# USB - SECTION BIOLOGY INSTITUTE OF BIODIVERSITY AND ECOSYSTEM RESEARCH – BAS



# SEMINAR OF ECOLOGY - 2017 WITH INTERNATIONAL PARTICIPATION 10<sup>th</sup> ANNIVERSARY 27-28 April 2017

UNION OF SCIENTISTS IN BULGARIA

Програма/Program Абстракти/Abstracts



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The Seminar of Ecology is 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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Contents:	
Program	1
Abstracts	12
Session I: Biodiversity and conservation biology	
<ul><li>Lectures</li></ul>	12
<ul> <li>Poster presentations</li> </ul>	19
Session II: Biotic and abiotic impact of the living nature and mechanisms of adaptation	
<ul><li>Lectures</li></ul>	30
<ul> <li>Poster presentations</li> </ul>	40
Session III: Ecosystem research, services and ecological agriculture	
<ul><li>Lectures</li></ul>	48
<ul> <li>Poster presentations</li> </ul>	51
Session IV: Landscape ecology	
<ul><li>Lectures</li></ul>	55
<ul> <li>Poster presentations</li> </ul>	56
Session V: Ecology and education	
<ul> <li>Lectures</li> </ul>	57
<ul> <li>Poster presentations</li> </ul>	59
Session VI: Other related topics	60
<ul> <li>Lectures</li> </ul>	60
<ul> <li>Poster presentations</li> </ul>	61
Index	63





# 10<sup>TH</sup> ANNIVERSARY "SEMINAR OF ECOLOGY – 2017" WITH INTERNATIONAL PARTICIPATION

27-28 April 2017PROGRAM27 April 2017

8<sup>00</sup> - 9<sup>00</sup> Registration (IBER-BAS)

9<sup>00</sup> - 10<sup>00</sup> Opening Ceremony

Family photo

#### THEMATIC SESSION BIODIVERSITY AND CONSERVATION BIOLOGY

Chairman: Akad. Atanas Atanasov and Prof. Boyko Georgiev

#### Plenary presentations

 $10^{00}$ - $10^{20}$ - BIO-MONITORING OR MONITORING OF BIODIVERSITY? <u>Yordan Uzunov-PL01\_01</u>

10<sup>20</sup>-10<sup>40</sup>- DRONES WITH ON-BOARD SENSORS AND FREELY AVAILABLE, UP-TO-DATE SATELLITE MULTISPECTRAL DATA HAVE CHANGED THE POSSIBILITIES FOR ECOSYSTEM ANALYSES IN BULGARIA <u>Valko Biserkov-</u> PL01\_02

#### Oral presentations

10<sup>40</sup>-10<sup>50</sup>- IMPACT OF PASTORALISM ON BIODIVERSITY IN THE CENTRAL BALKAN NATIONAL PARK'S TREELESS ZONE <u>Dimitar Ragyov</u>, Yordan Koshev, Rumyana Kostova, Tsvetoslav Mihovski, Evgeni Chehlarov, Kiril Vassilev, Borislav Naumov, Iva Apostolova, Tsvetelina Terzijska, Anna Ganeva, Borislav Guéorguiev, Valko Biserkov, Atanas Kirilov, Elena Ivanova, Magdalena Kircheva, Mariela Karapavlova, Rashid Rashid-L01\_01

10<sup>50</sup>-11<sup>10</sup> Discussion

11<sup>10</sup>-11<sup>30</sup>- Coffee break

- 11<sup>30</sup>-11<sup>40</sup>- GENETIC DIVERSITY AND POPULATION DIFFERENTIATION OF CENTAUREA PSEUDAXILLARIS (ASTERACEAE), A RARE AND ENDEMIC PLANT IN BULGARIA Galya Petrova, Stefan Petrov, Svetlana Bancheva- L01\_02
- 11<sup>40</sup>-11<sup>50</sup>- DISTRIBUTION OF LOTUS SPECIES IN BULGARIA AND ANALYSIS OF THEIR ECOLOGICAL CHARACTERISTIC <u>Kiril Vassilev</u>, Viktoriya Koleva, Mariya Georgieva, Iliana Dzhovanova, Media Gumus, Evelin Totev, Borislav Tsvetanov, Yusuf Ayan, Plamena Vassileva, Hristo Pedashenko, Antoaneta Petrova, Nikolay Velev- L01\_03
- 11<sup>50</sup>-12<sup>00</sup>- DISTRIBUTION AND CONSERVATION STATUS OF THE TORTOISES TESTUDO HERMANNI AND TESTUDO GRAECA IN PROTECTED AREA "ORANOVSKI PROLOM LESHKO" (BG0001022), BULGARIA <u>Nevena Malakova</u>, Lidia Sakelarieva, Alexander Pulev- L01\_04
- 12<sup>00</sup>-12<sup>10</sup>- THE USE OF BMWP AND ASPT INDICES FOR EVALUATION OF WATER QUALITY OF RIVER OREVOVECHKA REKA (REPUBLIC OF MACEDONIA) <u>Biljana</u> Rimcheska- L01 05

12<sup>10</sup>-12<sup>20</sup> **DEVELOPMENT OF WEB-BASED PORTAL AND INFORMATION SYSTEM FOR THE BIODIVERSITY OF SURFACE WATER BODIES IN THE REPUBLIC OF BULGARIA** *Galia Georgieva*, *Yordan Uzunov*, *Marieta Stanachkova*, *Dimitar Kozuharov*, *Pencho Yanev*, *Svetozar Stanachkov* **L01\_06** 

12<sup>20</sup>-12<sup>30</sup> **DISTRIBUTION AND DENSITY OF THE WILDCAT (FELIS SILVESTRIS SCHR.) IN NATIONAL PARK "PIRIN", BULGARIA <u>Neli Ivanova</u>, Elitsa Popova, Nikola Doykin, Valentin Zlatanov, Diana Zlatanova <b>L01\_07** 

12<sup>30</sup>-12<sup>50</sup> Discussion

12<sup>50</sup>-13<sup>50</sup>-Lunch

# THEMATIC SESSION ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE

Chairmen: Prof. Valko Biserkov and Assos.Prof. Ventsislav Karamfilov

#### **Plenary presentations**

13<sup>50</sup>-14<sup>10</sup> ECOSYSTEM SERVICES AND CLIMATE CHANGE OR WHAT IS THE COST OF DOING NOTHING AT ALL <u>Svetla Bratanova-Doncheva</u>, Nesho Chipev- PL03\_01

14<sup>10</sup>-14<sup>30</sup> THE INNOVATIONS IN ORGANIC FARMING – PROBLEMS AND PERSPECTIVES <u>Atanas Atanassov</u>, Maria Shishiniova, Goritsa Rakleova, Mariana Vlahova, Ivelin Panchev, Ventsislav Bardarov, Radoslav Russev- PL03 02

#### **Oral presentations**

- 14<sup>30</sup>-14<sup>40</sup> ECOLOGICAL EFFECTS OF THE CHANGE IN THE STRENGTH OF PRESSURES FROM A LOCAL POINT SOURCE OF ANTHROPOGENIC EUTROPHICATION IN A COASTAL ECOSYSTEM. A MODEL STUDY IN SOZOPOL BAY, BLACK SEA <u>Dimitar Berov</u>, Ventsislav Karamfilov-L03\_01
- 14<sup>40</sup>-14<sup>50</sup> THE CASE OF AIRSOFT GAMES IN MALA PLANINA AS AN UNCONVENTIONAL CULTURAL ECOSYSTEM SERVICES USE *Borislav Grigorov*, Assen Assenov- L03\_02
- 14<sup>50</sup>-15<sup>00</sup> DRIVERS OF CHANGES IN HIGH MOUNTAIN ECOSYSTEMS CASE STUDY: RILA HIGH MOUTAIN ECOSYSTEMS <u>Kostadin Katrandzhiev</u>, Svetla Bratanova-Doncheva, Gergana Georgieva-L03\_03

15<sup>00</sup>-15<sup>20</sup> Discussion

**15<sup>20</sup>-15<sup>40</sup>-Coffee break** 

#### THEMATIC SESSION LANDSCAPE ECOLOGY

Chairman: Prof. Svetlana Bancheva and Assoc. Prof. Assen Assenov

#### Plenary presentations

15<sup>40</sup>-16<sup>00</sup>- CHALLENGES IN BIOGEOGRAPHY DEVELOPMENT Assen Assenov-PL04\_01

#### **Oral presentations**

16<sup>00</sup>-16<sup>10</sup>- BRIDGING BETWEEN PHYSICAL GEOGRAPHY AND OCEANOGRAPHY: THE SCIENCE OF SEASCAPE ECOLOGY *Iliyan Kotsev*- L04\_01

16<sup>10</sup>-16<sup>30</sup> Discussion

16<sup>30</sup>-18<sup>50</sup> Poster sessions (*BIODIVERSITY AND CONSERVATION BIOLOGY; ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE; LANDSCAPE ECOLOGY)* and Discussion

Chairmen: Prof. Elisaveta Stoimenova and Assoc. Prof. Galina Radeva

#### POSTER SESSION BIODIVERSITY AND CONSERVATION BIOLOGY

P01\_01 FIRST REPORT OF DIATRYPELLA MALALEUCA (XYLARIALES) IN BULGARIA <u>Dimitar Stoykov</u>

P01\_02 RECENT FINDS OF LOBARIA PULMONARIA AND L. SCROBICULATA IN BULGARIA Dimitar Stoykov

P01\_03 ACUTE AND CHRONIC TOXICITY OF CADMIUM (CD) AND POLYAROMATIC HYDROCARBONS (PAH) ON ZEBRA MUSSEL (DREISSENA

- **POLYMORPHA PALLAS, 1771**) <u>Vesela Yancheva</u>, Elenka Georgieva, Stela Georgieva, Vesela Tsvetanova, Kostadinka Todorova, Iliana Velcheva
- P01\_04 NEW RECORDS OF HYPOGEOUS FUNGI FROM BULGARIA Boris Assyov, Monica Slavova
- P01\_05 BALKAN ENDEMIC PLANT LATHRAEA RHODOPEA ON MT FALAKRON, NORTH-EASTERN GREECE Asen Asenov
- P01\_06 NEST-SITE SELECTION OF LONG-LEGGED BUZZARD (BUTEO RUFINUS) IN THE BALKAN MOUNTAINS <u>Nadejda Djorgova</u>, Dimitar Ragyov, Borislav Naumov, Vulko Biserkov, Boris Nikolov
- P01\_07 TRANSPOSABLE ELEMENT-ASSOCIATED POLYMORPHISMS OF ARABIDOPSIS THALIANA ECOTYPES Mladen Naydenov, Elena Apostolova, Nikolaj Anachkov, Vesselin Baev, Mariyana Gozmanova, <u>Galina Yahubyan</u>
- P01\_08 EX VITRO ADAPTATION OF IN VITRO CULTURES OF ARTEMISIA ERIANTHA TEN. PLANTS <u>Gabriela Decheva</u>, Mariya Rogova, Lubov Hristova, Veneta Kapchina-Toteva
- P01\_09 BREEDING DENSITY AND HABITAT PREFERENCES OF SOMBRE TIT (POECILE LUGUBRIS) IN A KARST ENVIRONMENT <u>Marta Dimitrova</u>, Mattia Brambilla, Boris Nikolov
- **P01\_10 CUCKOO BUMBLEBEES IN BULGARIA** <u>Bilyana Stoykova</u>, Albena Lapeva-Gjonova
- P01\_11 ONE NEW GENUS OF ORDER DORYLAIMIDA (NEMATODA) FROM MARITIME ANTARCTIC Milka Elshishka, Stela Lazarova, Georgi Radoslavov, Peter Hristov, Vlada K. Peneva
- P01\_12 FIRST RECORD OF ANT-ATTENDED JUMPING PLANT-LICE ERYNGIOFAGA LOEWIANA (HEMIPTERA: PSYLLOIDEA) IN BULGARIA Ilia Gjonov, Albena Lapeva-Gjonova, Monica Pramatarova
- P01\_13 DISTRIBUTION OF SANGUISORBA SPECIES IN BULGARIA AND ANALYSIS OF THEIR ECOLOGICAL CHARACTERISTIC Kiril Vassilev, Viktoriya Koleva, Mariya Georgieva, Iliana Dzhovanova, Media Gumus, Evelin Totev, Borislav Tsvetanov, Yusuf Ayan, Plamena Vassileva, Hristo Pedashenko, Antoaneta Petrova, Nikolay Velev
- P01\_14 COMPARATIVE ANALYSES OF FLAVONOIDS IN ROOTS OF THREE BULGARIAN AND ONE UKRAINIAN GLYCYRRIZA GLABRA L. POPULATIONS <u>Asya</u> Kozhuharova, Milena Nikolova, Marina Stanilova

- P01\_15 POLLEN MORPHOLOGY OF SOME SPECIES FROM GENUS SILENE L. (CARYOPHYLLACEAE) <u>Atanas Tanev</u>, Dolja Pavlova
- P01\_16 WHAT DO WE KNOW ABOUT VEGETATION AND HABITAT DIVERSITY OF ELIN PELIN MUNICIPALITY? <u>Mediya Gumus</u>, Gana Gecheva, Nikolay Velev, Kiril Vassilev
- P01\_17 FIELD RELEASE OF ENTOMOPHAGA AULICIAE (ENTOMOPHTOROMYCOTA, ENTOMOPHTORALES) FOR CONTROL OF BROWN TAIL MOTH EUPROCTIS CHRYSORRHOEA (LEPIDOPTERA, EREBIDAE) <u>Daniela Pilarska</u>, Pencho Dermendzhiev, Rumen Nachev, Maria Dobreva
- P01\_18 BIOCONSERVATION STATUS OF THE CRITICALLY ENDANGERED ORCHIDS IN BULGARIA <u>Andrey Popatanasov</u>
- **EP01\_01 COLEOPTERA SPECIES OF SKOPJE CITY AND THE SURROUNDING AREA** Radmila Blazhevska, Aleksandra Cvetkovska-Gjorgjievska, Dana Prelić, Slavčo Hristovski, Julijana Arsovska, Jelena Hinić

# ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE

P03\_01 THE EFFECT OF FOLIAR TREATMENT OF SPRAY CARNATION WITH THE BIOMINERAL FERTILIZER PLANTAGRA Bistra Atanassova, Maria Yovkova

P03\_02 URBAN ECOSYSTEMS IN PLOVDIV PROVINCE: ASSESSMENT AND MAPPING OF PROVISIONING SERVICES RELATED TO GROUNDWATER <u>Zvezdelina Aydarova</u>, Bilyana Borisova, Miglena Zhiyansky, Stoyan Nedkov, Mariyana Nikolova

P03\_03 A RAPID METHOD FOR VULNERABILITY ASSESSMENT OF COASTAL PLANT COMMUNITIES FROM FLOODING CAUSED BY UNUSUAL STORMS <u>Stoyan Vergiev</u>, Mariana Filipova-Marinova, Ekaterina Trifonova, Iliyan Kotsev

P03\_04 MACROPHYTE COMPOSITION IN INLAND WETLANDS IN NORTHERN BULGARIA Borislava Gyosheva, Vladimir Valchev, Nevena Ivanova

#### LANDSCAPE ECOLOGY

P04\_01 ENVIRONMENTALLY RELEVANT ORGANIC COMPOUNDS FROM MARISA EAST DUMPS A. Kosateva, M. Stefanova, Z. Milakovska, S.P. Marinov

P04\_02 LAND COVER CLASSIFICATION OF SOUTHERN KRAISHTE REGION USING SATELLITE IMAGERY <u>Petko Bozhkov</u>

#### 28 April 2017

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

Chairmen: Prof. Stephka Chankova and Prof. Snezhana Grozeva

#### Plenary presentations

9<sup>00</sup>-9<sup>20</sup> THE EXOGENOUS FACTORS AND CARCINOGENESIS <u>Margarita Topashka-Ancheva</u>-PL02 01

#### **Oral presentations**

- 9<sup>20</sup>-9<sup>30</sup> PESTICIDES PERSISTENT ORGANIC POLLUTANTS IMPACT ON THE ENVIRONMENT AND HUMAN HEALTH *Irena Bogoeva* L02\_01
- 9<sup>30</sup>-9<sup>40</sup> VARIABILITY OF PSII FUNCTIONALITY AND CHLOROPLAST MEMBRANE LIPIDS OF SOME HALOPHYTIC AND GLICOPHYTIC REPRESENTATIVES FROM GENUS LACTUCA (ASTERACEAE) <u>Lilia Angelova</u>, Albena Ivanova, Dimitrina Koleva, Svetlana Momchilova, Sabina Taneva, Liliana Maslenkova-L02\_02
- 9<sup>40</sup>-9<sup>50</sup> IMPACT OF LIGHT STRESS AND SALINITY ON THE SPECTRAL PROPERTIES OF PHOTOSYNTHETIC APPARATUS IN PAULOWNIA <u>Stefanov Martin</u>, Yotsova Ekaterina, Markovska Yuliana, Apostolova Emilia- L02\_03
- 9<sup>50</sup>-10<sup>00</sup> INFLUENCE OF HEAVY METALS ON THE PHOTOSYNTHETIC APPARATUS OF CHLORELLA VULGARIS <u>Ekaterina Yotsova</u>, Martin Stefanov, Anelia Dobrikova, Emilia Apostolova- L02\_04
- 10<sup>00</sup>-10<sup>10</sup> ADAPTIVE CHANGES IN THE STRUCTURE AND THE FUNCTIONAL PERFORMANCE OF PHOTOSYNTHETIC APPARATUS OF MEDICINAL PLANT PETASITES HYBRIDUS FROM DIFFERENT HABITATS <u>Vesela Yordanova</u>, Albena Ivanova, Albena Momchilova, Svetlana Momchilova, Dimitrina Koleva, Liliana Maslenkova-L02 05
- 10<sup>10</sup>-10<sup>20</sup> CONTEMPORARY CONCEPTS IN THE ECOTOXICOLOGY OF CADMIUM (CD) IN TERRESTRIAL ECOSYSTEMS AND ITS BIOMONITORING IN TERRESTRIAL VERTEBRATES Peter Ostoich, Michaela Beltcheva, Roumiana Metcheva L02 06

10<sup>20</sup>-10<sup>30</sup> IMPACT OF BOTTOM SUBSTRATE AND ORGANIC QUANTITY IN SEDIMENTS ON TROPHIC STRUCTURE OF THE RIVER MACROZOOBENTHOS <u>Maria Kerakova</u>, Emilia Varadinova- L02\_07

10<sup>30</sup>-10<sup>50</sup> Discussion

10<sup>50</sup>-11<sup>10</sup> Coffee break

11<sup>10</sup>-11<sup>20</sup> HYPOXIA INDUCED FISH KILLS IN VARNA LAKE, BULGARIA. ASSESSMENT OF DEAD FISH BIOMASS AND DRIVING FACTORS FOR FISH MORTALITY <u>Stefan Kazakov</u>, Detelina Belkinova, Rumen Mladenov, Plamen Stojanov, Luchezar Pehlivanov-L02\_08

11<sup>20</sup>-11<sup>30</sup> THE ASSOCIATION OF ANEMIA AND CONCENTRATION OF LEAD, COPPER, ZINC AND IRON IN CHILDREN FROM ENVIRONMENTS CONTAMINATED WITH HEAVY METALS Snezana Veselinovska-L02 09

11<sup>30</sup>-11<sup>40</sup> DNA SUSCEPTIBILITY OF CHLAMYDOMONAS REINHARDTII AND SACCHAROMYCES CEREVISIAE TO NURELLE D <u>Petya Parvanova</u>, Teodora Todorova, Daniela Miteva, Zhana Mitrovska and Stephka Chankova L02\_10

11<sup>40</sup>-11<sup>50</sup> PHENOL DERIVATIVES DEGRADATION BY FREE LIVING AND ENTRAPPED IN CRIOGELS ENVIRONMENTAL BACTERIAL STRAINS <u>Satchanska G.</u>, Topalova Y., Dimkov R., Groudeva V., Petrov P., Tsvetanov Ch., Selenska-Pobell S., Golovinsky E. –**L02**\_11

11<sup>50</sup>-12<sup>00</sup> SCREENING FOR GENOTOXITY OF EXTRACTS OF AMARYLLIDACEAE PLANT ON THE CHLAMYDOMONAS REINHARDTII <u>Petya Parvanova</u>, Zhana Mitrovska, Boriana Sidjimova, Milena Nikolova, Strahil Berkov and Stephka Chankova L02\_12

12<sup>00</sup>-12<sup>10</sup> ADAPTIVE POTENTIAL OF TWO PHASEOLUS VULGARIS L. GENOTYPES TO SINGLE AND COMBINED PEG AND UV-B TREATMENTS <u>Tsveta Angelova</u> and Stephka Chankova-L02 13

12<sup>10</sup>-12<sup>30</sup> Discussion

12<sup>30</sup>-13<sup>30</sup> Lunch

# THEMATIC SESSION ECOLOGY AND EDUCATION

Chairman: Prof. Spasimir Tonkov and Prof. Daniela Pilarska

#### Plenary presentations

13<sup>30</sup>-13<sup>50</sup>- MODEL FOR ORGANIZING ENVIRONMENTAL EDUCATION THROUGH "REGION FOR KIDS"- A SUCCESSFUL BRAND AT REGIONAL LEVEL <u>Asya Asenova</u>-PL05 01

#### Oral presentations

13<sup>50</sup>-14<sup>00</sup>-CORRELATION-INTEGRATION METHODICAL SYSTEM IN PROCESSING CONTENTS FROM ENVIRONMENTAL EDUCATION DURING ENGLISH LESSONS Snezana Veselinovska, Snezana Kirova- L05\_01

#### THEMATIC SESSION OTHER RELATED TOPICS

Chairman: Prof. Spasimir Tonkov and Prof. Daniela Pilarska

#### **Oral presentations**

14<sup>00</sup>-14<sup>10</sup>-WATER MEMORY DISPLAYED BY THE CONTACT ANGLE DISTRIBUTIONS OF EVAPORATING DROPS Lidia Popova, Stefan Todorov L06 01

14<sup>10</sup>-14<sup>30</sup> Discussion

14<sup>30</sup>-14<sup>50</sup>-Coffee break

14<sup>50</sup>-17<sup>00</sup> Poster sessions (*BIOTIC AND ABIOTIC IMPACT OF THE LIVING NATURE AND MECHANISMS OF ADAPTATION; ECOLOGY AND EDUCATION; OTHER RELATED TOPICS*) and Discussion

