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FACULTY OF BIOLOGY  
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# FACULTY OF BIOLOGY

## YOUTH SCIENTIFIC CONFERENCE

### “KLIMENT’S DAYS”

16th–17th NOVEMBER 2017

# A B S T R A C T S



**Sofia University St. Kliment Ohridski – Faculty of Biology  
Union of Scientists in Bulgaria  
National Science Fund of  
Ministry of Education and Science of Bulgaria**

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FACULTY OF BIOLOGY  
SOFIA UNIVERSITY

## **YOUTH SCIENTIFIC CONFERENCE**

**“KLIMENT’S DAYS”**

**16th–17th NOVEMBER 2017**

# **A B S T R A C T S**

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**PROGRAMME**

**/16th NOVEMBER 2017 /THURSDAY**

**FACULTY OF BIOLOGY**

<b>Hour</b>	<b>SESSION</b>	<b>Auditorium</b>
08:00 – 12:00	Registration	Foyer II-nd floor
08:00 – 12:30	Poster mounting	Foyer II-nd floor
09:00 – 9:15	<b>OPENING CEREMONY</b> <b>Prof. Mariela Odjakova,</b> <i>Chairman of the Organizing Committee</i> <b>Assoc. Prof. Stoyan Shishkov,</b> <i>Dean of the Faculty of Biology</i>	239
<b>Hour</b>	<b>PLENARY LECTURES</b>	239
	Chairman – Prof. Yana Topalova Secretary – Assist. Prof. Daniel Todorov	
09:15 – 09:45	<b>Junmin Li,</b> Zhejiang Provincial Key Laboratory of Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China <b>The natural history of an invasive plant <i>Solidago Canadensis</i> in China and its potential control using native parasitic <i>Cuscuta</i> plants</b>	
09:45 – 10:15	<b>Ivailo Alexiev,</b> National Reference Confirmatory Laboratory of HIV National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria <b>Molecular epidemiology of HIV</b>	
10:15 – 10:30	<b>Dimitar Djilianov, Daniela Moyankova, Krasimir Rusanov, Stoyan Shishkov, Anita Tosheva,</b> Agrobiointitute and Faculty of Biology, Sofia University <b>STARBIOS2 – A HORIZON2020 Project, Focused on Responsible Research and Innovations in Biology</b>	
10:30 – 11:00	<b>COFFEE BREAK</b>	
<b>Hour</b>	<b>SEMINAR ELSEVIER</b>	239
	Chairman – Assoc. Prof. Ivan Traykov Secretary – Assoc. Prof. Yana Evstatieva	
11:00 – 12:30	<b>Katarzyna Gaca,</b> Elsevier Customer Consultant for Central Eastern Europe <b>How to write scientific papers?</b>	
12:30 – 17:30	<b>POSTER SESSION</b>	Foyer II-nd floor
13:30 – 15:30	<b>ROUND TABLE</b> <b>CAREER DEVELOPMENT</b>	239
	Chairman – Assoc. Prof. Dilyana Nikolova Secretary – Assist. Prof. Daniel Todorov	
BG Nauka; Biotechnology & Biotechnological Equipment; Bulgarian Journal of Agricultural Science; Elsevier; Pensoft		

15:30 – 17:30	<b>ROUND TABLE TRENDS IN E-LEARNING – TECHNOLOGICAL AND PEDAGOGICAL SOLUTIONS</b>	239
	Chairman – Assoc. Prof. Asya Asenova Secretary – Assist. Prof. Kamelya Yotovska	
<b>17th NOVEMBER 2017 /FRIDAY/ FACULTY OF BIOLOGY</b>		
<b>FEBS Workshop on Molecular Life Sciences Education</b>		
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10:00 – 10:30	<b>Wet Practicals</b> <b>JERKA DUMIC</b> - <i>University of Zagreb, Croatia</i>	239
10:30 – 11:00	<b>COFFEE BREAK</b>	
11:00 – 11:45	<b>Examples of Active Learning methods:</b> "Transforming Your Classroom with Team-Based Learning: Bringing Deep Engagement and the Joy of Learning to Your Students"	239
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POSTERS	
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MOLECULAR BIOLOGY AND BIOTECHNOLOGY - M1–M68	
ECOLOGY AND SUSTAINABLE DEVELOPMENT - E1–E21	
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B2	<u>Aleksandrina Boina</u> , Maria Dinkova <b>DATABASE ARCHIVING OF HERBARIUM COLLECTION – UNIVERSITY BOTANICAL GARDENS BALCHIK</b>
B3	<u>Aleksandra Sokolova</u> , Asen Stoyanov, Nikolay Petkov, Stefka Stoyanova <b>STUDY OF THE MEDICINAL PLANTS FROM THE BLACHIK UNIVERSITY BOTANICAL GARDENS - BALCHICK COLLECTION</b>
B4	Andrey Popatanasov <b><i>OPHRYS INSECTIFERA L.</i> – UPDATE OF THE STATUS OF A CRITICALLY ENDANGERED ORCHID IN BULGARIA</b>
B5	<u>Atanas Grozdanov</u> , Hristo Peshev and Miroslav Slavchev <b>POSITIVE EFFECT OF ARTIFICIAL MICRO-PONDS ON VERTEBRATE FAUNA</b>
B6	<u>Bilyana Stoykova</u> , Albena Lapeva- Gjonova <b>NEW DATA ON BUBLEBEE SPECIES FROM LOZENSKA MOUNTAIN</b>
B7	<u>Boriana Sidjimova</u> , Luba Evstatieva <b>MEDICINAL PLANTS IN RILOMANASTIRSKA GORA RESERVE</b>
B8	<u>Desislava Rozdina</u> , Galerida Raikova <b>SIZE STRUCTURE, L-W RELATIONSHIP AND CONDITION FACTOR OF <i>RUTILUS FRISII</i> (NORDMANN, 1840) IN THE RIVERS VELEKA AND REZOVSKA, BULGARIA.</b>
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## THE NATURAL HISTORY OF AN INVASIVE PLANT *SOLIDAGO CANADENSIS* IN CHINA AND ITS POTENTIAL CONTROL USING NATIVE PARASITIC *CUSCUTA* PLANTS

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**Keywords:** invasive plant, parasitic plant, abiotic factors, feedback, climate, *Solidago canadensis*, *Cuscuta* spp.

Interactions between invasive and native species determine the invasiveness of exotic plants and the invisibility of native resident communities. Native parasitic plants may restrict the growth of invasive plants and thus may be used as a novel biological agent to control their spread. In the first part, I will talk about the natural history of *Solidago canadensis*, a species highly invasive in China. I reconstructed its invasion history in China based on specimen information, literature and field investigation, and demonstrated its multiple origin and possible dispersal routes based on molecular data of cpSSR, chloroplast ITS sequence and GBS-sequencing data. I assessed plasticity of its geographic clines in response to water and temperature treatments and tested its relationships with latitude, longitude, and climatic conditions. In the second part, I will talk about the effects of parasitizing by *Cuscuta* spp. on the performance of on invasive and native plant species. *Cuscuta* showed higher preference for invasive plants than native ones, and had top-down effects on soil microbes, which further could feedback to plant growth. The results suggest that both high phenotypic plasticity and local adaptation to abiotic factors contribute to the spread of *S. canadensis* in China and the native *Cuscuta* plants can be used as a biological control agent for managing the invasion of this exotic species.

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## MOLECULAR EPIDEMIOLOGY OF HIV

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**Keywords:** HIV, molecular epidemiology, subtypes

HIV is responsible for one of the largest viral pandemics in human history. Despite a significant global response for prevention and treatment, the virus continues to spread. For public health planning to prove effective and successful, we need to understand the dynamics of regional epidemics and to intervene appropriately. HIV molecular epidemiology tools as implemented in phylogenetic, phylodynamic and phylogeographic analyses are powerful tools in public health planning across many studies. Numerous applications with HIV suggest that molecular methods can provide inferences about the transmission dynamics, critical epidemiological parameters, or spatiotemporal characteristics of epidemics. Molecular tools have been used to assess the impact of an intervention and outbreak investigation which is of great public health relevance. In some settings, molecular sequence data may be more readily available than HIV surveillance data, and can therefore allow for molecular analyses to be conducted more easily.

## **STARBIOS2 – A HORIZON2020 PROJECT, FOCUSED ON RESPONSIBLE RESEARCH AND INNOVATIONS IN BIOLOGY**

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**Keywords:** RRI, plant science, society, education

STARBIOS2 project is aiming on the idea to open biological science for society with particular focus on young people – students in high schools and universities. There are numerous questions to be raised and answered in respect to Responsible Research and Innovations practices in biological science and education. What structural changes are needed, how to engage the society, are there problems in plant science with gender issue, bioethics, open access, education etc.?

How the recently developed Plant Biotechnology Information Center of ABi and its web page [plantbiotech.bg](http://plantbiotech.bg) will answer to these and other challenges, in close collaboration with Faculty of Biology of Sofia University "St. Kliment Ohridski", will be discussed.

