoeas* - of Cd, Hg and As.

Material and Methods: For the purposes of the study four test sites were chosen at various distances from the smelter, according to the wind rose. Each site was subdivided to 5 sampling squares, and both soil and plant samples were collected simultaneously (BDS 17.4.5.01-85). Sample preparation and mineralization were performed according to standard ISO 11466.

Concentrations of As, Cd, Cu, Hg, Ni and Pb in the all samples were analyzed by ICP-MS method with instrument Agilent 7700 (2009). Concentrations of studied elements in soil samples were compared with the Bulgarian hygiene norms (Regulation Norm 3, 2008). Coefficient of soil technogenic pollution (C_t) and the coefficient of bioaccumulation (C_b) of the studied trace elements in plants were calculated. Raw data were processed using statistical software package Statistica 7.0 (StatSoft Inc., 2004).

Results: We found that the concentrations of studied trace elements were higher in sampling sites situated in South direction from the smelter (Site 2, 3 and 4) in comparison with those in North direction (Site 1). Distance from the emitter also had a considerable effect to the contamination level which is in agreement with other authors (Henderson *et al.*, 1998; McMartin *et al.*, 2002; Martley *et al.*, 2004; Sichorova *et al.*, 2004; Dimitrova & Yurukova, 2005). Coefficient of soil technogenic pollution was found as follows: As – from 0.1 (Site 1) to 16 (Site 4); Cd – from 1.7 (Site 1) to 30 (Site 2); Cu – from 0.35 (Site 1) to 1.4 (Site 2); Hg – from 0.06 (Site 1) to 0.94 (Site 2); Ni – from 1.3 (Site 1) to 2.3 (Site 3); Pb – from 2.9 (Site 1) to 63.8 (Site 3). Values of the coefficient of bioaccumulation of the studied trace elements confirmed the poor ability of Cu, Ni and Pb to move from soil to plants. *Plantago lanceolata* and *Carex divisa* showed similar bioaccumulation levels for Cu ($C_b=0.1-0.14$), Ni ($C_b=0.1-0.12$) and Pb ($C_b=0.04-0.08$). *Plantago lanceolata* was found as better accumulator of Cd ($C_b=1.3$), followed by *Papaver rhoeas* ($C_b=1.1$). From the studied plant species, only *Papaver rhoeas* showed good bioaccumulation of As ($C_b=2.6$), and Hg ($C_b=2.0$).

Conclusion: Soils in the studied area are contaminated with Pb, Cd, Ni and As. Of the studied plant species, *Plantago lanceolata* and *Carex divisa* were found as bio accumulators of Pb, Cd and Cu, and *Papaver rhoeas* – of Cd, Hg and As.

Keywords: trace elements, soil pollution, bioaccumulation, smelter “KCM 2000”-Plovdiv

P1_11

HYPOGLYCAEMIC EFFECTS OF GLYPHOSATE BASED HERBICIDE ON COMMON CARP (*CYPRINUS CARPIO* L.) AND BIGHEAD CARP (*ARISTICHTHIS NOBILLIS* RICH.) LIVER

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Aim: In the present work the main objective was to study the impact of a glyphosate based herbicide on glycogen storage in common carp (*C. carpio*) and bighead carp (*A. nobillis*) liver using PAS-reaction on cryosections. We used different concentrations of the test herbicide in laboratory conditions for 96 hours. Results showed glycogen storage depletion in the liver of both fish species with increasing the herbicide concentration. We observed a clearer tendency towards glycogen depletion in the bighead carp liver which indicated a higher sensitivity to glyphosate.

Material and Methods: Histochemical analysis was performed according standard хистохимична procedure described by Pearse (1972) at the Department of Anatomy, Histology and Embryology at Medical University of Plovdiv, Bulgaria. Positive PAS-reaction was

presented in purple-magenta staining and the degree of intensity of histochemical staining of all specimens, including control fish livers were appraised semi-quantitatively by using the grading system of Peebua (2006), which we slightly modified: (-) – negative reaction of histochemical staining; (+/-) – weak positive reaction of histochemical staining (weak purple staining); (+) – moderate positive reaction of histochemical staining (purple-magenta staining); (++) – strong positive reaction of histochemical staining (intense purple-magenta) ; (+++) – strong positive reaction of histochemical staining in the hepatocytes (intense magenta).

Results: We found positive PAS-reaction in all test groups, including control. In the fish group exposed to 20 mg L⁻¹ and the control we observed a similar degree of expression of PAS-reaction in both fish species. Degree of expression of histochemical staining in the common carp liver was moderate and in the bighead carp liver was strong positive. At the higher herbicide concentrations of 40 mg L⁻¹ and 72 mg L⁻¹ we found a weak positive PAS-reaction which indicates depletion of glycogen storage in the liver of both fish species.

Conclusions: Changes in liver glycogen indicate that carbohydrate metabolism in fish might be impaired under the effect of this herbicide (Shrivastava, 2007; Hassanein & Okail, 2008). Positive PAS-reaction which we observed in common and bighead carp showed a similar degree of expression of histochemical staining. Tendency was towards glycogen depletion with increasing the herbicide concentrations. We consider that the reduced intensity of PAS-reaction is probably a result of metabolic changes due to glycogen depletion. This trend was more pronounced in the liver of bighead carp which indicates that this fish species is more sensitive to the herbicide effects. We also think that this chemical may impact the whole organism, not just the liver. Therefore, we suggest that further investigations need to be carried out.

Keywords: glyphosate, Pas-reaction, glycogen, fish

P1_12

EFFECTS OF METAL-CONTAMINATED WATERS OF TOPOLNITSA RESERVOIR ON GILLS OF EUROPEAN PERCH (*PERCA FLUVIATILIS* L.)

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Aim: In the present study we measured As, Cd, Cu, Ni, Pb and Zn concentrations in surface water of Topolnitsa reservoir, which has been impacted in the last few decades due to copper ore extraction and smelting operations in the region. We also measured the metal concentrations in gills of European perch (*Perca fluviatilis* L.) and observed the gill morphological structure. Metal concentrations in the water samples varied in all three seasons but they were significantly higher in the summer. Furthermore, As and Cu were above the maximum permissible levels set by law. Gill metal concentrations were significantly higher than in the water. We also observed serious gill histological alterations such as lamellar lifting, oedema, proliferation of epithelial cells, vasodilatation in the secondary lamellae, and aneurysms which were also more pronounced in the summer.

Material and Methods: Water samples for metal analysis were collected according to ISO 5667-6:2012 standard using a boat once every season – spring, summer, and autumn in 2012. During the field trip pH, temperature (°C), and conductivity (μS cm⁻¹) were recorded, simultaneously, using a pH-meter (Multi 340i, WTW). Water was analysed for metals according to the ISO standard 17294-2 (22) with an Agilent 7500ce (Agilent Technologies, Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS), and the findings are reported as μg L⁻¹. Detection limit of the instrument was 0.5 μg L⁻¹ for As, 0.05 μg L⁻¹ for Cd, 0.5 μg L⁻¹ for Cu, 0.5 μg L⁻¹ for Ni, 10 μg L⁻¹ for Pb, and 10 μg L⁻¹ for Zn.

Fish samples were collected by the international standard procedures for determination of metal accumulation given in the EMERGE Protocol. Gills were analysed with ISP-MS (Agilent 7500ce, Japan) and reported as $\mu\text{g kg}^{-1}$ wet weight. Detection limit of the instrument was: $10 \mu\text{g kg}^{-1}$ for As, $1 \mu\text{g kg}^{-1}$ for Cd, $10 \mu\text{g kg}^{-1}$ for Cu, $10 \mu\text{g kg}^{-1}$ for Ni, $30 \mu\text{g kg}^{-1}$ for Pb, and $30 \mu\text{g kg}^{-1}$ for Zn.

For histological analysis gills were placed in vials with 10% neutrally buffered formaldehyde solution (pH = 7) for 12-24 h. They were rinsed in tap water, dehydrated in a graded series of ethanol concentrations, cleared in xylene, embedded in paraffin wax with melting point of 54-56°C, sectioned to a thickness of 5-7 μm using a rotary microtome and mounted on sterilized glass slides. Sections were then deparaffinised, stained with hematoxylin and eosin (H&E) for histological examinations and prepared for light microscopy analysis (Takashima and Hibiya, 1995). Histological changes in the gills were observed and photographed by using a microscope, mounted with a digital camera. Gill histology of all specimens were appraised individually and semi-quantitatively by using the grading system of Peebua et al. (2006) which we slightly modified. Evaluation of the histological changes was carried out and presented as an average value in percentages.

Results: Overall, water concentrations varied in the spring, summer and autumn. We measured higher metal concentrations in the summer which were significantly different than in the other seasons. In addition, As and Cd water concentrations in summer and Cu water concentrations in all three seasons were above the maximum permissible concentrations set by the Bulgarian law. In contrast, Pb water concentrations were below detection limit of the instrument during the whole period of study. Gill metal concentrations were significantly higher than in the water, but they did not differ significantly. Histological alterations were classified in three main groups: 1) proliferative changes – lamellar lifting, oedema, proliferation of epithelial cells, and lamellar fusion; 2) degenerative changes of gill lamellae, and 3) changes in the blood vessels – vasodilatation in the main blood sinus, secondary lamellae, and aneurysms. Extent and severity of each particular lesion varied in all three seasons, but in general they were more pronounced in summer.

Conclusions: We can conclude that Topolnitsa reservoir waters are metal-contaminated with As, Cd and Cu. Fish gills accurately reflected the impaired water quality and process of bioaccumulation. We also found that the gill morphological structure is seriously altered which we link with the negative metal effects. Thus, we consider that the fish gill metabolism and functions may be disturbed, and we suggest that further investigations need to be carried out in this particular area.

Acknowledgments: We would like to thank all colleagues from the regional accredited laboratory of the Executive Environment Agency of Bulgaria for helping with metal analyses.

Keywords: metals, freshwater, fish gills, histology

P1_13

CFGE A PERSPECTIVE METHOD FOR DSBs DNA DETECTION IN HUMAN LYMPHOCYTES *IN VITRO*

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A lot of environmental genotoxins could seriously damage DNA inducing DSBs which if not repaired can cause cancer or other serious diseases in the organism. Detection of DNA DSBs induction is of interest for many studies in which a variety of methods are used.

Aim: The main question we address in this study is to adapt and use constant field gel electrophoresis (CFGE) to investigate sensitivity of human lymphocytes from various donors to DNA DSBs inducer zeocin.

Material and Methods: Lymphocyte cultures were prepared by Phicol-paque gradient centrifugation from peripheral venous blood of clinically healthy donors (smokers and non-smokers) age between 27 and 45 years. CFGE (modified in our lab) were applied to obtain DNA DSBs induced by radiomimetic zeocin in human lymphocytes. The movement of DNA out of the starting wells into the electrophoresis gel has been measured using UV-gel scan and computer analysis of DNA-ethidium bromide fluorescence (Syngene software; GeneTools).