Chairmen: Prof. Mariyana Lyubenova and Assoc. Prof. Marina Stanilova

# POSTER SESSION BIOTIC AND ABIOTIC IMPACT OF THE LIVING NATURE AND MECHANISMS OF ADAPTATION

- P02\_01 ASSESSMENT OF THE IMPACT OF OF SALINITY ON THE GROWTH AND ANTIOXIDANT ACTIVITY OF TWO LYCIUM SPECIES <u>Velimira Dimitrova</u>, Teodora Georgieva, Maria Geneva, Yuliana Markovska
- P02\_02 RESPONSE OF GLUTATHIONE REDUCTASE TO OXIDATIVE STRESS IN INTACT PEA PLANTS <u>Elena Shopova</u>, Vera Alexieva, Valentina Bubova
- P02\_03 PROBING THE PHYCOBILISOME STABILITY IN SYNECHOCYSTIS CELLS BY MICROCALORIMETRY <u>Nia Petrova</u>, Svetla Todinova, Tomas Zakar, Tünde Tóth, Stefka Taneva, Zoltan Gombos, Sashka Krumova
- P02\_04 IMPACT OF GROWTH REGULATORS ON THE ANTIOXIDATIVE CAPACITY OF PISUM SATIVUM PLANTS Marina Alexeeva, Ivalena Ilieva, Miroslava Zhiponova, Veneta Kapchina-Toteva, Ganka Chaneva
- P02\_05 ENHANCEMENT OF SEED GERMINATION AND GROWTH OF ECHINACEA PURPUREA (ASTERACEAE) <u>Boryanka Traykova</u>, Marina Stanilova
- P02\_06 THE IMPACT OF SEA WATER IMMERSION ON THE VIABILITY OF PSAMMOPHILOUS SPECIES GALILEA MUCRONATA (L.) PARL. <u>Stoyan Vergiev</u>
- P02\_07 PINUS NIGRA L. AS A BIOMONITOR OF AIR POLLUTION IN URBAN AREAS (PLOVDIV, BULGARIA) <u>Zlatovesa Hristozova</u>, Megi Dakova, Kostadinka Todorova, Bogdan Nikolov, Iliana Velcheva, Slaveya Petrova
- PO2\_08 DISTRIBUTION OF BOTTOM FAUNA UNDER FISH FARMING CAGES IN KARDZHALI DAM <u>Stefan Kazakov</u>, Maria Yankova, Angelina Ivanova, Kostadin Dochin, Georgi Rusenov, Maria Gevezova-Kazakova, Angel Zaikov, Tanya Hubenova
- P02\_09 ACCUMULATED HEAVY METALS AND OXIDATIVE STATUS IN TISSUES OF THE BLACK SEA MUSSEL (MYTILUS GALLOPROVINCIALIS LAMARK, 1819)

  Lachezar Yakimov, Albena Alexandrova, Elina Tsvetanova, Almira Georgieva, Nesho Chipev
- P02\_10 CYTOTOXIC ACTIVITY OF SIDERITIS SCARDICA EXTRACTS AND FRACTIONS ON HUMAN BREAST ADENOCARCINOMA CELL LINE Petya Koleva, Elena Stoyanova, Kalina Alipieva, Ina Aneva, Ljuba Evstatieva, Kalina Danova

P02\_11 ROLE OF GLUTATHIONE IN CELL RESPONSE TO OXIDATIVE STRESS IN YEAST Anna Tomova, <u>Ventsislava Petrova</u>

P02\_12 CLIMATE CHANGES IMPACT ON THE POPULATIONS OF THE CRITICALLY ENDANGERED ORCHIDS IN BULGARIA <u>Andrey Popatanasov</u>

EP02\_01 BIOMASS OF PINE FORESTS IN THE NORTH-EAST OF EUROPEAN PLAIN IN DEPEND ON SOIL MOISTURE CONDITIONS Andrey Osipov, Ivan Kutjavin

#### ECOLOGY AND EDUCATION

P05\_01 CONTENT OF RADIOACTIVE ELEMENTS IN SEDIMENTS OF THE NORTHERN BLACK SEA COAST OF BULGARIA FOR THE PERIOD 2010-2016 <u>Ivanka Yordanova</u>, Donka Staneva, Eliza Pavlova

P05\_02 AGALMATIUM FLAVESCENS (HEMIPTERA, ISSIDAE) AND CAMPONOTUS AETHIOPS (HYMENOPTERA, FORMICIDAE) – AN UNKNOWN TROPHOBIOTIC ASSOCIATION <u>Ilia Gjonov</u>, Albena Lapeva-Gjonova

#### OTHER RELATED TOPICS

P06\_01 DRY MASS YIELD AND AMOUNT OF FIXED NITROGEN IN SOME FORAGE LEGUME CROPS AFTER TREATMENT WITH ORGANIC FERTILIZER HUMUSTIM Viliana Vasileva, Todor Kertikov, Anna Ilieva

**P6\_02 ANTIOXIDANT PROPERTIES OF CANCER PAGURUS HEMOCYANIN** <u>Yuliana</u> <u>Raynova</u>, Milka Mileva, Ivan Kindekov, Dimo Krastev, Krassimira Idakieva

 $17^{00}$ - $17^{30}$ Film

 $17^{30}$  AWARDS AND CLOSING PROCEDURE OF THE  $10^{\rm TH}$  ANNIVERSARY "SEMINAR OF ECOLOGY - 2017" WITH INTERNATIONAL PARTICIPATION



#### THEMATIC SESSION I BIODIVERSITY AND CONSERVATION BIOLOGY

PL01\_01

#### Bio-monitoring or monitoring of biodiversity?

Yordan Uzunov

Department «Aquatic Ecosystems», Institute of Biodiversity and Ecosystem Research-Bulgarian Academy of Sciences

The position and role of the monitoring as a substantial element within the process of management, including the environment, is discussed in this report. The basic steps/activities include: forecasting and planning; regulating; provisioning (financial, material, technical, staffing); controlling, monitoring and reporting. The importance of the monitoring as an independent and objective activity which provides feedback in managerial process is underlined. Differences between "control" and "monitoring" are under discussion in comparison with other assessment activities such as study, investigation, survey, surveillance, observation and other specific measurements/evaluations designed for operational purposes.

Biological monitoring represents the systematic registration of the biotic responses of living systems to external impacts. Further discrimination between anthropogenic and natural fluctuations of the environmental variables is possible in biological monitoring. The purpose of the biomonitoring is to provide regular data with the intent to use this information in quality control programs when comparing the results obtained with reference and/or standard values, for instance, biological assessment, of the state of the environment and its components. These biotic responses can be expressed at all levels of organization of life - from the molecular level to ecosystems and biomes. It is evident that the possible biotic responses even at supra-organismal levels (biocenoses, ecosystems, biomes) could outnumber the possible impacts, which may differ mostly in their intensity. The knowledge on this overwhelming diversity of possible biotic responses from molecular to ecosystem levels is still insufficient and poorly applied for monitoring observations.

As each monitoring system, this one of the bio-monitoring is built up also on several common fundaments: massive and complex of observations (networking of representative sites); complexity and coherence with analogous systems; unification and standardization of the methods and metrics; unification of measurement methods and parameters/metrics to be monitored; centralization of data obtained for further processing, storage, and forecasting for management purposes. Thus, the system of environmental biomonitoring represents a comprehensive, national-wide system for collection, processing, storage and reporting information about biophysical and/or ecological state of the objects.

There are several methods and techniques using the physiological or behavioral responses of the organisms for detection and measurement of the state of the environment. Such biotic responses have found practical implementation for bioindication while they send signals for further analysis to be undertaken. In this case such organisms could be defined as *biosensors* or *biomonitors*, rather than bioindicators. Another concept associated with the term 'bioindicator' is that of an organism that accumulates substances in its tissues, which, after chemical analysis, could estimate prevailing environmental concentrations. Such organisms are defined as *bioaccumulators*. On the other hand, the biological indicator or *bioindicator* seems to be an organism (species) known to have particular requirements with regard to some range of environmental variables. Once these are defined, the presence of a particular species in a habitat or the expression of some biotic response indicates that the given determinant or parameter is within the ecological tolerance limits of that species.

For the purpose of biomonitoring, two groups of evaluations are most applicable: 1. Parameters or indices expressed by an *integral* in time, for instance, reporting the results of some dynamic processes at the moment of their measurement, and 2. Parameters or indices expressed by a derivative in time (*differential*), for instance, rates of some dynamic processes. There is another, third group of evaluations that does not measure quantities but that have general importance in measuring, recording, or evidence of events such as phenological observations (first appearance of migrating birds or flowering of plants, etc.).

Three main groups of methods are commonly used for biomonitoring and bioassessment; they are mainly used for monitoring water and ambient air quality. These are: species-related methods; community-related methods; and habitat-related methods.

In terms of *monitoring the biodiversity*, the relation is likely between an object and a subject: it depends on the aims of monitoring - assessing the state of the environment by means of biological responses or the state/health of the species/populations and/or habitats.

#### PL01 02

# Drones with on-board sensors and freely available, up-to-date satellite multispectral data have changed the possibilities for ecosystem analyses in Bulgaria

#### Valko Biserkov

Institute of Biodiversity and Ecosystem Research-Bulgarian Academy of Sciences

The use of freely available, up-to-date multispectral satellite data combined with purposefully gathered multispectral data from quadcopters with on-board sensors enables new opportunities for remote monitoring of target ecosystems, natural habitats, conservationally important species and key abiotic environmental parameters.

Since March 7<sup>th</sup> 2017 the twin satellites Sentinel-2A and 2B of the European Space Agency's Copernicus Programme provide a 13-band multispectral imagery of Europe every five days. More than 60 meaningful combinations of bands can be produced. With a pixel resolution of 60 meters for coastal aerosol, water vapor and SWIR Cirrus, 10 meters for RGB+NIR bands and 20 meters for other bands, the Sentinel-2 products enable precise analyses of the ecosystem's condition. The archive of the Sentinel-2 satellite imagery enables the discovery of long-term trends in ecosystems. Properly planned ecological observations in the field can be linked to satellite data when solving different ecological tasks.

Drone imagery can provide higher detail and can be used to calibrate approaches for interpreting the satellite imagery. They can also provide more accurate information about local objects. The use of some these new capabilities is demonstrated with examples from the work performed by the "Conservation Biology", research group at IBER-BAS: 1) evaluation of damage to the Ponto-Sarmatian steppes by a 2016 fire in the Bolata dere area; 2) spatial distribution of seagrass in the Sozopol bay; 3) pilot inspection in 2015 of the "Srebarna" managed reserve with regards to a confirmed infection with bird influenza (H5N1) in the Dalmatian pelican colony.

**Keywords**: DJI drones, Inspire 1 Pro, Phantom 4, Sentinel-2, ecosystem

#### L01 01

# Impact of pastoralism on biodiversity in the Central Balkan National Park's treeless zone

<u>Dimitar Ragyov</u><sup>1</sup>, Yordan Koshev <sup>1</sup>, Rumyana Kostova<sup>2</sup>, Tsvetoslav Mihovski<sup>3</sup>, Evgeni Chehlarov <sup>1</sup>, Kiril Vassilev <sup>1</sup>, Borislav Naumov <sup>1</sup>, Iva Apostolova <sup>1</sup>, Tsvetelina Terzijska <sup>1</sup>, Anna Ganeva <sup>1</sup>, Borislav Guéorguiev <sup>4</sup>, Valko Biserkov <sup>1</sup>, Atanas Kirilov <sup>5</sup>, Elena Ivanova <sup>6</sup>, Magdalena Kircheva <sup>6</sup>, Mariela Karapavlova <sup>6</sup>, Rashid Rashid <sup>6</sup>

<sup>1</sup>Institute of Biodiversity and Ecosystem Research-Bulgarian Academy of Sciences

<sup>2</sup>Sofia University "St. Kliment Ohridksi"

<sup>3</sup>Institute of Mountain Pastoralism and Agriculture – AA

<sup>4</sup>National Museum of Natural History – BAS

<sup>5</sup>Institute of forage crops, Pleven – AA

<sup>6</sup>Fortis Facility Ltd.

**Aim:** To assess the impact of the mountain pastoralism on the biodiversity in the Central Balkan National Park's treeless zone.

**Material and methods:** Choice of model territories (GIS analysis) and model groups of species; Linear (point/square) transects for field data collection; Statistical analysis.

**Main results:** The data collected in this pilot study was not sufficient to definitively evaluate the mountain pastoralism impact on the biodiversity. However the study revealed several robust models:

- Mammals. The European ground squirrel abundance was highest in the places with the highest pastoralism intensity.
- Birds. The insectivores birds abundance was negatively affected by the highest and the lowest grazing intensity, while moderate grazing intensity affects it positively. The herbivorous birds' relative numbers and distribution were affected in an opposite way.
- Amphibians and reptiles. Theoretically, could be expected that overgrazing would cause diversity decline, due to the habitat degradation.
- Insects. The highest ground beetles species richness was recorded in the moderate grazing zones. The species richness decreased to the zones with lower or higher levels of grazing.
- Plants and habitats. The grazing intensity had a key impact on the structure and composition of the flora, vegetation and habitats. The sheep grazing had a lower impact on this group than cattle and horse grazing in relation to soil degradation and ruderalisation.

**Conclusion:** The mountain pastoralism (through its type and intensity) affects the biodiversity in different way according to the different species/species groups. Therefore a specific management approach should be applied in order to conserve the biodiversity.

**Acknowledgements:** This study was conducted in the frame of a public order "Choice of Consultant Natura" of OPE 2007-2013 project DIR5113325-12-109 "Central Balkan – Park for Everyone".

**Keywords:** Management, Protected area, Grazing, Bulgaria, NATURA 2000

#### L01\_02

# Genetic diversity and population differentiation of *Centaurea pseudaxillaris* (Asteraceae), a rare and endemic plant in Bulgaria

Galya Petrova<sup>a\*</sup>; Stefan Petrov<sup>b</sup>; Svetlana Bancheva<sup>a</sup>

<sup>a</sup> Institute of Biodiversity and Ecosystem Research-Bulgarian Academy of Sciences, Sofia, Bulgaria <sup>b</sup>Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria

**Introduction:** Centaurea s.l. (Asteraceae) is one of the most endemic-rich genera in the Bulgarian flora. Centaurea pseudaxillaris Stef.&T.Georgiev is a Bulgarian endemic, protected by the national Biodiversity Act, included in the Red List of vascular plants and in the Red Data Book of Bulgaria with conservation status "Critically endangered". In the course of previous studies, both ex situ and in situ measures were undertaken. However, the population genetic structure of this species had never been addressed.

**Aim:** The present study aims to determine whether habitat fragmentation and decreasing population size have influenced the genetic diversity of *C. pseudaxillaris*.

**Materials and Methods:** *C. pseudaxillaris* individuals were sampled from the populations of the species near the Dobrich village, Haskovo district and Besaparski Hills, south-east of the town of Pazardzhik. DNA was extracted from young leaves following a modified CTAB-procedure. The Inter-simple sequence repeat (ISSR) genetic patterns among populations were determined by STRUCTURE v.2.2. GenAlEx v.6.5 was used to calculate the partition of genetic variation between and within populations. Spatial genetic relationships among the samples were visualized by Principal Coordinate Analysis (PCoA) using Nei's genetic distance.

**Results:** Ten primers (Microsynth, Switzerland) were selected after testing 25 primers on the collected samples. Genetic diversity was measured based on the percentage of polymorphic loci (P), Shannon's information index (SI), genetic diversity (h), and unbiased genetic diversity ( $h_u$ ).

**Conclusion:** Our results indicate a general decline of *C. pseudaxillaris*' populations. However, more comprehensive molecular study is needed to get a clearer picture on the population status of the species. **Acknowledgments:** This work was supported by the Bulgarian NSF under Grant DFNI-B02/18.

**Keywords:** Centaurea pseudaxillaris, Asteraceae, Genetic diversity

#### L01\_03

### Distribution of *Lotus* species in Bulgaria and analysis of their ecological characteristics

<u>Kiril Vassilev</u><sup>1</sup>, Viktoriya Koleva<sup>2</sup>, Mariya Georgieva<sup>2</sup>, Iliana Dzhovanova<sup>3</sup>, Media Gumus<sup>5</sup>, Evelin Totev<sup>3</sup>, Borislav Tsvetanov<sup>3</sup>, Yusuf Ayan<sup>4</sup>, Plamena Vassileva<sup>2</sup>, Hristo Pedashenko<sup>1</sup>, Antoaneta Petrova<sup>6</sup>, Nikolay Velev<sup>1</sup>

<sup>1</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Department of Plant and Fungal Diversity and Resources, Sofia, Bulgaria

University of Sofia "St. Kliment Ohridski", Faculty of Geology and Geography, Sofia, Bulgaria
 University of Sofia "St. Kliment Ohridski", Faculty of Biology, Sofia, Bulgaria
 University of Forestry, Faculty of Forestry, Sofia, Bulgaria
 University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Plovdiv, Bulgaria
 Botanical Garden, Bulgarian Academy of Science, Sofia, Bulgaria

**Aim:** The aim of this study is to summarize the available data on the distribution of *Lotus* species in Bulgaria as well as their ecological preferences to abiotic conditions (altitudinal range, basic rock, exposition, etc.) and occurrence in certain vegetation and habitat types.

Material and methods: We collected all available data from floristic and phytocoenological literature sources, such as herbarium collections (SOM, SO, SOA) and phytocoenological databases (Balkan Dry Grassland Database, Balkan Vegetation Database, Bulgarian National Database) about all 8 species of the *Lotus* genus reported for the Bulgarian flora – *L. aegeus, L. angustissimus, L. corniculatus, L. strictus, L. tenuis, L. uliginosus, L. longesiliquosus, L. maritimus*. All localities were georeferenced using Google Earth *a posteriori*, when GPS data was missing. The mapping was done using ArcGis 10.0 software. The classification of vegetation types follows the concept of Mucina et al. (2016) about high rank vegetation types, whereas habitat types follow the EUNIS habitat classification and Directive 92/43/EEC. We also analyzed the applied floristic elements categories for the studied species.

**Results:** The study area is covering the whole territory of the country. We found a disproportion in available data for the studied species. *L. angustissimus* and *L. strictus* are locally found, whereas *L. corniculatus* has widest distribution, due to its wider ecological requirements. All species are accompanying species in grassland communities, whereas *L. corniculatus* is sometimes a co-dominant species in mesic grassland.

**Conclusions:** *Lotus* species are mainly found in sub-Mediterranean vegetation types in the country. Only *L. corniculatus* is widely distributed in different vegetation types and habitats.

**Keywords**: Flora, vegetation, spatial distribution, floristic elements

#### L01 04

# Distribution and Conservation Status of the Tortoises *Testudo hermanni* and *Testudo graeca* in Protected Area "Oranovski Prolom – Leshko" (BG0001022), Bulgaria

Nevena Malakova, Lidia Sakelarieva, Alexander Pulev

South-West University "Neofit Rilski", Faculty of Mathematics and Natural Sciences,
Department of Geography, Ecology and Environmental Protection,
Blagoevgrad, Bulgaria

**Aim:** To supplement and summarize data about the distribution of the Hermann's Tortoise (*Testudo hermanni*) and Spur-thighed Tortoise (*T. graeca*) in Protected Area BG0001022 "Oranovski Prolom – Leshko".

**Material and methods:** The two species and localities were registered during field trips in March 2010 – October 2016. The geographic coordinates have been determined with GPS. All localities (published and new) have been summarized in a table and marked on a map.

**Results:** Totally 60 new localities have been registered. The Hermann's Tortoise has been found in 44 localities and the Spur-thighed Tortoise in 16. The sex structure of the populations is different. The proportion between the male and female specimens of *T. hermanni* is 1.19:1, while the male specimens of *T. graeca* are 3 times more than the female ones (3.25:1). The two species are protected according to the national and international legislation.

**Conclusion:** Both tortoises have been recorded in the protected area. Data about the spread of the Spur-thighed Tortoise are reported for the first time, and the Hermann's Tortoise has been registered in many new localities. Both species have high frequency of occurrence. The number of specimens of *T. hermanni* has been almost 3 times more than the one of *T. graeca*.

**Key words:** Testudo, new locality, conservation status, protected zone, Natura 2000

#### L01 05

# The use of BMWP and ASPT indices for evaluation of water quality of river Orevovechka Reka (Republic of Macedonia)

#### Biljana Rimcheska

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria;

E-mail: rimceska@gmail.com

**Aim**: The goal was to assess the water quality according to BMWP and ASPT indices of the river Orevovechka Reka.

**Material and methods**: The survey was based on samples taken during March 2011-March 2012 from the hydrographic basin of the river Orevovechka Reka. Macroinvertebrate specimens from different substrates were collected by a Surber net and with an Eckman grab, following a standard methodology for collection of the bottom fauna. The data were analyzed using the ASTERICS software package version 3.3.

**Results**: During the researched period a total of 146 macroinvertebrate taxa were recorded. The highest BMWP score (149) was noted near the spring region of Orevovechka Reka in March 2012, while the lowest water quality was estimated at the lowest part of the stretch in August 2011. The ecological status on the most upstream sampled site was improved from moderate (during March, May, August and September 2011) to good (in November 2011 and March 2012). Contradictory, on the middle stretch of the river we noted deteriorated water quality from good (March and May 2011), trough pure (August 2011) to moderate in the next mounts. At the most downstream site (T3) in general the lowest ecological status was observed (moderate-pure). The gained ASPT scores varied from 7.2 (May 2011 on T2) to 4.3 (November on T3).

**Conclusion**: This analysis shows the existing misbalance in the semi-mountainous rivers especially at extreme conditions and dry seasons, where in, during postponed rain periods there is a risk of the river bed to over dry.

**Keywords**: Macroinvertebrates, BMWP, ASPT, Orevovechka Reka River, Republic of Macedonia.

#### L01 06

# Development of web-based portal and information system for the biodiversity of surface water bodies in Bulgaria

<u>Galia Georgieva</u><sup>1</sup>, Yordan Uzunov<sup>1</sup>, Marieta Stanachkova<sup>2</sup>, Dimitar Kozuharov<sup>2</sup>, Pencho Yanev<sup>3</sup>, Svetozar Stanachkov<sup>3</sup>

<sup>1</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria

<sup>2</sup>Department of General and Applied Hydrobiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

<sup>3</sup>Jar Computers, Business Center Serdika, 83 Gyueshevo Str., Sofia, Bulgaria

**Aim:** This paper is part of a larger project which objectives are to combine, digitalize and visualize the biodiversity of Bulgarian surface water bodies (SWB). The database was worked out through a content management system and serves data retrieval by queries, the results of which are presented in a tabular form.

Materials and methods: It was essential to gather information on both taxa occurrence and ecological information on these taxa. For the pilot version of the database data on Oligochaeta Limicola and zooplankton communities was used. The database management system (DBMS) used MySQL server. For the web-site

development PHP 5, HTML, CSS, jQuery, AJAX queries and Java scripts based on the free Framework web CodeIgniter were used.

At the moment the query results are visualized in a tabular view, but in the future will be visualized using the Google Graph. For the map view of results the Google maps library will be used.

**Results:** The database is a catalogue of the studied SWB and contains taxonomic and ecological information as a "living document". As a result of the collected data different queries are set. In response to search queries georeferenced maps of the distribution of species are created, as well as graphs resulting from different statistical analyzes. Furthermore a virtual library of scientific papers in the field of hydrobiology was organized.

**Conclusion:** At present, there is no such database on the hydrobionts developed in Bulgaria. This product could combine the available information and serve as a tool for increasing the awareness of scientists and students in the field of hydrobiology.

**Keywords:** data base, biodiversity, Bulgaria, oligochaeta, zooplankton

#### L01 07

# Distribution and density of the wildcat (Felis silvestris Schr.) in National Park "Pirin", Bulgaria

Neli Ivanova<sup>1</sup>, Elitsa Popova<sup>1</sup>, Nikola Doykin<sup>1</sup>, Valentin Zlatanov<sup>2</sup>, Diana Zlatanova<sup>1</sup>

<sup>1</sup>Department of Zoology and Anthropology, Faculty of Biology, Sofia University St. Kliment Ohridski, Sofia, Bulgaria, 
<sup>2</sup>Advanced Wildlife Technologies and Management, Sofia, Bulgaria

**Aim:** The wildcat's distribution and density are poorly studied in Bulgaria. The objective of this study was to collect this data for the territory of National Park "Pirin", Bulgaria through camera trapping.

Materials and Methods: We set up 24 camera traps between April and November 2014 in 7 areas of NP "Pirin" according to a predetermined grid (1x1 km). The collected data was processed with Camera Base 1.6 and GIS instruments. 23 adults and 2 sub-adults were individually recognized and were used in Capture-Mark-Recapture (CMR) analyses using the program Capture. The density was estimated using the results of the CMR and areas derived by buffers around each camera trap with radius equal to the half Mean Maximum Distance Moved of each recapture.