## **STUDENT INITIATIVE CONTRIBUTION TO THE AVIFAUNA RESEARCHES IN EASTERN SOFIA PLAIN FOR 2016**

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**Keywords:** bird monitoring, conservation, student activities

The following work presents the results of year-round avifauna monitoring of selected parts of Eastern Sofia plain, executed by mentor and members of students' club Skorec, Faculty of biology, University of Sofia. The area was selected for its high biodiversity levels, good accessibility and at the same time strong human influence on all habitats. A transect route with length of 55 km. was monitored for two times every month by vehicle and partly by walking (the latter in the area of Ognyanovo reservoir). Information of species, numbers, behavior, weather conditions and conservation threats was gathered. As a results we observed 120 bird species for 2016. Highest diversity was registered in the area of Ognyanovo reservoir (101 species). Monthly dynamics of species and number of individuals were also presented.

This research aimed to contribute to the current biodiversity knowledge for Sofia plain, a valuable and highly urbanized nature area, but also to improve the research skills of the biology students. Involving them in scientific activities is very important educational and motivational tool, which should be included as an official activity option in the university programs. The monitoring of the area should continue in order to identify the full species lists and trends of their presence, the conservation threats and the possible solutions for them. The widening of the research period will also provide the opportunity for inclusion of new young researchers.

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## **DATABASE ARCHIVING OF HERBARIUM COLLECTION – UNIVERSITY BOTANICAL GARDENS BALCHIK**

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**Keywords:** university botanical gardens, Balchik, herbarium collection.

During the volunteering program in the UBG-Balchik, Sofia university 'St. Kliment Ohridski' (July 2017) a database archive of the part of the herbarium collection were conducted. Totally 298 herbarium specimens from 196 species, 159 genera and 58 families were established: Compositae (21), Leguminosae (18), Lamiaceae (15) and Poaceae (15). Collecting data of archived specimens are ranged from 1936 to 1959 and were mostly collected from northeast Bulgaria floristic region (Varna and Balchik district).

As a result the database was developed as a structure and content and has to be enlarged with future studies.

## **STUDY OF THE MEDICINAL PLANTS FROM THE BLACHIK UNIVERSITY BOTANICAL GARDENS - BALCHICK COLLECTION**

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**Keywords:** medicinal plants, taxonomy, chemical composition, application, biodiversity, University Botanical Gardens, Balchick

Over the course of a week during August 2017, the collection of medicinal plants of the Balchik University Botanical Garden has been studied. A total of 137 taxa from 50 families have been described. The most plentiful categories are the Lamiaceae family (30 taxa; 21,9%), followed by Compositae (19 taxa; 13,9) and Rosaceae (8 taxa, 5,8%). In accordance with this we have found that most common are plants containing chemical substances from the following chemical classes: essential oils (61 taxa; 44,5%), flavonoids (43 taxa; 31.4%) and vitamins (40 taxa; 29,2%). This reflects on the taxa's broader applications outside their medicinal use, the largest groups comprised of ornamental (56 taxa; 40,9%), followed by seasoning (31 taxa; 31,6%) and edible and food (27 taxa; 19,7%) plants. The collection's diversity is further outlined by the taxa's origins: (65 taxa; 47,4%) plants being native to Bulgaria and (72 taxa; 52,6%) alien. The goal of this study is to outline the contribution of the Balchik University Botanical Garden towards maintaining biodiversity and providing the necessary means towards creating an interest in the study of plant taxa.



## ***OPHRYS INSECTIFERA* L. – UPDATE OF THE STATUS OF A CRITICALLY ENDANGERED ORCHID IN BULGARIA**

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**Keywords:** *Ophrys insectifera*, bioconservation, orchids.

*Ophrys insectifera* L. due to its peculiar biology and environmental requirements is among the most threatened and endangered vascular plant in Bulgaria. On the territory of Bulgaria the species fulfills the IUCN criteria for critically endangered [CR C2a(i); D] and is included also in the Red Data Book and Biodiversity Act. The present study aimed to explore and evaluate the status of its populations and their threats.

Exploration and monitoring of the populations of the critically endangered orchid *O.insectifera* in Bulgaria was done from 2012 till 2017. The shoot count and some morphometric parameters were recorded for evaluation of their distribution and population dynamics.

At two locations *Ophris insectifera* can be considered extinct. However two new locations were found so the total number of known locations is preserved. Approximately over 50% of the occupied territories fall outside protected sites and many of the habitats are endangered by anthropogenic or other factors.

At all of the known locations there are less than 50 shoots per place. These facts put a high stress on the perspectives of their populations' survival in Bulgaria.

## **POSITIVE EFFECT OF ARTIFICIAL MICRO-PONDS ON VERTEBRATE FAUNA**

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**Keywords:** amphibians, birds, reptiles, water bodies

The artificial water ponds are among the effective tools for conservation of many vertebrate species worldwide. Their role is probably increasing in the context of climate anomalies, which put in risk different stages of the species development, e.g. the metamorphosis of amphibians. This research presents results of building four experimental micro-ponds in different country regions with different climate, elevation, urbanization and microclimate characteristics. After the start of the ponds functioning, they were inhabited by 6 amphibian species and visited by 5 reptile and 16 bird species. For all amphibian species successful breeding was confirmed and for the reptiles and birds ponds were identified as sources of food and/or water. The temperature conditions during the hibernating period of the reptiles and amphibians were measured. In one of the ponds introduction attempt of two species of newts and fire-bellied toads was made, but without success.

Building of artificial micro-ponds could compensate partly the loss of wetlands, caused by human activities or climate changes. The present results show that this practice improve the biodiversity conditions of the sites, supporting the survival of many species. Therefore, the investigations on the topic should continue, with inclusion of more monitoring methods.

## **NEW DATA ON BUBLEBEE SPECIES FROM LOZENSKA MOUNTAIN**

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**Keywords:** *Bombus*, distribution records, Lozenska mountain, Bulgaria

The data available about bumblebee species (genus *Bombus*) (*Hymenoptera*, *Apidae*) from Lozenska mountain are old and incomplete. The present study aims to update the existing records. Thus we present new distribution data of *Bombus* species. The current study is based on materials from the Sofia University Zoological collection. The specimens are collected by the authors and students during summer field practices.

## **MEDICINAL PLANTS IN RILOMANASTIRSKA GORA RESERVE**

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**Keywords:** medicinal plants, "Rilomanastirska gora" Reserve, conservation

The knowledge of the diversity and distribution of medicinal plants in the protected areas has strong relation to the preservation of their genetical resources. This study was conducted to determine the current number of medicinal plant species and their conservation status in the "Rilomanastirska gora" Reserve. The collected data will contribute to the implementation of adequate protection measures of medicinal plants in the Management plan of "Rilomanastirska gora" Reserve. As a result of our study a systematic list of medicinal plants, distributed on the territory of "Rilomanastirska gora" Reserve was prepared for the first time. The systematic list includes 264 species of medicinal plants belonging to 194 genera of 70 families. The current conservation status at national and international level was indicated. The main threats for the medicinal plants are established. Biodiversity conservation measures for the medicinal plants are proposed. This information was a part of the development of the Management plan of "Rilomanastirska gora" Reserve and was financed by OP Environment 2007-2013.

## **SIZE STRUCTURE, L-W RELATIONSHIP AND CONDITION FACTOR OF *RUTILUS FRISII* (NORDMANN, 1840) IN THE RIVERS VELEKA AND REZOVSKA, BULGARIA.**

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**Keywords:** size structure, condition factor, L-W relationship, *Rutilus frisii*, Veleka River, Rezovska River

The size structure and length-weight relationship of *Rutilus frisii* (Nordmann, 1840) in the Rivers Veleka and Rezovska, Bulgaria was studied. The smallest individual caught in Veleka River was with length 6.6 cm and weight 5.2 g. The largest individual was with length 15.5 cm and weight 57.4 g. The smallest individual caught in Rezovska River was with length 10.3 cm and weight 18.8 g and the largest one was 13.2 cm in length and 31.5 g in weight. The caught fish were divided in size classes over 1 cm each. Altogether 9 size classes were established in Veleka River and 4 in Rezovska River. The smallest size class in Veleka River was 6.1-7 cm and the biggest 15.1-16 cm. The smallest size class in Rezovska River was 10.1-11 cm and the biggest 13.1-14 cm. In the both rivers the most abundant was the size class 10.1-11 with 24 individuals in Veleka river and 12 individuals in Rezovska River. The L-W relationship for the catchment from Veleka River was  $W = 0,017L^{2,9664}$ ,  $R^2 = 0,9805$ . The L-W relationship for the catchment from Rezovska River was  $W = 0,1624L^{2,0152}$ ,  $R^2 = 0,8432$ . The condition factor for the fish from Veleka River was calculated as 1.7 and for Rezovska River 1.5.

## **NEW FAUNISTIC DATA ON ORDER LEPIDOPTERA (INSECTA) FROM THE REGION OF SLAVEYNO VILLAGE, BULGARIA**

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**Keywords:** butterflies, moths, light trap, conservation value

The first detailed butterfly and moths studies in the area of Slaveyno village (Western Rhodopi, Bulgaria) were made in the period 17-25 July 2016, with a total of sixty-three species of butterflies and moths from twelve families collected using entomological aerial nets. The current study, done in July 19-26, 2017, aims to amplify the faunal list by applying additional methods - two types of light traps. New species of butterflies and moths were established for the region and two species - *Limenitis populi* (L.) and *Apatura iris* (L.) are with high conservation value. n factor for the fish from Veleka River was calculated as 1.7 and for Rezovska River 1.5.