Results: We found that zeocin (100-200 µg/ml) induces DSBs in all donors – both smokers and non-smokers. The effectiveness of action of the radiomimetic depends on the concentration.

Conclusion: This study indicated that CFGE is effective and appropriate for DNA DSBs detection in human lymphocytes *in vitro*.

Acknowledgements: This work is supported by the grant of the Bulgarian Ministry of Education, Youth and Science project: “Evaluation of DNA protective potential of bioactive natural compounds towards DNA damaging agents”, BIONATPROT, BSTC/ Slovakia/01/1.

Keywords: DSBs, CFGE, human lymphocytes, zeocin

P1_14

POTENTIAL THREATS FOR AIR POLLUTION IN WESTERN RHODOPES: A REVIEW

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Western Rhodopes were especially selected as a study area because are free of local industrial emissions and incorporate background monitoring station ‘Rozhen’. Nevertheless, traditional sources of atmospheric pollution together with cross-border pollution have a serious potential impact. A review of existing assessment and ecological monitoring programs dealing with air quality issues in the territory of Western Rhodopes has been performed. It allows the identification of the most dangerous sites for the air health damages which should be used as relevant sites for biological monitoring. Further identification of biomonitors for air evaluation will facilitate the monitoring operation, its development and harmonization in the Western Rhodopes region.

The review process has taken into account 12 literature sources, which could be classified into 3 broad categories: reports on the state of environment of the Regional Inspectorate of Environment and Water - Smolyan, municipal programs and reports under the Conservation of Globally Significant Biodiversity in the Landscape of Bulgaria’s Rhodope Mountains Project (Rhodope Project).

This review registered around 3 potential threats (solid fuels for domestic heating, *vehicle pollution and combustion plants*) and a large number of point and diffuse sources of pollution. This preliminary work will pave the way for further biomonitoring with mosses in the Western Rhodopes region.

Keywords: air pollution, Western Rhodopes, monitoring

THEMATIC SESSION II

MECHANISMS OF ADAPTATION OF LIVING SYSTEMS

L2_01

INTERDISCIPLINARY INTERACTION FOR THE BIOTECHNOLOGICAL DEVELOPMENT OF BALKAN MEDICINAL PLANT SPECIES

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Aim: Develop biotechnological approaches for the controlled delivery of biologically active compounds of medicinal and aromatic plants characteristic for the Balkan region.

Material and methods: Investigated plant species were chosen on an ethnobotanical principle. Representatives of the *Hypericum* and *Pulsatilla* genera, *Sideritis scardica* Sofia 2 cultivar, *Inula britannica*, *Artemisia alba* were collected from Bulgaria. Tissue culture initiation and media optimizations were performed at the facilities of the Institute of Organic Chemistry with Centre of Phytochemistry, BAS. Polyphenolic contents, enzymatic activities, molecular markers of oxidative stress were measured spectrophotometrically. Structural and functional alterations of photosynthetic membranes were characterized by 77 K fluorescent microscopy, electrophoretic profile by 10% SDS-PAGE. Essential oils were prepared by micro-steam distillation and non-volatile chemical constituents by ultrasonic extraction.

Results and Discussion: Through modification of plant growth regulators supplementations and vitamin content, optimizations were achieved affording stimulation of polyphenolic content in the *in vitro* cultured plants. Studies of physiological status provided evidence of interrelations between enzymatic and non-enzymatic defense of the plants regarding polyphenolics biosynthesis *in vitro*. Three different culture systems were established for production of essential oils with modified terpenoid profile for *A. alba*. Physiological studies indicated relations between morphogenetic response to plant growth regulators, structure and function of photosystem II and electrophoretic profile of the plant.

Conclusions: Obtained results will be used for scientifically based targeted delivery of plant material with defined secondary metabolite profile. Further research is in progress to evaluate the potential biological activity of extracts, fractions and individual compounds of the studied *in vitro* culture systems.

Acknowledgment: Swiss National Science Foundation in the Framework in the Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZO_142989; DO2-1153)

Keywords: *in vitro* culture optimization, secondary metabolite production, Balkan medicinal and aromatic plants

L2_02

ANTIMUTAGENIC AND ANTICARCINOGENIC EFFECT OF AMYGDALIN IN *SACCHAROMYCES CEREVISIAE*

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Aim: To evaluate the antimutagenic and anticarcinogenic effect of Amygdalin in *Saccharomyces cerevisiae*

Materials and methods: Frequencies of three genetic events - mitotic gene conversion in the *trp5* locus, mitotic crossing-over in the *ade2* locus, and reversion of the *ilv1-92* allele in *Saccharomyces cerevisiae* strain D7ts1 were measured. Frequency of Ty1 retrotransposition expressed as increasing the number of histidine prototrophic colonies (His⁺) in haploid strain 551 rho⁺ of *Saccharomyces cerevisiae* was established. The standard MTT-dye reduction assay for *in vitro* activity testing of amygdalin was performed on cancer cell cultures (HepG2, HT-29) and normal (3T3, BJ) cell lines. Amygdalin was used as antimutagen and anticarcinogen, methylmethane sulphonate as mutagen and chromiumVI (CrVI) as carcinogen.

Results: Pretreatment with 25, 50 and 100 µg/ml amygdalin decreased the frequencies of gene conversion 2-3 fold, reverse mutations 3-fold (only 100 µg/ml) and total aberrants 2-3-fold (50 and 100 µg/ml) induced by MMS.

Treatment with 10 mg/ml amygdalin reduced the survival of cancer cell lines HepG2 и HT-29 to 60% at the 96th hour. The survival of the normal human cell line BJ at the 96th hour decreased 95%, and for the mouse line BALB/c 3T3 - 80%.

Amygdalin at concentrations 25, 50, 100 and 200 µg/ml significantly decreased the levels of transposition from 3 to 5 times respectively in comparison with control and stimulated the cell survival.

Conclusion: The results obtained in this study confirm the literature data about the ambiguous actions of amygdalin.

L2_03

STRESS RESPONSE OF *CHLAMYDOMONAS REINHARDTII* STRAINS TO ZEOCIN DEPENDS ON THE GENOTYPE

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Aim: evaluation of the stress response to zeocin of strains of *Chlamydomonas reinhardtii* with different genotype.

Material and Methods: Four *C. reinhardtii* genotypes with different DNA repair capability were used - two DNA repair proficient (137C, WT and CW15, cell-wall-less) and two DNA repair deficient (UVS-10, *recombination*-repair deficient and UVS-14, *mismatch*-repair deficient). Different endpoints were applied: cellular - spot-test, micro-colonies survival assay; biochemical (MDA, H₂O₂ and carotenoids quantity) and molecular – double-strand DNA breaks (DSBs) induction. Radiomimetic zeocin was used as an inducer of oxidative stress.

Results: Response to zeocin was shown to vary depending on the genotype and concentrations. Genotypes differ in their sensitivity to zeocin. 2-10 fold higher concentration was found to induce LD₅₀ in a WT, comparing with concentrations inducing the same level of lethality in both DNA repair deficient genotypes and cell-wall-less genotype. Differences in DNA susceptibility of genotypes were also obtained. The most pronounced increasing of DSBs after low doses zeocin (2 - 10 µg mL⁻¹) treatment was measured for *rec*-repair deficient genotype (P < 0.01) - about 5-fold higher than the level of induced DSBs in *mismatch*-repair deficient genotype (P < 0.01). The strongest oxidative stress response to zeocin, measured as highest MDA (P < 0.01) and H₂O₂ contents (P < 0.001) was obtained in *mismatch*-repair deficient genotype.

Conclusions: Two DNA-repair deficient genotypes and cell-wall-less genotype have shown to express higher susceptibility to zeocin in comparison with WT. The DNA of the *rec*-repair deficient genotype was more susceptible to low doses of zeocin. Biochemical data demonstrate that *mismatch*-repair deficient genotype undergoes the strongest oxidative stress.

Acknowledgements: This work was funded by the Bulgarian Ministry of Education and Science (B-1520 and BioCORE), and joint research project between BAS and RAS “Molecular mechanisms of induced resistance to oxidative stress in plants”.

Keywords: genotype susceptibility, *C. reinhardtii*, zeocin, oxidative stress

L2_04

PROTECTIVE ACTIVITY OF DIFFERENT EXTRACTS OF *CLINPODIUM VULGARE L.*

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Aim: To test the potential of *Clinopodium vulgare L.* (Wild basil) to scavenge free radicals and to protect plasmid DNA.

Materials and methods: Phytochemical analysis of wild basil was performed with gas chromatography–mass spectrometry (GC-MS). Four *Clinopodium vulgare L.* water extracts (total, leaves, flowers, stems) and BuOH extract from the above-ground parts of the plant were studied using DPPH assay and DNA topology assay.

Results: *Clinopodium vulgare L.* extracts showed strong antioxidant activity compared to standard - ascorbic acid. Based on the results obtained extracts could be arranged as followed: leaves, total, flowers, stems, BuOH. No DNA damaging activity of extracts was revealed using DNA topology assay. All of them were able to protect plasmid DNA against damaging action of Fe³⁺.

Discussion: Here it was shown that different extracts of *Clinopodium vulgare L.* possess strong scavenging and DNA protective activities. The most pronounced scavenging and DNA protective activities were observed for leaf extract.

Conclusion: Our preliminary results provide information for potential protective properties of different *Clinopodium vulgare L.* extracts. The further investigation of their antimutagenic and anticarcinogenic activities is in a progress.

Acknowledgements: This study was supported by project DNTS/Slovakia/01/1 and „Ecological and genetic risk: methods and strategies for overcoming“ – BAS.

Keywords: *Clinopodium vulgare L.*, DPPH, DNA topology assay, gas chromatography–mass spectrometry

L2_05

ANTIGENOTOXIC EFFECT OF SALVIA EXTRACT AGAINST OXIDATIVE DNA DAMAGE INDUCED BY ZEOCIN

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Aim: The aim of the present study was to investigate the anticytotoxic and anticlastogenic potential of *Salvia officinalis* water extract against DNA damage induced by the radiomimetic zeocin on two types of experimental test-systems – *Hordeum vulgare* (root tip meristem cells) and human lymphocytes *in vitro*.

Material and Methods: the cytotoxic effect was assessed by the value of the mitotic index, and DNA damage - by the induction of chromosome aberrations and micronucleus.

Results: *Salvia* water extract alone has none to low cytotoxic and clastogenic activities depending on the tested concentrations. The *Salvia* extract decreases the frequencies of chromosome aberrations induced by zeocin. These data were obtained using two types of experimental schemes - conditioning treatment with non-toxic concentrations of *Salvia* extract prior to high damage concentrations of zeocin with inter-treatment time and treatment without any inter-treatment time.

Conclusion: Our results indicated that water extract of *Salvia officinalis* possesses potential to reduce the cytotoxic and clastogenic effects of zeocin in human lymphocytes as well as in root tip meristem cells of barley. Data could be useful for further investigations in order to use in pharmacology.