**Results:** The results indicate that the wildcat is evenly distributed in NP "Pirin". The mean density of the species in the Park was estimated as 0.14 ind./km². The highest wildcat density (0.24 ind./km²) was observed in the south and southwestern parts of the park which are less inhabited by humans. We found that wildcats avoid areas with intense presence of humans and dogs, caused by the network of settlements and resorts around the park, especially in the northeastern part.

**Conclusions:** The wildcat in NP "Pirin" has a relatively stable population with unfragmented distribution. Our density estimations are similar to those reported for other European countries. During our study some direct threats were recorded.

**Acknowledgements:** Camera traps were provided by the Directorate of NP "Pirin", acquired through Project № DIR-5113325-3-91 of Operational Program Environment 2007-2013.

**Keywords**: camera trapping, density estimation, threats, wildcat

#### First report of Diatrypella malaleuca (Xylariales) in Bulgaria

Dimitar Stoykov

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

**Aim**: Contribution to the knowledge of the fungi from Xylariales in Bulgaria.

**Material and methods**: The materials were collected during 2016 in the Eastern Forebalkan region. Information on the substrata was gathered in the field and documented with the aid of Canon Power Shot A460. The exact locations of the findings were taken using Garmin Etrex 10. Dry materials were preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Sofia (SOMF).

**Results**: First find of *Diatrypella malaleuca* (*Xylariales*) on fallen twigs of *Fagus sylvatica* was reported in the Eastern Forebalkan, Golyama Zhelyazna village (along Toplya river, above the natural landmark 'Peshtera Topya'), while in the surroundings of the adjacent Staro Selo village (foothill of Vasiljovska Mt) the closest *D. quercina* was documented for the first time from the Forebalkan on old twigs of *Quercus cerris*. Brief description of *D. malaleuca*, supported with macro- and microscope photos of the finds made under the Boeco LM, was applied.

**Conclusion**: The first record of *Diatrypella malaleuca* expands the current knowledge on the fungi with diatrypelloid stromata in the country. *Quercus cerris* was recorded as new host plant for Bulgaria of *D. quercina*. Brief overview on the known data about the genus *Diatrypella* in Bulgaria was made.

**Acknowledgements**: The present work was conducted within the frame of the project 'Taxonomy, conservation and sustainable use of fungi'.

**Keywords**: *Diatrypella*, *Fagus*, fungal diversity, new host

#### P01 02

#### Recent finds of Lobaria pulmonaria and L. scrobiculata in Bulgaria

Dimitar Stoykov

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

**Aim:** Additions to the data on the ecology and distribution of the foliose lichens.

**Material and methods:** Field trips for collecting of the lichen thalli were arranged during 2015-2016, following the linear transect method. Single finds of *L. pulmonaria* were dated from 2004-2009. Information on the substrata was collected in the field and documented with the aid of Canon PS, while positions of recent localities, indicated on the presented UTM-grid map with 10 km<sup>2</sup> per cell were taken with the help of Garmin 62S, Garmin Etrex 10.

**Results:** The most rich populations of the lung lichen (mature and juvenile individuals) were found in Rilomanastirska Gora Reserve, in the area of Rilski monastery (along Ilijna Reka; towards Brichebor peak; Kalugerski Dol - in direction Ivan Vazov chalet; Kirilova Polyana locality), on bark of beech, maple, oak, rocks and in Parangalitsa Reserve, on beech. Single new locations were reported in Trevnenska Mt (Balgarka Nature Park), on beech; along Devinska Reka river (above Devin, Rhodopi), on oak; along Krajna Reka river (Rila National Park), on maple. *Lobaria scrobiculata* is recorded from Rila Mts: Rilomanastirska Gora Reserve (Ilijna Reka), on rock, and in Parangalista Reserve - on beech.

**Conclusion:** Recent localities are situated mainly in the mountain areas at altitudes between 1100 and 1700 m (Stara Planina, Rila Mts). The finds, exposed on the UTM-grid map, are essential for future conservation strategies and bring additional information on their habitats.

**Acknowledgements:** This work was supported by the management plan of the Rilomanastirska Gora Reserve and the update for the plan of the National Park Rila. The author is grateful to his colleagues from IBER, BAS (Sofia) for the gathered materials of *L. pulmonaria*.

**Keywords:** distribution, ecology, *Lobaria*, tree lungwort

#### P01 03

# Acute and chronic toxicity of cadmium (Cd) and polyaromatic hydrocarbons (PAH) on zebra mussel (*Dreissena polymorpha* Pallas, 1771)

<u>Vesela Yancheva<sup>1\*</sup></u>, Elenka Georgieva<sup>2</sup>, Stela Georgieva<sup>2</sup>, Vesela Tsvetanova<sup>3</sup>, Kostadinka Todorova<sup>4</sup>, Iliana Velcheva<sup>1</sup>

<sup>1</sup>Plovdiv University "Paisii Hilendarski", Faculty of Biology, Department of Ecology and Environmental Conservation, Plovdiv

<sup>2</sup>Plovdiv University "Paisii Hilendarski", Faculty of Biology, Department of Developmental Biology, Plovdiv <sup>3</sup>Regional Accredited Laboratory at Executive Environment Agency, Ministry of Environment and Water, Plovdiv <sup>4</sup>Plovdiv University "Paisii Hilendarski", Lyuben Karavelov Branch, Kardzhali

**Aim:** The main objective in the present experiment was to study the possible negative effects which Cd and PAH could have on the lysosomal membrane stability in haemocytes of the invasive mollusc zebra mussel (*Dreissena polymorpha*) by applying the neutral red retention assay (NRRA).

**Materials and methods:** The mussels were exposed to different concentrations of Cd and PAH in laboratory conditions for 96 hours and 31 days. These toxicants are considered as priority substances in surface waters according to Directive 2008/105/EO of the European parliament and the Council. The concentrations were prepared as 50% above and 50% below the maximum permissible levels (100 %) set by national and EU law.

**Results:** We found that the acute and chronic exposure to both toxicants decreased significantly the lysosomal membrane stability of zebra mussels compared to the control. In addition, PAH toxicity was more severe compared to Cd as the PAH exposure led to lower retention time (NRRT).

**Conclusions:** Overall, we consider that such experiments can be successfully applied in risk assessment and monitoring programs on contaminated aquatic systems with both, metal and organic pollutants, and the obtained results in the field of water policy, respectively.

**Acknowledgements:** We thank the Regional Accredited Laboratory at Executive Environment Agency, Ministry of Environment and Water, Plovdiv for providing the toxicants which were needed for this study.

**Keywords:** mussels; Cd; PAH; lysosomes; haemocytes

#### P01\_04

#### New records of hypogeous fungi from Bulgaria

Boris Assyov<sup>1</sup>, Monica Slavova<sup>2</sup>

<sup>1</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria <sup>2</sup>4 Krivolak Str., 1000 Sofia, Bulgaria

**Aim:** The aim of this work was to expand the knowledge on hypogeous fungi in Bulgaria, contributing records of new species.

**Materials and methods:** Fungi were collected mostly with the aid of trained dogs. Specimens were preserved in air-dried state after thorough examination. Observations of microscopic features were held with light microscope. Widely used methods for study of those fungi were applied.

**Results:** Twenty species were recorded for the first time from Bulgarian localities, namely *Balsamia vulgaris*, *Elaphomyces anthracinus*, *Genea pseudoverrucosa*, *Gymnomyces xanthosporus*, *Hydnotrya michaelis*, *Hymenogaster hessei*, *Hysterangium coriaceum*, *H. epiroticum*, *Lactarius borzianus*, *Protoglossum aromaticum*, *Pyrenogaster pityophilus*, *Radiigera atrogleba*, *Sclerogaster hysterangioides*, *Sepultaria sumneriana*, *Tuber foetidum*, *T. fulgens*, *T. magnatum*, *T. mesentericum*, *T. nitidum*, and *Wakefieldia macrospora*.

Members of genera *Genea, Gymnomyces, Hysterangium, Protoglossum, Pyrenogaster, Radiigera, Sclerogaster* and *Wakefieldia* are discovered for the first time in this country. *Gymnomyces xanthosporus, H. michaelis, L. borzianus, P. aromaticum,* and *R. atrogleba* also constitute first records for the Balkan Peninsula. *Sepultaria sumneriana* is a neomycete, spreading in the country through the cultivation of *Cedrus* spp.

**Conclusion:** Data collected show unequivocally that Bulgarian hypogeous mycota is rich, hosts uncommon species, and thus merits further exploration.

**Acknowledgements:** Studies of the first author are held within project 'Taxonomy, conservation and sustainable use of fungi'.

**Keywords:** Balkan mycota, sequestrate fungi, truffle-like fungi, truffles

#### P01\_05

#### Balkan endemic plant Lathraea rhodopea on Mt Falakron, North-Eastern Greece

Asen Asenov

Sofia University, Faculty of Bilology, 8 "Dragan Tsankov" Blvd, 1164 Sofia, Bulgaria

**Aim:** Investigation on distribution of the Balkan endemic plant *Lathraea rhodopea* Dingler on Mt Falakron.

**Introduction:** Mt Falakron (2232 m) is situated between Mt West Rhodope and Aegean Sea, North-Eastern Greece. Rocks base is calcareous (marbles), soils are rendzinas. The climate is Transitional Mediterranean. Plant diversity is very rich.

Areal of Balkan endemic plant *Lathraea rhodopea* covers territory of south Bulgaria (Mt Slavianka, Mt Rhodopes, Tracian plane) and North-Eastern Greece (Mt Rhodopes, Mt Falakron and some other mountains close to Mt Rhodope).

Material and methods: Field trips

**Result**: The investigations discover only one fragment numbering 15 vegetative individuals growing on area 2 m², parasititic on *Salix triandra* L., situated on valley of River Soushitsa, close to Pirgi village, on 543 m, south side of Mt Falakron.

Conclusion: Distribution of this endemic species on Mt Falakron is very poor, which is prerequisite for the disappearance of this species from the flora of Mt Falakron. Perspectives for the survival of this species are negative.

**Keywords:** Balkan endemic plant, *Lathraea rhodopea*, distribution

#### P01 06

#### Nest-site selection of Long-legged Buzzard (Buteo rufinus) in the Balkan Mountains

Nadejda Djorgova, Dimitar Ragyov, Borislav Naumov, Vulko Biserkov, Boris Nikolov

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

**Aim:** Long-legged Buzzard (*Buteo rufinus*) is among the least studied diurnal birds of prey in Europe and our study aims to identify natural factors, which influence its nest-site selection.

**Materials and methods:** Data analyses are based on 38 nests from Balkan Mountain Range, Bulgaria. Various parameters describing their location are measured in ArcGIS. To define the position index of the nests the ratio between the elevation of nests and the maximum elevation of the surrounding area in three circular plots with different radius is calculated. The size assigned to the circular plots speculatively represents the immediate nesting surroundings (radius 500 m), the core area (2000 m) and the whole breeding territory (4000 m) of a pair. **Results:** The mean elevation of nests of the species is 823.2 m. Most of them fall in the low mountain zone (600-1000 m) – 47.4%. The nests are exclusively on cliffs, mainly on a steep slope – mean 23.4° (44.7%), with exposures comprising mostly southerly component (57.8%). Position index of the nests varied between 0.83 (500 m-plot) and 0.61 (4000 m-plot). Nesting cliffs were surrounded by various habitat types, in most cases transitional woodland-shrub (44.7%), followed by broad-leaved forest (34.2%), mixed forest (7.9%), bare rocks (7.9%), natural grasslands (2.6%) and sparsely vegetated areas (2.6%).

**Conclusion:** Long-legged Buzzard is exclusively petrophilic in the Balkan Mountains, with a dominant position of the nests within its territories. The species shows an ecological plasticity in terms of nest-site location and breeding habitats.

**Keywords:** diurnal raptors, petrophilic species, nest position index, Bulgaria

#### P1 07

#### Transposable element-associated polymorphisms of Arabidopsis thaliana ecotypes

Mladen Naydenov, Elena Apostolova, Nikolaj Anachkov, Vesselin Baev, Mariyana Gozmanova, Galina Yahubyan

Department of Plant Physiology and Molecular Biology, University of Plovdiv, Plovdiv, Bulgaria

**Aim:** Plant adaptation to local environments has been associated with gene polymorphism caused by insertions. Transposable element-induced alleles were mostly related to gene bodies, and a few of them to promoter areas. In this study, the promoter regions of 9 stress-related genes were searched for insertions of transposable elements in 12 ecotypes of *A. thaliana* spanning different geographic regions.

**Materials and methods:** The *A. thaliana accessions* Col-0, Ws, Landsberg, Shah, C24, N2, Nok-0, Pf-0, Cal-0, Cvi-0, Yo-0, and Pirin were grown under controlled conditions. The promoter screening for the transposable element presence was performed via PCR amplification with primers designed to flank the insertion of transposable element in the promoter areas of the reference accession Col-0.

**Results:** Insertion/deletion (indel) polymorphisms, associated with transposable elements, were identified in 7 of the 12 promoter loci across studied ecotypes. Notably, the length of a particular transposable element does not differ across the ecotypes in which this transposable element is present in the respective promoter region.

**Conclusions:** The identified indel polymorphisms can be developed further as molecular markers for distinguishing the natural populations of *A. thaliana*. On the other hand, the collected data can be a starting point for gene expression profiling studies under conditions resembling the natural habitats of accessions.

**Acknowledgements:** This work was partially supported by the Bulgarian Ministry of Education and Sciences, Grant DH06/6.

**Keywords:** transposable elements, gene promoter, ecotypes

#### P01 08

#### Ex vitro adaptation of in vitro cultures of Artemisia eriantha Ten. plants.

Gabriela Decheva<sup>1</sup>, Mariya Rogova<sup>1</sup>, Lubov Hristova<sup>2</sup>, Veneta Kapchina-Toteva<sup>1</sup>

Department of Plant Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov blv.,
 1164 Sofia, Bulgaria, +359 2 865 66 41, <a href="mailto:mrogova@abv.bg">mrogova@abv.bg</a>
 Institute of Agriculture –Kyustendil, Sofiisko shose str., 2500 Kyustendil, Bulgaria, +359 78 522 612,
 Ihristova2@gmail.com

*Artemisia eriantha* Ten. (Asteraceae) is an aromatic perennial plant under the protection of Biodiversity Act of Bulgaria and it is European endemic species (LC) in European red data list of IUCN. Its Bulgarian population is restricted in National Parks "Central Balkan", "Rila" and "Pirin". Moreover, the small Bulgarian population and the low *in vivo* reproductive potential worsen the status of this species.

**Aim:** The present study aims to develop a protocol for successful *ex vitro* adaption of *in vitro* shoot cultures of *A. eriantha*.

**Material and methods:** Intact plant material of *A. eriantha* was collected at its natural habitat near Kabata, National Park "Pirin", Pirin Mountain, Bulgaria. The voucher specimen SO106477 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 vivo* growing wild plant. The survived shoots were successfully grown on MS medium supplied with Gamburg B5 vitamines, 3% (w/v) sucrose and 0.8 g/L agar. Regenerated plants with well-developed root system and plentiful leaf biomass were fell under *ex vitro* adaption in a greenhouse. After 21 days, 20% of all used plants were successfully *ex vitro* acclimatized.

**Results:** The protocol of *ex vitro* adaption of *in vitro A. eriantha* plants in greenhouse has been done.

**Conclusion:** A collection from *ex vitro* adapted plants, which is an approach for preservation of *A. eriantha* has been established.

**Keywords:** *Artemisia eriantha, in vitro* cultivation, *ex vitro* adaption

#### P01 09

### Breeding density and habitat preferences of Sombre Tit (*Poecile lugubris*) in a karst environment

Marta Dimitrova<sup>1</sup>, Mattia Brambilla<sup>2, 3</sup>, Boris Nikolov<sup>1</sup>

**Aim:** Sombre Tit (*Poecile lugubris*) is one of the least studied passerine bird species in Europe, and the least known among Paridae. The aim of the study is to assess its breeding density and habitat preferences in a karst environment.

**Material and methods:** The study took place in 2016 within the Special Protection Area "Rayanovtsi" in Western Bulgaria – a limestone terrain featured by sinkholes, bare rocks, caves etc. A total of 51 territories were identified by means of point counts and territory mapping. In order to quantitatively describe the habitat structure and the fine-scale land-cover 17 habitat variables were measured in the field at territory and control

<sup>&</sup>lt;sup>1</sup> Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria

<sup>&</sup>lt;sup>2</sup> Fondazione Lombardia per l'Ambiente, Settore Biodiversità e Aree protette, Largo 10 Luglio 1976 1, I-20822, Seveso, MB, Italy;

<sup>&</sup>lt;sup>3</sup> Museo delle Scienze, Sezione di Zoologia dei Vertebrati, Via Calepina 14, I-38122 Trento, Italy

plots (the latter were located at 51 unoccupied sites), within a radius approximating the territory size of the species. We built a logistic regression model according to an information-theoretic approach to identify the main predictors of species occurrence.

**Results:** Five habitat variables drove Sombre Tit occurrence according to the model – solar radiation, number of solitary trees (DBH>10 cm), number of trees (DBH>10 cm) within the 5m-woodland edge, cover of karst and distance to woodland. Those results highlight the ecotonal habits of the species, which occurred within the study area with a mean density of 0.38 pairs/ 10 ha in a karst-dominated environment with sparse tree cover (solitary trees and patchy woodlots).

**Conclusion:** Our findings can help improve the monitoring schemes targeted (also) at this species, and may be used to inform the management plans of mosaic landscapes in Natura-2000 zones and other protected areas.

**Keywords:** Sombre Tit, Poecile lugubris, breeding density, habitat preferences, karst

P01\_10

#### Cuckoo bumblebees in Bulgaria

Bilyana Stoykova, Albena Lapeva-Gjonova

Department of Zoology and Anthropology, Faculty of Biology, Sofia University, 8 Dragan Tsankov blv., 1164 Sofia, Bulgaria

**Aim**: Cuckoo bumblebees (genus *Bombus*, subgenus *Psithyrus*) are obligate social parasites in colonies of the other bumblebee species. They are therefore completely dependent on hosts to rear their offspring. The defining traits in cuckoo bumblebees are lack pollen-collecting corbiculae on the hind legs and a worker caste. The data available on *Psithyrus* species in Bulgaria suggest the need for actual recording in advancing our understanding of species distribution and host range.

The present study aims to summarize the information on composition and distribution of seven *Psithyrus* species in Bulgaria.

**Material and methods:** The study is based on literature and unpublished records from the museum collection of NMNH-Sofia. The bibliographic references and faunistic data are used to build the online platform Scratchpads on the bumblebees in Bulgaria.

**Results**: Nowadays the following seven species of subgenus *Psithyrus* are supported by faunistic data from Bulgaria: *Bombus bohemicus* (Seidl, 1838), *B. vestalis* (Geoffroy, 1785), *B. barbutellus* (Kirby, 1802), *B. rupestris* (Fabricius, 1793), *B. campestris* (Panzer, 1801), *B. quadricolor* (Lepeletier, 1832) and *B. sylvestris* (Lepeletier, 1832). The available literature on *Psithyrus* species in Bulgaria was surveyed and the data therein used and in some cases reviewed due to taxonomic changes. Additionally, new records were included based on the material, preserved in the museum collection of NMNH-Sofia. Maps for each cuckoo bumblebee species were prepared.

**Conclusion**: However more actual data in study of the species distribution, variation and host ranges of cuckoo bumblebees in Bulgaria is needed.

Keywords: Bombus, bumblebees, Bulgaria, Scratchpads

#### P01 11

#### One new genus of order Dorylaimida (Nematoda) from Maritime Antarctic

Milka Elshishka, Stela Lazarova, Georgi Radoslavov, Peter Hristov, Vlada K. Peneva

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

Nematodes are one of the key components of the soil biodiversity. In extreme habitats like polar deserts they occupy central position in the soil micro-food webs. Order Dorylaimida is the most diverse taxon in Maritime Antarctic, presented by 6 genera and 12 species with all the species and two of the genera being endemic.

The objective of this work was to study a new dorylaimid genus from Livingston and King George Islands.

**Materials and methods:** Nematodes were extracted from soils and plant materials by using a Baerman funnel method, killed by gentle heat and fixed in 4% formalin.

**Results:** Based on morphological, including SEM study, morphometric and sequence data (18S rDNA and the D2-D3 expansion fragments of 28S rDNA), the new genus is placed in family Nordiidae. The DNA data and phylogeny reconstructions showed that it is evolutionary closer to *Pungentus* spp. (family Nordiidae). The new genus is characterized by cuticle with discernible transverse striations, lips with well-developed refractive four platelets, wide stoma, short (as long as or slightly longer than labial diam.), slender odontostyle with small aperture, pharyngeal expansion occupying about half of pharynx length, amphidelphic female genital system, short and simple uterus, transverse vulva, elongate conoid tail, ventrally curved.

**Conclusion:** Nordiidae n. gen., n. sp., a monotypic and endemic genus, member of the dorylaimid fauna of the Maritime Antarctic was characterized morphologically and molecularly. Hitherto, it has limited distribution associated with mosses and soils of Antarctic higher plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl.

**Acknowledgements:** The present study was supported by the project №64/27.04.2016, Program for support of young scientists, Bulgarian Academy of Sciences.

**Keywords:** Taxonomy, morphology, SEM, 18S, D2-D3 28S rDNA

#### P01 12

# First record of ant-attended jumping plant-lice *Eryngiofaga loewiana* (Hemiptera: Psylloidea) in Bulgaria

Ilia Gjonov, Albena Lapeva-Gjonova, Monica Pramatarova

Department of Zoology and Anthropology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov blv., 1164 Sofia, Bulgaria

**Aim**: The present study aims to provide new data on fauna and biology of poorly known Psyllids in Bulgaria. The jumping plant-lice *Eryngiofaga loewiana* (Šulc, 1913) (Hemiptera: Psylloidea) is reported for the first time from Bulgaria.

**Material and methods:** Specimens of adults, eggs and all nymphal stages were sampled by hand in two sites, respectively in the vicinity of Belogradchik (20.09.2011) and Bryagovets village (Eastern Rhodopes) (06.09.2016).

**Results**: Both observations were on the host plant *Eryngium campestre* L. where psyllids are involved in an unknown trophobiotic interaction with ants – *Lasius paralienus* Seifert, 1992 and *Formica cunicularia* Latreille,

1798. They were observed taking honeydew from both nymphs and adults of *Eryngiofaga loewiana*. Generally, there are very few records of this type of trophobiosis in psyllids.

Several behavioural traits related to myrmecophilous life habits as close physical contact between ants and the psyllids, no escape reactions, neither jumping by the psyllids and direct honeydew collecting by the ants were observed. In addition color photographs of insect aggregations are provided.

The psyllid is currently known from Czech Republic, Slovakia, Hungary, Moldova, Ukraine, Turkey and Georgia. It joins *Eryngiofaga mesomela* (Flor, 1861) as the second member of the genus in Bulgaria. Based on our finding, the total species number of Psylloidea reach 79.

**Conclusion**: This result support the idea that more surveys are needed in order to increase the number of known jumping plant-lice species from Bulgaria.