## **ESTIMATING BROWN BEAR POPULATION DENSITY WITH CAMERA TRAPS IN CENTRAL BALKAN MOUNTAIN, BULGARIA**

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**Keywords:** *Ursus arctos*, camera traps, high density, supplemental feeding

Estimating population size and density for mammals which are not individually recognizable is particularly challenging. However, these indicators are vital for species management. In this study we make the first attempt to estimate the population density of the endangered in Bulgaria brown bear (*Ursus arctos* L.) using camera traps. 38 camera traps were set up at random locations (spaced at approx. 1 km from each other) between July and August 2017 in the State hunting enterprise "Rositsa", located on the Northern slopes of the Central Balkan Mountains, Bulgaria. The recorded 50 independent registrations of brown bears were analyzed through Capture-Mark-Recapture Models (CMR) without the need for individual recognition – a method proposed by Rowcliffe. The results indicate local population density of 1.78 ind./km<sup>2</sup>, which is unusually high especially in comparison with the observed densities in the neighboring National Park Central Balkan (0.1-0.2 ind./ km<sup>2</sup>). This is most likely due to the supplemental feeding in the hunting area which attracts many individuals and increases the density locally. The proposed method is cost-effective and can be used to replace or enhance current monitoring schemes.

## **REMARKS ON THE PLANTHOPPER FAMILY DELPHACIDAE (HEMIPTERA, FULGOROMORPHA) FROM ALDOMIROVCI AND DRAGOMAN MARSHES**

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**Keywords:** Delphacidae, faunistics, host-plants, Natura 2000, Bulgaria

The Dragoman and Aldomirovci marshes are part of the Dragoman protected area (Natura 2000, Habitats Directive No BG0000322) and they are the largest wet zone in the inland part of Bulgaria. Up to date there are no faunistic studies on the Fulgoromorpha fauna of the region. The present study is carried out between March and October 2017. Some early collected and unpublished materials were included. Several species were documented by macro photographs "*in vivo*". Chorology, phenology and host-plant relation of the species were commented.



## **THE SOKOLSKI MONASTERY PROTECTED SITE, CENTRAL BALKAN RANGE – HABITAT CHARACTERIZATION**

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**Keywords:** Sokolski Monastery, protected site, habitats, EUNIS, NATURE 2000

The Sokolski Monastery is a Bulgarian Orthodox monastery located 15 km southwest of Gabrovo on the northern slopes of the Central Balkan Range in the Balgarka Nature Park. It was founded in 1833 and played an important role in the development of the Bulgarian society. The Sokolski Monastery was declared a historical site in 1973 and re-categorized in a protected site, 6<sup>th</sup> category of protected area according to the Protected Areas Act in 2002.

Sokolski Monastery protected site occupies 75.5 ha area. Forest and grassland communities form its vegetation cover. **The aim of the present study** is the determination of the habitats distributed at the territory of the protected site on the basis of the floristic composition and ecological features of the relevant plant communities. Standard methods of the Braun-Blanquet's phytosociological school were used for the phytocoenological characteristic. Habitats are determined according to EUNIS classification system and their correspondence and significance in NATURE 2000 is pointed. The results of the terrain work and processing of the data show the distribution of four main types of habitats at the territory of Sokolski Monastery – these are G1.A322 Dacio-Moesian hornbeam forests (9170), G1.661 Middle European dry-slope limestone beech forests (9150), E1.234 Moesio-Carpathian meadow steppes, (6110) and E2.7 Unmanaged mesic grasslands (no relevance with NATURE 2000). The forests occupy larger area in the protected site and are habitat for some rare and endangered plant species.

**Acknowledgements:** *The present study is implemented within a Master's Degree Program in Environmental Protection in the Department of Ecology and Environmental Protection.*

## **HEMOLYTIC EFFECTS OF THE VENOM OF VARIOUS VIPERIDAE SPECIES**

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**Keywords:** true vipers, snake venom, hemolysis

Although there are a lot of studies on the toxicology of *Viperidae* family, little data exist on the *Vipera ammodytes transcaucasiana* ssp. (enlisted as nearly threatened by IUCN) specific toxicological profile. This subspecies shows little morphological differences with the European true vipers (*V. ammodytes* ssp.), but there are some reports on the different pathophysiological effects of its venom, therefore we decided to evaluate some features of its venom potential, in comparison with two other well researched viper species, one of them with well-expressed hemolytic activity. Hemolysis was investigated by a photo spectroscopy assay.

## **INVESTIGATIONS ON TERRESTRIAL SNAILS FROM THE UNIVERSITY BOTANICAL GARDEN OF BALCHIK DURING STUDENT VOLUNTEER ACTIVITIES**

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**Keywords:** terrestrial snails, conservation, student activities

The present research continues the investigations on the terrestrial gastropods diversity in the Sofia University Botanical Garden in Balchik and was made during the annual volunteer activities in the area. The site is a suitable place for research and education due to its abundance of animal and plant species and the availability for students. As a result of the current work 19 species of terrestrial snails were found, 14 of which were present in the previous researches, with the most common species being *Pomatias elegans*. In comparison with the study from 2016, new research locations were added and 5 new species were identified for the area. We investigated a variety of habitats in the garden's territory, characterized by different vegetation and gardening regimes. The material was gathered by hand and from collected soil probes. During the gathering the following parameters were marked: date, sea level, vegetation, temperature and soil type. Material was also collected from cracks, tree stems, under rocks and other locations, differently exposed to sunlight and respectively with different humidity levels. No snails were found in parts of the garden that were exposed to a variety of gardening practices, including pesticide treatment.

The University botanical garden in Balchik holds a significant biodiversity, due to its geographical position and diverse vegetation and also play an important educational functions for the biology students. This theory is confirmed also by the present research, which should be used to encourage such scientific and educational activities in the future.

**Acknowledgements:** *The authors wish to thank to the University Botanical gardens and the Balkan Ecological Centre for the organized volunteer activities and the provided opportunities for scientific work.*

## **FIRST RECORD OF *TRIOZA RHAMNI* (SCHRANK, 1801) (HEMIPTERA, PSYLLOIDEA) FROM BULGARIA**

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**Keywords:** new record, *Rhamnus saxatilis*, *Trioza rhamni*, Psylloidea, host-plant, Bulgaria

The current study presents the first record of a jumping plant-lice *Trioza rhamni* (Schrank, 1801) from Bulgaria. Adult specimens, both males and females, were collected in April 2017 in Besaparski Hills. The region is located in the foothills of the Rhodope Mountain and includes limestone treeless ridges with typical steppe vegetation. The species spreads across Europe, Anatolia and Caucasus. Until now the two host plants - the buckthorns *Rhamnus cathartica* L. and *R. pallasii* Fisch. & C.A. Meyer are associated with *T. rhamni*. A new host-plant *R. saxatilis* Jasq. is recorded for the jumping plant-lice in Bulgaria. This research is a part of a project of a Student Entomological Club of Sofia University in order to research the entomological fauna of the Besaparski Hills.

## **EFFECT OF THE LURE ON THE WILD CAT (*FELIS SILVESTRIS* SCHR.) AND OTHER MAMMALS IN “VITOSHKO-STUDENA” HUNTING AREA, VITOSHA MT.**

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**Keywords:** lure, wild cat, mammals, activity, behaviour

Lures are often used in camera trap studies with the aim to maximize the number of registrations of the focal species. This is especially important when rare species are concerned. We compared the data from 20 camera traps set up in 2016 (without baiting) and 2017 (with non-rewarding olfactory bait, aiming to attract wild cats), located at the same spots on the territory of “Vitoshko-Studena” Hunting Area. In order to analyze the effect of the lure, we studied the detection rate (DR), the shifts in the activity patterns of some of the species and the general activity (i.e. the number of observed individuals and species) in the two study periods. The results indicate that most of the registered species react to the bait – they sniff it, rub or mark on it. The herbivores (roe deer and red deer) are more active in the morning when the humidity is higher which enhances the effect of the lure. The number of species and individuals that are registered does not decline with time after bait renewal. The lure does not affect the focal species (the wild cat), but influences the behavior of most of the other mammals and can be valuable in attracting them for research purposes. Our results suggest that the herbivores are more frequently registered without bait, unlike the omnivores (except for the wild boar) and the carnivores (except for the wild cat).

## **EFFECTIVENESS AND SUITABILITY OF DEFRAGMENTATION AND FENCING FACILITIES ON STRUMA MOTORWAY, LOT 1**

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**Keywords:** fragmentation, road facilities, wildlife crossings, landscape and ecological engineering

Road constructions have a serious impact on environmental components, mainly through fragmentation and direct loss of habitats, collision mortality, barrier effect and general pollutions. Wildlife crossings and fences, which have entered recently in motorway constructions, are only part of the measures to mitigate their negative effects. One of the newest motorway project in Bulgaria – "Struma" has also an improved system for wildlife permeability and fences, reducing wildlife and human mortality. The main emphasis was placed on the first stretch - Lot 1 (from km 305 + 220 to km 322 + 000) as it has various structures for wildlife protection and it has a long enough adaptation period, which is an important condition for reporting the facilities effectiveness.

In addition, the study assessed the suitability and technical implementation of specialized and non-specialized facilities for negative impact mitigation of road constructions. Also we analysis the connectivity of the model species to their abundant content in the studied area. The main methods used in the survey include photographic capture by photo traps, trace and excrement report, local population opinion survey, an analysis of the technical and natural features of the facilities, as well as a transect method for reporting the abundance of the model species in the area.