Acknowledgements: This work is supported by the grant of the Bulgarian Ministry of Education, Youth and Science project: "Evaluation of DNA protective potential of bioactive natural compounds towards DNA damaging agents", BIONATPROT, BSTC/ Slovakia/01/1.

Keywords: *Salvia officinalis*, anticlastogenic effect, test-systems, zeocin

L2_06

COMPARISON OF BIOCHEMICAL AND MOLECULAR RESPONSES OF *PHASEOLUS VULGARIS* CULTIVAR AND THREE MUTANT LINES TO DROUGHT STRESS

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Aim: To compare the stress response of *Phaseolus vulgaris* cultivar and three mutant lines to drought stress.

Material and Methods: Three mutant lines (D₂-0,0031 M NEU – №2; D₂-0,0062 M EMS – №3 and D₂-0,0125 M EMS - №6) derived by the methods of chemical mutagenesis from cultivar Dobrydjanski 2 were studied. Plants at the third leaf phase were split into three groups: control group and treated with two concentrations of polyethylene glycol (PEG - MW 10 000) for 24h.

Biochemical and molecular markers - malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline (Pro) and heat shock protein content (HSP70B) were analyzed.

Results: Dose-dependent accumulation of MDA and H₂O₂ was found for all treated genotypes. Change of content of these markers could be considered as oxidative damages, or as a signal function. Mutant line 2 and 6 were characterized with higher levels of MDA and H₂O₂ compared with Dobrudjanski 2 and line 3. The levels of MDA and H₂O₂ in Dobrudjanski 2 and line 3 were similar. The results showed that concentration of 16 % PEG induced stronger response than 8 % PEG concentration in all genotypes.

Conclusions: Based on the lower levels of MDA and higher levels of proline and HSP70B after the treatment with two concentrations of PEG it could be speculated that Dobrudjanski 2, Line 2 and Line 6 are more drought resistant compared with Line 3. Higher content of HSP70B after the treatment with 8 % PEG in Line 3 could be considered as a compensatory mechanism.

Acknowledgements: This study was funded by the projects: DDVU_02/87 “Complex morphometric, physiological, biochemical and molecular assessment of drought tolerance in Bulgarian common bean genotypes (*Phaseolus vulgaris* L.)”, „Ecological and genetic risk: methods and strategies for overcoming“ – BAS and “Biochemical and molecular markers of drought tolerance in Bulgarian common bean genotypes” – scientific cooperation between RAS and BAS.

Keywords: biochemical and molecular markers, drought stress, *Phaseolus vulgaris* L.

P2_01

EFFECT OF THE SALINITY OF THE SOIL ON THE FUNCTIONS OF THE PHOTOSYNTHETIC APPARATUS OF TWO LINES OF *PAULOWNIA*

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Aim: The functional activity of the photosynthetic apparatus of *Paulownia tomentosa x fortunei* and *Paulownia elongate x elongata* grown in two soils with different salinity were studied.

Material and methods: Pulse Amplitude Modulated (PAM) chlorophyll fluorescence and determination of the oxidation-reduction kinetics of P700 were used for characterization of the studied lines of *Paulownia*.

Results: Analyses of the PAM chlorophyll fluorescence measurements revealed that the photochemical quenching (q_p), non-photochemical quenching (q_N), the quantum yield of the primary photochemistry of the photosystem II in dark adapted state (F_v/F_m) and in light adapted state (F_v'/F_m') are similar in both lines of *Paulownia*. In addition, the data showed that salinity does not affect on the above parameters. On the other hand the rate constant of the cyclic electron transport around the photosystem I is higher in *Paulownia elongate x elongate* in comparison to *Paulownia tomentosa x fortunei* and strongly influenced by the degree of salinity.

Conclusion: Data in the present investigation revealed increased stability of the photosynthetic apparatus of the studied lines of *Paulownia* to high salinity as well as clarified some of the reasons for this stability.

Key words: *Paulownia*, salinity, PAM chlorophyll fluorescence, oxidation-reduction kinetics of P700

P2_02

***IN VITRO* DETERMINATION OF ANTIOXIDANT CAPACITY OF PEA (*PISUM SATIVUM* L. CV.RAN) PLANTS**

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The purpose of the study is to estimate the antioxidant capacity (AOC) along the pea plant; to obtain a picture of organ distribution of non-enzymatic antioxidants and their protective efficacy during pea vegetative growth.

Methods. AOC was determined by HORAC (hydroxyl radical averting capacity), ORAC (oxygen radical absorbance capacity) and F–C (Folin–Ciocalteu) assays which assess different functions of certain non-enzymatic antioxidants.

Results. Pea plant developed all leaf stages before flower formation during the studied growth period. Root and shoot biomasses enhanced which were accompanied by AOC increase. Highest AOC was localized in the green organs such as leaves, especially those in the shoot apex; stem and root AOC were lower. The AOC increase during the organ growth was expressed in different magnitude by the assays because the assays estimate the antioxidant function of different compounds; their identification is needed. The contribution of potential non-enzymatic antioxidants in the assays is discussed.

Conclusion. By means of three different assays we demonstrate that the green pea organs especially the youngest one (shoot apex), possess high AOC, resp. these are antioxidant-rich source. The AOC of pea non-enzymatic antioxidants is dynamic, and has potential to change in response to growth conditions.

Key words: *Pisum sativum* L. cv. Ran, antioxidant capacity (AOC) assays.

THEMATIC SESSION III

ECOSYSTEM RESEARCH AND SERVICES

L3_01

ASSESSMENT OF ECOSYSTEM FUNCTIONS AND SERVICES AT LANDSCAPE LEVEL OF SREBARNA LTER SITE

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The concept of **Ecosystem Services (ESS)** refers to the benefits provided by specific ecosystem structures and functions for the human society. Therefore the concept is suitable for identifying the consequences of long-term changes in ecosystems. This concept became generally known by its application in the Millennium Ecosystem Assessment of the United Nations.

The regulating services, provisioning services and cultural services will be assessed. A total of 39 individual ecosystem services can be assessed by means of appropriate indicators.

CORINE Land Cover (CLC) is a project of the European Commission with the objective of assessing the land cover and land use types covering the EU using satellite images. CLC is subdivided in 44 land use classes and data are available for 1990, 2000 and 2006. With a matrix the individual ecosystem services will be referred to the different CLC land use classes by ranking each land use type's capacity to supply different ESS. The case-study of Srebarna LTER site will be presented.

L3_02

VALUE OF CULTURAL ECOSYSTEM SERVICES PROVIDED BY ECO-TRAIL "CANYON FALLS" NEAR SMOLYAN

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Aim: The main aim of this work is to discuss the future development of eco-trail "Canyon Falls" and the need to introduce an admission fee to generate funds for maintenance.

Material and Methods: A survey of visitors to the eco-trail is conducted, which contains 12 questions. In a single day 25 respondents have been interviewed. A comparison is made with other similar sites in Bulgaria and Europe, where admission fee is collected.

Results: The survey results show that only 16% of the respondents believe that a fee should not be collected. 44% of the respondents visit the eco-trail twice a year, and one of them 3 times a year. The average value of the admission fee determined by the people that replied with "yes" to the question about the need of introducing a fee is 3.58 BGN. Survey results show that eco-trail visitors predominantly agree with the necessity of introducing an admission fee.

Conclusions: Maintenance of eco-trails in Bulgaria requires generating of financial resources to ensure the quality and quantity of ecosystem goods and services provided to people by these natural sites.

Acknowledgements: This research is sponsored by the "National, European, and Civilizational Dimensions of the Culture – Language – Media Dialogue" Program of the "Alma Mater" University Complex for the Humanities at Sofia University "Saint Kliment Ohridski", funded by the Bulgarian Ministry of Education, Youth, and Science Scientific Research Fund.

Keywords: biodiversity, ecosystem goods and services, eco-trail, admission fee.

L3_03

AIR QUALITY IN REPUBLIC OF MACEDONIA DEPENDIG ON PRESENCE OF SULFUR DIOXIDE

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Aim: In accordance with the EU directives on quality of air, the member countries are obliged to monitor the air quality in their region by generally accepted methods and principles of

evaluation. The requirement as to continuous measurement of the quality of air depends on the level of quality of air and the population in a certain area. Although not an EU member country, Republic of Macedonia has undertaken the obligations in compliance with the EU directives.

Material and methods: To evaluate the quality of air, agglomeration zones have been established in R. Macedonia. Two zones (east and west zone) and Skopje region agglomeration have been defined for the main polluting substances: sulfur dioxide (SO₂), nitrogen dioxide (NO₂), nitrogen oxides (NO_x), suspended particles < 10 micrometres in a diameter of (PM₁₀), carbon monoxide (CO) and ozone (O₃).

Using the available data, the evaluation, the classification of the main polluting substances, the zones and the agglomeration as well as the minimal measurement requirements have been reviewed. To undertake the obligations as to evaluation of air quality and management of heavy metals given in Directive 2004/107/EC, available data on those polluting substances, in this case, sulfur dioxide, have been analyzed. The methods of measurement of different parameters of air quality are given. Emissions of sulfur dioxide and quantity measured are also discussed.

Results and Conclusion: Based on the research, a conclusion has been drawn that there are no sufficient data for establishment of zones and agglomerations for heavy metals.

Key words: agglomeration zones, emissions of sulfur dioxide, concentrations of sulfur dioxide

P3_01

ASSESSMENT OF OAK DENDOCHRONOLOGICAL SERIES FOR EUSTRESS IDENTIFICATION IN PROTECTED ZONE

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Aim: The work presents a new concept for trees' eustress as a set of years with low stem growth, caused by unfavourable ecological impacts.

Material and Methods: The developed SPPAM application for identification and assessment of eustress is demonstrated by analysis of oak chronologies from protected zone. The tree ring width sequences are approximated with polynomials with $R^2 > 0.45$. The indexes as ratios between measured and approximated values are computed. Years, where it is lower than a threshold value, are categorized as eustress ones. Specified in the study climatic years are studied as adverse years according to the obtained eustress. A four grade scale for assessment of frequency, duration and negative deviation of eustress periods and their comparative analysis is proposed.

Results: The predominance of hot and dry adverse years is established. The scaled characteristics of eustress were compared. The potential risk for studied forest vegetation is estimated.

Conclusions: The proposed holistic approach is convenient for the fast diagnosis of forest state. The capability of approach allows the expression of reactive functional types, which may support the vegetation modelling.

Acknowledgements: Co-funded project (MES) "Application of information technologies for modelling of forest ecosystems as an approach for developing of DGVMs" to COST Actions ES0805 and FP1106 and project "Dendrochronological studies of oak forests in SCI "Z.St.Pl. i Predbal." at the Faculty of Biology, 2014.

Key words: dendrochronology, eustress, SPPAM, oak, SCI

P3_02

LACTIC ACID CONTENT IN COMMERCIAL AND HOMEMADE YOGURT

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Aim: The aim of the study was to examine commercial and homemade yogurt for lactic acid content.