**Keywords:** Psylloidea, Eryngiofaga, new records, ant-attendance, Bulgaria

#### P01 13

### Distribution of Sanguisorba species in Bulgaria and analysis of their ecological characteristic

<u>Kiril Vassilev</u><sup>1</sup>, Viktoriya Koleva<sup>2</sup>, Mariya Georgieva<sup>2</sup>, Iliana Dzhovanova<sup>3</sup>, Media Gumus<sup>5</sup>, Evelin Totev<sup>3</sup>, Borislav Tsvetanov<sup>3</sup>, Yusuf Ayan<sup>4</sup>, Plamena Vassileva<sup>2</sup>, Hristo Pedashenko<sup>1</sup>, Antoaneta Petrova<sup>6</sup>, Nikolay Velev<sup>1</sup>

<sup>1</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Department of Plant and Fungal Diversity and Resources, Sofia, Bulgaria

University of Sofia "St. Kliment Ohridski", Faculty of Geology and Geography, Sofia, Bulgaria
 University of Sofia "St. Kliment Ohridski", Faculty of Biology, Sofia, Bulgaria
 University of Forestry, Faculty of Forestry, Sofia, Bulgaria
 University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Plovdiv, Bulgaria
 Botanical Garden, Bulgarian Academy of Science, Sofia, Bulgaria

**Aim:** The aim of this study is to summarize the available data on distribution of *Sanguisorba* species in Bulgaria as well as their ecological preferences to abiotic conditions (altitudinal range, basic rock, exposition, etc.) and occurrence in certain vegetation and habitat types.

**Material and methods:** We collected all available data from floristic and phytocoenological literature sources, such as herbarium collections (SOM, SO, SOA) and phytocoenological databases (Balkan Dry Grassland Database, Balkan Vegetation Database, Bulgarian National Database). Two species of *Sanguisorba* genus were reported in Bulgarian flora – *S. minor, S. officinalis*. All localities were georeferenced using Google Earth *a posteriori*, when GPS data was missing. Mapping was done using ArcGis 10.0 software. The classification of vegetation types follows the concept of Mucina et al. (2016) about high rank vegetation types, whereas habitat types follows EUNIS habitat classification and Directive 92/43/EEC. We also analyze the applied floristic elements categories for studied species.

**Results:** The study area is covering the whole territory of the country. *S. minor* is widely distributed in Bulgaria from sea level to subalpine zone. Its ecological optimum is related to dry grassland vegetation types (*Festuco-Brometea, Koelerio-Corynephoretea, Helianthemetea guttati* classes) and rarerly found in other vegetation types. It is a companion species in phytocoenosis. On the other hand *S. officinalis* is locally found mainly in semi-mountainous and mountainous regions in the country. It is widespread in mesic and wetland grasslands. Its communities are also frequently found along river banks.

**Conclusions:** *Sanguisorba* species have wide distribution in the country. *S. minor* has wider ecological niche and is found in different vegetation types and habitats, whereas ecological optimum of *S. officinalis* is related to mesic and wet vegetation types.

**Keywords**: Flora, vegetation, spatial distribution, floristic elements

#### P01\_14

#### Comparative analyses of flavonoids in roots of three Bulgarian and one Ukrainian Glycyrriza glabra L. populations

Asya Kozhuharova, Milena Nikolova, Marina Stanilova

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

**Aim:** The study was aiming at determination of the total flavonoid content in the commercially important medicinal plant *Glycyrrhiza glabra*, and screening for occurrence of aglycones and glycosides.

**Material and Methods:** Root samples were gathered from four populations of *Glycyrrhiza glabra*: Dolni Vit, Koilovtsi, and Beltsov, near Danube River in Bulgaria, and one Ukrainian, in October 2016 during the fruition. Total flavonoids were determined spectrophotometrically according to the European Pharmacopeia. Methanolic extracts were examined by TLC for occurrence of nonpolar (aglycones) and polar (glycosides) flavonoids, and semi-quantitative assessment.

**Results:** Total flavonoids were similar for three of the tested populations, over 0.2% while the sample from Dolni Vit contained only 0,04 %. The TLC analysis showed that the extracts were richer in flavonoid aglycones than in glycosides. Eight spots with TLC data (Rf -values and color) of flavonoid aglycones and two of flavonoid glycosides were detected. Flavonoid profiles of the four samples relating to glycosides were the same in qualitative and quantitative terms. The profiles of flavonoid aglycones showed greater variability mainly in terms of quantity. One compound (aglycone) was determined only in the Dolni Vit sample.

**Conclusion:** The studied samples of *Glycyrrhiza glabra* showed a complex flavonoid profile concerning flavonoid aglycones. Additional identification of the components will be made to accomplish the study.

**Acknowledgement:** Authors are grateful to Bioprograma EAD for the financial support of the study.

**Keywords:** Licorice, Flavonoids, Medicinal plants

#### P01\_15

#### Pollen morphology of some species from genus Solene L. (Caryophyllaceae)

Atanas Tanev, Dolja Pavlova

Faculty of Biology, Department of Botany, Sofia University, 8 Dragan Tsankov blv., 1164 Sofia, Bulgaria, <a href="mailto:atanas.tanev@abv.bg">atanas.tanev@abv.bg</a>

**Aim:** To examine the pollen morphological characters of the species and their variation at population level in relation to species similarities and relationship.

**Materials and Methods:** The pollen morphology of populations of *Silene supina* Bieb., *S. thymifolia* Sm., *S. fetlerii* D. Pavlova and *S. spergulifolia* (Willd.) M. Bieb. (section *Spergulifolia* Boiss) was studied with light microscopy (LM) and scanning electron microscopy (SEM). The data were statistically analyzed.

**Results:** It was established that the pollen grains of all studied species belong to *Lychnis*-type. The pollen morphology is rather homogeneous, the pollen grains are sphaeroidal, 39-52 μm in diameter, polypantoporate, with number of pores between 20 and 35, the annulus width is below 1.5 μm, the ornamentation is microechinate with many small puncta. The echinae are small and low, inordinately arranged all over the surface; puncta are of the same size or smaller than echinae. The columellae are distinct, variable in size, but more or less uniform in shape, irregularly arranged, but sometimes in short rows. The species could be divided in two groups: one with

*S. thymifolia*, and the other including the rest of the species. Within the second group *S. fetlerii* is placed in a separate subgroup because of its larger pollen grains and larger pores.

**Conclusions:** The results support the similarities between the quite variable species *S. spergulifolia* and *S. supina*, and also show that the recently discovered species *S. fetlerii* is well distinguished from the other taxa by pollen morphological characters.

**Keywords:** Caryophyllaceae, pollen morphology, Silene, taxonomy, variation

#### P01 16

# What do we know about vegetation and habitat diversity of Elin Pelin municipality?

<u>Mediya Gumus<sup>1</sup></u>, Gana Gecheva<sup>1</sup>, Nikolay Velev<sup>2</sup>, Kiril Vassilev<sup>2</sup>

<sup>1</sup>University of Plovdiv "Paisii Hilendarski", Faculty of Biology Department of Ecology and environmental protection, Plovdiv, Bulgaria, <sup>2</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences Department of Plant and Fungal Diversity and Resources

**Aim:** Analysis and characteristics of existing knowledge about vegetation and habitat diversity on the territory of Elin Pelin municipality

**Material and methods:** We checked all phytocoenological publications from Bulgaria and we found only a few related to vegetation and habitat diversity on the territory of municipality. We also used available information about NATURA 2000 sites. Two sites fall into the study area – BG0001043 Etropole-Baylovo and BG0001389 Sredna gora. In addition, we used information about vegetation diversity from the Balkan Vegetation Database (EU-00-019) and the Balkan Dry Grassland Vegetation Database (EU-00-013).

**Results:** Study area covers 433.049 km<sup>2</sup> and only 5,15 % of its territory fall into NATURA 2000 network in Bulgaria. A total of 14 habitat types protected by the Habitat Directive 92/43/EEC were found on the territory of municipality. They cover an area of 2232 ha. Woodland vegetation has widest distribution – 3,96 % (1716.7 ha) as *Galio-Carpinetum* oak-hornbeam forests (9170), Moesian beech forests (91W0) and Pannonian-Balkanic turkey oak-sessile oak forests (91M0). Shrubland and grassland habitat types cover 514.5 ha or 1,19 % from the study area. Syntaxonomical diversity is poorly studied and is represented by 3 classes (*Molinio-Arrhenatheratea*, *Festuco-Brometea* and *Trifolio-Geranietea*), 3 orders, 5 alliances and 5 associations.

**Conclusion:** The territory of Elin Pelin municipality is characterized with different habitat types, which are determined by wide variety of abiotic conditions (altitude, slope, geology, soil types, etc.). This syntaxonimcal and habitat diversity analyses show that the vegetation and habitats on the territory are still poorly studied.

**Keywords**: Directive 92/43/EEC, NATURA 2000 sites, Bulgaria, syntax

#### P01 17

# Field release of *Entomophaga auliciae* (Entomophtoromycota, Entomophtorales) for control of brown tail moth *Euproctis chrysorrhoea* (Lepidoptera, Erebidae)

Daniela Pilarska<sup>1, 2</sup>, Pencho Dermendzhiev<sup>3</sup>, Rumen Nachev<sup>3</sup>, Maria Dobreva<sup>3</sup>

<sup>1</sup>New Bulgarian University, Department of Natural Sciences, <sup>2</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, <sup>3</sup>Forest Protection Station – Plovdiv

**Introduction:** Brown tail moth, *Euproctis chrysorrhoea* is important insect pest of deciduous trees in Bulgaria. It is characterized with large fluctuations in its density and calamities. Entomophagous insects and

entomopathogens play an important role for suppression of the pest populations. In May, 2016 in the region of Asenovgrad mass mortality of brown tail moth larvae was observed. The microscopic analyses showed that it was caused by the entomopathogenic fungus *Entomophaga auliciae*.

**Aim:** The success of introduction of another entomopathogenic fungus *Entomophaga maimaiga* in populations of the insect pest, the gypsy moth, *Lymantria dispar*, initiated the idea for release *E. auliciae* in a population of *E. chrysorrhoea* with high density in the region of Kardzhali.

**Material and Methods:** Dead larvae of brown tail moth collected in June, 2016 from the region with epizootics (Asenovgrad) were placed in a plastic bag stored in soil humus layer prior to the release.

**Results:** The release of *E. aulicae* was performed in the beginning of November 2016. Crushed dead larvae of the pathogen containing azygospores were spread around 6 trees with fresh nests with pest larvae in the experimental site. The base of release trees was watered with 2-3 L of water to achieve adequate humidity. The results from the release will be recorded in June, 2017.

**Conclusion:** The release of *E. auliciae* in a site with brown tail moth infestation using the methodology for introduction of *E. maimaiga* is an attempt to conduct biological control against one of the most important insect pests.

**Keywords:** brown tail moth, Entomophaga auliciae

#### P01 18

#### Bioconservation status of the critically endangered orchids in Bulgaria

Andrey Popatanasov

Faculty of Biology, Plovdiv University "Paisii Hilendarski", Plovdiv, Bulgaria.

**Aim**: Orchids due to their peculiar biology and environmental requirements are among the most threatened and endangered vascular plants in Bulgaria. On the territory of Bulgaria are found 70 species, 10 of which fulfill the IUCN criteria for critically endangered or regionally extinct and are included also in the Red Data Book and Biodiversity Act. The present study aimed to explore and evaluate the bioconservatory status of their populations.

**Materials and methods:** Exploration and monitoring of the populations of the critically endangered orchids in Bulgaria was done from 2012 till 2016. The shoot count and GPS coordinates were recorded for mapping of their distribution and evaluation of the status of their habitats was done.

**Results**: Among the current 2 species with regionally extinct status there should be added two more, namely *Epipactis geuteri* and *Orchis spitzelii*. Additionally at two locations *Ophris insectifera* is extinct. The new (and total known) locations that were found are as follows: one for *Cypripedium calceolus* (2), one for *Orchis provincialis* (4), two for new locations of *Ophris insectifera* (4), two new for *Traunsteinera globosa* (4). Approximately over 60% of the occupied territories fall outside the protected sites and many of the habitats are endangered by anthropogenic activities.

**Conclusion**: At all of the known locations there are less than 50 shoots per place, exception of this "rule" are some of the populations of *Traunsteinera globosa*, *Cephalantera epipactoides and Dactylorhiza kalopisii*. These facts put a high stress on the perspectives of their populations' survival.

**Keywords**: bioconservation, orchids, distribution mapping.

#### **EP01 1**

#### Coleoptera species of Skopje city and the surrounding area

Radmila Blazhevska, Aleksandra Cvetkovska-Gjorgjievska\*, Dana Prelić, Slavčo Hristovski, Julijana Arsovska, Jelena Hinić

Institute of Biology, Faculty of Natural Science and Mathematics, Ss. Cyril and Methodius University, P.O. Box 162, 1000 Skopje, Republic of Macedonia

\*corresponding author: acgorgievska@yahoo.com

**Aim:** This paper presents an overview of the species list of the *Coleoptera* fauna from 3 different parts (urban, rural and forest) of Skopje city.

**Materials and methods:** The research was carried out during the summer of 2015, with a monthly dynamics, by using pitfall traps placed along a transect.

**Main results:** As a result, 77 taxons of *Coleoptera* belonging to 20 families and 37 genera were registered. Overall, ground-beetle representatives (*Carabidae*) had the highest species richness (46 taxons). Compared by localities the highest share of species was registered in the urban parts of the city (30 taxons), with ground-beetles being the most species rich (19).

**Conclusion:** This data gives updated information for the beetle fauna of Skopje Valley and Vodno Mt.

**Keywords:** taxonomy, Coleoptera, Skopje city

# THEMATIC SESSION II BIOTIC AND ABIOTIC IMPACT OF THE LIVING NATURE AND MECHANISMS OF ADAPTATION

#### PL02 01

#### The exogenous factors and carcinogenesis

Margarita Topashka-Ancheva

Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria

Evaluation and control of the factors of living environment with potentially harmful effects on human health, accompanied prevention of the "Eco-diseases".

People are exposed to various environmental factors that can come into contact with the human body through air, water and food. So every year the environment and health science have increasingly intersections.

**Aim:** The aim is to present the role of the some exogenous factors in the tumorigenic processes.

The different rates of incidence of malignant tumors in populations from different parts of the world suggests that environmental factors and lifestyle not less than internal factors of the organism determined the risk of tumorigenic processes.

An important part of the exogenous factors of carcinogenesis has anthropogenic origin and their share is increasing. One of the most worrying problems for example is the widespread use of pesticides in agriculture and forestry. Environmental pollution by them attains global significance. Many of these substances have a direct carcinogenic effect or convert into nitroso compounds.

The majority of such factors identified as human carcinogens, have appeared in the environment in the past 100 years. The statistical data showing that cancer incidence in the human populations is higher in cities compared to smaller towns and villages are explained with the role of anthropogenic pollution of the living environment. This

accounts the fact that some tribes living in the high plateaus of the Himalayas, as close as possible to natural conditions, not know cancer. Very important are living and food habits of the population, leading to the emergence of one or other malignant neoplasms.

**Keywords:** human health, carcinogenesis

L02\_01

#### Pesticides-persistent organic pollutants. Impact on the environment and human health

Irena Bogoeva

Risk Assessment Center on Food Chain, Ministry of Agriculture and Food

Persistent Organic Pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate in agricultural products and in the food and pose a risk of causing adverse effects to human health and the environment. They are organic compounds that are resistant to natural degradation through chemical, biological, and photolytic processes. Many POPs are currently or were in the past used as pesticides, solvents, pharmaceuticals, and industrial chemicals. Humans can be exposed to POPs through diet, occupational accidents and from the environment (including indoor). Exposure to POPs, either acute or chronic, can be associated with a wide range of adverse health effects, including illness and death. Although their use as plant protection products has been banned in Europe and several other countries in the world many years ago, residues of POPs can still be found in agricultural commodities. One of the sources of their presence in the food is probably the storage of obsolete pesticides in the deserted and dilapidated warehouses. Adequate management measures are necessary, to find resources for the safe and environmentally sound destruction of this danger.

**Keywords:** Persistent Organic Pollutants (POPs), plant protection products, obsolete pesticides

#### L02 02

# Variability of PSII functionality and chloroplast membrane lipids of some halophytic and glicophytic representatives from genus *Lactuca* (Asteraceae)

<u>Lilia Angelova<sup>1</sup></u>, Albena Ivanova<sup>1</sup>, Dimitrina Koleva<sup>2</sup>, Svetlana Momchilova<sup>3</sup>, Sabina Taneva<sup>3</sup>, Liliana Maslenkova<sup>4</sup>

<sup>1</sup>Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria 
<sup>2</sup>Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia 1164, Bulgaria 
<sup>3</sup>Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria 
<sup>4</sup>Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

**The aim** of this study was to investigate the structural-functional peculiarities of chloroplast membranes influenced by the environmental conditions in different natural habitats of some halophytic and glicophytic *Lactuca* species. Chlorophyll thermoluminescence, photosynthetic oxygen evolution and fatty acid composition of thylakoid membranes were used to evaluate the sensitivity and adaptive mechanisms of the investigated species.

**Materials and methods:** Leaves of halophytic species *Lactuca tatarica* were collected from the coast and of glicophytic species *Lactuca quercina* and *Lactuca serriola* from oak forests and fields, respectively.

The pigment content was determined spectrophotometrically. Light microscopy was carried out by Nikon Eclipse 50i (Tokyo, Japan). Thylakoid membranes were isolated and re-suspended in Hepes, pH 7.6 by standard procedure. The analyses of total lipophylic extracts were performed using thin-layer and gas chromatographic techniques. Specific changes in PSII functionality and recombination events were assessed by alterations in thermoluminescence (TL) emission parameters, flash-induced oxygen yield patterns and oxygen induction curves under continuous irradiation.

**Results:** Comparative histological analysis of the leaves showed specific anatomical characteristics of the *Lactuca* species from different locations. TL glow curves and oscillation pattern of isolated chloroplasts followed particularly well the TL parameters recorded with intact leaves of the investigated plants. The observed species variations in the kinetics of the initial oxygen burst and oxygen flash pattern might be a result of conformational changes of PSII due to some modification of membrane lipid composition. The comparative analysis of fatty acids composition of thylakoid membranes confirmed the existence of qualitative and quantitative differences.

**Conclusion:** The results of this study show specific structural and functional characteristics of photosynthetic membranes in halophytic and glicophytic *Lactuca* species, reflecting different adaptive strategies of the studied species to environmental conditions in their natural habitat.

**Acknowlegement:** This work was completed in the frames of bilateral project between Ukrainian Academy of Sciences and Bulgarian Academy of Sciences (2014-2018).

**Keywords:** *Lactuca tatarica* L., *Lactuca quercina* L., *Lactuca serriola* L., fatty acid composition, PSII oxygenevolving activity, histological analysis, thermoluminescence.

#### L02 03

# Impact of light stress and salinity on the spectral properties of photosynthetic apparatus in *Paulownia*

Stefanov Martin<sup>1</sup>, Yotsova Ekaterina<sup>1</sup>, Markovska Yuliana<sup>2</sup>, Apostolova Emilia<sup>1</sup>

<sup>1</sup>Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, "Acad. G. Bonchev "21, Sofia 1113, Bulgaria

<sup>2</sup>Faculty of Biology, University of Sofia, 8 Dragan Tsankov Blvd., Sofia 1164, Bulgaria martin@bio21.bas.bg

**Aim:** The goal of this investigation was to assess the effects of the light stress on the photosystem II photochemistry, absorbance and fluorescence spectra of two lines of *Paulownia (Paulownia tomentosa x fortunei, TF* and *Paulownia elongata x elongata, EE)* grown on soils with different salt content.

**Materials and methods:** Pulse amplitude modulated (PAM) chlorophyll fluorescence, low temperature chlorophyll fluorescence and absorption spectroscopy were used for characterization of the studied lines of *Paulownia*. The measurements are made on isolated thylakoid membranes.

**Results:** The experimental results showed that salinity influences (i) the light-induced inhibition of the quantum yields of the primary photochemistry of the photosystem II in the dark adapted state (Fv/Fm) in both hybrid lines; (ii) the ratio of photochemical to nonphotochemical processes (Fv/F<sub>0</sub>) only in EE; (iii) the light-induced changes in energy transfer between chlorophyll-protein complexes only in EE line.

**Conclusion:** Data in the present investigation revealed some of the reasons for light sensitivity of the studied salt tolerant *Paulownia* lines.

**Acknowledgements:** This work was supported by the Bulgarian Academy of Sciences and the Program for career development of young scientists, BAS (Contract ДΦΗΠ-138/12.05.2016).

**Keywords:** Paulownia, chlorophyll fluorescence emission spectra, light stress, salinity

#### L2 04

#### Influence of heavy metals on the photosynthetic apparatus of Chlorella vulgaris

Ekaterina Yotsova, Martin Stefanov, Anelia Dobrikova, Emilia Apostolova

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Science, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

**Aim:** This study aims to investigate comparatively the influence of heavy metals (Cd, Zn, Pb) on the photosynthetic apparatus of the green alga *Chlorella vulgaris*.

**Material and methods:** The effects of different heavy metal concentrations on the photosynthetic activity of *Chlorella* cells were studied by pulse-amplitude-modulated (PAM) chlorophyll fluorescence, photosynthetic oxygen evolution measured with polarographic oxygen electrodes (Joliot-type and Clark-type) and pigment analysis.

**Results:** The results showed a significant inhibition of the oxygen evolution, the primary photochemistry of PSII (Fv/Fm), the effective quantum yield of photochemical energy conversion of PSII ( $\Phi_{PSII}$ ), the maximum efficiency of PSII photochemistry (Fv'/Fm'), the photochemical quenching (qP) and the electron-transport rate (ETR). Data also demonstrated stronger impact of the studied heavy metals on the oxygen evolution in comparison to the photochemistry of PSII. All these changes were connected with a decrease of the pigment content.

**Conclusion:** The sensitivity of *Chlorella* cells to Pb was lower than that observed for Cd and Zn. The obtained data revealed that the photosynthetic oxygen evolution could be used for assessing the impact of heavy metals on the water ecosystems.

**Acknowledgements:** This work was supported by the Program for career development of young scientists, BAS (Contract  $Д\Phi H\Pi$ -137/12.05.2016).

**Keywords:** heavy metals, photosynthetic activity, photosystem II, Chlorella vulgaris

#### L02 05

# Adaptive changes in the structure and the functional performance of photosynthetic apparatus of medicinal plant *Petasites hybridus* from different habitats

<u>Vesela Yordanova</u><sup>1</sup>, Albena Ivanova<sup>2</sup>, Albena Momchilova<sup>1</sup>, Svetlana Momchilova<sup>3</sup>, Dimitrina Koleva<sup>4</sup>, Liliana Maslenkova<sup>1</sup>

<sup>1</sup> Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria 
<sup>2</sup> Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria 
<sup>3</sup> Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria 
<sup>4</sup> Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia 1164, Bulgaria

**The aim** of this study was to investigate the influence of environmental conditions in different natural habitats of the medicinal plant *Petasites hybridus* (common butterbur) on the structural organisation and functional characteristics of the photosynthetic apparatus.

**Materials and methods:** Leaves of *Petasites hybridus* were collected after the flowering period from plants growing at different florogeographical regions in Bulgaria. The pigment content was determined spectrophotometrically. Light microscopy was carried out by Nikon Eclipse 50i (Tokyo, Japan). Thylakoid membranes were isolated in Tricine pH 7.8 by standard procedure and re-suspended in MES 6.5. The analyses of total lipophylic extracts were performed using thin-layer and gas chromatographic techniques. Specific changes in PSII photochemistry was assessed by alterations in thermoluminescence (TL) emission parameters.

**Results:** Comparative histological analysis showed specific anatomical characteristics of the *Petasites* leaves from different locations. TL glow curves and oscillation pattern of isolated chloroplasts followed particularly well the TL parameters recorded with intact leaves of the *Petasites* from different growth habitats. The main fatty acids (FA) are lauric, palmitic, linoleic and linolenic, but strikingly, a substantial quantity of unsaturated very-long FA was observed.

**Conclusion:** The comparative structural and functional studies showed that the specific environmental conditions in the different natural habitats of *Petasites hybridus* in Bulgaria greatly affect the structural and functional characteristics of the photosynthetic apparatus. The current work is a part of a complex study on the correlations between the variability of photosynthetic performance and modulation in the profile and accumulation of secondary metabolites with therapeutic activity in this medicinal plant in order to elucidate the mechanism of plant acclimation towards environmental factors.