The monitored facilities are large mammal ecoduct at km 314 + 070, small and large mammal's subway at km 315 + 900, non-specialized viaduct at km 314 + 400, bridges with dry paths, reptile and amphibian tube drainages. We also investigate the suitability of "rabbit fences" and guiding concrete fences for amphibian.

## **NEW RECORDS OF MICROSPORIDIA (MICROSPORIDIA) FROM BLACK FLIES (DIPTERA: SIMULIIDAE) FROM BULGARIA**

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**Keywords:** Simuliidae, microsporidia infection, *Janacekia*, *Polydispyrenia*, *Amblyospora*

During the period August-October 2017, a total of 856 black flies larvae (Diptera: Simuliidae) were collected in river habitats near Jeleznitsa Village, SW Bulgaria. Microsporidia infection was found in a total of 120 host larvae from several species of genus *Simulium* Latreille, 1802. Based on the morphological characteristics of the spores microsporidia of the genera *Janacekia* Larsson, 1983, *Amblyospora* Hazard & Oldacre, 1975 and *Polydispyrenia* Canning & Hazard, 1982 were identified. The infection rate in the most intensively studied host population ranged from 10.0% to 24.0% between the individual samples.

## **ETHNOBOTANY OF MEDICINAL PLANTS USED IN SOME PARTS OF THE NORTHERN BLACK SEA COAST REGION - VARNA REGION (BULGARIA)**

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**Keywords:** Ethnobotany, medicinal plant, North Black Sea Coast.

This study is part of an ethnobotanical investigation of the medicinal plants in the Northern Black Sea coast region, which includes the area from the village of Durankulak to the town of Obzor. The boundaries were determined using the map of the floristic regions in Bulgaria. Field work was conducted in the period from June and July 2017. Surveyed were 74 people from 2 communities. The study was carried out on the basis of the survey methodology. Interviews with the local population were conducted using original questionnaires prepared upfront. The respondents belong to different gender, ethnicity, age and education groups. The data from the different applications of medicinal plants in the life of the local population were processed and summarized.



## **MEDICINAL PLANTS ON THE TERRITORY OF LOZEN MOUNTAIN**

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**Keywords:** medicinal plants, substances, conservation status, map, species

The current study is a floristic investigation of the medicinal plants on the territory of the Lozen Mountain (80 km<sup>2</sup>) conducted in the period 2003-2017. The results have identified 326 species and 11 subspecies belonging to 235 genera and 75 families or 44.1% of the taxa included in the Bulgarian Medicinal Plants Act. The families with the richest number of medicinal species are Lamiaceae (39), Asteraceae (34), Fabaceae (22) and Rosaceae (19). The genera with the highest number of species are *Veronica* (6), *Geranium* (5), *Mentha* and *Orchis* (5 species each). Most of the medicinal plants are located in the southwestern part of the mountain (73.6%) and the least of them are in the northeastern part (49.2%). Five species fall under restrictive collection regime and for 12 the collection is forbidden. Seventeen of the medicinal plants are species with conservation status, 4 of which are included in the Red Data Book of the Republic of Bulgaria, 4 are in the lists of IUCN and Bulgarian Red List of Vascular Plants, and 15 are in the Annex 4 of the Bulgarian Biodiversity Act. A map with the distribution of the species of conservation significance on the territory of the mountain was prepared.

## **NEW RECORDS OF ANTS (HYMENOPTERA, FORMICIDAE) FROM STRANDZHA MOUNTAIN (BULGARIA)**

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**Keywords:** Formicidae, new records, Strandzha, Bulgaria

The ant fauna of the Bulgarian part of Strandzha Mountain and its Black Sea coast was investigated by pitfall trapping from 2009 to 2011. New records join previous ant research of the region when mainly direct sampling was applied. Current study reports first records of two ant species *Temnothorax solerii* (Menozzi, 1936) and *Lasius lasioides* (Emery, 1869) for Bulgaria. The former species is currently known from Greece and Italy as the latter is present in southern Europe to Middle East, Central Asia and North Africa. We present short descriptions of the studied plots and a list of ant species as well.

## **MACROPHYTE INVENTORY OF THE NEGOVAN LAKE, BULGARIA**

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**Keywords:** sandpit lakes, macrophytes, restoration, reintroduction, conservation

The beginning of the XX century marked increased pressure on most of the lowland wetlands in Bulgaria. Alongside the Lesnovska River once stood one of the most biologically diverse wetland in western Bulgaria – the Negovan Swamp. The regulation of the Lesnovska River in the 1930s resulted in the cut-off of the wetland from the river. Additionally, dikes were dug out to drain water from the swamp. Subsequently, the area was developed as a gravel and sand pit lake that provided materials for the constructions in the region, mainly for the capital city of Sofia. After the exploitation of bottom material was ended, the resultant lake started gradually to regain its wetland appearance and conservation value for birds, especially waterfowl along the migratory route Via Aristotelis. Since 2013 several restoration projects have been undertaken to restore and improve the natural appearance of the lake as a wetland priority habitat for biodiversity conservation. As a result, a macrophyte inventory of the Negovan Lake was compiled. We found 42 species of hydro- and hygrophytes in different parts of the lake, as a result of both natural and intended (re)introduction. So far, eight species of conservation value were selected for reintroduction (*Acorus calamus*, *Aldrovanda vesiculosa*, *Hippuris vulgaris*, *Marsilea quadrifolia*, *Menyanthes trifoliata*, *Nymphaea alba*, *Nuphar lutea*, *Nymphoides peltata*), as well as six more broadly distributed aquatic plants (*Butomus umbellatus*, *Callitriche cophocarpa*, *Callitriche platycarpa*, *Lemna trisulca*, *Potamogeton lucens*, *Ranunculus trichophyllus*). The effects of the invasive species on the post-restoration development of the wetland was evaluated, with special emphasize on the influence of *Elodea nuttallii*. A complete inventory of the macrophyte in 20 sampling plots/transects was used to produce a map of the macrophyte in the lake.

## **STRUCTURE ANALYSIS OF THE PHOTOSYNTHETIC APPARATUS OF TWO PLATANUS ORIENTALIS L. ECOTYPES**

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**Keywords:** leaf tissues, *P. orientalis*, drought stress

The leaf structure of *Platanus orientalis* L. plants from two ecotypes – Bulgarian and Italian, grown from seeds in controlled conditions in greenhouse were studied. Leaf transverse sections of experimental plants from three variants: control, under drought-stress, and after recovery were analyzed by light microscopy method. Also, the thickness of the leaf lamina, mesophyll, palisade and spongy parenchyma were measured. The comparative study showed similar leaf histological organization and cell morphology in the control plants of the Bulgarian and Italian ecotypes. Under drought-stress conditions a reduction of the leaf lamina thickness was registered only in Bulgarian ecotype's plants. After recovery the plants of both ecotypes showed same leaf structure as the control ones. The experimental results suggest that the leaf anatomy markers define *P. orientalis* as a drought resistant species.

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## **CETACEAN MORTALITY ALONG THE BULGARIAN BLACK SEA COAST DURING 2017**

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**Keywords:** Cetaceans, marine mammals, The Black Sea, *Tursiops truncatus*, *Phocoena phocoena*, *Delphinus delphis*

The present study provides information on the stranding of cetaceans along the Bulgarian Black Sea coast during 2017. The study covers 4 annual seasons - spring, summer, autumn and winter. A total of 3 cetacean species - harbor porpoise (*Phocoena phocoena*), bottlenose dolphin (*Tursiops truncatus*), and common dolphin (*Delphinus delphis*) were found. Observations were made through covering transects along the Bulgarian Black Sea coast. In total, 117 stranded cetaceans have been found. Of the cetaceans, the most numerous is the harbor porpoise - 48% followed by the Bottlenose Dolphin - 15% and common dolphin - 8%, which is registered as rare. During the winter, no cetacean carcasses were found. The first dead cetaceans are thrown to shore during the last decade of March. The most significant number of strandings was observed in August (61 cases).

## **NEGATIVE FACTORS AFFECTING THE BAT POPULATIONS IN STRANDZHA NATURE PARK**

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**Keywords:** Bas, negative factors, Strandzha Nature Park

Strandzha Nature Park is the largest protected area in Bulgaria. It covers a territory of 1,161 square kilometres in the Strandzha Mountain situated at the extreme south-eastern corner of the country near the Turkey border. The region is one of the richest of bat diversity in Bulgaria. Studies on the negative effects on bats on the territory of Strandzha Nature Park were carried out in the period 2013-2017. Studies were conducted in the main underground shelters of the bats in which the majority of the populations of the Park territory are concentrated. These are the caves Elenina hole, the Bratanova cave, Big Vupa Cave, Kaleto Cave, the Lake, the Cave along the Greec's path and others. Outside the park is explored the Tangaruchkata Hole, which offers an important underground shelter for the bats. Shelters of anthropogenic type - abandoned buildings and bat houses were also studied. An analysis of the impact of felling as a negative factor on the bats was carried out by assessing the number of destroyed potential bat shelters in the cut logs. As the most significant negative factors on bats have been assigned to treasure hunting and digging of caves, the destruction and theft of cave formations, the burning of fire in the cave, the disturbance of people entering the caves, felling, traffic, and finally the impact of natural enemies.