Materials and methods: Six trade mark yogurt samples (Vereya, Na baba, Elena, Elbi, Parshevitza and Rodopeya) and four homemade yogurt samples (sheep, bull and two cow yogurts) were analyzed. Lactic acid concentration (in %) was determined *via* titration method with phenolphthalein and 0.1 N NaOH. The acidic degrees of Toerner (°T) were obtained using specific calculation. Analysis was performed three months after the samples collection in order to find out if the fermentation goes on.

Results: Lactic acid (2-hydroxypropanoic acid, C₃H₆O₃) plays important role in cytokines synthesis. Its production depends on the bacterial strain as well as on the culture conditions. Our results demonstrated the highest lactic acid concentration in homemade yogurt samples - sheep – 28 %, bull - 20 % and cow – 14-17 %. Trade mark yogurt samples contain less lactic acid described as follows: Elbi – 16 %, Vereya and Elena - 13 %, Na baba - 12 %, Rodopeya – 11 % and Parshevitza – 10 %.

Conclusions: Homemade yogurt samples contain significantly higher concentration of lactic acid than the trade mark yogurt. We expect that the high lactic acid content in homemade yogurts is due to the ongoing fermentation while in the trade mark samples the fermentation was inhibited by the preservative substances, hence the lactic acid production was stopped.

Key words: lactic acid content, trade mark yogurt, homemade yogurt, health effects

P3_03

SIZE-AGE AND DIET COMPOSITION OF SOME OF THE MOST IMPORTANT COMMERCIAL FISH SPECIES IN THE BLACK SEA BASIN

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In the early 1970s, after reduction of apex predators (bonito, mackerel, bluefish, dolphins), the industrial fisheries in the Black Sea started targeting mainly small pelagic fish species like sprat, anchovy and horse mackerel. By their position and linkage within the food webs, the small pelagic fish species, have essential roles in the functioning and dynamics of the marine ecosystems.

The aim of the investigation was to determine the age-size structure of the most important commercial fish species in the Black Sea. Besides targets of the industrial fisheries, the small pelagic fishes (sprat, anchovy) are also under the predatory pressure of the horse mackerel and the blue fish. The knowledge of the food spectrum of these species obtained from stomach content analysis, will allow to make an estimate of the amounts of consumed fish and fish mortality due to predation.

Material and methods: The samples were collected from commercial fishing vessels, trawling along the southern Black Sea fishing area. The samples were processed for determination of population parameters (total length, weight, age) and for stomach content analysis.

Result: The analyses showed that the sprat population in the coastal zone is dominated by 1st age-class. On the other hand the dominant age-classes in the anchovy population are the 2nd and 3rd. The most important food item among the bluefish diet was found to be the horse mackerel, which in turn feeds mainly on benthic invertebrates in the spring months.

Acknowledgements: The research was carried out with the PERSEUS project of the 7FP.

Keywords: Black Sea, diet composition, population parameters, small pelagic fish

P3_04

ON THE APPLICATION OF LIQUID CRYSTALS IN ECOLOGY AND THEIR ROLE AS BUILDING BLOCKS FOR BIODIVERSITY

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The **aim** of the work is, on the basis of the unique properties of liquid crystals, to analyze some important up to date applications in the field of ecology and a modern view of their role as building elements of biodiversity.

Materials and methods flexoelectricity spectral analysis was performed on the liquid crystal specimens by one of the authors (among other methods reviewed from the literature).

The **main results** include application of liotropic liquid crystals as detergents, or alternatively (if misused) as contaminators of the environment; liquid crystals as detectors of contamination in water with a precision exceeding up to ten times the known methods. Furthermore the sensitivity of liquid crystals and their ability to interplay between mechanical, electrical and optical external influences (as revealed in particular by up to date experiments on flexoelectricity analysis) make them able to incorporate actively in the living organisms.

As a **conclusion**, the liquid crystals are widely found as effectively functioning building blocks of the living organisms, and have numerous applications in the field of ecology.

Key word: Adaptation mechanisms, liquid crystals, contamination detection.

THEMATIC SESSION IV

LANDSCAPE ECOLOGY

L4_01

ALGORITHM FOR LANDSCAPE ECOLOGICAL RESEARCH IN MOUNTAIN AREAS

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Aim: To propose a new algorithm for landscape ecological research in mountain areas.

Material and Methods: Applying the classic landscape research "genetic approach" for systematization and classification all possible factors of landscape differentiation (natural and

anthropogenic) are considered. The digital landscape maps are developed by using methods of spatial analysis and mapping in GIS environment. Work on the maps includes overlaying of vector layers obtained in the initial processing of the data (morphological types of relief based on morphometric parameters of ASTER GDEM; lithological units; climate types based on Thornthwaite moisture index; vegetation; soils). The study uses watersheds as natural systems that have functional integrity and naturally determined borders to generalize the information and create a new perspective on landscape function's analysis. The hemeroby index is integrated in the evaluation of landscape anthropogenization

Results: Analytical database for evaluation of landscape structure and functions for adaptive management of mountain areas has been developed.

Acknowledgements: This research is sponsored by the "National, European, and Civilizational Dimensions of the Culture – Language – Media Dialogue" Program of the "Alma Mater" University Complex for the Humanities at Sofia University "Saint Kliment Ohridski ", funded by the Bulgarian Ministry of Education, Youth, and Science Scientific Research Fund.

Keywords: landscape ecology, landscape classification, GIS

THEMATIC SESSION V

BIODIVERSITY AND CONSERVATION BIOLOGY

L5_01

POPULATION STATE AND CONSERVATION OF THE BULGARIAN ENDEMIC *Verbascum tzar-borisii* (SCROPHULARIACEAE)

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Aim: The aim of the study is to investigate the biology, ecology and the population state of the Bulgarian endemic plant *Verbascum tzar-borisii* (Dav. ex Stoj.) Stef.-Gat. (Scrophulariaceae Juss.) in order to reveal the main threats for the populations of the species and to indicate the measures necessary for its conservation.

Material and Methods: The study has been carried out mainly *in situ* following the Protocol for Monitoring of plant species elaborated by the Executive Agency of Environment.

Results: In the Bulgarian flora genus *Verbascum* (Scrophulariaceae) is represented by 45 species and is the second richest in endemics. One of the rarest taxa is the Bulgarian endemic *Verbascum tzar-borisii* with very local distribution in the Northeast Bulgaria floristic region – only two populations are currently known – from Sivri tepe locality, near to the Chernevo village and Pobit kamak locality, near to the Ravna village, Varna district. It grows in very open grassland communities on clayey marls slopes and poor soils, at 130-200 m alt. and numbers about 1000 individuals (539 in the first population and 515 in the second one). The following threats have been identified to directly affect the populations of the target species: forest felling, fires, grazing, succession changes, low germination rate, etc.

Conclusions: In order to improve the population state *in situ* and *ex situ* conservation measures have been undertaken and two protected areas have been designated.

Acknowledgements: The study was completed during the implementation of the project 'A pilot network of small protected sites for plant species in Bulgaria is using the Plant Micro-reserve

model' supported by the EU's financial instrument for environmental and nature conservation LIFE.

Keywords: Bulgaria, Endemic plant, Scrophulariaceae, *Verbascum tzar-borisii*.

L5_02

ECO-BIOLOGICAL ASSESSMENT OF SOME FOREST TREES IN “GOVEDARCI” AND “JASENKOVO” EXPERIMENTAL STATIONS

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Aim: This research aims to determine the current state (growth and survival) of the different provenances and progenies of the species involved in the experiment using dendrometrical and dendrochronological methods.

Material and Methods: The research is based on provenance trials and progeny tests of forest tree species established in two ecologically different locations. In Govedarci near the town of Samokov at 1250 m a.s.l. including *Picea abies* (L.) Karst. and *Pinus sylvestris* L. from 1973, 1974, 1977.

In Jasenkovo near Shumen at 450 m a.s.l. where provenance trials of *Larix decidua* Mills, *Picea abies* (L.) Karst., *Quercus robur* L., *Quercus sessiliflora* Salisb., *Quercus cerris* L., *Quercus frainetto* Ten., *Pseudotsuga menziesii* Franco ssp. *menziesii* were carried out in 1974.

Key words: forest trees, ex-situ, provenance trials, progeny tests

L5_03

CONTRIBUTION TO THE RESEARCH ON MYRIAPODS (CHILOPODA, DIPLOPODA) IN THE MADARA PLATEAU, SHUMEN REGION, NORTH-EASTERN BULGARIA

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Aim: This study is the first attempt at systematic research on the taxonomic structure and the ecological characteristics of myriapods fauna (Chilopoda, Diplopoda) in the Madara Plateau (12 km east of the town of Shumen), North-eastern Bulgaria.

Material and Methods: The material was collected at 7 sampling sites through pitfall traps recorded on a monthly basis in the period June – October 2012, July – December 2013.

Results: A total of 1116 millipedes were collected: 975 Diplopoda and 141 Chilopoda. 10 species of 4 orders were recorded: Glomerida, Polydesmida, Chordeumatida, and Julida (class Diplopoda), and 12 species of the orders Scutigermorpha, Lithobiomorpha, Scolopendromorpha, and Geophilomorpha (class Chilopoda). There are two new species for the region: the Diplopoda *Glomeris balcanica* Verhoeff, 1906 and the Chilopoda *Pleurolithobous patriarchalis* (Berbese, 1984), which increased the number of millipedes in the region to 61 species.

Conclusions: Individuals from the order Julida (class Diplopoda) and from the order Lithobiomorpha (Chilopoda) were predominant in the region. Eurytopic and polytopic mesothermal, mesophilic, and mesohygrophilic species prevailed. In terms of zoogeographic structure both classes featured highest number of species from the Northern complex followed

by the species distributed in the Balkans. The Southern complex was represented by only two East Mediterranean species of Chilopoda – *Lithobius nigripalpis* and *Pleuroolithobous patriarchalis*.

Acknowledgements: This work is financially supported by the projects BG051PO001-3.3.06-0003 and RD 05-248/2012 and RD 08-266/2013, funded by Konstantin Preslavsky University of Shumen. BG0000104

Keywords: Madara Plateau, Chilopoda, Diplopoda

L5_04

MICROBIOLOGICAL ANALYSIS OF THE WATER OF THE RIVER LUMËBARDHI (KOSOVO) DURING THE WINTER SEASON

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Aim: The objective of this study is to assess the quality of the water of the Lumëbardhi river during the winter season in year 2012, by means of microbiological analysis.

Material and Methods: Samples for microbiological analyses were collected in three localities along the river. The microbiologically analyzed parameters were: Total coliform bacteria, SS (*Salmonella* and *Shigella*), heterotrophic *Streptococcus faecalis* and fungi.

Results: We determined a higher number of microorganisms in the waters of the Lumëbardhi river during the winter season. The locality three is more polluted with microorganisms, compared with the other locality (1 and 2). The number of total coliform bacteria (14.000 cfu/100 ml) is higher at the third locality, compared with the first locality (5.000 cfu) and second locality (10.000 cfu).

Conclusions: The bacteriological analysis showed that the waters of the river are polluted microbiologically.