**Acknowledgments:** This work was supported by grant № DFNP-131/12.05.2016 financed by Bulgarian Academy of Sciences.

**Keywords:** Petasites hybridus L., isolated chloroplasts, fatty acid composition, photosynthetic activity, histological analysis, thermoluminescence.

## L02\_06

# Contemporary concepts in the ecotoxicology of cadmium (Cd) in terrestrial ecosystems and its biomonitoring in terrestrial vertebrates

Peter Ostoich, Michaela Beltcheva, Roumiana Metcheva

Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences Sofia, 1 Tzar Osvoboditel blvd. Sofia, Bulgaria E-mail: p.ostoich@gmail.com

**Aims:** The present study is a review of the most current perspectives on the general toxicity and ecotoxicity of cadmium and the contemporary achievements in the biomonitoring of this trace metal in terrestrial animals.

**Results and discussion:** Cadmium is a well-established toxicant and carcinogen in mammals. Recent years have seen a decrease in the production and applications of the metal within the European Union. However, due to the problems of disposal, and its presence in polymetallic ores, it still remains a contaminant of concern for the environment.

The carcinogenicity of cadmium has been demonstrated in mammals. However, it has been shown to be only weakly mutagenic in prokaryotic test systems. The present article discusses the most contemporary achievements in the study of the genotoxicity of cadmium, exploring in particular the mechanisms of oxidative stress induction and the deregulation and inhibition of key mammalian DNA repair systems. In addition, ecological aspects are discussed: the ecotoxicity of cadmium in terrestrial ecosystems, the kinetics of the toxicant in the bodies of mammals, and past and current achievements in the biomonitoring for Cd in terrestrial ecosystems, including the use of monitor species of rodents and non-invasive biomonitoring with the use of avian feathers.

**Conclusion:** Cadmium is confirmed as a powerful toxicant, its genotoxicity is explored in detail, and examples of its biomonitoring are given.

**Keywords:** ecotoxicology, genotoxicity, cadmium, biomonitoring, review

## L2 07

# Impact of bottom substrate and organic quantity in sediments on trophic structure of the river macrozoobenthos

Maria Kerakova, Emilia Varadinova

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences 2 Gagarin Street, 1113 Sofia, Bulgaria

**Aim:** The purpose of this study is to identify the influence of heterogeneity of substrate and the amount of organic carbon in sediment surface layer on trophic structure of river benthos in four Bulgarian rivers.

**Material and Methods:** The trophic structure of benthic communities was studied in rivers Mesta, Tundza, Vit and Veleka during three seasons of the years 2011 and 2012. After processing of the samples, taxa established were referenced to six functional feeding groups/FFG (shredders, scrapers, collectors, filterers, deposit feeders and predator). Analyzes of sediments were made by drying and burning of the organic component.

**Results:** Our results show a substrate-dependent distribution of FFG. Limited role of the bottom substrate is expressed in preferences of the predators from stony substrate, filterers and deposit feeders from vegetation and muddy substrate, shredders and scrapers from gravel substrate and collectors from sandy substrate. Analyzes of organic quantity in the sediments showed an increase in the percentage of organic carbon along the rivers, as the highest value was recorded at stations with organic load. In most cases, higher levels of the amount of the organic component in the sediments correspond to a higher biodiversity and abundance of representatives of deposit feeders. The species of this trophic group feed namely organic matter in river sediments and some use it as a living environment.

**Conclusion:** Changes in the characteristics of the substrate, natural or anthropogenic organic layering and values of environmental factors in different seasons, influence the formation of food resources, trophic preferences of the macrozoobenthos and structuring of trophic composition of benthic fauna.

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

### L02 08

# Hypoxia induced fish kills in Varna Lake, Bulgaria. Assessment of dead fish biomass and driving factors for fish mortality

Stefan Kazakov<sup>1</sup>, Detelina Belkinova<sup>2,3</sup>, Rumen Mladenov<sup>3,4</sup>, Plamen Stojanov<sup>3,4</sup>, Luchezar Pehlivanov<sup>1</sup>

**Aim:** Fish kills occurred at the south coast of Varna lake on 16 August 2016. Assessment of dead fish biomass was made and driving factors for fish mortality were analyzed.

<sup>&</sup>lt;sup>1</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2, Gagarin Str., Sofia 1113, Bulgaria <sup>2</sup> Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 23, Acad. G. Bonchev Str., Sofia 1113, Bulgaria;

<sup>&</sup>lt;sup>3</sup> Department of Botany and Methods of Biology Teaching, Faculty of Biology, University of Plovdiv 'Paisiy Hilendarski', 24 Tzar Asen Str., Plovdiv 4000, Bulgaria

<sup>&</sup>lt;sup>4</sup> Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 120 Bratya Bukston Blvd. Plovdiv 4000, Bulgaria

**Materials and Methods:** Fish count was conducted using photographic images and fish biomass was calculated by length-weight relationship. Dissolved oxygen, temperature, transparency, chlorophyll *a* concentration and phytoplankton bio volume were measured in order of algal bloom assessment.

**Results:** Dead fish along the observed 1.125 km shoreline was assessed as about 2.79 tons, mostly represented by benthic species from fam. Gobiidae. Considering the large area of the lake, the real biomass of dead fish could be several times higher. Mortality was caused by hypoxia, as far as the mean oxygen content for the investigated littoral zone was 1.95 mg/l (SD=0.65) and 2.5 mg/l (SD = 0.29) for the epilimnion of the adjacent pelagic zone. Measured surface water temperature was below average  $T = 24.4^{\circ}C$  (SD = 0.26), while transparency (Secchi depth = 2.13 m, SD = 0.12), chlorophyll A (11.43 mg/m³, SD=1.15) and phytoplankton bio volume (PhB = 6.33 g/m³, SD = 0.36) revealed algal bloom occurrence.

**Conclusion:** Weather series analysis suggests that the west wind transported large amount of algal biomass from Bleoslavsko Lake in the western part of Varna Lake through their connecting channel. Process was followed by rapid extinction of the phytoplankton due to adverse weather conditions and degradation of the dead matter causing oxygen depletion.

**Acknowledgements**: Study was carried out accidentally in some relation to contract № 3260/19.07.2016 with Executive Agency of Environment.

**Keywords:** fish kills, Gobiidae, hypoxia, algal blooms, climate change, nutrients

# L02\_09

# The association of anemia and concentration of lead, copper, zinc and iron in children from environments contaminated with heavy metals

Snezana Stavreva Veselinovska

Faculty of Educational Sciences, University "Goce Delcev", Stip, R. Macedonia

**Aim:** Heavy metal poisoning can cause a variety of hematologic disorders. Exposure to heavy metals is ubiquitous in the industrial environment and must be considered in the differential diagnosis of many types of anemia. The heavy metals most commonly associated with hematologic toxicity are lead, copper, and zinc. A few distinctive clinical features characterize the hematologic manifestations of many occult heavy metal poisonings.

Deficiency of certain trace elements generally causes hypochromic microcytic anemia. Iron deficiency not only causes hypochromic microcytic anemia, but also increases the absorption of other elements such as lead (Pb) and cadmium (Cd). Therefore, in patients with hypochromic microcytic anemia, the serum levels of these elements may increase causing deterioration of anemia. Generally, heavy exposure to (Pb and Cd) causes hypochromic microcytic anemia. Iron absorption occurs predominantly in the duodenum and jejunum.

**Material and Methods:** The study is a cross-sectional performed on 50 children. Venous blood samples were taken from the studied population for estimating hematological parameters as well as iron and ferritin levels. The concentrations of zinc, copper, and lead were measured. The studied population was divided into anemic and non-anemic (control) groups. The anemic group was further classified into mild, moderate and severe anemia. The study subjects were also categorized into low and high blood lead level groups.

**Results:** Approximately 55.13% of children had blood lead levels  $\geq 10 \, \mu g/dl$ . At the blood lead level range of 10-20  $\mu g/dl$ , a significant association was found for mild and severe anemia. The blood level of iron and ferritin was found to be significantly lower in high blood lead level and anemic groups than those of the low blood lead level and control groups. Lead level in drinking water was higher than the permissible limit.

Conclusion: Lead level  $\geq 10~\mu g/dl$  was significantly associated with anemia, decreased iron absorption and hematological parameters affection. High blood lead levels were associated with low serum iron and ferritin. Lead level in drinking water was found to be higher than the permissible limits.

**Keywords:** anemia, heavy metals, children, blood, lead, cadmium, iron, environments contaminated.

## L02\_10

# DNA susceptibility of *Chlamydomonas reinhardtii* and *Saccharomyces cerevisiae* to Nurelle D

Petya Parvanova, Teodora Todorova, Daniela Miteva, Zhana Mitrovska and Stephka Chankova

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

**Aim:** to compare DNA susceptibility of *Chlamydomonas reinhardtii* and *Saccharomyces cerevisiae* to nurelle D. **Material and Methods:** The commercial products nurelle D was applied. The nurelle D is organophosphorus insecticide containing two active substance chlorpyrifos and cypermethrin. Two test systems: yeast *S. cerevisiae* diploid strain D7ts1 (*MATa/a; ade2-119/ade2-40; trp5-27/trp5-12; ilv1-92/ilv1-92; ts1/ts1*) and unicellular green algae *Ch. reinhardtii* strain 137 C+ were utilize. Cell suspensions of both genotypes with a density 1x10<sup>6</sup> cells/ml were treated for 30 min. Two different concentration ranges were applied: for *S. cerevisiae* - 0.05 mg/ml; 0.5 mg/ml and 5 mg/ml and for *Ch. reinhardtii* - 0.05 mg/ml; 0.10 mg/ml; 0.15 mg/ml; 0.20 mg/ml and 0.25 mg/ml. Induced DSBs levels were measured by constant field gel electrophoresis (CFGE).

**Results:** Our results demonstrate that although significantly lower treatment concentrations, DSBs levels in *Ch. reinhardtii* was about 3-5 fold higher comparison with those in *S. cerevisiae*.

**Conclusion:** Our result show more pronounced DNA susceptibility of *Chlamydomonas reinhardtii* 137 C+ strain compared with *Saccharomyces cerevisiae* diploid strain D7ts1.

**Acknowledgements:** This study was funded by the projects "Strategies for overcoming the genetic risk of anthropogenic pollution with organophosphorus pesticides" and "Ecological and genetic assessment of the environmental - management and strategies for overcoming the risk".

**Keywords:** DNA DSBs, *Chlamydomonas reinhardtii*, organophosphate insecticide, *Saccharomyces cerevisiae* 

## L02 11

# Phenol derivatives degradation by free living and entrapped in criogels environmental bacterial strains

Satchanska G.<sup>1</sup>, Topalova Y.<sup>2</sup>, Dimkov R.<sup>2</sup>, Groudeva V.<sup>2</sup>, Petrov P.<sup>3</sup>, Tsvetanov Ch.<sup>3</sup>, Selenska-Pobell S.<sup>4</sup>, and E. Golovinsky<sup>5</sup>

New Bulgarian University, Montevideo Blvd, 21, 1618 Sofia, Bulgaria
 Sofia University "St. Kliment Ohridsky", D. Tzankov Blvd, 8, 1164 Sofia, Bulgaria
 Institute of Polymers, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria,
 Helmholz Zentrum Dresden-Rossendorf, 01314 Dresden, Germany
 Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

**Introduction:** Two xenobiotic degrading bacteria - KCM R<sub>5</sub> and KCM RG<sub>5</sub> were isolated from heavy metals and aromatics polluted industrial area in South Bulgaria. Both strains were studied as free living cells for their

capability to grow on phenol and five chloro- and nitrophenols as sole carbon and energy source. Further the strains were immobilized on polyethylene oxide (PEO) cryogels to assess their capability for phenol degradation. **Aim:** To assess phenol degradation by free living and entrapped in criogels environmental bacterial strains.

**Materials and methods:** PCR, ARDRA, sequencing and BLAST analysis were applied for the strains' molecular-genetic characterization. Metal (Mn, Pb, Cu, Zn and Cd) and phenol derivatives tolerance of the strains as free living cells was examined in mineral media for one week. High molecular PEO cryogels were prepared according to an original method. Bacteria entered and entrapped the cryogels *via* shaking for 48 h resulting in two biofilters formation - PEO-KCM R<sub>5</sub> and PEO-KCM RG<sub>5</sub>. Sequencing batch process was performed for 30 days at 28 °C *via* feeding of biofilters at every 24 h with 250 mL of 300 to 1000 mg/L phenol solution. At the end of the degradation process biofilters with bacteria inside were studied using SEM.

**Results:** 16S rDNA analysis of the environmental bacterial isolates showed they belong to the genera *Pseudomonas* (KCM R<sub>5</sub>) and *Bacillus* (KCM RG<sub>5</sub>). Both strains showed at different extent metal tolerance and capability to grow on phenol and chloro- and nitrophenols as free living cells. The biofilter PEO-KCM R<sub>5</sub> removed phenol at concentration of 1000 mg L<sup>-1</sup> while the biofilter PEO-KCM RG<sub>5</sub> was able to degrade phenol at concentration up to 600 mg L<sup>-1</sup>. After 30 days of biodegradation process, the PEO-biofilters remained compact, porous and elastic, containing compact microbial biofilms as shown by SEM analysis.

**Conclusions:** PEO cryogel-bacteria biofilters are highly effective and sustainable for phenol degradation and are relevant for application in detoxification technologies of industrial waste waters.

**Acknowledgements:** This work was supported by Grant VUH-302 of The National Science Fund, Bulgarian Ministry of Education and Science.

**Keywords**: environmental bacterial strains, 16S rDNA gene, phenol degradation

## L02\_12

# Screening for genotoxity of extracts of Amaryllidaceae plant on the Chlamydomonas reinhardtii

Petya Parvanova, Zhana Mitrovska, Boriana Sidjimova, Milena Nikolova, Strahil Berkov and Stephka Chankova

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

**Aim:** to analyze the potential genotoxic capacity of 10 Amaryllidacea plant extracts.

**Material and Methods:** Ten total leave's extracts of Amaryllidacea plants were derived prof. Berkov and his team using the following procedure: dried leaves were extracted with CH<sub>3</sub>OH for 24h in proportion 1:10.

Clamidomonas reinhardtii strain 137 C+ (WT) was applied as a test-system. Cells at the end of exponential and the beginning of stationary growth phases were treated for 2h and 24h with concentrations of  $5\mu$ g/ml to  $1000\mu$ g/ml. The Spot test was utilized for the identification of extracts genotoxity. The potential genotoxic capacity of extracts was compared with those in control cells - DMSO (0.1%), Sager-Granick liquid medium (SG) and positive control PO (5  $\mu$ M).

**Results:** Our preliminary results show that three total leaves extracts of Amaryllidacea plants probably have genotoxic potential:

- Hippeastrum papilio exhibits genotoxic effect at 1000 μg/ml and exposition of 24h;
- *Spikelia formosissima* show genotoxic effects at 1000 μg/ml and exposition 2h as well as at 5 μg/ml; 50 μg/ml; 100 μg/ml; 500 μg/ml, 1000 μg/ml and exposition 24h;
- Narcissus triandrus L. genotoxic effect at  $1000 \,\mu\text{g/ml}$  and exposition 2h, as well as at  $500 \,\mu\text{g/ml}$ ;  $750 \,\mu\text{g/ml}$  and  $1000 \,\mu\text{g/ml}$  and exposition 24h.

**Conclusion:** Genotixic activity of 10 extracts was checked. Three of them -H. papilio; S. formosissima and N. triandrus demonstrated some genotoxic potential. Further experiments must be done to elucidate the mode of action.

**Acknowledgements:** This study was funded by the "Program for career development of young scientists, BAS" and "Ecological and genetic assessment of the environmental - management and strategies for overcoming the risk".

**Keywords:** Amaryllidaceae plants, *Clamidomonas reincardtii*, extracts, genotixity, spot test

## L02 13

# Adaptive potential of two *Phaseolus vulgaris* L. genotypes to single and combined PEG and UV-B treatments

Tsveta Angelova and Stephka Chankova

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

**Aim:** to compare adaptive potential of two genetically closely related genotypes of *Phaseolus vulgaris* L to single and combined polyethylene glycol (PEG) and UV-B treatments.

Material and methods: Two *Phaseolus vulgaris* L. genotypes were studied. Seed germination was performed in growth chamber (GC 400) at standard conditions (t=23±0.2°C, 60% humidity, in continuously light) for 3-7 days. Seedlings were grown to the cotyledon phase. Plants were separated into eight groups −controls, single treatment with 16% PEG and UV-B-100 J/m<sup>-2</sup>, 250 J/m<sup>-2</sup>; 500 J/m<sup>-2</sup>; combined treatment - 16% PEG+100 J/m<sup>-2</sup>, 16% PEG+250 J/m<sup>-2</sup>, 16% PEG+500 J/m<sup>-2</sup>. PEG, Alfa Aesar, Germany, cat. № B21955; MW 10 000 was used for 24h to induce osmotic stress in plants. At 12 h of the PEG treatment, cotyledons were radiated with UV-B. After that cotyledons were kept for 12 h in PEG. SOD and CAT were chosen as endpoints because it is well known that these enzymes act as antioxidants and protect cellular components from reactive oxygen species (ROS). ANOVA GraphPad Prism, version 5.00 was used to assess differences among samples.

**Results:** Ten days after the removal of UV-radiation induced stress SOD levels were 2.5-3 fold higher after single 250 J/m<sup>-2</sup> and 500 J/m<sup>-2</sup> UV-B. The profound increse of SOD activity was found after the combined treatment with 16% PEG and 100 J/m<sup>-2</sup> for Dobrudjanski 7. Higher levels of SOD (about 5-fold) were found after 250 J/m<sup>-2</sup> and after 16% PEG+500 J/m<sup>-2</sup> treatments for Dobrudjanski ran.

Ten days after the removal of stress stimulli CAT activity in Dobrudjanski 7 samples were not statistically proven. Interestingly, higher levels of CAT were measured after single PEG (about 2,5 fold), after single UV-B treatments (about 2-3,5 fold) and after combined 16% PEG+500 J/m<sup>-2</sup> (about 4,5 fold) treatment for Dobrudjanski ran, ten days after stress removal.

**Conclusion:** It could be concluded that probably both genotypes use different protective mechanisms (Dobrudjanski 7-SOD; Dobrudjanski ran-SOD and CAT activity).

**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.)" and "Ecological and genetic risk: methods and strategies for overcoming"– BAS.

**Key words:** *Phaseolus vulgaris* L., polyethylene glycol, UV-B

## P02\_01

# Assessment of the impact of salinity on the growth and antioxidant activity of two *Lycium* species

<u>Velmira Dimitrova<sup>2</sup></u>, Teodora Georgieva<sup>2</sup>, Maria Geneva<sup>3</sup>, Yuliana Markovska<sup>1\*</sup>

<sup>1</sup>Faculty of Biology, University of Sofia, 8 Dragan Tsankov Blvd., Sofia 1164, Bulgaria

<sup>2</sup>Biotree, 7 Bansko shose str., 1331 Sofia, Bulgaria

<sup>3</sup>Department of Plant Soil Interactions, Institute of Plant Physiology and Genetics, Bulgarian, Academy of So

<sup>3</sup>Department of Plant Soil Interactions, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev str., Bl.21, Sofia, Bulgaria

**Aim:** Lycium barbarum and Lycium chinense are two species from which the goji berry was harvested. The impact of salinity on growth parameters and antioxidant activity in these species, grown ex vitro in soils with different salinity was studied.

**Material and Methods:** Seeds and in vivo explants from L. barbarum and L. chinense were used for developing of  $in\ vitro$  multiplication protocol, licened by Bio Tree Ltd., Bulgaria. Plant adaptability is traced after  $ex\ vitro$  cultivation of both plants in pots filled with non-saline (type 0 – peat) and two saline soils (type 1 and 2) under green house conditions for two years.

The root and shoot dry mass of plants was measured gravimetrically after heating at 60°C for 48 h to a constant weight.

Spectrophotometric quantification of water- (WS - AOC) and lipid-soluble (LS - AOC) antioxidant capacity was performed through the formation of phosphomolybdenum complex. The total antioxidant potential of samples was determined spectrophotometrically by DPPH and FRAP assay.

**Results:** The ratios fresh mass/dry mass of roots and shoots of *Lycium barbarum* rose in higher degree after cultivation on saline soil in comparison with *Lycium chinense*. LS – AOC antioxidant capacity was enhanced at the same conditions of cultivation in both plants, but total antioxidant activities measured by DPPH and FRAP methods changed in a different manner.

**Conclusions:** Our results suggest that *Lycium chinense* was more salt tolerant than *Lycium barbarum* because it responded to high salinity level with increasing antioxidant activity and improved dry mass production.

**Keywords:** *Lycium*, salinity, growth, antioxidant activity

E-mail for corresponding: pmmaster2001@yahoo.com

## P02\_02

# Response of gluthathione reductase to oxidative stress in intact pea plants

Elena Shopova, Vera Alexieva, Valentina Bubova

Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences

**The aim** of this study was to evaluate the effect of two different active oxygen species (AOS) - singlet oxygen ( $^{1}O_{2}$ ), and superoxide radical ( $^{\bullet}O_{2}^{-}$ ) on the activity and isoenzyme profile of glutathione reductase (GR) in intact pea plants.

**Material and methods:** Pea plants (*Pisum sativum* L. cv. Skynado) were grown as water culture (photoperiod 12/12, light intensity 70–90  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, temperature 25±2°C, relative humidity 60–70%). The roots of pea plants, with fully expanded third leaf (14-day old), were removed. Shoots were placed in dishes with selected concentrations of photosensitizing dye eosin and herbicide paraquat (producing  $^{1}O_{2}$  and  $^{\bullet}O_{2}^{-}$ , respectively).

Plants were exposed to continuous light for 20 h. Parallel experiments were carried out in darkness. GR activity and GR isoenzyme profile were determined.

**Results:** Treatment with eosin and paraquat in light conditions increased GR activity. A strong dose-response relationship in eosin treated plants was observed. Native PAGE showed new bands displaying glutathione reductase activity in eosin and paraquat treated plants.

**Conclusion:** In vivo production of  ${}^{1}O_{2}$  and  ${}^{\bullet}O_{2}^{-}$  in pea plants affected significantly GR activity. Probably, the new GR isoforms are AOS inducible.

**Acknowledgements:** This work is a part from the project of DFNP-205/ 16.05.2016.

**Keywords:** pea plants, glutathione reductase, singlet oxygen, superoxide radical

## P02\_03

# Probing the phycobilisome stability in Synechocystis cells by microcalorimetry

Nia Petrova<sup>1</sup>, Svetla Todinova<sup>1</sup>, Tomas Zakar<sup>2</sup>, Tünde Tóth<sup>2</sup>, Stefka Taneva<sup>1</sup>, Zoltan Gombos<sup>2</sup>, Sashka Krumova<sup>1</sup>

<sup>1</sup>Institute of Biophysics and Biomedical Engineering, BAS, Sofia, Bulgari <sup>2</sup>Institute of Plant Biology, Biological Research Center, HAS, Szeged, Hungary

**Aim**: Phycobilisomes are the major light-harvesting antennae of cyanobacteria. These multi-protein complexes attach and may migrate on the thylakoid surface in a manner that is not fully understood. For their efficient operation they require specific pigment content and stable assembly of the structural units. It was shown that although not included in the phycobilisome structure the level and type of carotenoids do influence (indirectly) its assembly. In this work we aim at probing the structural stability of phycobilisomes in intact *Synechocystis* cells as a function of the carotenoid content of their thylakoids.