**CONTRIBUTION TO THE SPECIES COMPOSITION,  
DISTRIBUTION, BEHAVIOR AND VOCALIZATION OF BATS IN  
THE AREA OF PANAGIURISTE MUNICIPALITY AND SREDNA  
GORA PROTECTED AREA - BG0001389**

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**Keywords:** , Bats distribution, Panagiuriste, SCI BG0001389, *Myotis aurascens*,

The present study have been carried out during July 2014 - September 2015 period. The research was conducted at the territory of Panagiuriste municipality and partially at the surrounding SCI BG0001389. The habitats here are farmlands, industrial mine area, settlements and most important for bats old beech and oak forest, that are large homogeneous area including mountain rivers and streams. The bat species object for preservation in the protected area according the standard data form are *M. myotis*, *Rh. ferrumequinum*, *M. schreibersii*, *M. capaccinii*, *M. bechsteini*, *Rh. hipposideros*, *M. blythii*, *Rh. blasii*, *M. emarginatus*, *B. Barbastellus* and *Rh. Euryale*. In our study the bat detectors survey and mist netting bats methods (with appropriate authorization) have been applied. The following species have been found: *Barbastella barbastellus*, *Eptesicus serotinus*, *Nyctalus leisleri*, *N. noctula*, *Myotis daubentonii*, *Myotis aurascens*, *Pipistrellus pipistrellus*, *P. nathusii*, *P. pygmaeus*, *Rhinolophus ferrum-equinum*, *Rh. hipposideros*, *Vespertilio murinus*.. The most interesting finding is the location in the region of one young female individual of Steppe whiskered bat. Some behavioral peculiarities of bats have been described using ad libitum sampling method. The sonograms of bat sounds have been presented and analyzed.

## **PRELIMINARY STUDY OF BIRDS AND BATS IN THE AGROECOSYSTEMS OF KRAYMORSKA DOBRUDZHA**

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**Keywords:** Birds and bats a distribution, agro-ecosystems, Dobrudja

The present study has been carried out during April 2016 and May 2017 in the North-Eastern part of Dobrudzha, on the territory of General Toshevo municipality. The habitats here are farmlands and agro-ecosystems of different types, separated by safety belts. The breeding birds and bat species have been studied using the line transect method. Especially for the bats, Petersson 240X bat detectors were used. The breeding avifauna in agricultures and grassland habitats is predominated by *Alauda arvensis*, *Miliaria calandra* and *Melanocorypha calandra*. It is also a foraging habitat for *Merops apiaster*. In safety belts, the dominant species is *Luscinia megarhynchos*. The European turtle dove (*Streptopelia turtur*) and the Black-headed bunting (*Emberiza melanocephala*) are also common. In the region, there is a strong population of *Phasianus colchicus*. From the raptors, the most common is *Buteo buteo*. Some important for conservation species have been recorded as possible breeders – *Milvus milvus*, *Pernis apivorus*, *Circus aeruginosus*. The agro-ecosystems are one of the most unfavorable environment for bats, so the bat fauna was very scanty, presented of only two species – *Nyctalus noctula* recorded in safety belts and *Pipistrellus pipistrellus* in the settlements and their surroundings.



## **INNOVATION PROGRAM OF EDUCATION OF PLANT BIODIVERSITY**

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**Keywords:** Plant biodiversity, international experience, young researchers

A group of students from Sofia University "St. Kliment Ohridski" took part in the pilot program "Higher Education Innovation in Plant Diversity: flexible learning paths for emerging labor market - HEI PLADI" offering a network of training courses to young cadres to continue their future professional development in the field of plant biodiversity.

The project combines the theoretical and practical training developed by an international team from the Universities of Molise, Cagliari, Lisboa, Malta, and Sofia, the Mediterranean Agronomic Institute in Chania and the Polish Academy of Sciences - Centre for Biological Diversity Conservation.

The participation of the young researchers in these courses gave them the opportunity to acquaint themselves with the ecology and conservation of specific European flora elements, through the application of classical and modern methods.

The selected courses have enabled the students to develop their potential for communication in a multicultural environment and to improve their adaptability, creativity and presentational skills.

## **PSAMMOPHILOUS AND GEOBIONT SCARABAEIDAE SPECIES IN BULGARIA**

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**Keywords:** Orphninae, *Chaetonyx*, Aphodiinae, *Leiopsammodius*, distribution, cryptic species, SEM, DNA barcoding

Psammophilous and geobiont species of family Scarabaeidae (Coleoptera: Scarabaeoidea) are insufficiently studied in Bulgaria as are the species in subterranean habitats in general. For investigating the local distribution of psammophilous Aphodiinae and geobiont Orphninae standard samples were collected in riverside and mountain sites. The samples from the genera *Leiopsammodius* Rakovich, 1981 and *Chaetonyx* Shaum, 1862 were investigated for the presence of cryptic species. The specimens were identified and selected structures were examined with scanning electron microscopy. With the research grant 80-10-213/2017 of Sofia University CoI gene of selected specimens is sequenced for the purposes of the DNA barcoding and for molecular phylogenetic analysis.

## **BIG PICNIC: BIG QUESTIONS – ENGAGING THE PUBLIC WITH RESPONSIBLE RESEARCH AND INNOVATION ON FOOD SECURITY**

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**Keywords:** big picnic, food security, urban gardening, biodiversity, botanic garden

The BigPicnic project, funded under the EU's Horizon 2020 program, includes international partnership of nineteen partner organizations: botanic gardens, universities, a science shop, an institute for art, science and technology and an international NGO. Co-ordinated by London based Botanic Gardens Conservation International (BGCI) the Big Picnic will work with the public to open up the debate on the future of our food. The project will encourage collaboration and conversation to build public understanding of food security issues and enable adults and young people to articulate their views to decision makers.

The partners are developing a set of traveling exhibitions, practical activities, science cafes and events to generate dialogue and collaboration with the local community, sharing ideas and encouraging activity. "Big picnics" were held in 12 countries in Europe and one in Africa. This year, three traveling exhibitions on "urban gardening" topic took place in Bulgaria. There will be science cafes to discuss different aspects of the topics food availability, food access and food utilization.

**Acknowledgements:** *Horizon 2020, BGCI*

## PLANT GROWING BIOLOGICAL EFFECTS OF STRAIN *AZOTOBACTER VINELANDII*

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**Keywords:** *Azotobacter vinelandii*, plant growth metabolites, seed germination

Plant growth promoting rhizobacteria (PGPR) are a group of free-living bacteria that colonize the rhizosphere and contribute to increased growth and yield of crop plants. PGPR possess many traits that make them well suited as biofertilizer and biocontrol agent, such as: rapid growth and utilization of seed and root exudates, colonization as well as multiplication in the rhizosphere.

Among the PGPR group, *Azotobacter* is a free-living N<sub>2</sub>- fixer diazotroph that has several beneficial effects on the crop growth and yield. It helps in the synthesis of growth regulating metabolites like auxins, cytokinin and giberellic acid (GA) and also stimulates rhizospheric microbes, protects the plants from phytopathogens and improves boost up nutrient assimilation.

Strain morphology and cultivation characteristics were investigated with modification of Ashby's media, *Azotobacter* media and Yensen media. Colony forming units per ml were used as main control parameter in the process of cultivation and were measured on Yensen media with bromphenol blue addition. The biochemical profile of strain *Azotobacter vinelandii* was determined by API 20NE and API ZYM systems (BioMericux, France). The results from the cultivation experiments demonstrated that modified Yensen media was optimal for culture growth.

The effect on seed germination of strain *Azotobacter vinelandii* was studied *in situ* on *Lactuca sativa* seeds.

## **MODULATING OF NANOCOMPOSITES THIN FILMS FOR MEDICAL APPLICATION**

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**Keywords:** thin films, nanocomposites, myconoside, epithelial cells, antibacterial activity

In our previous study we showed that plant extracts increase surface properties of some toxic nanocomposites. The aim of this study is to modulate the toxicity of TiO<sub>2</sub>:Ag:Cu thin film with glycoside myconoside. The thin films of TiO<sub>2</sub>:Ag:Cu are deposited on glass substrates without heating during the deposition by r.f. magnetron co-sputtering of TiO<sub>2</sub> target and pieces of Ag and Cu. The studied films, thickness about 60 nm, were deposited and annealed.

The specific glycoside *myconoside* is isolated from the Haberleardhodopensis extract and under normal conditions tend to show high antioxidant capacity at stress and normal conditions and cytotoxic effect (concentration up to 15 µg/ml).

Crystal violet staining and phase-contrast microscopy observation for epithelial cell lines (A549 and MDSK) were used to determine changes in morphology and cytotoxicity. Our investigations show that toxic thin films of TiO<sub>2</sub>:Ag:Cu in presence of 10 µg/ml myconoside increase cell viability. The thin films have good antibacterial activity in regard to bacteria - E.coli and Staphylococcus epidermidis. The addition of myconoside to nanocomposite material does not alter the antibacterial activity.

In conclusion, the glycoside myconoside improves the surface properties of the nanocomposite material and increases its biocompatibility used for application in medicine such as wound dressings, catheters, bone implants in medical application.