The river section examined during this investigation, demonstrates that the river water belongs to the fourth class of quality.

Keywords: technique, plate, microbiological, analysis, water

L5_05

ALGOCENOSIS OF RIVER VALBONË (ALBANIA), DURING WINTER SEASON 2012

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Aim: The aim of this study is to determine the algocenosis of the Valbona river, nearby the city of Bajram Curri, and the quality of the water, through algal bioindicators during the winter season of the year 2012.

Material and Methods: Samples for algological analyses were collected at three localities along the river. They were conserved in 40 % formalin. Determination was done according to the algal keys.

Results: During our investigation we determined the different species of algae and bioindicators, through which can evaluate the quality of water and class of bonity.

At the river of Lumëbardhi, during the winter we determined 31 species of algae.

Conclusions: The Bacillariophyta dominate with 24 species, following by Chlorophyta with 3 species, Cyanophyta with 2 species, Euglenophyta and Xanthophyta with 1 species.

Keywords: algocenosis determination, algae, water, river, Valbona, Albania.

L5_06

GENETIC POLYMORPHISMS OF BULGARIAN AND PORTUGUESE COMMON BEAN (*PHASEOLUS VULGARIS*) GERMPLASM

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Aim: to study the genetic variation of Bulgarian and Portuguese common bean (*Phaseolus vulgaris*) germplasm.

Material and Methods: Total genomic DNA from young leaves of 17 different bean genotypes including 9 Portuguese, 7 Bulgarian and 1 Mexican was isolated. AFLP analysis with eight primer combinations was used. Seven microsatellite loci were used to perform the genetic diversity analysis of the selected accessions. For the ALFP and SSR analyses all PCR products were analyzed by capillary electrophoresis. Genetic diversity parameters were estimated using GenALEX software.

Results: A total of 506 polymorphic bands were detected with the ALFP analysis. Percentage of polymorphic loci was 51.98% (74.31% for Portuguese genotypes, 81.62% for the Bulgarian and 0% for the Mexican ones). According to the SSRs results it was observed that the loci are highly homozygotic, what is expected considering that the common bean is a selfer. Furthermore, the seven microsatellites were found to be polymorphic.

Conclusions: Preliminary results from the two analyses showed:

- The ALFP analysis revealed two main groups – Portuguese and Bulgarian. Mexican genotype clustered with the Portuguese.
- The SSR analysis revealed a few differences between the Portuguese and Bulgarian germplasm.

Keywords: genetic diversity, ALFP, SSR, *Phaseolus vulgaris*

Acknowledgements: This study was financed by the projects: BG051PO001-3.3.05-0001 „Science and business“ – Ministry of Education and Science, Bulgaria; DDVU_02/87 “Complex morphometric, physiological, biochemical and molecular assessment of drought tolerance in Bulgarian common bean genotypes (*Phaseolus vulgaris* L.)” and Ecological and genetic risk: methods and strategies for overcoming – BAS.

L5_07

COMPARISON OF VASCULAR FLORAS OF TREE LIMESTONE TERRAINS IN BULGARIA-ZEMENSKA MOUNTAIN, GOLO BARDO AND BESAPARSKI RIDOVE

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Aim: The aim of this study was compare the systematical and phytogeographical structure of tree vascular limestone floras from zones with different altitude in Bulgaria. Also their endemic plants, Tertiary relicts and rare and threatened plants were compared.

Material and methods: The floristic analysis of the Zemenska Mountain was made by Asen Asenov during 2006-2013 (Asenov & Dimitrov, in press). Comparison with Golo Bardo is according to Apostolova-Stojanova & Stoyanov (2009). Comparison with Besaparski ridove is according to Stanev (1975).

Result: The analysis of the flora of Zemenska Mountain showed that it is middle European, temperately continental flora with submediterranean influence. It has submediterranean flora with high percent of endemic, rare and threatened plants, as well as Tertiary relicts.

According to Apostolova-stojanova & Stoyanov (2009) and Stanev (1975) Golo Byrdo and Besaparski ridove also have submediterranean flora and possess a high percentage of endemic plants, Tertiary relicts, rare and threatened plants.

Conclusion: Comparison between the tree floras shows that the flora of the lowest and smallest zone (Besaparski ridove) has the higher percent of endemic plants and Tertiary relicts. Despite its small area it has a high percentage of species. It has the highest botanical value. The flora of the highest and largest zone (Zemenska Mountain) has lowest percent of endemic plants and Tertiary relicts, and lowest botanical value.

Key words: vascular flora, Zemenska Mountain, Golo Bardo, Besaparski ridove, comparison
This research was made on private bill.

P5_01

BULGARIAN COLLECTIONS AND FIRST MOLECULAR DATA OF *ZEUS OLYMPIUS* (ASCOMYCOTA, FUNGI)

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Aim: The first Bulgarian records are reported of *Zeus olympius* Minter & Diamandis, an unusual ascomycete, described and so far known only from a single locality in Greece and related to *Pinus heldreichii* Christ. The aim of this study was to provide further data on this peculiar fungus and obtain molecular data, establishing its phylogenetic position.

Materials and methods: Specimens from four localities in Pirin and Slavyanka mountains were studied by standard methods employed in ascomycete taxonomy. The molecular methods included CTAB-based DNA extraction, PCR amplification of ITS and 28S rLSU regions, sequences alignment in MEGA 5.0, analysis in PAUP* 4.0b10, model testing in Mr.Modeltest 2.3 and bayesian analysis in MrBayes 3.1.

Results: *Zeus olympius* is unambiguously identified due to its peculiar, bright colored ascomata, specific substrate, and ascospores with characteristic mucous sheaths. All Bulgarian collections agree with the two previous descriptions of the species. Mean values for spore length and width, as well as their quotient ratio, are established for the first time. The present results are the first molecular data on the genus suggesting close relationship to the *Hemiphacidium* clade and not to *Rhytismatales*, where it is currently placed.

Conclusion: The new collections suggest that *Z. olympius* may be more widespread in the Balkan Peninsula, since the geographical range of *P. heldreichii* includes Albania, Bosnia, Macedonia, and Serbia, reaching Italy to the west.

Acknowledgements. The work was held within the project 'Taxonomy, conservation and sustainable use of fungi'. The authors thank to Dr Pablo Alvarado (Alvalab) for performing the molecular analysis.

Key words: Bulgarian mycota, endemic fungi, *Hemiphacidium* clade, *Rhytismataceae*, *Zeus*

P5_02

DETERMINATION OF THE ALKALOID GLAUCINE IN FOUR BULGARIAN POPULATIONS OF *GLAUCIUM FLAVUM* (PAPAVERACEAE)

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Bulgaria

The study **aimed** to update the information about the content of the main alkaloids of the valuable medicinal plant *Glaucium flavum* Crantz. (yellow hornpoppy), a herbage used as raw material for the production of several trademarks, among them Glauvent®, Glauterpin® and Broncholytin® (Sopharma AD).

Material and methods: Plant collection from the natural populations of the species was forbidden by the MEW due to the permanent downward tendency of its resource. A few cultivars created about 30 years ago were lost and the plant quality of the scanty present plantations is doubtful. To overcome the resource deficiency, trials on *in vitro* multiplication are in progress; however, it's important to use individuals of high glaucine content for culture initiation.

Plant samples, each consisting of the aboveground part of several plants, were gathered in July 2013 from four Bulgarian populations of the species located near Varvara, Arkutino (along the south Black Sea coast), Shkorpilovtsi (further north along the Black Sea coast), and Sofia. The raw alkaloid mixture was obtained from the plant material by usual chemical procedure. The percentage content of the glaucine in these mixtures was determined by densitometry program Quanti Scan from Biosoft (2004).

Results: The percentage of the glaucine in the raw alkaloid mixture of three of the tested populations (Varvara, Arkutino and Sofia) was high and similar: between 70.2% and 74.2% while that of Shkorpilovtsi was only 31.2% which suggested some different chemotype. The glaucine content was highest in Varvara: 35.8 mg/g DW and lowest in Shkorpilovtsi: 4.4 mg/g DW.

Key words: yellow hornpoppy, glaucine, Bulgarian populations

P5_03

ESSENTIAL OIL COMPOSITION OF *IN SITU* AND *IN VITRO* CULTIVATED *ACHILLEA THRACICA* VELEN. PLANTS

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Aim: We aimed to assess comparative analysis of essential oil composition of *in situ* and *in vitro* cultivated *A. thracica* plants.

Material and methods: Intact plant material of *A. thracica* was collected at its natural habitat near Manole village, Plovdiv, Bulgaria. The voucher specimen SO107385 has been deposited in the Herbarium of the Department of Botany, Faculty of Biology, Sofia University. *In vitro* shoot cultures were induced from sterilized mono-nodal stem segments of the *in situ* growing wild plant. The species was successfully grown on Murashige and Skoog medium with 30 g/l sucrose and 8 g/l agar. Hexane oil extracts of *in vitro* and *in vivo* grown plants were used in order to examine their terpene and carbon composition.

Results: The *in situ* grown plants contained 21 terpenes and hydrocarbons and the content of these compounds increased about two-times reaching 42 during *in vitro* cultivation. Trans-2,7-Dimethyl-4,6-octadien-2-ol and cineole were the main constituents respectively in the oils of *in situ* and *in vitro* plants.

Conclusion: The observed discrepancy between *in situ* and micropropagated plants suggests that the hydrocarbons and terpenes amount and content are very sensitive to the growth conditions.

Key words: *Achillea thracica*, *in vitro* cultivation, terpenes

P5_04

FIRST RECORD OF *POLYDESMUS SCHAESSBURGENSIS* VERHOEFF, 1989 (DIPLOPODA, POLYDESMIDA, POLYDESMIDAE) FROM BULGARIA

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Aim: To report on *Polydesmus schaeessburgensis* and its habitat preferences in Shumen region and the Shumen Plateau, Northeastern Bulgaria.

Material and Methods: Pitfall traps were used in 8 sampling plots distinguished in terms of their origin, composition of plant formations, and anthropogenic impact. The sites were situated in Shumen and its suburbs and in the rural habitats located in the Nature park Shumen Plateau. The catch was collected on a monthly basis from May 2013 to December 2013.

Results: A total of 108 specimens of *P. schaeessburgensis* (83 ♂, 24 ♀, 1 subad) were collected in the period of study. The species were registered only in the suburban area of Shumen (Park Kyoshkovete) and the finds were recorded exclusively in the months of May and June.

Conclusions: *P. schaeessburgensis* Verhoeff, 1989 is a new species to the millipede fauna of Bulgaria. Carpathian species known to date in Europe are allocated only in Hungary, Serbia, Romania, Moldova, and Ukraine. The species is generally defined as a xeromesophilic woodland form reported mainly from deciduous forests, clearings and groves but also from meadows, hawthorn thickets and rocky places.

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Keywords: *Polydesmus schaessburgensis*, distribution, habitat preferences, Diplopoda, Bulgaria.