**Materials and methods**: Synechocystis sp. PCC 6803 wild type, PSI trimer-less  $\Delta psaL$  and xanthophyll-less  $\Delta crtRO$  mutants were grown photoheterotrophycally at 25, 30 or 35°C. The intact cells were harvested and their thermograms were recorded on DASM-4 microcalorimeter with scanning rate of 1 °C/min.

**Results**: The phycobilisome structure was not affected in the  $\Delta psaL$  mutant that had largely reduced β-carotene level, probably because its absence was compensated by increased accumulation of myxoxantophyll and zeaxanthin. The structural stability of phycobilisomes decreased at high (35°C) growth temperatures in wild-type cells but not in  $\Delta crtRO$  cells possibly due to lower carotenoids content in the wild-type compared to that in  $\Delta crtRO$  cells at that temperature.

**Conclusion**: Our data confirm that the carotenoid composition of thylakoid membranes affects the stable phycobilisome assembly and demonstrate that microcalorimetry is suitable technique to characterize the effect of various factors on the structural stability of phycobilisomes *in vivo*.

**Acknowledgements**: This work is supported by the bilateral program for scientific exchange between the Bulgarian and Hungarian Academy of Sciences.

**Keywords**: phycobilisomes, differential scanning calorimetry, carotenoids, ΔpsaL mutant, ΔcrtRO mutant

### P02 04

# Impact of growth regulators on the antioxidative capacity of Pisum sativum plants

Marina Alexeeva<sup>1,2</sup>, Ivalena Ilieva<sup>1,2</sup>, Miroslava Zhiponova<sup>1\*</sup>, Veneta Kapchina-Toteva<sup>1</sup>, Ganka Chaneva<sup>1</sup>

Department of Plant Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov blvd.,

1164 Sofia, Bulgaria

<sup>2</sup> these authors contributed equally

**Aim:** Investigation of the effects of plant growth regulators abscisic acid (ABA) and gibberellic acid (GA) on growth and antioxidative potential of *Pisum sativum* plants.

**Materials and methods:** *P. sativum* seeds were germinated in dark at 22°C for 4 days. Seedling hydroponic cultures were grown under continuous light at 22°C for 6 days. Then three different concentrations of plant growth regulators ABA and GA were added and the plants were cultivated for 7 more days. The seedlings were analyzed for their morphometric characteristics and leaf pigment content, total antioxidant activity (TAA), total phenolic and flavonoid contents (TPC, TFC).

**Results:** The stems and the internodes of the plants treated with GA were longer than the control plants, while treatment with ABA caused only a slight effect. The pigment content did not change significantly. The plants treated with GA had increased TPC correlating with higher TAA. The opposite tendency was observed for plants treated with ABA. Addition of GA resulted in decreased TFC.

**Conclusion:** The results indicate concentration-dependent effects of ABA and GA on growth and antioxidative status, which can be applied to improve the quality of peas.

**Keywords:** pea, plant, growth, regulator

## P02 05

# Enhancement of seed germination and growth of *Echinacea purpurea* (Asteraceae)

Boryanka Traykova, Marina Stanilova

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

**Aim**: The study deals with stimulation of seed germination of the medicinal plant *Echinacea purpurea* (eastern purple coneflower) and enhancement of the seedlings growth.

**Material and methods**: Mature seeds from the *ex situ* collection of IBER were stored in a cool chamber during the winter. Seeds were triple dipped consecutively in hot and ice-cold water, then soaked for one hour in GA<sub>3</sub> (0,1% or 0,35%) or 0,2% KNO<sub>3</sub>; control seeds in distilled water. Each variant consisted in 30 shelled and 30 intact seeds (4 repetitions 15 seeds). Seeds were put in terrines with soil mixture under control conditions for a month. In addition, seed germination was tested on airoponic system "Green Diamond" with 120 intact seeds using the same stimulats.

**Results**: Best results were obtained in the variant with intact seeds soaked in 0,35% GA<sub>3</sub> where 46% of the seeds germinated in the terrine and almost all of them survived. Seedlings growth was twice faster on the airoponic. **Conclusion**: The results improving seed germination and seedling growth could be applied in the practice.

Keywords: Eastern purple coneflower, Seed germination, Hydroponics, Medicinal plants

## P02 06

# The impact of sea water immersion on the viability of psammophilous species Galilea mucronata (L.) Parl.

Stoyan Vergiev

Museum of Natural History Varna, 41, Maria Louisa Blvd., 9000 Varna, Bulgaria

**Aim:** This study aims to determine the effects of flooding stress on whole plants of *Galilea mucronata* and how long its rhizomes can remain viable in sea water in order to investigate its capacity as dune stabilizer.

**Material and methods:** Two simulated flooding experiments were conducted. In the first experiment, whole plants were immersed in sea water for 20 days. Visible morphological changes of leaves, stems and roots are recorded and assessed in 12 parameters. In the second experiment, rhizomes were immersed in sea water, then planted and allowed to grow for one month before harvesting in order to establish rhizomes viability, biomass and root/shoot ratio.

**Results:** Conducted flooding experiments established that *G. mucronata* was very tolerant to immersion impact and salt stress. Whole plants stayed viable longer than the flood with a maximum duration along the Bulgarian Black Sea Coast, and rhizomes were able to regenerate after 30 days in seawater. Statistical analysis of experimental data demonstrates that immersion in sea water increases rhizomes viability, biomass and allocation to root biomass, whereas other factors, such as duration of immersion and temperatures of sea water have not significant effect.

**Conclusions:** *G. mucronata* is less tolerant to water immersion than other psammophytes, but demonstrates a high potential to be a key species for dune stabilization.

**Keywords**: Immersion tolerance, viability, Galilea mucronata.

## P02 07

# Pinus nigra L. as a biomonitor of air pollution in urban areas (Plovdiv, Bulgaria)

Zlatovesa Hristozova, Megi Dakova, Kostadinka Todorova, Bogdan Nikolov, Iliana Velcheva, Slaveya Petrova\*

University of Plovdiv "Paisii Hilendarski", FAcultty on Biology, Department of Ecology and Environmental Conservation, 24 Tzar Asen Str., 4000-Plovdiv, Bulgaria
\*Corresponding author: sl.petrova@abv.bg

**Aim:** Aim of this biomonitoring study was to assess the air pollution in Plovdiv, one of the most damaged European cities, on the base of heavy metals and toxic elements content in needles of *Pinus nigra*.

**Material and Methods:** Seedlings of *Pinus nigra* were purchased from certified greenery and planted in four plots during the spring of 2015, as follows: Plot 1 - Central part (NE direction); Plot 2 - Central part (SE direction); Plot 3 - "Smirnenski" suburb (SW direction), near busy road junction; Plot 4 - City park "Recreation and Culture" (NW direction), low anthropogenic impact. Leaf samples were taken in September 2015 and the content of Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, U and Zn was measured by ICP-MS.

**Results:** The content of Cd in pine needles varied from 0.07 (Plot 3) to 0.14 (Plot 2), content of Cr - from 0,4 (Plot 4) to 0.58 (Plot 2) mg kg<sup>-1</sup> and the content of Cu was between 2.2 (Plot 4) and 3.5 (Plot 3) mg kg<sup>-1</sup>. Highest content of Mn (10 mg kg<sup>-1</sup>) and Zn (29 mg kg<sup>-1</sup>) was found in plant samples from Plot 2 and lowest – in those from Plot 4. Fe, Pb and U had also maximums in Plot 2 but minimums were found in Plot 1 (Fe) and Plot 3 (Pb and U).

**Conclusions:** *Pinus nigra* showed significant differences in element accumulation between the studied plots. Changes in the each element content corresponded to the degree of anthropogenic impact in the area. **Acknowledgements:** We are grateful for the financial support to Fund "Scientific researches", University of Plovdiv "Paisii Hilendarski", Contract SP15-BF14, managed by Assoc. Prof. Iliana Velcheva

**Keywords:** air pollution, biomonitoring, Pinus nigra, Plovdiv

## P02 08

## Distribution of bottom fauna under fish farming cages in Kardzhali dam.

Stefan Kazakov<sup>1</sup>, Maria Yankova<sup>2</sup>, Angelina Ivanova<sup>2</sup>, Kostadin Dochin<sup>2</sup>, Georgi Rusenov<sup>2</sup>, Maria Gevezova-Kazakova<sup>2</sup>, Angel Zaikov<sup>2</sup>, Tanya Hubenova<sup>2</sup>

**Aim:** The influence of cage fish farming on benthic community structure of Kardzhali dam was investigated. **Materials and methods:** The sampling was conducted from August to October of 2016. Spatial and temporal features of the benthic community were represented by multidimensional scaling (MDS plots). Biodiversity indices as well as descriptive statistics were used to outline the patterns of dissimilarity between the samples. The assessment of fish farming impact on benthic community was analyzed by correlation tests between benthic community structure and a set of environmental variables (size and age of the farm, depth of the water column, water flow and sediment's organic content).

**Results:** At first there was a clear difference between benthic community from control samples and samples under fish cages. Under the cage facilities were registered decreased biodiversity and significant abundance of class Oligochaeta. Correlation analysis revealed significant positive relationship between Oligochaeta abundance and farm's age and size. A negative relationship was established between the direction of the cumulative loading and biotic variables as number of taxa, total abundance and Shannon diversity index. Later in October an increased level of similarity within the benthic community was registered. Usually in the summer the dam experience its maximum water abstraction resulting in increased water flow velocity.

**Conclusion:** The impact of fish farming in Kardzhali dam on benthic community structure was expressed in reduced biodiversity and increased abundance of class Oligochaeta. Water flow rate seems to be important factor mitigating the strength of that impact affecting the entrainment, transport and mixing of the bottom sediments and biota.

**Aknowledgements:** The authors would like to express their gratitude to all fish producers involved in the project "Evaluation of water quality in Kardzhali reservoir in connection with the development of sustainable aquaculture" for their help and support.

**Keywords:** fish farming cages, impact assessment, benthos community response, deep reservoir, Kardzhali dam

## P02 09

# Accumulated heavy metals and oxidative status in tissues of the Black Sea mussel (Mytilus galloprovincialis Lamark, 1819)

<u>Lachezar Yakimov</u><sup>1</sup>, Albena Alexandrova<sup>2</sup>, Elina Tsvetanova<sup>2</sup>, Almira Georgieva<sup>2</sup>, Nesho Chipev<sup>1</sup>

**Background:** It is well known that the alterations in oxidative status of marine organisms could be used to evaluate stressful environmental conditions, especially the toxic effects of metal pollutants.

<sup>&</sup>lt;sup>1</sup>Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2, GagarinStr., Sofia 1113, Bulgaria <sup>2</sup>Institute of Fisheries and Aquaculture, Vasil Levski 248 Str., Plovdiv 4000, Bulgaria

<sup>&</sup>lt;sup>1</sup> Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria <sup>2</sup> Institute of Neurobiology, Laboratory of Free Radical Processes, BAS, 23 Acad. G. Bonchev str., 1113 Sofia, Bulgaria

**Aim:** To assess the oxidative status of three tissues of Black Sea *M. galloprovincialis*, which is known to accumulate high levels of persistent pollutants from the marine environment.

Material and Methods: One year grown specimens of *M. galloprovincialis* were used. They were obtained from maricultures, located in areas considered as "clean" and reference sites. Hepatopancreas, gills and foot from the mussels were excised. In each tissue 1) the analytical determination of Cu, Pb, Zn, Cd and Ni content was made by using atomic emission spectrometry and 2) the measurement of oxidative stress markers: lipid peroxidation (LPO), glutathione levels (GSH) and the activities of enzymes catalase (CAT), superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G6PD) was performed spectrophotometrically.

**Results:** The gills showed the highest metal's accumulation (1.65 - 131.82  $\mu$ g/g dry w), lower SOD activity and higher LPO level in comparison to other tested tissues. The hepatopancreas was characterized by intermediate accumulation of metals (0.30 – 106.67  $\mu$ g/g dry w) and lower G6PD activity. The lowest metal content (1.6 – 53.85  $\mu$ g/g dry w) was detected in foot muscle along with high SOD and G6PD activities and low LPO.

**Conclusions:** The results of this pilot study indicated that the different tissues of *M. galloprovincialis* accumulate different amounts of metals, as their content appears to correlate with the LPO levels as a marker of oxidative stress.

Keywords: Mytilus galloprovincialis, oxidative stress, Black Sea, heavy metals

### P02 10

# Cytotoxic activity of *Sideritis scardica* extracts and fractions on human breast adenocarcinoma cell line

Petya Koleva<sup>1</sup>, Elena Stoyanova<sup>2</sup>, Kalina Alipieva<sup>1</sup>, Ina Aneva<sup>3</sup>, Ljuba Evstatieva<sup>3</sup>, <u>Kalina Danova<sup>1\*</sup></u>

<sup>1</sup> Institute of Organic Chemistry with Centre of Phytochemistry, BAS
 <sup>2</sup> Institute of Biology and Immunology of Reproduction, BAS
 <sup>3</sup> Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences

Sideritis scardica Griseb. (Lamiaceae) is a Balkan endemic species. Different types of its extracts have been shown to possess cytotoxic effect on murine melanoma B16, human leukaemia HL-60 cells, as well as C6 rat glioma cells, these effects being attributed to reactive oxygen species induction by the chemical constituents present in the studied preparations.

**The aim** of the present work was to investigate the cytotoxicity of extracts and fractions of the plant on human breast adenocarcinoma cell line.

**Materials and methods:** Hexane, chloroform and methanol extracts were prepared by sonification from commercial samples of the plant. Ethyl acetate, butanol and water fractions were obtained by the methanol extract. The effect of the extracts was tested on MCF7 human breast adenocarcinoma cell line in concentrations of 0.2, 0.1 and 0.05 mg/l. Preliminary characterization of the chemistry of the tested preparations was performed by thin layer chromatography.

**Results:** While marked inhibitory activity of the ethyl acetate fraction was established, no effect on cell growth was recorded upon methanol and butanol preparations treatments. The TLC characterization of the samples showed that while a mixture of phenyethanoid (verbascoside) and flavonoid (isoscutellarein and hypolaetine type) diglycosides were characteristic for the total methanolic extract and butanol fraction, the ethylacetate fraction exhibited predominantly the presence of only the two flavonoid diglycosides.

**Conclusion:** The results are indicative of the possible selectivity of the latter compounds towards the tested cell lines and might provide evidence on the mechanism of action of the different polyphenolics present in the aerial parts of Balkan endemic *Sideritis scardica*.

**Acknowledgements:** This work was partially financed by the PhytoBalk project (BSRP, grant No. IZEBZ0\_142989)

**Keywords:** Sideritis scardica Griseb., cytotoxicity, adenocarcinoma cell line

## P02 11

# Role of glutathione in cell response to oxidative stress in yeast

Anna Tomova, Ventsislava Petrova

Faculty of Biology, Sofia University, "St. Kliment Ohridski"

Glutathione is a unique cellular antioxidant with multiple functions. Its growing biotechnological application as an antioxidant in a range of nutritional, cosmetic and pharmaceutical products stimulates the intensive research work directed to its synthesis and function in the cell.

**Aim:** The present work aimed to study the effect of hydrogen peroxide  $(H_2O_2)$  on cell survival and glutathione synthesis in yeast species differing in their metabolic type.

**Materials and Methods:** Two yeast strains are used - *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*. They were grown for 48h at  $30^{\circ}$ C on YPD solid media with and without the addition of  $H_2O_2$ . The cell's metabolic status was assessed through the measurement of glutathione, ROS, oxidized proteins and lipids.

**Results:** Survival of both yeast strains was not affected significantly by 3 mM concentration of  $H_2O_2$ . The detected sub-lethal concentration of  $H_2O_2$  was 4mM. At 5 mM  $H_2O_2$  a better survival was observed for K. marxianus  $90_3$  (60%) in comparison to S. cerevisiae 584 (40%). When growing in the presence of 4 mM  $H_2O_2$  fermentative S. cerevisiae 584 showed higher levels of ROS, oxidized proteins and lipids. Contrary, K. marxianus  $90_3$  was characterized with better adaptive response and increased glutathione levels.

**Conclusions:** The adaptive cellular response to  $H_2O_2$  in the studied strains includes the activation of the non-enzymatic antioxidant defense system and synthesis of high levels of intracellular glutathione. The respiratory yeast *Klyuveromyces marxianus*  $90_3$  showed higher adaptive capacity, better resistance to 4 and 5 mM  $H_2O_2$  and elevated concentrations of glutathione. This gives grounds to further investigate these yeasts as a system for industrial production of glutathione.

**Keywords:** S. cerevisiae, K. marxianus, adaptive response, glutathione

## P02 12

# Climate changes impact on the populations of the critically endangered orchids in Bulgaria

Andrey Popatanasov

Faculty of Biology, Plovdiv University "Paisii Hilendarski", Plovdiv, Bulgaria.

**Aim**: Orchids are considered by many amongst the evolutionary youngest vascular plants and at the same time they are among the most endangered plants worldwide in the current industrialized consumar world. The aim of the study was to explore the effects of some of the factors related/influenced by climate changes upon the critically endangered orchids in Bulgaria.

**Materials and methods**: Monitoring of the population status was done from the spring of 2011 till the autumn of 2016. The morphometric characteristics and number of flowering individuals as well the successfully pollinated and the ones that reached seed dispersal phase were recorded. The values of the environmental variables were taken from the WorldClim database.

**Results**: The recorded data were analyzed statistically and the results indicate that the precipitation values and peak shifts before or during the flowering phase have greatest impact upon the number of flowering individuals and number of flowers. The impact of the precipitation during the seed ripening phase was less pronounced. The shifts of temperature have less pronounced effects. The most affected were the species from the subfamilies Epidendroideae and Cypripedioideae.

**Conclusions**: The climate changes have some beneficiary impact during the flowering phase in most of the monitored species but the impact is mostly neutral to negative in the late phases of vegetation. Especially devastating is the impact upon the late flowering species.

**Keywords**: *climate changes, orchids, vegetation.* 

### **EP02 01**

# Biomass of pine forests in the North-East of European plain in depend on soil moisture conditions

Andrey Osipov, Ivan Kutjavin
Institute of Biology of the Komi Science Centre of the Ural Division Russian Academy of Science,
Syktyvkar, Russian Federation

**Aim**: The aim of study was to the estimate biomass of pine forests in the North-East of the European plain in dependence of the soil moisture conditions.

**Materials and methods:** The objects under study were eleven pine stands located in the North-East of the European plain. The biomass of the stands was evaluated using equations derived from the analysis of 5–10 sample trees on each plot. The biomass of shrub and moss layers was determined by cutting 30–50 samples of 625 cm<sup>2</sup>. The data are presented in absolutely dry weight.

**Results:** The total biomass of the pine forests varies from 79.6 to 182.5 Mg/ha. The highest concentrations were observed in overmature *Myrtillus* type pine forests and the lowest – in middle-aged pine forest of the *Sphagnosa* type.

The pine stands of the *Lichen* type contain 124–150 Mg/ha. The ecosystems in the mesic soil moisture conditions (*Myrtillus* type) have 100-182 Mg/ha, and in overwetting conditions (*Sphagnosa* type) -79-125 Mg/ha. The soil moisture conditions determine the role of ground cover plants in the total biomass. We found a positive correlation (R=0.64; p=0.03) between soil moisture and the biomass of ground cover plants. This impact was confirmed by ANOVA (F=34.9; P<0.05).

**Conclusion:** The biomass is an important component of the present climate change investigations. It is a base for further studies of the carbon cycle of the pine ecosystems (occupying about  $7.1 \times 10^6$  ha) located in the North-East of European plain.

**Acknowledgements**: This study was partly supported by project of Russian Federation President for young scientists' MK-6670.2016.5.

**Keywords**: biomass, soil moisture conditions, pine forest

# THEMATIC SESSION III ECOSYSTEM RESEARCH, SERVICES AND ECOLOGICAL AGRICULTURE

## PL03 01

# Ecosystem services and climate change, or what is the cost of doing nothing at all

Svetla Bratanova-Doncheva, Nesho Chipev

Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences

Human well-being depends on the good condition of the planet's systems, which provide services such as food, materials, clean water, fresh air, regulation of climate, floods, erosion, diseases etc., and aesthetical, intellectual, physical and cultural interaction with nature. The socio-ecological systems are based on numerous regulating and maintaining services requiring clean and productive forests, lands, seas, fertile soils, a supply of fresh water, pollination of fruits and crops, natural flood protection and/or climate regulation. However, the ecosystems, habitats and species that provide these services are under serious pressure by human activity (EEA, 2015b, 2016a; Newbold et al., 2015). The capacity of ecosystems to deliver ecosystem services for human well-being depends on the condition of ecosystems, i.e. the quality of their structure and functionality - ecosystem integrity. There is growing understanding of the importance of biodiversity in ecosystem functioning and service delivery, which represents our natural capital (Harrison et al., 2014). Consequently, biodiversity is an important element in ecosystem and ecosystem service assessment, and this activity is implemented as part of the EU Biodiversity Strategy to 2020. Recently, climate change is becoming a serious issue in ecosystem management. Average global surface temperatures have risen by approx. 0.8°C in the period between 1861 and 2005 and are expected to continue to increase until the end of this century by 1.5-5.8°C above the 1990 value. The complex effects of global climate change on ecosystems limit our ability to predict biological responses in a standard way and may compromise ecosystems management with respect to potential changes. An integrated view on the possible effects of global climate change is provided while taking into account that not only the rising average temperature is likely to impact natural ecosystems but also that increased variation around the mean and higher frequency of extreme events will be important. The European assessment of ecosystem condition for the implementation of the EU Biodiversity Strategy to 2020 considers five major pressures, as identified in the MAES, EEA, (2016), climate change being one of them. Many impacts have been reported for terrestrial and freshwater ecosystems, and knowledge about marine systems is rapidly growing. However, even if the impacts of climatic changes have become increasingly visible, other pressures are often as important as or even more important than climate change. It should be noted that the current knowledge about the impacts of climate change has not yet been fully integrated into ecosystem service assessments and hence, development of new adaptation strategies are needed.

## Key messages:

- On the global level the main drivers for changes in the ecosystems condition are climate change and land cover changes.
  - Climate change significantly affects ecosystems, their biodiversity and consequently their potential to provide services for human well-being; it could have already provoked regime shifts in ecosystem functioning transition from one state to another.
  - There is still limited knowledge about the combined effects of climate change and other pressures on ecosystems and their potential to provide services, but the knowledge base is improving.
  - The relative importance of climate change compared with other pressures depends on the environmental sector (terrestrial, freshwater, marine) and geographical region. Europe's marine and alpine ecosystems are recognized as being most sensitive to climate change.

**Acknowledgements:** Project Methodological support for ecosystem service assessment and biophysical valuation (**MetEcoSMap**), funding by FM of EEA

Keywords: ecosystem services, climate change, pressures, impact, vulnerability, mitigation, adaptation

## PL03\_02

# The Innovations in Organic Farming – Problems and Perspectives

Atanas Atanassov<sup>1</sup>, Maria Shishiniova<sup>1</sup>, Goritsa Rakleova<sup>1</sup>, Mariana Vlahova<sup>1</sup>, Ivelin Panchev<sup>2</sup>, Ventsislav Bardarov, Radoslav Russev

<sup>1</sup> Joint Genomic Center to Sofia University "St. Kl. Ohridski" <sup>2</sup> Biology Faculty to Sofia University "St. Kl. Ohridski"

At present time the organic farming turn out to be a suitable and alternative system for promoting the production of high quality agricultural products; to keep the biodiversity and soil fertility alive and to lead to the CO<sub>2</sub> and other emissions diminished.

Since 1990 the market of the organic products is growing permanently reaching the size of 69 billions of US dollars in 2005 for all over the world.