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## **ANTIOXIDATIVE POTENTIAL IN IN VITRO AND IN VIVO PLANTS PLANTAGO ATRATA**

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**Keywords:** *Plantago atrata, in vivo and in vitro, antioxidative activity, phenolics*

Plants produce a large variety of secondary compounds that contain a phenol residue. Many phenolics serve in the defense against herbivores and pathogens. Another function is attracting pollinators or absorbing harmful ultraviolet radiation. *Plantago atrata*, also known as mountain plantain, belongs to the *Plantaginaceae* family and it grows as a high-altitude mountain perennial plant. *In vitro*-propagated plants were grown for 5 weeks on plant growth medium. *In vivo* leaves were collected at mountain Vitosha (2200 m) during the period of flowering. Extracts of *in vitro* and *in vivo* cultured *P. atrata* leaves were evaluated regarding the contents of total phenolics, flavonoids and total antioxidant activity. The study showed that phenolic concentrations in *in vivo* plants are 2 times higher than in *in vitro* ones. Further total antioxidant activity test indicated correlation with the total content of phenolic compounds. Flavonoids concentration did not change due to the growing conditions. An important conclusion is that the amount of specific secondary metabolites is related to environmental changes. Future experiments should involve a profound metabolic analysis to find the exact phenols that play role in the adaptation of high-altitude mountain species.

## SMICRONYX GALLS ON CUSCUTA CAMPESTRIS SHOW DIFFERENTIAL ISOENZYMES PROFILE

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**Keywords:** antioxidant enzymes, biotic stress, hydrolytic enzymes, insect galls, parasitic plants

*Cuscuta campestris* Yunck. is a stem parasitic flowering plants, causing significant growth inhibition of various hosts. It is considered a major agricultural pest and invasive species on a global scale. Weevils of genus *Smicronyx* are widely distributed parasites on *Cuscuta*, causing the formation of characteristic galls. Thus, they are considered as potential biological control approach. However, the metabolic changes, occurring in *Smicronyx* galls are largely unknown. The aim of the present study was to establish the isoenzymatic profiles of several antioxidant and hydrolytic enzymes in the inner and outer layer of the galls as well as in non-infected stems. Galls were collected from a wild population of *Cuscuta campestris* in the Sofia area. Enzymes were studied by zymographic analyses after separation on semi-denaturing polyacrylamide gel electrophoreses. Interestingly, antioxidant enzymes - glutathione reductase, ascorbate peroxidase and catalase tend to decrease in activity in the inner layer of the gall. Superoxide dismutase showed no apparent change and guaiacol peroxidases increased in activity and number of isoforms in the gall. The activity of the hydrolytic enzymes – chitinases,  $\alpha$ -amylases and proteases were significantly increased in activity in the gall. In summary, *Smicronyx* galls showed distinct enzymatic profiles, different from uninfected stems, with a notable decrease of several antioxidant enzymes activities.

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## **DETECTION OF EARLY STAGE COLORECTAL CANCER IN *IN VIVO* EXPERIMENTAL MODELS BY DIFFERENTIAL SCANNING CALORIMETRY**

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**Keywords:** colorectal cancer, *in vivo* experimental models, differential scanning calorimetry, albumin

Colorectal cancer is one of the leading causes of cancer related mortality in humans, therefore the development of markers for early disease diagnostics are of high interest. In this work we employ differential scanning calorimetry (DSC) to examine the thermal behavior of blood plasma proteome of *in vivo* experimental models and to identify the changes in the calorimetric profiles (thermograms) associated with the development of precancerous lesions on the colon.

To induce colorectal cancer 1,2-dimethylhydrazine treatment was applied to 10 male/female animals albino Wistar rats, while another 10 males/females were left untreated (controls). The histomorphological analysis revealed that one month after the applied treatment mild dysplasia of the glandular epithelium or/and epithelial hyperplasia on the colon appeared in all treated animals. No major changes in the levels of the major plasma proteins were found however the thermograms of the treated and untreated animals showed significant differences. The biggest effect was related to a decrease of the ratio of the excess heat capacities of albumin and immunoglobulins due to shift of the albumin assigned transition towards higher temperatures.

Our data demonstrate that DSC is capable to detect early signs of development of colorectal cancer in experimental models that can be used as calorimetric markers of pre-neoplastic lesions.

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## CHANGE IN CELL SURFACE PROPERTIES OF LACTIC ACID BACTERIA UNDER MEDROL TREATMENT

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**Keywords:** lactic acid bacteria, zeta potential, DLS, immunomodulator (methylprednisolon)

In the present work the LAB would be used as a model system for study the surface properties of the membranes and to examination the mechanisms by which the Medrol<sup>®</sup> (methylprednisolon) affect the membrane. In order to understand the bacterial interactions, electrokinetic and optic approaches would be applied with a special focus on the environmental conditions (pH, ionic strength) and concentration affecting surface electrical properties of bacteria. Several techniques have been implemented to evaluate bacterial surface and methylprednisolon interaction, such as particle microelectrophoresis, dynamic light scattering (DLS) and oxidation reduction potential.

The preliminary investigations on the influence of immunomodulators were made. It was observed the effect of Medrol on yeasts of *Saccharomyces boulardii*, medicine from Biocodex, France (Enterol). Medrol activates the anti-inflammatory program of the immune system and organism is protected by the inflammatory processes, which could concern the mucous membrane and tissues of the respiratory organs in depth, to provide necrosis or liquid keeping.

The small effect of increase in negative zeta potential by about of 9% at 0.04 ng/ml and 0.4 ng/ml and approximately 7% at other concentrations of 0.2, 1 and 2 ng/ml was established at isotonic ionic strength (PBS, pH 6.0).

It is important to be noticed the large difference in the electrokinetic behavior of *S. boulardii* yeasts in water solution. It was observed an increase of zeta potential ( $\zeta$ ) by 5 mV at acting concentration of 0.2 and 0.4 ng Medrol/ml, where  $\zeta$  reaches - 43 mV in comparison to control value of - 39 mV. These data could be due to a sharp increase in volume of *S. boulardii* yeasts of 23% and 58%, respectively, under the upper doses of Medrol. Dose of 1 ng Medrol/ml, however, significantly decreased the zeta potential with approximately 5 mV, like reaching to - 34.39 mV. It was connected by the strong decrease in particles volume of 33% and decrease in particle size with 16%.

Data for electrophoretic mobility (EPM) of LAB (SM11) of - 1.66 x 10<sup>-8</sup> m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup> by the method of microelectrophoresis is according to the value of bacterial surface (Grare et al.,2007). Surface electric charge is calculated to be - 6.78 mC/m<sup>2</sup> and is characterized for low ionic strength. Lower concentrations of immunomodulator lead to change in negative electrokinetic potential and surface charge of LAB.

Knowledge on the surface characteristics of LAB could contribute to designing new medicine and more efficient dairy food, and to a better inderstand relationship between LAB and medium components

## IRON BACTERIA FROM VITOSHA MOUNTAIN ISOLATION OF NOVEL STRAINS OF NEUTROPHILIC

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**Keywords:** neutrophilic iron-oxidizing bacteria, cultivation scheme, *Lepthothrix*, sheath-forming bacteria

Neutrophilic iron-oxidizing bacteria include various species with different morphological and physiological characteristics. All of them possess the ability to oxidize  $\text{Fe}^{2+}$  at neutral pH and to form insoluble ferric oxides/(oxy)hydroxides which are object of great interest for application in different applications. The aim of the recent study is focused on the optimization of the cultivation scheme for isolation and differentiation of bacteria, related to *Lepthothrix* species and subsequent facilitation of the formation of ferroxides-containing sheaths. The optimization included investigations of the types of cultivation, used of the different nutrient media and the duration of the cultivation. Bacterial biomass collected from Vitosha mountain natural springs, presumably containing sheath-forming bacteria of the genus *Lepthothrix* was used for the isolation procedure. After the application of the optimized cultivation protocol, pure cultures of seven novel strains were obtained and subjected to taxonomical identification. Both classical and molecular methods were applied. 4 of the isolates were related to *Lepthothrix ochracea* and 3 to *Lepthothrix discophora*.

## **A MODEL OF PRIMARY CULTURE FROM COLORECTAL CANCER**

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**Keywords:** colorectal cancer, primary cell culture, bacterial contamination. Antibiotics

Colorectal cancer (CRC) is a significant medical and scientific problem. In order to understand better the biology of colorectal cancer and search for new diagnostic, treatment and prognostic strategies, there is a need of experimental models, especially primary cell cultures and permanent cell lines, that can cover all the variety of CRC subtypes.

To establish a primary cell culture (PCC) from human CRC explant and to determine the optimal conditions for cultivation of these cells.

The primary human CRC PCC was obtained using standard approaches for cultivation of tumor tissue explants and subsequent propagation of proliferating cells. In order to reduce the risk of bacterial contamination (one of the major problems in the establishment of CRC cell cultures), the following measures were performed: i) the cell medium was supplemented with a mixture of antibiotics (изброй ги кои са); ii) increased concentration (5x) of antibiotics in medium for transport CRC explants; iii) pretreatment of CRC explants (cut into pieces with a diameter of approximately 0.5 cm) with braunol - an antiseptic and disinfectant containing povidone iodine. The cells were grown in DMEM medium supplemented by fetal bovine serum (10 or 20%). The cell cultures were passaged using a mixture of 0.05% trypsin – 0.02% ethylenediaminetetraacetic acid (EDTA).

A strategy for preventing bacterial contamination and obtaining primary cell cultures from human colorectal cancer was successfully introduced in our laboratory

The application of antibiotics belonging to different groups / with a different mechanism of action at the earliest stages of CRC primary cell culture establishment (starting from transportation of cancer samples) is crucial to avoid the risk of bacterial contamination.