P5_05

SPATIAL NICHE PARTITION AMONG FIVE SYMPATRIC LIZARDS IN NORTH-WEST BULGARIA

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The **aim** of the present study is to give more data about the habitat use, share of spatial niche and ecological requirement of lizard species.

Materials and methods: A total of 620 individual locations were investigated, which fall among seven general type of habitats – stony shore of the dam, sandy shore with low vegetation, oak forest, meadows and grasslands, ecotone between the forest and the meadow, pine forest and road sides covered with shrubs. To express the diversity of species by habitats, and also the diversity of used habitats by species, Shannon diversity index was used.

Results: The green lizard is the species with the widest niche and is found in all of the habitats but is most common in grasslands with shrubs. The rest of the species, in view of the competition with the dominant *L. viridis*, have narrowed spatial niches and the meadow lizard is the species with the narrowest one, being confined mainly to oak forests. The highest number of species is found in the grasslands – all of the five species because of the intermediate position between the other habitats.

Conclusion: All of the species display specific habitat preferences and in this way competition among them is avoided.

Acknowledgement: We are greatly indebted to Vladislav Vergilov and Georgi Hristov for their help during the field work.

Key words: habitat use, lizards, ecology, Lacertidae, Scincidae

P5_06

NEW DATA ON AQUATIC OLIGOCHETS (OLIGOCHAETA LIMICOLA) IN THE BURGASKO (VAYA) LAKE

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Aim: The main scope of the study was to present new data on species diversity and abundance of the aquatic oligochets in Vaya Lake (South-Eastern Bulgaria) - a Ramsar site and an Important Bird Area.

Materials and methods: The survey was conducted in 8 sampling points; samples were taken in every season during the period 2004-2007 from the exact lake bottom, outside the area of the fringing communities. Qualitative samples of benthic macroinvertebrates were taken according to the standard method ISO 9391:1995. Field and cameral work was done in accordance with the standard method EN/ISO 5667-3:2003/AC:2007. The species diversity, density and biomass of oligochaets were measured.

Results: The oligochaete complex was dominated by two species: *Potamothrix hammoniensis* (Michaelsen, 1901) and *Limnodrilus hoffmeisteri* Claparede 1862. Findings of oligochaets from fam. Naididae (single speices from genera *Nais* and/or *Dero*) were sporadic during the whole studied period.

Conclusion: When comparing to the earlier studies, the community structure showed lasting changes where the euribiont species *Tubifex tubifex* (Müller 1774) was dominant while in nowadays it was replaced by ponto-caspian species *Potamothrix hammoniensis* (Michaelsen, 1901).

Key words: Vaya Lake, macrozoobenthos, aquatic oligochaets, *Potamothrix hammoniensis*

P5_07

DIVERSITY AND CONSERVATION STATUS OF VASCULAR PLANTS OF THE ROCKY HILLS EAST- AND WESTWARD FROM TRIGRAD VILLAGE

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Aim: This article presents the distribution and conservation status of the vascular plants growing on the rocky and eroded hills around Trigrad village. The existence of a diverse and rich endemic and sub endemic flora is of great importance for conservation of biodiversity in the studied region. The consequences of anthropogenic pressure are discussed and recommendations for conservation of these habitats of national importance are provided.

Materials and Methods: The field observations were carried out during July, August, September and October three years ago (2011 - 2013). The transect method was used for establishing the distribution of vascular plant species of the two localities. The number of plants in the population of *Sideritis scardica* Griseb. was determined. Field observations were carried out in sample plots, 100 m² in size. During the observations in the field GPS coordinates, altitude, some biotic and abiotic factors were described.

Results and Discussion: Together with the fill floristic inventory of the localities studied, particular attention is given to the populations of *Sideritis scardica* Griseb., a Balkan endemic and valuable medicinal plant. Population size and structure of this species are described. The elaborated list of floristic composition indicates that besides the endemic and sub-endemic species (15%), elements with Mediterranean origin or related to Mediterranean is important (40%). The ecological factors that have the highest impact on the floristic composition in the studied area are intensive light, air temperature and low humidity, limestone rock base, soil type, its depth and quantity.

Acknowledgements: The authors are grateful to the financial support provided by 7. Contract № 2370/13.06.2013 on a separate lot 5 with subject "Field studies of vascular plants, mosses and fungi," concluded between the Environmental Protection Agency and the Institute of Biodiversity and Ecosystem Research, BAS.

Key words: flora, conservation, endemic species, *Sideritis scardica* Griseb.

P5_08

***PSEUDOMONAS CORRUGATA* – CAUSE OF TOMATO PLANTS DIEBACK IN GREENHOUSES IN BULGARIA**

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Aim: This study aimed to identify the causal agent of tomato plants dieback in greenhouses in different regions in Bulgaria in order to provide relevant advices for growing tomatoes.

Material and methods: Bacterial strains were isolated from diseased plant parts and subjected to pathogenicity tests. The biochemical scheme of Schaad and Biolog™ system were used for characterization and identification.

Results: The isolates were positive for the hypersensitivity reaction test and induced the formation of watery brown spots on the 3rd day after artificial inoculation in tomato stems. The spots later turned to gaps with necrotic rings. On the basis of conducted tests the strains were identified as *Pseudomonas corrugata* – a pathogen known worldwide to cause pith necrosis of tomato. The full metabolic profile for the Bulgarian isolates was described. To our knowledge this is the first report of this pathogen in Bulgaria.

Conclusion: *Pseudomonas corrugata* was established as causal agent of tomato plants dieback (‘pith necrosis’) in greenhouses in Bulgaria.

Key words: *Pseudomonas corrugata*, tomato, disease, greenhouses, Bulgaria

P5_09

SPECIES COMPOSITION OF EARTHWORMS (LUMBRICIDAE) IN THE BOYANA LAKE SURROUNDINGS (VITOSHA MOUNTAIN)

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Aim: A survey was conducted around the Boyana Lake and a forested site on the Vitoshka Mountain. The results of the earthworm’s fauna were presented. Research was carried out during May-September 2010 and May-September 2011, included a small semi-artificial headwater wetland from the essential part of Vitoshka Natural park, the first park of this kind on the Balkans. The results of an earthworm survey of 6 different sites around the Boyana Lake are following 6 species belonging to 3 different genera of the Lumbricidae family.

Material and Methods:

Site Description

Boyana Lake is a small semi-artificial lake, situated on the northern slopes of the Vitoshka Mountain at 1035 m.a.s.l. The lake is a small ($V = 5160 \text{ m}^3$; $A = 5180 \text{ m}^2$) and shallow ($Z_{\text{max}} = 2.1 \text{ m}$, $Z_m = 1 \text{ m}$) forest lake, fed by rain and groundwater, and the outflow is regulated by a small artificial canal. Ice forms from mid-November and persists until the end of the March, reaching a depth of up to 50 cm.

The soil in the investigated area is leached cinnamonic forest soil with a slightly alkaline to acid reaction. The mechanical composition of the soil is with sandy clay texture.

Sampling procedure

The samples were collected from Boyana Lake's surroundings by digging (0.4 x 0.4 m quadrates) and hand sorting. Collected specimens were cleaned and immediately fixed in 4% formalin and transferred to 90 % ethanol. Identification of species was done in accordance to: BLAKEMORE (2004), MRŠIĆ (1991), ZICSI (1982), ŠAPKAREV (1978), CSUZDI, ZICSI (2003).

Results: In this study, the identification results of earthworm samples, collected in 6 different locations of the Boyana Lake's surroundings for two years, are presented. In all, 6 species belonging to 3 genera of Lumbricidae were identified.

Conclusion: The Boyana Lakes's surroundings are exclusively inhabited by one species - *Octolasion lacteum* for 2010. Individuals of this species were found in each location during the whole investigated period for the first year (2010) of the survey. For 2011 the prevalents were the *Aporrectodea* species, *A. caliginosa trapetosoides* and *A. rosea*, distributed in the most of the sampling sites.

Key Words: Boyana Lake, Earthworms, Lumbricidae

P5_10

NEW DATA ON THE LICHENIZED FUNGI (*ASCOMYCOTA*) IN BALGARKA NATURAL PARK

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Aim: The purpose of this work is to study the lichen diversity in the area of Balgarka Natural Park. This was the first comprehensive investigation on lichenized fungi, carried out in Balgarka Natural Park during a period of 2012-2013.

Materials and methods: The preparation of the cross sections of the lichen thalli was made with the help of a razor blade, checking of the color reactions was made in laboratory conditions following the accepted methods, all of the studied specimens were housed at the Mycological Collection, Institute of Biodiversity and Ecosystem Research (SOMF). Spores were measured with the help of specialized software for digital pictures Carnoy 2.0 (© Peter Schols, 2001). Microscopic observations in LM were done using standard methods. Most of the pictures of lichen thalli were taken with the help of Canon PS A460 and Canon PS A1400 HD under Boeco microscope.

Results: *Micarea lignaria* (Ach.) Hedl. is found as new species to Bulgaria; several ones are reported with new localities in the Balkan Mts. Brief historical information on the studies on lichens conducted in the territory of the Park 'Balgarka', performed by Ö. Szatala, B. Zhelezova and V. Shivarov, was included also. All of the 46 recorded taxa are presented in alphabetic order, including short data on collection's localities and valuable site information.

Conclusion: During the field studies, arranged in Central Balkan Mt (Shipchenska and Trevnenska Mts) new data on 46 species of lichenized fungi, inhabiting various substrata - bark of trees, stones, rocks and mosses are recorded. They represent nearly 1/3 of the known lichen diversity of the Park area.

Acknowledgements: This work is held within the frame of the project 'Taxonomy, conservation and sustainable use of fungi'.

Key words: *Cladonia*, lichens, *Lecanora*, *Leptogium*, *Micarea*, *Verrucaria*

TROPHIC STATE VS. ECOLOGICAL STATUS – A SURVEY OF DIFFERENT WATER BODY TYPES IN BULGARIA

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Aim: The objectives of the study were to describe the physicochemical parameters and to assess the trophic state and the ecological potential of the water bodies according to the macrophyte assemblages within selected reservoirs.

Material and methods: We investigated 10 reservoirs distributed throughout Bulgaria. The reservoirs varied greatly in location, size, depth, alkalinity and trophic status. There were distinct differences in species composition and abundance among the reservoirs, which were related to both physical and biological disturbances of the macrophyte communities and not so to the apparent trophic state of the reservoirs.

Sampling was conducted between 2009 and 2013 in the deepest part of the water bodies, from 0.5 m bellow the water surface using a Limnos type water sampler (1.0 L). On site measurements of water transparency (Secchi disc visibility), water temperature (T) and dissolved oxygen (DO) were measured by OXI- 196 WTW, and pH and electrical conductivity by a HI 98129 pH-meter. The concentrations of nutrients (NO₃-N, NH₄-N, PO₄-P, TN and TP) were determined spectrophotometrically with standard MERCK kits and total alkalinity (TA) after ISO 9963-1. The Carlson’s trophic state indices were used to assess the trophic conditions in the reservoirs.