During the period 2001 - 2015 the area of organic producing crop species is growing with 8.9 % annually. In 2013 the total area of organic production reached 43 mln ha which is equally to 1.1 % of whole area utilized for agriculture.

Europe and particularly Bulgaria have strong orientation for enhancing the share of organic farming in term of conventional agricultural production. Bulgaria has all the natural conditions for the realization of this goal.

A number of issues will be considered in relation of the national strategy and policy as well as specific questions in term the innovation developments in the organic farming, namely – omics technologies; precise and digital agriculture and others.

Some arguments why the organic farming should have a priority in the Bulgarian agricultural production as an important socio-economic and environmental importance will be also considered.

## L03 01

# Ecological effects of the change in the strength of pressures from a local point source of anthropogenic eutrophication in a coastal ecosystem. A model study in Sozopol Bay, Black Sea.

Dimitar Nikolov Berov, Ventsislav Kostadinov Karamfilov

Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences

**Aim:** The goal of this study was to evaluate the changes in the state of a coastal ecosystem after the construction of a waste-water treatment plant (WWTP) and the stopping of decades-long release of untreated nutrient-rich waters.

Materials and methods: Water nutrient concentrations and water column properties were monitored in a station network in the Sozopol Bay, during the period pre-WWTP construction period of increased eutrophication pressures (June-September). The structure and ecological status of macroalgal and seagrass communities in the area were also evaluated, using WFD methods (EEI-c,EI) and MSFD GES criteria.

**Results:** Nutrient concentrations and water transparency in the Sozopol Bay decreased significantly in comparison with the period prior to WWTP functioning, resulting in a higher ecological status of waters towards the boundaries for good environmental status even at monitoring stations the vicinity of the discharge point. Despite this improvement, the ecological status and depth structure of the Cystoseira-dominated macrolagal communities did not change significantly in one year, but is likely in the near future.

**Conclusion:** The stop of release of untreated waters in the study area resulted in immediate improvement in water quality. This did not lead to instant improvement in the ecological status of phytobenthic ecosystems, which need several vegetative seasons to improve and recover.

**Acknowledgements**: This work was funded by the Bulgarian Academy of Sciences Program for support of young scientist's contract number 201/14.05.2016

**Keywords**: eutrophication, water quality, macroalgae, recovery

## L03 02

# The Case of Airsoft Games in Mala Planina as an Unconventional Cultural Ecosystem Services Use

Borislav Grigorov, Assen Assenov

Faculty of Geology and Geography, University of Sofia "St. Kliment Ohridski", 15 Tzar Osvoboditel Blvd., 1504 Sofia, Bulgaria.

**Aim:** The main aim of the current research is to investigate the provision of ecosystem services in the western part of Mala Planina. The work examines the cultural ecosystem services in particular.

Materials and methods: The study is based on cameral and terrain research and the focus is a specific type of sport in the form a live action role-playing game (LARP), called Airsoft. The game is based on the elimination of your opponents by firing non-harmful Airsoft pellets launched via replica weapons. The specific area of the LARP falls within the boundaries of the abandoned military unit near Ponor village. A thorough open-ended interview with events arranger, called a game master is applied, as a qualitative research method for gathering the necessary information, which is processed in order to fulfill the aims of the investigation.

**Results:** Around 640 players (a major share of them participate more than once) per season take part in the game, organized at least one time per month and the fact that a lot of the players come back again proves undoubtedly the quality of the natural capacity of Mala Planina.

**Conclusion:** The study is demonstrating the fact that the western part of Mala Planina attracts visitors by cultural ecosystem services that it provides.

**Keywords:** Mala Planina, cultural ecosystem services, live action role-playing game, Airsoft

L03 03

# Drivers of Changes in High Mountain Ecosystems Case study: Rila High Moutain Ecosystems

Kostadin Katrandzhiev, Svetla Bratanova- Doncheva, Gergana Georgieva

Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences

**Aim:** Mountain ecosystems undergo different changes. We present a conceptual framework of two hypotheses for drivers causing them – land use and climate changes, causing regime shifts in Europe's highland ecosystems. These areas are considered to be the most vulnerable zones existing. The aim of the study is to examine these drivers and prioritize their influence on the condition of high mountain ecosystems.

**Materials and methods:** By set of criteria a small territory in Bulgarian Rila Mts was chosen as study area for our research. It is located in SE slopes of Rila Mt and occupies a territory of 400 ha in a range of 1800 m up to 2485 m a.s.l. Using GIS- based data, field data and literature, we defined and described following drivers of changes in these ecosystems – land use and climate change.

**Results:** The results confirmed that for the condition of high mountain ecosystems essential drivers are land use and climate change. Prioritizing them our results shows that these ecosystems have always been under law protection. This fact make us expect reduction the influence of land use and give priority to climate change as driver of change the condition of Rila high mountain ecosystems.

**Conclusion:** Our results show high Rila Mtn ecosystems' vulnerability to land use and climate change in different degree, so additional research also of their complex action is needed to prioritize them. Taking into account that high mountain areas are law protected, the conclusion is that climate change is expected driver which influences more the condition of Rila high mountain ecosystems.

**Acknowledgements:** This study was supported by project: Enhancing the resilience capacity of SENSitive mountain FORest ecosystems under environmental change (SENSFOR)

**Keywords:** land use, climate change, high mountain ecosystems

## P03\_01

# The effect of foliar treatment of spray carnation with the biomineral fertilizer Plantagra

Bistra Atanassova, Maria Yovkova

Institute of Ornamental Plants – Sofia1222, Sofia, Negovan

**Aim:** The objective of the present study was to explore the effect of the different number of treatments with the biomineral fertilizer Plantagra on the growth and development of spray carnation mother plants.

**Material and Methods:** In 2013-2014, a pot trial with two Bulgarian spray carnation cultivars – Russalka and Naslada was carried out in glasshouse conditions at the Institute of Ornamental Plants, Sofia. The purpose was to study the effect of different number of foliar treatments (double, triple and four times) with 0.14% solution of the biomineral fertilizer Plantagra on the growth and development of mother plants.

**Results and discussion**: The growth rate increased with the increase of the number of treatments and varied with in 5.6%-25.9% in cv. Russalka and 15.8%-19.3% in cv. Naslada.

The number of treatments also had a positive effect on the development of the lateral branches in spray carnation in all the trial variants. The effect was expressed better in cv. Russalka with the highest growth rate reported in four times treatment and in cv. Naslada – in triple treatment with the growth rate values exceeding those of untreated plants with 28.6% and 15.0%, respectively.

The positive effect of foliar treatment on spray carnation cuttings productivity in both cultivars was expressed better with more applications with a higher relative percentage vs. the control, namely 15.4% and 10.2% in cv. Russalka, respectively and 25.0% and 21.9% in cv. Naslada.

**Conclusions:** The plants of Bulgarian spray carnation cultivars Russalka and Naslada developed better following the foliar treatment with the biomineral fertilizer Plantagra – they were higher, with a bigger number of lateral branches and more productive cuttings compared to the untreated plants.

The best results were obtained after four times treatment of the mother plants with 0.14% solution of Plantagra, where the productivity of cuttings exceeded that of the untreated plants with 15.4% in cv. Russalka and 25.0% in cv. Naslada.

**Acknowledgments:** Project № R158 / 2014 Biodiversity conservation, improving of varietal structure, optimization of technologies for growing ornamental plants and testing the reaction of ornamentals to stress factors, Institute of Ornamental plants plants - Sofia, Agricultural Academy.

**Keywords:** spray carnation, leaf treatment, fertilizer, growth, productivity

## P03 02

# **Urban Ecosystems in Plovdiv Province: Assessment and Mapping of Provisioning Services Related to Groundwater**

<u>Zvezdelina Aydarova<sup>1\*</sup></u>, Bilyana Borisova<sup>2</sup>, Miglena Zhiyansky<sup>3</sup>, Stoyan Nedkov<sup>4</sup>, Mariyana Nikolova<sup>5</sup>,

\*Corresponding author: zvezdi 15.pz@abv.bg

Urban ecosystems are complex, heterogeneous, and dynamic systems, heavily influenced by human activity. The main challenges to their sustainability is the maintaining of the delicate balance between the ecosystems potential to provide services and their constantly increasing demand.

**Aim:** The research aims to assess and to map the provisioning ecosystem services, recognized as essential for human well-being in the context of urban development - groundwater for drinking and for nondrinking purposes (EEA CICES 4.3), in the territorial scope of urban ecosystems in the Plovdiv region outside the NATURA 2000 zones.

<sup>&</sup>lt;sup>1</sup>Faculty of Geology and Geography, Sofia University "St. Kliment Ohridski", Sofia 1504, 15 Tzar Osvoboditel blvd, Bulgaria

<sup>&</sup>lt;sup>2</sup> Faculty of Geology and Geography, Sofia University "St. Kliment Ohridski", Sofia 1504, 15 Tzar Osvoboditel blvd, Bulgaria

<sup>&</sup>lt;sup>3</sup>Forest Research Institute, Bulgarian Academy of Sciences, 132, St. Kliment Ohridski Blvd, 1756 Sofia, Bulgaria <sup>4</sup>National Institute of Geophysics, Geodesy and Geography, Bulgarian Academy of Sciences, Sofia 1113, Acad. G.

Bonchev Str., bl. 3, Bulgaria

<sup>&</sup>lt;sup>5</sup> National Institute of Geophysics, Geodesy and Geography, Bulgarian Academy of Sciences, Sofia 1113, Acad. G. Bonchev Str., bl. 3, Bulgaria

**Materials and methods:** The survey is conducted in accordance with the national methodology for urban ecosystems mapping and assessment (Zhiyansky et al., 2015). The assessment procedure uses the indicator "Exploitation index" applied by national River Basin Directorates for water abstraction reporting and human pressure restriction. GIS spatial analysis of ecosystem services distribution was conducted for each urban ecosystem types on a scale from 1 to 5 (1 - very low potential, 2 - low potential, 3 average potential, 4 - high potential 5 - very high potential).

**Results:** The final maps demonstrate spatial differences in urban ecosystems potential to provide particular services. In 78% of the urban areas in the Plovdiv region possess very high potential for the provision of groundwater services. 16% have a low potential and 6% are the territories with a very low potential.

**Conclusion:** Ecosystems services are highly correlated to the geographical factors and local social-environmental interrelations. The results are oriented towards a better understanding of the ecosystem services concept in support of territorial management and planning.

**Acknowledgments:** The study was performed within the project "Toward Better Understanding of Ecosystem Services in Urban Environments through Mapping and Assessment" TUNESinURB (FM of EEA 2009-2014) www.tunesinurb.org

**Keywords:** Urban ecosystems, Ecosystem services, Ecosystem services assessment, Exploitation index, Groundwater bodies, Bulgaria

## P03 03

# A rapid method for vulnerability assessment of coastal plant communities from flooding caused by unusual storms

Stoyan Vergiev<sup>1</sup>\*, Mariana Filipova-Marinova<sup>1</sup>, Ekaterina Trifonova<sup>2</sup>, Iliyan Kotsev<sup>2</sup>

<sup>1</sup>Museum of Natural History Varna, 41, Maria Louisa Blvd., 9000 Varna, Bulgaria

<sup>2</sup>Institute of Oceanology, Bulgarian Academy of Sciences, POBox. 152, 9000, Varna, Bulgaria

**Aim:** This work proposes a rapid method for vulnerability assessment of coastal plant communities to flooding caused by unusual storms over the Bulgarian Black Sea Coast.

Material and methods: In order to create a dynamic GIS model, data from experimental results, hydrodynamic modelling, detailed topography, bathymetry surveys and detailed GIS mapping on the Kabakum beach (Varna) were incorporated. As a result of a simulated flooding experiment, Critical Decomposition Time (CDT) was obtained. Whole plants of 6 species were immersed in sea water for 20 days. CDT is defined as the time point at which plants show signs of irreversible decomposition of vegetative organs and indicates that will not survive and their communities will not be able to recover.

**Results:** Integration of results from inventory, distribution and altitudinal spreading of plants and flooding maps shows that only 6 species are potentially threatened by negative impacts during storm events. Linking flood duration and CDT for these species determines that *Artemisia vulgaris* L., *Eryngium maritimum* L. and *Crambe maritima* L. are vulnerable to storms. This approach has allowed experimental results to be applied directly to a field-based situation to predict the effects of future storm events on the beach vegetation.

**Conclusions:** Plant communities in Kabakum beach are not threatened by complete destruction, even during a storm with return period of 100 years. Habitat recovery does not require human intervention, and full recovery is likely within a season, therefore Environmental Vulnerability Index could be estimated to be equal to 1.

**Acknowledgments:** The support of the European Commission through FP7.2009-1, Contract 244104 THESEUS ("Innovative technologies for safer European coasts in a changing climate"), is gratefully acknowledged.

**Keywords**: Vulnerability assessment, GIS, floods.

## P03\_04

# Macrophyte composition in inland wetlands in Northern Bulgaria

Borislava Gyosheva, Vladimir Valchev, Nevena Ivanova

Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences

**Aims:** One hundred and forty polygons, situated in Northern Bulgaria, are verified during the project *Wetland ecosystem services mapping and assessment in Bulgaria*. These polygons are inland/terrestrial wetlands which are out of the NATURA 2000 network. The aim of the study is to determine the macrophyte composition in verified polygons and to assess their wetland ecosystem condition based on plant biodiversity, plant species richness and invasive species presence.

**Material and methods:** The methodology that is used is based on the project "Methodology for assessment and mapping of wetlands ecosystems condition and their services in Bulgaria". The study was conducted in July-September 2016. The studied wetlands belong to D5 EUNIS habitat classification: Sedge and reedbeds, normally without free-standing water. These types of wetlands are poorly studied in Bulgaria.

**Results:** This is the first attempt to systematically study the macrophyte diversity in these wetlands. Sixty-two of the polygons are validated on the field. The remaining seventy-eight polygons are excluded for various reasons. Seventy-six macrophyte species were determined during the study.

According to the plant species richness criteria used to assess the wetlands condition, the biggest percentage of the polygons are in *moderate condition* (score 3) and the smallest percentage are in good condition (score 4).

Conclusion: In our opinion the obtained results reflect the climate specifics and the small water budget in these sites

**Acknowledgments:** This report is developed within the project Wetland ecosystem services mapping and assessment in Bulgaria (WEMA)", supported by the Financial Mechanism of the European Economic Area 2009-2014, Program BG03 Biodiversity and ecosystem services, D-33-86/27.08.2015.

Keywords: macrophytes, wetlands ecosystem assessment, D5 EUNIS classification

# THEMATIC SESSION IV LANDSCAPE ECOLOGY

PL04\_01

# Challenges in biogeography development

Asen Assenov

Faculty of Geology and Geography, University of Sofia "St. Kliment Ohridski", Sofia, Bulgaria

**Aim:** The aim of the work is to reveal the challenges that research methodology in Biogeography faces today. **Materials and methods:** The interdisciplinary nature of biogeography as a synthetic science creates a prerequisite for the use of a wide range of methods that are typical of the main natural sciences – biology, geography, ecology, paleontology, physics, chemistry, mathematics, even medicine. Emerging new subdisciplines in biogeography are analyzed as they are a challenge for the development of biogeography's subject of research.

**Results:** Periodization of the development of biogeography based on the analysis of the progress of biogeographic sub-disciplines and changing philosophical views in the development of science is made. The parallel comparison of achievements in different biogeographical sub-disciplines globally with the development of biogeography in Bulgaria reveals a significant gap and tight scientific bias of the authors in Bulgaria compared to wide-aspect horizontal interactions of biogeographers in Europe, North and South America, Japan, Australia, New Zealand and many of the Commonwealth countries. Outlined are the necessary prerequisites for the development of biogeography here in order to make the transition to follow the leading countries practices if not to catch up with them.

**Conclusion:** The reflection of the challenges lying ahead of modern biogeography in Bulgarian science has comparable general negative traits with almost all natural science fields in the country, except the existing momentum in developing information technologies. A particularly important conclusion for overcoming the not so positive scientific image of Bulgaria is the lack of horizontal common interactions between different scientific fields.

**Keywords:** *object, subject, paradigm, sub-disciplines, biogeography* 

### L04 01

# Bridging between physical geography and oceanography: the science of seascape ecology

Iliyan Kotsev

Institute of Oceanology "Fridtjof Nansen" – BAS, Varna, Bulgaria

Having originated as a science studying the relationship between pattern and process, the interdisciplinary field of landscape ecology has been expanded beyond the traditional terrestrial focus and its research principles are widely applied in oceanography nowadays.

**Aim:** To trace chronologically the main development stages of seascape ecology; to summarize its achievements; to classify its main sub-fields; to identify the science's principal areas of research; to point out differences and similarities among the leading Eastern and Western scientific schools.

Materials and methods: The study was carried out as a literature review of available publications.

**Results:** The development of seascape ecology can be chronologically grouped into four main stages, i.e.: late  $19^{th}$  – mid  $20^{th}$  c.; early 1950s – early 1970s of the  $20^{th}$  c.; mid 1970s – early 1990s of the  $20^{th}$  c.; early 1990s – present day. A few among the principal sub-fields are: submarine landscape (seascape) morphology, (geo) dynamics and evolution; coastal landscape ecology and dynamics; sea-islandscape ecology, etc. The focal areas of this interdisciplinary field are numerous, ranging from benthic habitat characterization, through stock assessment, to marine spatial planning and integrated coastal zone management.

**Conclusions:** A relatively young discipline, seascape ecology is nowadays seen as an applied interdisciplinary field rather than an individual branch of oceanography. There are considerable differences between the Eastern and the Western scientific schools, reflecting their conceptual dissimilarities in the perception of the landscape and its marine equivalent, the seascape. Nevertheless, seascape ecology is ubiquitously considered an innovative complex-based approach to studying the coastal and marine environment.

**Keywords**: (sub) marine landscape, landscape (seascape) pattern, seafloor mapping; coastal zone, Sea Islands

## P04 01

# Environmentally relevant organic compounds from Marisa East dumps

Angelina Kosateva<sup>1</sup>, M. Stefanova<sup>1</sup>, Z. Milakovska<sup>2</sup>, S.P. Marinov<sup>1</sup>

<sup>1</sup>Laboratory Fuel Chemistry, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

<sup>2</sup>Department of Paleontology, Stratigraphy and Sedimentology, Geological Institute, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

**Aim:** The timeliness of the study was based on the scarcity of information on organic geochemistry of dump materials from open pit coal mines. Recent investigations proved that hydrocarbons, phenols etc. move through/release out of the dump area as a result of alteration processes of the organic matter (OM) caused by the wash-out and/or drainage processes. These data and processes established give the reason to turn to study the dumps in Marisa East coal basin.

**Materials and Methods:** Two dumps sample (ca. 30 g) were placed in a Soxhlet thimbles and extracted by chloroform ( $\sim$ 300 ml), under reflux for 30 h. Chloroform-soluble organic matter (SOM) was concentrated and asphaltenes were precipitated by pouring the toluene solution into cold *n*-hexane (1:100 v/v), while the soluble portions (maltenes) were concentrated and subsequently separated *via* column chromatography. Mini-glass column ( $10\times1$  cm) with Kieselgel (35-70 mesh ASTM) was used. The following fractions were prepared: neutral, aromatic/slightly polar and polar. Likewise SOMs of the two dumps were fractionated. Subsequently they were studied by gas-chromatography-mass spectrometry.

**Results:** In the aromatic/slightly polar fractions a broad set of components was determined, i.e. phthalates (dominant), *i*-propyl palmitate, *i*-propyl myristate, *n*-hexyl benzoates, etc. These organic contaminants could be regarded more likely as anthropogenic (originating from plasticizers, industrial pollutants, pesticides, etc.).

**Conclusion:** Plasticizers, antioxidants, stabilizers and other wastes of the modern chemical industry are present in Maritsa East dumps. They move through/out and could be potential contaminants for the waters and soils in the area. Their nature and amounts should be under regulation.

Acknowledgements: The study was funded by NSF, Ministry of Education and Science under DN 04/5 project.

**Keywords:** *lignite, dumps, anthropogenic contaminants* 

## P04 02

# Land cover classification of Southern Kraishte region using satellite imagery

#### Petko Bozhkov

Faculty of Geology and Geography, Department of Climatology, Hydrology and Geomorphology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

The study object is part of the Struma valley between Zemenska, Konjavska and Rudini Mountains. Presented results characterize the southern parts of the Kraishte region and upper Struma valley including the Zemen Gorge.

**Aim**: The aim of presented research is to create and evaluate a land cover map of the Southern Kraishte. Therefore, a digital satellite image classification is performed using supervised approach. This technique is used to group pixels with similar values in several image bands into land cover classes. High resolution imagery and data from field work is used for the visual assessment of the derived land cover map.

**Materials and Methods**: Multispectral image from satellites Landsat 8 OLI/TIRS from 2016 is used an input data. The classification process is performed using an open source GIS software – QGIS 2.18 and the Semi-Automatic Classification Plugin (version 5.3.6). Selected representative samples (also known as training sites) for each land cover class in the digital image are evaluated using statistics.

**Results**: The end result of the image classification is a land cover map showing the spatial pattern of each land cover class. The area of each class is calculated both in absolute in relative units (% of the whole study area).

**Conclusion**: Presented results show locally relevant information for land cover and landscape management. Land cover maps could be used in ecoforcasting and sustainable territorial development.

**Keywords**: supervised classification, Landsat, remote sensing, QGIS

# THEMATIC SESSION V ECOLOGY AND EDUCATION

### PL05 01

# Model for organizing environmental education through "Region for kids"- a successful brand at regional level

## Asya Asenova

Sofia University "St. Kliment Ohridski", Faculty of Biology, Methodology of Biology Teaching, 1164 Sofia, 8 Dragan Tsankov Blvd.

This work provides a detailed analysis of the extracurricular activities in biology education in terms of national policies, programs and strategies. Presented and analyzed is a successful model for organizing the environmental education and sustainable development through concrete tools for extracurricular activities.

The educational product, which is presented in the article, is training programmed "Region for kids", presented in the form of training aids, including handbook for teachers, a small guidebook in the biological diversity of the concrete region and a CD with special materials from the aid. The topics and activities are orientated to the age range of the target audience and the instruments proposed for performance reflect the modern trends in

environmental education- interactive methods, a combination of theory and practice, inclusion of all the senses, applying the principles of natural interpretation.

**Acknowledgments:** The "Region for kids" brand and related projects is being financed by the Mitsubishi Corporation Fund for Europe and Africa. The programme is being worked out by the Regional Environmental Center for Central and Eastern Europe.

**Keywords:** environmental education, Region for kids, extracurricular activities

## L05 01

# Correlation-integration methodical system in processing contents from environmental education during English lessons

Snezana Veselinovska<sup>1</sup>, Snezana Kirova<sup>2</sup>

<sup>1</sup>Faculty of Educational Sciences, University "Goce Delcev", Stip R. Macedonia <sup>2</sup>Faculty of Philology, University "Goce Delcev", Stip R. Macedonia

**Aim:** Since the last quarter of the twentieth century, the world has been increasingly working on connecting the teaching contents from different subjects (cross-curricular approach), on integration of subjects, and on linking the teaching of foreign languages with other subjects. Common processing does not necessarily refer only to the topic, but also to smaller or larger or teaching units. We will direct our attention to the connection between the teaching contents of environmental education and of English language, and the advantages and disadvantages of this approach to teaching.

## **Material and Methods:**

Example of a practical lesson:

Teaching subject – Environmental education

Teaching unit: "Vegetative organs of plants"

Teaching subject – English language Teaching content: "Our Environment"

### **Results:**

- Increasing of student motivation,
- Facilitating access to foreign literature and various media for expanding knowledge,
- Improving self-image,
- Creatingan opportunity to teach some of the contents of environmental education during English language lessons prior to their treatment in mother tongue.