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## **PLANT RESPONSE ON BIOTIC AND ABIOTIC FACTORS**

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**Keywords:** *Pisum sativum*, *jasmonic acid*, *salinity*, *physiological responses*

Pea plant (*Pisum sativum* L.) is an annual legume plant with high nutritional value. The aim of our research was to examine the impact of jasmonic acid (JA) and salinity (NaCl) on physiological parameters in pea. JA is a phytohormone that is involved in various physiological processes such as growth, development, metabolism and biotic stress responses. Salinity is considered as a major abiotic stress affecting germination, seedling growth and crop production. In the present study, pea plants were grown as hydroponic cultures for 2 weeks. They were treated between day 7 and 14 with JA or NaCl. We tested the effects on growth-related characteristics, stress markers, and antioxidative capacity. JA did not increase plant growth but stimulated the photosynthetic potential in leaves. Correspondingly, the phenolics content increased while the total antioxidative activity remained as in the control. In the presence of JA, the malondialdehyde (MDA) stress marker was not changed and the decreased hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) level could reflect increased peroxidase activity specific for developing tissues. On the other hand, NaCl reduced plant growth and slowed down the photosynthetic potential in leaves. This correlated with a stressed status with MDA accumulation and decreased production of secondary metabolites. NaCl treatment decreased H<sub>2</sub>O<sub>2</sub> level, which could reflect high activity of H<sub>2</sub>O<sub>2</sub>-deactivating enzymes (peroxidase, catalase, superoxide dismutase) rather than role of phenolic compounds. In support, the total antioxidative activity increased, too. In conclusion, physiological studies could very well contribute to the understanding how plants respond to environmental changes.

## ANTIBACTERIAL EFFECT OF PEPTIDE AND PROTEIN EXTRACTS FROM MUCUS OF CORNU ASPERSUM AGAINST GRAM POSITIVE AND GRAM NEGATIVE TEST MICROORGANISMS

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**Keywords:** antibacterial activity, peptides and protein extracts, mucus from *Cornu aspersum*

Regardless of the indisputable positive effects of antibiotics, their mass usage leads to resistance of the microorganisms, which presents a threat to the human health. The rising number of bacteria resistant to conventional antibiotics forces scientists to focus on discovering alternative substances possessing antibacterial qualities. Such qualities may be found in a number of peptides and protein extracts.

The aim of the study is to investigate the antibacterial activity of peptides and protein extracts, isolated from the mucus of garden snail *Cornu aspersum*, against three bacterial strains: *Pseudomonas aureofaciens* AP9 (Gram -), *Brevibacillus laterosporus* BT271 (Gram +) и *Escherichia coli* NBIMCC 878 (Gram -). Those microorganisms were used as model organisms for different groups of bacteria. The isolated extract from mucus of garden snail was divided in to three fractions with different molecule masses by means of membrane filters. Fraction 1 (0 to 10 kDa), Fraction 2 (30 to 50 kDa) and Fraction 3 (above 50 kDa). Further filtration separates Fraction 1 in to additional fractions with masses below 3 kDa, 3 to 5 kDa and 5 to 10 kDa. To evaluate their antibacterial activity an experiment was conducted using two types of cultivation methods: inoculation of bacteria on the surface and in-depth of the agar. These variations aim to estimate the future application of the extract (by pervasion or by biofilm formation). The quantity of the inoculated bacteria was standardized. The results were processed using a method adapted for our research (mm<sup>2</sup>/mg Pr/μl ). The amount of protein in the studied peptides and protein fractions was determined according to two methods- Micro biuret method and Lowry method. The size of the formed sterile area was measured and then the data retrieved from both methods for protein determination was compared. That comparison allows us to gain information about the amount of peptide bonds and aromatic amino acids.

Complex protein fraction with molecule mass >30 kDa expressed inhibiting activity against all bacterial strains, in both cultivation methods and also in both protein determination methods. Fraction with mass 10 kDa presented lower, but distinct antibacterial qualities against all strains, according to the deep cultivation method. Fractions with masses 3 kDa and 3-5 kDa showed activity against *Brevibacillus laterosporus* BT-271, cultivated on the surface of the agar.

The results affirmed the following substantial tendencies: More complex mucus fractions express higher and broader antibacterial effect, while more purified fractions with lower masses can be narrowly targeted, depending on their application as therapeutic and cosmetic agents.

**Acknowledgments:** This study was financially supported by the project 01/14 "New natural peptides with antimicrobial activity of snail *Cornu aspersum*", financed by NSF to the Ministry of Education and Science of Republic of Bulgaria.

## **CYTOTOXIC ACTIVITY OF TWO COPPER (II) COMPLEXES WITH SCHIFF BASES IN HUMAN CERVICAL CARCINOMA CELLS**

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Various Schiff bases and their metal complexes have been reported to be express promising antitumor properties. These compounds are very attractive because they can attack more than one molecular target in the cell decreasing in this way risk of drug resistance development and undesired side reactions.

The aim of our study was to evaluate the influence of two copper (II) complexes on viability/proliferation of HeLa human cervical carcinoma cells. The following two complexes were examined: [Cu(VanTrpt)(4,4'-bipy)(H<sub>2</sub>O)] and [Cu(VanSer)(4,4'-bipy)(H<sub>2</sub>O)], where 4,4'-bipy = 4,4'-bipyridine; VanTrpt and VanSer are Schiff base ligands derived from condensation reaction between o-vanillin (Van) and DL tryptophan (Trpt) or L-serine (Ser).

The experiments were performed using MTT test and neutral red uptake cytotoxicity assay.

The results obtained revealed that applied at a concentration range of 1 – 200 µg/ml for 24h, 48h and 72h both complexes decreased in a time- and concentration dependent manner viability and proliferation of the treated cells.

**Acknowledgements:** *This study was supported by Grant ДКОСТ 01/16 from 17.08.2017, COST Action CA15135 and a bilateral project between Bulgarian Academy of Sciences and Romanian Academy.*

## **CHARACTERIZATION OF NEW ISOLATED *BACILLUS* STRAINS APPLICABLE IN ORGANIC FARMING**

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**Keywords:** organic farming; biofertilizers; *Bacillus*; rhizobacteria; molecular identification; enzymatic profile; PGPR

The study was carried out to characterize 3 newly isolated rhizosphere bacteria in the case of their potential use as biofertilizers. The studied strains were identified through 16S rDNA sequences analysis with BLAST algorithm. Strains TRL16 and SUB1 show 97% and 98% identity with *Bacillus subtilis* species, respectively. Strain R4 shows 97% identity with *Bacillus amyloliquefaciens* species. The ability of the strains to utilize 49 carbohydrates was studied through API 50 CH™ test. A number of enzymatic activities were studied by the API ZYM™ test. *In vivo* experiments with *Pelargonium peltatum* showed noticeable growth improvement on plants treated with the strains compared to the control.

Their interesting enzyme profile and the observed plant growth promotion effect of this *Bacillus* strains show a potential for use in organic farming, but further study is needed.

**PROTECTIVE, MODULATORY AND REACTIVATORY FUNCTION OF L-ARGININE ON ACETYLCHOLINESTERASE (AChE) IN THE FRACTION OF *VESPULA GERMANICA* AND *APIS MELIFERA*, DAMAGED BY VARIOUS FACTORS. MECHANISMS OF INHIBITORY ACTION ON THE ENZYME ACTIVITY FROM Na-NITROPRUSSIDE**

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**Keywords:** L-Arginine, Na-Nitroprusside, Acetylcholinesterase, antidote, reactivation, Invertebrates, mechanisms

In our other studies, was found that L-Arginine at concentrations of 1,0 to 50 mM significantly stimulated AChE and butyrylcholinesterase (BChE) in different brain regions in vertebrates. This message details the effects of this reagent on the activity of AChE in invertebrate fractions. In the case of *V. germanica* a stimulation of the enzyme is negligible – only 6-10%, but in this condition the L-Arginine (50 mM) protect significantly AChE – about 350% relative to the control, where Na-Nitroprusside (1.0 mM) inhibits about 90% baseline cholinesterase activity. Similar is the protector efficiency of L-Arginine in case of poisoning the enzyme with a specific anticholinesterase agent – Eserine salicylate.

The activity of AChE in *A. mellifera* is many times higher than that of *V. germanica* compared to baseline protein content. This indicates the presence of significant differences in the content or activity of this enzyme.

L-Arginine affects on AChE of *A. mellifera* is biphasic: insignificant inhibition (about 10% at a concentration of 1.0 mM) and concentration-dependent stimulation with progressive increase of concentration (e.g., about 80% at a concentration of L-Arginine at a 50 mM).

The dataset of the effect of L-Arginine on cholinesterase activity in invertebrates and vertebrates indicate that this amino acid and other sources of nitric oxide are powerful protectors, reactivators and antidotes in case of poisoning by anticholinesterase agents.



## **RESEARCH ON CANNABINOID AND PHYTOHORMONAL PROFILES IN PLANTS FROM GENUS *CANNABIS***

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**Keywords:** Plant Growth Regulators, Rhizospherical microflora, Cannabinoids

In most higher plants, photoperiodism and temperature affect the rate of progression to flowering, but the process itself is also controlled by other factors. In the present study we discuss the impact of PGRs and other physiological substances on this process by analyzing the effect of cannabis treatment with products containing cytokines and auxins of natural origin (microalgae, kelp extract), GA<sub>3</sub>, HA, and FA, as well as with rhizospherical microflora releasing significant quantities of PGR in the area of the root system, which are known to stimulate plants at different stages of their development. Furthermore, we've been searching for substances able to modulate the cannabinoid profile and increase CBD or THC production.