Main results: The trophic state of the studied reservoirs varied between oligo-mesotrophic to eutrophic state, with the majority being mesotrophic. Moderate positive correlation was observed between the transparency trophic state index and the reference index for the aquatic plant communities. On the other hand, no correlation was observed when comparing the trophic state and the ecological status of the water bodies, as only half of the studied reservoirs had corresponding assessments on both scales. The relationship between the trophic state and ecological status seems to be size dependent, with stronger disagreements in the bigger water bodies.

Conclusion: Trophic state assessment, traditionally, is focused mostly on the “open water” conditions and not so at the littoral aquatic plant communities. The macrophyte community reduces the phosphorus inputs to the water bodies, contributing to clearer lake water. Thus, the trophic state may be underestimated in water bodies with dense macrophyte communities. On the other hand, aquatic macrophytes are considered as one of the biological quality elements for which an assessment of the ecological status must be performed. The two assessment schemes have different ecological meanings as the former assesses the algal biomass and the latter – the deviation from reference conditions. Our results show the need to further investigate the relationship between the different biological quality elements, phytoplankton and macrophytes, and their response to the changes in the supporting physicochemical parameters.

Key words: WFD, aquatic macrophytes, reservoirs, bioindication, monitoring, eutrophication

EX SITU CONSERVATION OF THREATENED AND ENDANGERED PLANTS OF BULGARIAN FLORA IN THE TERRITORY OF UNIVERSITY BOTANIC GARDEN ECOPARK - VARNA

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Aim: to research *ex situ* conservation of threatened and endangered plants of Bulgarian flora on the territory of the University Botanic Garden Ecopark – Varna.

Material and methods: This research was conducted on the territory of the University Botanic Garden Ecopark – Varna during the vegetation period 2012-2013.

Sites for conservation significant plant species of the University Botanic Garden have been identified and mapped. Scientists have prepared a card file which provides background data and number of protected plants. Large picture database of all endangered species has been established.

Results: Research already done shows that 45 plant species listed as threatened and endangered occur on the territory of the University Botanic Garden.

The distribution of taxa in categories is as follows: 1 Balkan endemic, 23 species, listed in the Red Data Book of Bulgaria, 8 of which in the category “rare”, 14 – “endangered” and one species in category “extinct”; 34 are protected by the Law of the biodiversity; 1 species is listed in the List of Rare, Endangered and Endemic Plants in Europe; 5 species are subject of the Convention on the International Trade in Endangered Species of Wild Flora and Fauna, 2 species are listed in the Black Sea Red Data Book, 10 species are relict – 9 of them Tertiary and 1 Glacial.

Most of the protected species are introduced on the territory of the University Botanic Garden. Naturally preserved habitats define the presence of autohtonic plants with conservation significance.

Conclusion:

- University Botanic Garden has the value of *ex situ* collection of conservation significant species of Bulgarian flora (45 species). This collection can be enriched and used for scientific, educational and exhibition purposes.
- University Botanic Garden can be a source of genetic material of successfully introduced and adapted species.

THEMATIC SESSION VI

ECOLOGICAL AGRICULTURE

IMPLEMENTATION OF GOOD AGRICULTURAL PRACTICES AND THE BENEFITS FOR AGRICULTURAL PRODUCERS *Kliment Petrov* (see last page)

L6_02

INDUCTION OF RESISTANCE OF PEPPER TO POTATO VIRUS Y BY ACTIVATION OF NATURAL DEFENSE MECHANISMS OF THE HOST PLANT

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Aim: Our aim is to induce Systemic acquired resistance (SAR) to PVY in pepper plants using natural products.

Material and methods: Pepper cultivar Kurtovska Kapiya was grown at 22-25 C°, 75-85 % relative humidity, constant photo-period of 16/8 hours, light intensity 3000 lux. Symptoms were reported 7-21 days after virus inoculation. Plants were treated three days before and after artificial infection with Potato virus Y (PVY) by EXIN and BION at different concentrations. Combination with EXIN and BION were performed. Sprays were conducted in a greenhouse with a dose of 25 ml solution of compounds for a plant in 1-day interval and 3 treatments. Confirmation of the results was made with DAS-ELISA.

Results: Symptoms of PVY were mostly visible on the vegetative parts of the plants but rarely on fruits. They consisted of mosaic and necrotic symptoms on leaves and necrotic symptoms on stems. Treatment of the pepper plants with the two elicitors of Systemic acquired resistance (SAR) induced resistance against PVY resulting in reduction of the virus symptoms. We received different results when treating the plants before and after virus inoculation.

Conclusion: From the different treatment schemes we received best results from plants treated three times before virus inoculation with 3.6 mM BION. For the plants treated after virus inoculation the best scheme is the spatter with the combination of BION and EXIN. Reduction of the virus symptoms is not always associated with its reduction of the virus titer.

Key words: PVY, SAR, pepper

L6_03

MONITORING OF FUNGICIDE USE AGAINST THE POWDERY MILDEW AND APPLE SCAB IN NONCOMMERCIAL ORCHARDS IN SOFIA'S FIELD

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Aim: The most important diseases on apple, reducing the yields of the orchards and aggravating its quality, are apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*). There are different plant protection products (fungicides) against these diseases on the market. They have different active substances and different mode of action to the pathogens. Each company proposes its product as a solution of the problem caused by mentioned diseases but fungicide use is under the requirements concerning not only the efficacy to the target but also the safety for people, animals and environment. This imposes the necessity of periodical monitoring directed both to the efficiency and to the safety of realized fungicide treatment in the agricultural farms.

Material and methods: The monitoring was accomplished during one year. It included itinerary observations in the fruit orchards, visual and microscopic diagnosis of plant diseases and inquiry for fungicides already used.

Results: The paper presents results of one monitoring of fungicide use realized during the period October 2012 – September 2013. The fungicides registered in use were based on different active substances. Some of them define protective and other therapeutic mode of action. The treatments were orientated accordingly to main phases of the development of fruit trees. Pesticides out of the official list of permit for Bulgaria were not registered.

Conclusions: The main conditions for minimized but efficient fungicide use are: treatment in appropriate phenological phase, planting of cultivars resistant to pathogens and prevention against the initial infection.

Key words: fungicide efficiency, minimized fungicide use

P6_01

WHEAT VARIETIES AND WHEAT LINES AND THEIR REACTION DEGREE OF SENSITIVITY TO TAKE-ALL DISEASE CAUSED BY *OPHIOBOLUS GRAMINIS* (SACC.)

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Take-all disease, caused by *Ophiobolus graminis* (Sacc.) is the third most important disease of the complex pathogens, causing root-rot in wheat.

The main **aim** is to determine the degree of sensitivity to the pathogen in different wheat varieties.

Material and methods: Based on the greenhouse experiment were treated 25 wheat varieties and 17 wheat lines with aggressive strain of *Ophiobolus graminis* w-type /*Gaeumannomyces graminis var. tritici*/ according to Karjin (1978), Gerlagh (1968) methods.

Results: Highest reaction resistance NS and N showed 3 wheat varieties /Laska, Zora and Enola/ and 3 wheat lines / III-212, A-39⁹-3-6-1-3-3-1, V-8⁶-10/.

Key words: wheat, take-all, root-rot, disease, strain

P6_02

VIRUS DISEASES ON MEDICINAL PLANT *SILYBUM MARIANUM* (L.) GAERTN

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Aim: The objective of the research was the establishment of diseases, caused by widespread plant viruses on *Silybum marianum* – milk thistle.

Material and methods: Samples from leaves with symptoms resembling *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Tomato spotted wilt virus* (TSWV) were collected from the plantations of the Institute of Roses, Essential and Medicinal Cultures near Kazanlak”, Bulgaria in the period of 2009 to 2011. The samples were tested by the serological ELISA method with kits, purchased from the German company LOEWE and by the indicator

method, using test plants. All samples with extinction values two and a half times higher than the negative controls were assumed as virus positive.

Results: Three important plant viruses on milk thistle were established: CMV in 37 % of the analyzed plants; AMV in 20 % and TSWV in 19 %. The viruses caused chlorotic spots on the leaves, turned in necrotic spotting of individual leaves or entire yellowing of the leaf laminae. This yellow spotting followed to necrotic spotting, wilting and perishing of leaves and entire plants impeded to obtain good yield of seeds for pharmaceutical industry.

Conclusion: The results from the ELISA method and from the indicator method testify that the yellowing of a part of milk thistle plants on the field could be caused from virus diseases.

Key words: *Silybum marianum*, CMV, AMV, TSWV.

P6_03

INFLUENCE OF DOUBLE MICROBIAL ASSOCIATIONS WITH AM-FUNGI AND RHIZOBIUM ON THE GROWTH OF ALFALFA AND RED CLOVER AND ON THE SOIL STRUCTURE

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The aim of the study was to determine the effect of inoculation with mycorrhizal biofertilizer “Mycosym TRI-TON” applied alone or in combination with N-fixing Rhizobial bacteria on the growth of alfalfa and red clover, the N and P content of plant biomass and on the soil structure.

Materials and methods: A pot and a field experiments with the two forage grasses on slightly eroded Leached cinnamonic soil were carried out. The following parameters were determined: shoot and root dry weight, their N and P content, the percentage of root mycorrhization, the acid phosphatase activity and the percentage of water-stable aggregates in the soil.

Results: Positive effect of the inoculation on the studied plant parameters and on the content of water-stable aggregates in the soil was established. Specificity in the phosphorus translocation and its distribution in the plant parts were obtained.

Conclusions: The results obtained outline the possibility of application of forage grasses treatments with “Mycosym TRI-TON” and symbiotic N-fixing bacteria in systems for soil erosion protection in contemporary agriculture.

Key words: AM-fungi and Rhizobium inoculation, legume grasses, plant biomass, water-stable soil aggregates

P6_04

STUDY OF THE RADIUM-226 AND THORIUM-234 ACCUMULATION IN COTTON PLANTS FROM CONTAMINATED SOILS OF BUHOVO AREA

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Aim: The aim of the work is to study the effect of application of modified and natural zeolites for the assimilation of radioactive elements in agricultural products.

Material and Methods: We present and interpret the results of a vegetation experiment carried out in vessels with cotton of variety “Chirpan-603” on soil from the tailing pond of Buhovo, contaminated with natural radioactive isotopes. Two kinds of zeolites were folded in the soil – natural zeolite from the Beli Bair deposit and modified organic zeolite based on the natural calcite. The plants were air-dried and analyzed by gamma-spectrometry with multichannel analyzer CANBERRA DSA 1000 and high-purity germanium detector with 20 % efficiency and energy resolution of 1.3 keV for ^{60}Co γ -ray energy line at 1332 keV. The spectrum was analyzed by GENIE-2000 software with measurement uncertainties less than 10%. Typical counting times were 19–24 h. ^{234}Th concentration was derived from the weighted mean of the photopeaks at 63.5 keV and 92.6 keV and ^{226}Ra was evaluated at its 186.1 keV line taking into account the contribution of the overlapping line at 185.72 keV of ^{235}U .