**Conclusion:** As educators of future educators of young generations we should approach our work seriously with clearly set goals ahead. Such instruction and cooperation between two teachers of different subjects meaningfully reduces the speed of processing and linksthe teaching contents of two subjects, and it relieves students of some load because the knowledge acquired in one subject is positively used for acquiring new knowledge in the other subject, which in turn facilitates permanent acquisitions of certain notions.

**Keywords:** Environmental education, English language, correlation-integration methodical system, knowledge, vegetative organs of plants, our environment.

### P05 01

# Content of radioactive elements in sediments of the northern Black sea coast of Bulgaria for the period 2010-2016

Ivanka Yordanova<sup>1</sup>, Donka Staneva<sup>1</sup> Eliza Pavlova<sup>2</sup>

<sup>1</sup>Institute of Soil Science, Agrotechnology and Plant protection "Nikola Poushkarov", 7, Shousse Bankya Str, 1080 Sofia, Bulgaria

<sup>2</sup>Institute of Fisheries, 4, Primorski boul., 9000 Varna, Bulgaria

Radionuclides are present in the living and nonliving components of each aquatic environment. The accumulation of hazardous radioactive elements deposited with the fallout in marine environment started with the first nuclear tests and continues till nowadays. Rivers flowing into the Black Sea are a main transmission environment of radioactive materials discharged into land areas around the sea.

**The Aim** of the study is to investigate the radioactive contamination of the Bulgarian Black Sea Coast. In the period between 2010 and 2016 sampling of sediments from Varna bay, Varna Lake, Galata, Emine and other sites at the northern Black sea coast of Bulgaria was done.

**Materials and methods**: The samples were analyzed by gamma-spectrometry for mass activity determination of the following radionuclides – artificial <sup>137</sup>Cs and natural <sup>234</sup>Th, <sup>235</sup>U, <sup>226</sup>Ra, <sup>228</sup>Ac amd <sup>40</sup>K.

The gamma-spectrometry analyses was done with 20% efficiency Ge detector and multichannel analyzer DSA 1000 production of CANBERRA.

**Results**: The values obtained for the specific mass activity of natural radionuclides in the studied sediments are within the values detected in soils of Northeast Bulgaria. The artificial <sup>137</sup>Cs is detected in all analyzed samples. Most likely the presence of this radionuclide in marine sediments is mainly due to the accident at the Chernobyl nuclear power plant in April 1986.

**Conclusions:** The specific mass activities of the radionuclides determined in the sediments are within the range of background values and are not hazardous for contamination of sea water.

**Keywords:** Black sea, radioactivity, marine sediments, caesium - 137, strontium - 90.

## P05\_02

# Agalmatium flavescens (Hemiptera, Issidae) and Camponotus aethiops (Hymenoptera, Formicidae) – an unknown trophobiotic association

Ilia Gjonov, Albena Lapeva-Gjonova

Department of Zoology and Anthropology, Faculty of Biology, Sofia University, Bulgaria

**Aim**: The knowledge on trophobiosis between ants and planthoppers of family Issidae is limited to studies of individual cases from Argentina, Mexico, India, the island of Naxos (Cyclades) and an anecdotal report from Italy.

This work reports a previously undescribed ant attendance of *Agalmatium flavescens* (Olivier, 1791) (Hemiptera, Issidae) by *Camponotus aethiops* (Latreille, 1798). Brief literature review on associations between issid species and ants as well as some ecological aspects related to the new finding were included.

**Material and methods:** We observed and took photographs on the case of trophobiosis on 9<sup>th</sup> July 2016 in the vicinity of Ivan Vazovo Village (Plovdiv district).

**Results**: Three specimens of Agalmatium flavescens ( $2 \circlearrowleft$  and  $1 \hookrightarrow$ ) were found being tended and annuenated by ants of Camponotus aethiops on a field elm (Ulmus minor Mill.). The ant workers actively followed the

planthoppers and antennated them, stimulating in this way the secretion of honeydew. Issids, in turn, showed no sessile behavior and moved up and down the branches of the elm.

**Conclusion:** *A. flavescens* is a common species and lack of such observation until now suggests its facultative relationships with ants in contrast to opportunistic relations in some other hemipterian species where ants collect honeydew from the substrate. However, in the case presented here we observed antennation and honeydew drops directly collected by anal opening of issids.

**Keywords:** trophobiosis, ants, Fulgoromorpha, Issidae, Bulgaria

# THEMATIC SESSION VI OTHER RELATED TOPICS

## $L06_{01}$

# Water memory displayed by the contact angle distributions of evaporating drops

Lidia Popova<sup>1</sup>, Stefan Todorov<sup>2</sup>

<sup>1</sup>Initute of Solid State Physics, Bulgarian Academy of Sciences, 72 Tsarigradsko shosse Blvd., Sofia, Bulgaria ttlidia@yahoo.com

**The aim:** of the paper is to demonstrate the effect of water filtration on the contact angle evaporation distribution of a sample of a sessile drop.

**Material and methods:** A nuclear filter with holes with diameter of 0.15 micrometers was used to transport a water sample through its holes. The contact angles of the sessile drop are measured during the process of drop evaporation.

**Results and discussion:** The distributions before and after the filtration are shown to be manifestly different which implies a change of water properties due to filtration. Moreover, a difference between the filtered and unfiltered sample's distributions is maintained in time, implying existence of an effect of water "memory".

**Conclusions:** The water filtration trough a nuclear filter causes changes in some water structural properties which tend to be preserved in time.

**Keywords:** water filtration, nuclear filter, contact angle evaporation distribution, water memory.

<sup>&</sup>lt;sup>2</sup>Institute of Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tsarigradsko shosse Blvd., Sofia, Bulgaria, todofov\_st@yahoo.com

## P06 01

# Dry mass yield and amount of fixed nitrogen in some forage legume crops after treatment with organic fertilizer Humustim

Viliana Vasileva, Todor Kertikov, Anna Ilieva

Institute of Forage Crops, 89 "General Vladimir Vazov" Str., 5800 Pleven, Bulgaria

**Aim:** The study aimed to access some changes in agronomic performance of spring forage pea (*Pisum sativum* L., variety Pleven 4) and vetch (*Vicia sativa* L., variety Obrazets 666) after application of organic fertilizer Humustim.

**Material and methods:** Experiment was carried out on the experimental field of the Institute of Forage Crops, Pleven, Bulgaria in three consecutive years. The fertilizer was applied through i) presowing treatment of seeds, ii) during vegetation and iii) combination of both.

**Results:** Our observations showed that dry mass yield in both crops increased significantly after presowing treatment of seeds with Humustim at the dose of  $1.2 \, \text{L/t}$  + one treatment during vegetation (in vetch with 19.8%, and in pea with 20.1%), under high sustainable yield index. Amount of fixed nitrogen in pea was found increased to 20.8% (presowing treatment of seeds at the dose of  $1.2 \, \text{L/t}$  + one treatment during vegetation), and in vetch, to 21.3% (presowing treatment of seeds at the dose of  $1.2 \, \text{L/t}$  + two treatments during vegetation). Treatment with Humustim increased the efficiency of utilization of nutrients of peas. Pea's plants accumulated in their dry mass to  $4.25 \, \text{kg/da}$  more nitrogen after presowing treatment of seeds with Humustim at the dose of  $1.2 \, \text{L/t}$  + one treatment during vegetation. Treated plants, both peas and vetch was more effective over nontreated with regard to important agronomic characteristics.

**Conclusion:** Based on the positive results, it is recommended the use of Humustim in the technology of cultivation of peas and vetch in modern trends of organic farming.

**Keywords:** organic fertilizer, spring forage pea, vetch, nitrogen fixation

## P06\_02

# Antioxidant properties of Cancer parugus hemocyanin

<u>Yuliana Raynova</u><sup>1</sup>, Milka Mileva<sup>2</sup>, Ivan Kindekov<sup>3</sup>, Dimo Krastev<sup>4</sup>, Krassimira Idakieva<sup>1</sup>

<sup>1</sup>Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences

<sup>2</sup>The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

<sup>3</sup>Scientific Laboratory of Radiation Protection, Radiobiology and Cell Radiobiology,

Military Medical Academy

<sup>4</sup>Department of Anatomy and Histology, College of Medicine "Yordanka Filaretova"

**The aim** of the present study was to investigate the antioxidant properties of hemocyanin isolated from the marine crab *Cancer pagurus* (CpH) in prooxidant model systems *in vitro*.

**Material and Methods:** Native CpH was obtained from the haemolymph, collected from the species *Cancer pagurus*, by ultracentrifugation and subsequent gel filtration chromatography. The free radical scavenging activity was determined against three radicals – superoxide, hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The protection by CpH against oxidative damages of two model lipid membranes – rat liver supernatant and liposomal suspension in conditions of iron-induced lipid peroxidation was tested.

Results: In general, antioxidants compounds can interact with free radicals, to scavenge them, removing catalytic metal ions, and inhibit or retard the process of lipid peroxidation. CpH exhibited good DPPH and

hydroxyl radicals scavenging activities in a concentration-dependent manner and a lower ability to capture superoxide radicals at physiological value of pH. CpH showed very good capacity to inhibit Fe<sup>2+</sup>- induced lipid peroxidation in applied systems, as well as chelating activity toward iron ions.

**Conclusions:** This study reveals that CpH has an ability to act as iron chelating protein, as well it could provide protection against oxidative stress and in this manner to decline the risk of destruction of biomolecules, caused by initiation of harmful free radicals - mediated chain reactions.

Keywords: hemocyanin, antioxidant activity, Cancer pagurus

# **INDEX**

Name	Code	E-mail	Student/ PhD	Page
Alexandrova, Albena	P02_09		student	44
Alexeeva, Marina	P02_04	marinaalexeeva93@gmail.com		41
Alexieva, Vera	P02_02	marmanexecva/3@gman.com	•	40
Alipieva, Kalina	P02_10			45
Anachkov, Nikolaj	P01_07			22
Aneva, Ina	P02_10			45
Angelova, Lilia	L02_02	lilia_stoickova@abv.bg		31
Angelova, Tsveta	L02_13	angelova_ts@abv.bg		39
Apostolova, Elena	P01 07			22
Apostolova, Emilia	L02_03, L02_04			32, 33
Apostolova, Iva	L01_01			14
Arsovska, Julijana	EP01 01			30
Asenov, Asen	P01_05	asenasenov71@yahoo.com		21
Assenov, Assen	L03_02, PL04_01	asseni.assenov@gmail.com		50, 55
Asenova, Asya	PL05_01	asya_asenova@yahoo.com		57
Assyov, Boris	P01_04	contact@boletales.com		20
Atanassov, Atanas	PL03_02	atanas_atanassov@jgc-bg.or		49
Atanassova, Bistra	P03_01	iop_sofia@abv.bg		51
Ayan, Yusuf	L01_03, P01_13			15, 26
Aydarova, Zvezdelina	P03_02	zvezdi_15.pz@abv.bg		52
Baev, Vesselin	P01_07	•		22
Bancheva, Svetlana	L01_02	sbancheva@yahoo.com		15
Belkinova, Detelina	L02_08			35
Beltcheva, Michaela	L02_06			34
Bardarov, Ventsislav	PL03_02			49
Berkov, Strahil	L02_12			38
Berov, Dimitar	L03_01	dimitar.berov@gmail.com		50
Biserkov, Valko	PL01_02, L01_01, P01_06	v@biserkov.com		13, 14, 21
Blazhevska, Radmila	EP01_01	radmila.blazevska@outlook.com		30
Bogoeva, Irena	L02_01	iren.bog@abv.bg		31
Borisova, Bilyana	P03_02			52
Bozhkov, Petko	P04_02	petko_bozhkov@abv.bg		57
Brambilla, Mattia	P01_09			23
Bratanova-Doncheva, Svetla	PL03_01, L03_03	sbrat@abv.bg		48, 51
Bubova, Valentina	P02_02			40
Chaneva, Ganka	P02_04			41
Chankova, Stephka	L02_10, L02_12, L02_13	Stephanie.chankova@yahoo.com		37, 38, 39
Chipev, Nesho	PL03_01, P02_09	nchipev@abv.bg		48, 44
Chehlarov, Evgeni	L01_01			14

Cvetkovska-Gjorgjievska,	EP01_01		30
Aleksandra			
Dakova, Megi	P02_07		43
Danova, Kalina	P02_10	к_danova@abv.bg	45
Decheva, Gabriela	P01_08		23
Dermendzhiev, Pencho	P01_17		28
Dimitrova, Marta	P01_09	marta_hansova@abv.bg	23
Dimitrova, Velmira	P02_01	pmmaster2001@yahoo.com	40
Dimkov, R.	L02_11		37
Djorgova, Nadejda	P01_06	djorgova@gmail.com	21
Dobreva, Maria	P01_17		28
Dobrikova, Anelia	L02_04		33
Dochin, Kostadin	P02_08		44
Doykin, Nikola	L01_07		18
Dzhovanova, Iliana	L01_03, P01_13		
Elshishka, Milka	P01_11	melshishka@gmail.com	25
Evstatieva, Ljuba	P02_10	5	45
Filipova-Marinova,	P03_03		53
Mariana	_		
Ganeva, Anna	L01_01		14
Gecheva, Gana	P01_16		28
Geneva, Maria	P02_01		40
Georgieva, Almira	P02_09		44
Georgieva, Galia	L01_06	tsambi@abv.bg	17
Georgieva, Gergana	L03_03		51
Georgieva, Elenka	P01_03		20
Georgieva, Mariya	L01_03, P01_13		
Georgieva, Stela	P01_03		20
Georgieva, Teodora	P02_01		40
Gevezova-Kazakova, Maria	P02_08		44
Gjonov, Ilia	P01_12, P05_02		25, 59
Golovinsky, E.	L02_11		
Gombos, Zoltan	P02_03		41
Gozmanova, Mariyana	P01_07		22
Grigorov, Borislav	L03_02	borislav.g.grigorov@gmail.com	50
Guéorguiev, Borislav	L01_01		14
Groudeva, V.	L02_11		37
Gumus, Media	L01_03, P01_13,	med_i@abv.bg	15, 26,
	P01_16		28
Gyosheva, Borislava	P03_04	borislavagyosheva@gmail.com	54
Hinić, Jelena	EP01_01		30
Hristov, Peter	P01_11		25
Hristova, Lubov	P01_08	lhristova2@gmail.com	23
Hristovski, Slavčo	EP01_01		30
Hristozova, Zlatovesa	P02_07	sl.petrova@abv.bg	43
Hubenova, Tanya	P02_08		44
Idakieva, Krassimira	P06_02		61

Ilieva, Anna	P06_01			61
Ilieva, Ivalena	P02 04			41
Ivanova, Albena	L02_02, L02_05		-	31, 33
Ivanova, Angelina	P02_08			44
Ivanova, Elena	L01_01			14
Ivanova, Neli	L01_07	neli_bonev@abv.bg		18
Ivanova, Nevena	P03 04	nen_coneve dov.og	•	54
Kapchina-Toteva, Veneta	P01_08, P02_04			23, 41
Karamfilov, Ventsislav	L03 01			50
Karapavlova, Mariela	L01_01			14
Katrandzhiev, Kostadin	L03_03	kmkatrandjiev@gmail.com		51
Kazakov, Stefan	L02_08, P02_08	sakazak@mail.bg		35, 44
Kerakova, Maria	L02_07	mariqkerakova@abv.bg		35
Kertikov, Todor	P06_01			61
Kindekov, Ivan	P6_02			61
Kircheva, Magdalena	L01_01			14
Kirilov, Atanas	L01_01			14
Kirova, Snezana	L05_01			58
Koleva, Dimitrina	L02_02, L02_05			31, 33
Koleva, Petya	P02 10			45
Koleva, Viktoriya	L01_03, P01_13			15,
Kosateva, Angelina	P04_01	ani_popova@orgchm.bas.bg		56
Koshev, Yordan	L01_01			14
Kostova, Rumyana	L01 01			14
Kotsev, Iliyan	L04_01, P03_03	ikotsev@io-bas.bg		55, 53
Kozuharov, Dimitar	L01_06			17
Kozhuharova, Asya	P01_14	asya.kozhuharova@mail.bg		27
Krastev, Dimo	P6_02			61
Krumova, Sashka	P02_03			41
Kutjavin, Ivan	EP02_01			47
Lapeva-Gjonova, Albena	P01_10, P01_12,	gjonova@abv.bg		24, 25,
3	P05_02			59
Lazarova, Stela	P01_11			25
Malakova, Nevena	L01_04	ven90@abv.bg		16
Marinov, S. P.	P04 01	<u> </u>		56
Markovska, Yuliana	L02_03, P02_01			32, 40
Maslenkova, Liliana	L02 02, L02 05			31, 33
Metcheva, Roumiana	L02_06			34
Mihovski, Tsvetoslav	L01_01			14
Milakovska, Z.	P04_01			56
Mileva, Milka	P6_02			61
Miteva, Daniela	L02_10			37
Mitrovska, Zhana	L02_12, L02_10			37, 38
Mladenov, Rumen	L02_08			35
Momchilova, Albena	L02_05			33
Momchilova, Svetlana	L02_02, L02_05			31, 33
Nachev, Rumen	P01_17			28

Naumov, Borislav	L01_01, P01_06		14, 21
Naydenov, Mladen	P01 07		22
Nedkov, Stoyan	P03_02		52
Nikolov, Bogdan	P02 07		43
Nikolov, Boris	P01_06, P01_09		21, 23
Nikolova, Mariyana	P03 02		52
Nikolova, Milena	P01_14, L02_12		27, 38
Osipov, Andrey	EP02_01	a.f.osipoff@gmail.com	47
Ostoich, Peter	L02_06	p.ostoich@googlemail.com	34
Panchev, Ivelin	PL03_02	p.ostoren e googreman.com	49
Parvanova, Petya	L02_10, L02_12	petq_parvanova@abv.bg	37, 38
Pavlova, Dolja	P01 15	perq_par variova e aov.og	27
Pavlova, Eliza	P05 01		59
Pedashenko, Hristo	L01_03, P01_13		15, 26
Pehlivanov, Luchezar	L02_08		35
Peneva, Vlada K.	P01 11		25
Petrov, P.	L02_11		37
Petrov, Stefan	L01 02		15
Petrova, Antoaneta	L01_03, P01_13		15, 26
Petrova, Galya	L01_02	galiaty@abv.bg	15
Petrova, Nia	P02_03	petrova_nia@yahoo.com	41
Petrova, Slaveya	P02_07	sl.petrova@abv.bg	43
Petrova, Ventsislava	P02_11		46
Pilarska, Daniela	P01 17	dpilarska@yahoo.com	28
Popatanasov, Andrey	P01_18, P02_12	and_atanasov@abv.bg	29, 46
Popova, Elitsa	L01_07		18
Popova, Lidia	L06_01		60
Pramatarova, Monica	P01 12	1	25
Prelić, Dana	E-P01_01		31
Puley, Alexander	L01 04		16
Radoslavov, Georgi	P01_11		25
Ragyov, Dimitar	L01_01, P01_06	dimitar.ragyov@gmail.com	14, 21
Rakleova, Goritsa	PL03_02		49
Raynova, Yuliana	P6_02	yulianaraynova@hotmail.com	61
Rashid, Rashid	L01_01		14
Rimcheska, Biljana	L01_05	rimceska@gmail.com	17
Rogova, Mariya	P01_08	mrogova@abv.bg	23
Rusenov, Georgi	P02_08		44
Russev, Radoslav	PL03_02		49
Sakelarieva, Lidia	L01_04		16
Satchanska, G.	L02_11	gsatchanska@nbu.bg	37
Selenska-Pobell S.	L02_11		37
Shishiniova, Maria	PL03_02		49
Sidjimova, Boriana	L02_12		38
Shopova, Elena	P02_02	kostei@abv.bg	40
Slavova, Monica	P01_04		20
Stanachkov, Svetozar	L01_06		17
Stanachkova, Marieta	L01_06		17
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Stanilova, Marina	P01_14, P02_05		27, 42
Staneva, Donka	P05_01	donastaneva@abv.bg	59
Stefanov, Martin	L02_03, L02_04	martin_12.1989@abv.bg	32, 33
Stefanova, M.	P04_01	materia_12/1969 e de vieg	56
Stojanov, Plamen	L02_08		35
Stoyanova, Elena	P02_10		45
Stoykov, Dimitar	P01_01, P01_02	stoykovdimitar@abv.bg	19
Stoykova, Bilyana	P01_10	stoyno vanimur e ue vieg	24
Tanev, Atanas	P01_15	atanas.tanev@abv.bg	27
Taneva, Sabina	L02_02	unimasimine ( o me ( reg	31
Taneva, Stefka	P02_03		41
Terzijska, Tsvetelina	L01_01		14
Todinova, Svetla	P02_03		41
Todorov, Stefan	L06_01	todorov_st@yahoo.com	60
Todorova, Kostadinka	P01_03, P02_07	todorov_st@yanoo.com	20, 43
Todorova, Rostadiika Todorova, Teodora	L02_10		37
Tomova, Anna	P02_10	aderekova@yahoo.com	46
Tonova, Anna Topalova, Y	L02_11	aderekova @ yanoo.com	40
Topashka-Ancheva,	PL02_01	topashka.mn@lycos.com	30
Margarita	1 L02_01	topasiika.iiiii@iycos.com	30
Totev, Evelin	L01_03, P01_13		15
Tóth, Tünde	P02_03		41
Traykova, Boryanka	P02_05	borianka_traikova@abv.bg	42
Trifonova, Ekaterina	P03 03	borianka_trankova@abv.bg	53
Tsvetanov, Borislav	L01_03, P01_13		15,
Tsvetanov, Ch.	L02_11		13,
Tsvetanova, Elina	P02_09		44
			20
Tsvetanova, Vesela	P01_03	umugay@aaalah haa ha	12
Uzunov, Yordan	PL01_01, L01_06	uzunov@ecolab.bas.bg	54
Valchev, Vladimir	P03_04		35
Varadinova, Emilia	L02_07	1-1-15014@-11-	
Vassilev, Kiril	L01_01, L01_03,	kiril5914@abv.bg	14, 15,
Vassilava Plamana	P01_13, P01_16		26, 28
Vassileva, Plamena Vasileva, Viliana	L01_03, P01_13	viliana.vasileva@gmail.com	20, 26
Vasileva, Viliana Velcheva, Iliana	P06_01 P01_03, P02_07	viiiana.vasneva@gman.com	20, 43
	L01_03, P01_13,		
Velev, Nikolay	P01_16		15, 26, 28
Vargiov Stoven		atwaraiay@aby.ba	
Vergiev, Stoyan	P03_03, P02_06	stvergiev@abv.bg	53, 43 36, 58
Veselinovska, Snezana Vlahova, Mariana	L02_09, L05_01 PL03_02	snezana.veselinovska@ugd.edu	49
-		galina yahuhyan@amail aam	22
Yahubyan, Galina Yakimov, Lachezar	P01_07	galina.yahubyan@gmail.com	44
· · · · · · · · · · · · · · · · · · ·	P02_09	yakimovlachezar@abv.bg	\
Yancheva, Vesela	P01_03	veselayancheva@yahoo.com	20
Yanev, Pencho	L01_06		17
Yankova, Maria	P02_08		44
Yordanova, Ivanka	P05_01		59
Yordanova, Vesela	L02_05	v.v.yordanova.bul@abv.bg	33

Yotsova, Ekaterina	L02_03, L02_04	ekaterina_yotsova@abv.bg	32, 33
Yovkova, Maria	P03_01		51
Zaikov, Angel	P02_08		44
Zakar, Tomas	P02_03		41
Zhiponova, Miroslava	P02_04		41
Zhiyansky, Miglena	P03_02		52
Zlatanov, Valentin	L01_07		18
Zlatanova, Diana	L01_07		18