Results: The transfer coefficients for the different plant organs were determined. The results obtained show for both isotopes least accumulation in the plant seeds. The considerable accumulation in the cotton wadding should be remarked. The highest accumulation for both isotopes was observed in the plant stems. The application of zeolites in the soil has not reduced significantly the accumulation of isotopes in the plants.

Conclusions:

- The accumulation in the cotton seeds is less than that in the leaves, stems and wadding of the cotton.
- The results obtained show that the application of zeolites in the soil does not influence noticeably the accumulation in the plants of the observed radioactive elements.

Key words: plants, soil, radioactivity, transfer coefficient, Radium-226, Thorium-234

P6_05

EFFECT OF PLANT EXTRACTS TO *CLAVIBACTER MICHIGANENSIS* - CAUSAL AGENT OF BACTERIAL CANKER OF TOMATO

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Aim: The aim of this study was to test the effect of different plant extracts to the causal agent of bacterial canker of tomato - *Clavibacter michiganensis* subsp. *michiganensis*.

Material and methods: Plants materials from *Artemisia absinthium*, *Ambrosia atemisifolia*, *Conium maculatum*, *Clematis vitalba*, *Datura stramonium*, *Hedera helix*, *Melilotus officinalis* were oven-dried to absolute dry weight and ground. Methanol and hexane extracts were prepared in Soxhlet extractor and concentrated in vacuum evaporator. The soft and the solid fractions were diluted in water using dimethylsulfoxide. *In vitro* tests were conducted by the agar diffusion method.

Results: Water solutions of soft and liquid fractions of five of the tested plants showed no effect on the growth of *Clavibacter michiganensis*. Good results were observed by using water solutions of soft fractions (5%) of *Datura stramonium* and *Melilotus officinalis* which suppressed bacterial growth.

Conclusion: Methanol extracts from *Datura stramonium* and *Melilotus officinalis* showed a potential to be used for control of *Clavibacter michiganensis*.

Key words: *Clavibacter michiganensis*, *Datura stramonium*, *Melilotus officinalis*, plant extracts

P6_06

REDUCTION OF PLANT AND FRUIT DAMAGES CAUSED BY TOMATO MOSAIC VIRUS AND CUCUMBER MOSAIC VIRUS IN TOMATOES THROUGH NATURAL INDUCERS OF RESISTANCE

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Aim: Our aim was to reduce the virus symptoms and damages caused by Tomato mosaic virus and Cucumber mosaic virus in tomatoes with environmentally friendly products.

Material and methods: In our study we used two ecological substances to induce Systemic Acquired Resistance (SAR): EXIN and BION. They are salicylic acid analogs and are naturally present in the plants. They were tested on three tomato cultivars – Buffalo heart, Giant and Nazareth. Plants were treated with the substances in three different schemes before and after virus inoculation. Cucumber mosaic virus (CMV) and Tomato mosaic virus (ToMV) symptoms observation was done every week and confirmation with DAS-ELISA test was performed.

Results: Tomato phenophase at the time of infection was a crucial factor that determined the degree of damage of the diseased plants. Our results indicated relatively good protection of tomato cultivars against Tomato mosaic virus and Cucumber mosaic virus. We received different percentage of reduction of the virus symptoms and damages of the fruits. Treating the plants with these substances expressed good results against ToMV for the cultivars Giant and Nazareth and better for Buffalo heart. CMV virus titer in the treated plants was slightly reduced unlike virus symptoms and damages on the fruits which were significantly reduced.

Conclusion: Different cultivars expressed different immune reactions to these plant viruses according to the genomic characteristics of the plant cultivar and virus. In mixed infections where damages were higher, treatment with these SAR elicitors reduced losses to almost zero.

Key words: ToMV, CMV, SAR, tomato resistance

P6_07

BULGARIAN SORTS OF FIELD BEANS WITH EXCELLENT TASTE

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The beans are the main grain-leguminous crop in the country, grown as a stew. For years it has been an important export item with unlimited market and one of the most profitable agricultural products with high currency returns in the range of 550-600\$ / ton. Despite the availability of good Bulgarian sorts and good market, the area and production of beans in Bulgaria have decreased about ten times in the past 10 years (in 2001, 107 606 ha planted with beans and in 2013 - only 15 414 ha). The reasons for this collapse are left with comment.

The purpose of the scientific report is to showing the advantages of the two new sorts of field beans, created in IASP "Obraztzov Chiflik" - Russe by an author team with the tutor Prof.Dr. Dobri Dobrev.

Key words: beans, sorts, agricultural products

THEMATIC SESSION VII

INVASIVE SPECIES

L7_01

IMPORTANT INVASIVE INSECT SPECIES IN BULGARIAN FAUNA

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For more than 100 years the **Colorado potato beetle** *Leptinotarsa decemlineata* (Say), also known as the Colorado beetle, is a serious pest on potatoes and has also been causing significant damage to tomatoes and eggplants. It is native to North America.

The **green peach aphid**, *Myzus persicae* (Sulzer), is found throughout the world. It attacks plants in the field, and readily infests with plant viruses vegetables and ornamental plants grown in greenhouses. Most probably, this species is native to Asia.

The **fall webworm**, *Hyphantria cunea* (Drury), is a pest principally during its larval stage, creating the characteristic webbed nests on the tree limbs of a wide variety of hardwoods. It's a common pest of hardwood trees not only in its native North America but also in Europe.

The **horse-chestnut leaf miner** (*Cameraria ohridella*) (Deschka & Dimic) is a leaf-mining moth. The species was first observed in Macedonia in 1984, and was described as a new species in 1986. Its larvae are leaf-miners on the Common Horse-chestnut (*Aesculus hippocastanum*). Probably *C. ochridella* is native to Greece, Macedonia and Albania.

Oxycarenus lavaterae (Fabricius) is Mediterranean bug to family Lygaeidae. In Bulgaria this bug was found for the first time in 1998. The species forms a big winter aggregation on lime trees (*Tilia*). To the present time we have no data this bug to be a pest or to transmit plant viruses.

Native to Asia the **harlequin ladybird** *Harmonia axyridis* is the new invasive alien species in North America and Europe. In Bulgaria it was found for the first time in 2009. It is a successful biological agent for some aphids. However like other exotic natural enemies that have had some negative impact after establishment, this ladybird is showing adverse impact in its new range too. These negative impacts can be classified into three categories: impact on non-target arthropods; impact on fruit production; impact as a household invader.

P7_01

INVASIVE PLANT SPECIES ALONG THE MAJOR RIVERS IN STRANDJA NATURE PARK

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Aim: We investigate the spread of invasive plant species along the major rivers in Strandzha Nature park – Rezovska, Veleka and Mladezhka.

Material and methods: Object of the study are five invasive plant species: *Ailanthus altissima* (Mill.) Swinge, *Amorpha fruticosa* L., *Bidens frondosa* L., *Erigeron annuus* (L.) Desf. and *Robinia pseudoacacia* L. The research was conducted on the route method in 2013.

Main results: We have mapped the spread of the invasive plant species along the river Rezovska, Veleka and Mladezhka. We have determined the number of locations and the number of invasive plant species: *Ailanthus altissima* – 1011 individuals in 125 localities, *Amorpha fruticosa* – 14556 individuals in 161 localities, *Bidens frondosa* – 137348 individuals in 97 localities, *Erigeron annuus* – 233 individuals in 12 localities and *Robinia pseudoacacia* – 10618 individuals in 264 localities. We have identified risk areas of occurrence and distribution of the invasive species: sediments (sand and gravel), the mouth of the tributaries of the rivers, river branches that dry up in summer, riverbanks that were deforested during the flood in 2006, fishing grounds, roads, crossing rivers and edge of forest roads located near rivers. The number of the species was highest near the villages.

Conclusion: Special measures are required to limit the spread of the invasive plant species and achieve a gradual recovery of the affected habitats.

Acknowledgements: The study was funded by project Sustainable Management and Planning Strandzha Nature park, OP Environment 2007-2013, Contract NoDIR-5113326-C-004 by procedure BG161PO005/11/3/3.2/06/27.

Keywords: Invasive plants, rivers, Strandzha Nature park

THEMATIC SESSION VIII

ECOLOGY AND EDUCATION

L8_01

“THE HUMAN – AN INSEPARABLE PART FROM NATURE” ENVIRONMENTAL EDUCATION MODEL

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The aim of this paper is to present a project-based learning (PBL) model engaged in the field of ecology and education. The interdisciplinary connections between ecology, arts (photography), psychology, etc. have a key role in the project. The target groups are high-school students from ninth to eleventh grade in arts schools as their interests overlap with the project-based activities. The project will take form as a competition between workgroups (teams) from the same class. The students have to craft a portfolio, containing photos, shot by each member of the group. The photographs must be on the topic of “The Human – An Inseparable Part From Nature” and should be accompanied by a short description (when and where they were taken and what the author’s motive was). As the photos must not be a collage the participants are encouraged to examine the nature around them as they rely on their imagination and creativity to produce provocative and innovative materials for the portfolio. This PBL would develop in the participants not only biological competences but also key competences such as: communicative skills in the native language, basic knowledge in the field of science and technologies, digital competence, skills for self-dependent learning and researching and cultural competences.

Key words: ecology, education, photography, project-based learning, human, nature

L8_02

PROJECT- BASED LEARNING ON "SOIL EROSION PROTECTION"

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Formation of ecological awareness in students through activities for global environmental issues and skills for environmental protection is one of the main goals of biology and health education. The education, learning and training in the modern school is unthinkable without project-based learning. It gives an opportunity to use various interactive and integrative learning techniques in an original and different form of training.

The **purpose** of this work is to present a model of project- based learning, in the interdisciplinary topic "Soil erosion protection". In this process interdisciplinary links between chemistry, environmental geography and information technology will be realized.

This model will develop in the students biological and chemistry competences, communicative skills, basic knowledge in the field of science and technologies, digital competence, skills for self-dependent learning and researching and cultural competences.

Key words: ecology, education, project-based learning, soils

P8_01

„I PRACTISE SOME SPORT = I AM HEALTHY AND FIT” (MODEL FOR BIOLOGY AND HEALTH EDUCATION)

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Obesity is one of the most serious issues facing society today. According to leading medical associations, more than one third of children and adolescents worldwide are overweight or obese as a result of unhealthy food and sedentary lifestyle. Excessive amount of body fat causes some serious health problems, such as myocardial infarction, cerebrovascular insult, and noninsulin-dependent diabetes mellitus – disorders that are normally rare among young people.

The purpose of this project is to encourage students in the 9th or 10th grade to take up some physical activity and combine it with healthy diet for a certain period of time (2 months). By observing the results, they should conclude that if they take proper care of their bodies, they would feel and look good. The project involves interdisciplinary relationships between biology and PE. The project has two phases – in the first, the students will search some information about different types of sports and their effect on human body. The second phase consists of making a survey – students will have to find people who practice some uncommon sport or physical activity, and ask them a few questions about it.

Key words: project-based learning, biology and health education, physical activity, healthy diet

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