For this purpose two varieties of cannabis plants were grown in a phytochamber and treated with the above mentioned preparations. After the end of the tests the yield of the different variants was measured and chromatographic analyses were performed. High Performance Liquid chromatography tandem mass spectrometry was used to determine the PGR-producing ability of selected strains of PGRPB; the cannabinoid profiles of treated plants were determined by gas chromatography.

## **THE HSP90 INHIBITOR GELDANAMYCIN ACCELERATES THE REPAIR OF RADIATION-INDUCED DNA DOUBLE-STRAND BREAKS IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS**

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**Keywords:** molecular chaperone Hsp90, ionizing radiation, DNA damage and repair

Hsp90 is a molecular chaperone involved in the folding, assembly, stabilization and activation of hundreds proteins, including kinases involved in the cell cycle checkpoint and DNA repair. The Hsp90 inhibitor a natural antibiotic geldanamycin (GA) and some of its analogues, used either on their own, or in combination with chemotherapy and/or radiotherapy represent promising therapeutic agents. Recent studies imply that in contrast to their killing effect on tumor cells, Hsp90 inhibitors have proven useful for radioprotection of normal cells from gamma *in vitro* and *ex vivo* irradiation.

The present study analyzed the effect of the Hsp90 inhibitor GA on the radiation induced DNA damage in human blood cells. It was shown that the treatment of cells with GA can accelerate DNA repair and reduce the residual DNA damage levels. The stimulating effect exerted by GA on DNA repair kinetics was shown to be linked to DNA-PK<sub>CS</sub> kinase, essential component and central player in the Non-Homologous End Joining repair pathway (NHEJ). As DNA-PK<sub>CS</sub> belongs to the substrates of Hsp90, blocking the ATP binding site of Hsp90, GA may induce dissociation of the Hsp90/DNA-PK<sub>CS</sub> complexes and activate DNA-PK<sub>CS</sub> kinase, thus stimulating the cellular DNA damage repair.

## **THERMODYNAMIC PROPERTIES OF RED BLOOD CELLS OF WOMEN WITH REPRODUCTIVE PROBLEMS**

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**Keywords:** red blood cells, hemoglobin, differential scanning calorimetry

Human red blood cells (RBC) are among the most studied biological systems. In structural terms the cell is maintained by a system of cytoskeletal proteins that are connected to the RBC plasma membrane where two major proteins are found - glycophorin and band 3. The main protein constituent of RBC is the oxygen-transport metalloprotein hemoglobin that accounts for ca. 96% of the RBC's dry content.

It was suggested that there is a relationship between the level of the free hemoglobin, the membrane bound hemoglobin and the hematocrit, and complications during pregnancy, however the structural stability of hemoglobin in those cases was not evaluated.

In this study we apply differential scanning calorimetry to study the thermal stability of hemoglobin and band 3 protein during ageing of RBC derived from pregnant women with reproductive problems and healthy controls. The calorimetric profiles exhibit a complex shape consisting of four well resolved endothermic thermal transition centered at 50 °C, 57 °C, 63 °C and 72 °C, assigned to spectrin, bands 2.1, 4.1, 4.2 proteins, band 3 protein and hemoglobin, respectively. Freshly collected RBC of the studied patients and the controls did not differ, however in the course of ageing we observed that the amplitude of the hemoglobin transition decreased more rapidly for the patients than for the controls and the transition assigned to band 3 protein disappeared at an earlier stage than controls. Our data strongly suggest that the ageing of RBC proceeds faster in the studied patients compared to healthy individuals.

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## **BIOAUGMENTATION EFFECT OF PSEUDOMONAS AUREOFACIENS AP-9 ON MODEL BIOREMEDIATION OF PHENOL-CONTAMINATED SEDIMENTS**

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**Keywords:** bioremediation, sediments, *Pseudomonas*, phenol, biodegradation

Industry development has a major role to make people's living standards dramatically improve over the last century. Along with human progress, however, industry is seriously damaging the environment and water ecosystems. The section of the Iskar River at the cascade of the Middle Iskar sHPP is an example of such ecosystem. In the surface waters, river bed and sediments in this cascade, critical pollution control points were investigated and a number of hazardous pollutants such as heavy metals, petroleum products and organic pollutants were identified as a potential risk factor for high water, floods, construction and other similar events. In this section of the river, because of this ecological problem, large quantities of highly degradable pollutants were accumulated in the sediments. One of the possible ways to solve this problem is the application of specific bioremediation technologies to improve the status of sediments and their processes. Another very important approach is the application of bioaugmentation with microorganisms for the acceleration of biodegradation processes, which is important for increasing the effectiveness of the bioremediation technologies. These issues will be addressed in this study.

Phenol has been used as a model pollutant in model bioremediation sites, as it is one of the most widespread and risky pollutants of water and sediment. It is a by-product of the manufacture of medicines, plastics, dyes and others. It has an adverse effect due to its high toxicity as well as its easy oxidation, which reduces the oxygen content of the water. The aim of the study was to trace the bioaugmentation effect of the *Pseudomonas aureofaciens AP-9* strain on the biodegradation processes of phenol as a model toxic pollutant in laboratory conditions at model bioremediation sites. In the bioremediation sites were simulated models with sediments from the dam Tserovo at sHPP Tserovo. The processes were monitored through a variety of chemical (chemical oxygen demand and residual concentration of phenol), microbiological (total number of aerobic heterotrophs, bacteria from genus *Pseudomonas* and phenol-degrading bacteria) and kinetic (effectiveness of phenol eliminations and rate of phenol biodegradation) indicators.

The results of the study demonstrated the positive bioaugmentation effect of the added strain of *Pseudomonas aureofaciens AP-9*, which is clearly visible in the final phase of the process (24-48 hours). The pseudomonads that have been added enhance the biodegradation of phenol, which was of significant practical importance to the bioremediation technologies of contaminated sediments. This was associated with an important techno-economic benefit and could be used in the development of future experimental pilot bioremediation sites for sediments, soils and waters.

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## PREVALENCE OF RESPIRATORY VIRUSES IN CASES OF PEDIATRIC ACUTE RESPIRATORY TRACT ILLNESSES IN BULGARIA

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**Keywords:** respiratory viruses, acute respiratory infections

Acute respiratory illnesses (ARI) are one of the most prevalent infectious diseases in pediatrics. In most cases, they are self-limiting but in susceptible individuals, such as newborns and children below 5 years of age, the symptoms can impact on the lower airways or central nervous system (CNS), resulting in laryngotracheitis, bronchiolitis, pneumonia or neurological complications. This study aims to determinate the frequency and clinical significance of the most often respiratory viruses detected in specimens of children aged <5 years with ARI during the 2016/17 winter season in Bulgaria.

During the period 10.2016 – 04.2017, a total of 398 nasopharyngeal specimens of young children from different country regions were collected and tested. *Real-Time PCR* analysis was performed for detection of influenza viruses A/B, respiratory-syncytial virus (RSV), metapneumovirus (MPV), parainfluenza viruses (PIV 1/2/3), rhinovirus (RV), adenovirus (AdV) and bocavirus (BoV). Samples with negative results were tested for other respiratory viruses: human coronaviruses (HCoV), parainfluenza virus (PIV4) and enteroviruses (EV) by *Multiplex PCR* method using a *Seeplex RV15 ACE Detection* (fast-track diagnostic) kit.

*Real time PCR* analysis indicate 365 respiratory viruses among 398 samples tested and the numerical distribution of A (H1N1)pdm09, A (H3N2), B, AdV, RV, RSV, HBoV, HMPV, PIV 1, PIV 2, PIV 3 were obtained as: 4 (1,1%), 85 (23%), 4 (1,1%), 40 (11%), 33 (9%), 142 (39%), 12 (3,3%), 25 (7%), 0 (0%), 5 (1,4%), 15 (4,1%), respectively. A total of 91 negative samples were tested for HCoV, PIV 4 and EV by Multiplex PCR. Among them, HCoV (NL63/OC43), EV, PIV 4 accounted for 8%, 1% and 1%, respectively. The detection rate of HCoV among patients with neurological complications was 25%, laryngotracheitis - 22%, bronchiolitis – 15% and pneumonia – 0%. Children positive for EV and PIV 4 were diagnosed with meningitis and laryngotracheitis.

In conclusion, according to study results, RSV, influenza A (H3N2) and AdV are the most frequently detected viruses during the winter period and affected at most 0-4 age group. In negative samples where the most frequently identified respiratory viruses lacking, HCoV were associated with the development of laryngotracheitis and neurological complications.

## **WILD CHINESE STRAWBERRY (*FRAGARIA SPP.*) POLYPLOID CREATION AND IDENTIFICATION**

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**Keywords:** wild strawberry, polyploid induction; primary meristem treatment technology; identification; transcriptome

Strawberry is known as the "fruit queen.", not only because of its beauty and delicious but also aromatic and high nutritious value. At present, the whole strawberry cultivation still has the problems of poor resistance, rapid degradation of varietal characters, serious diseases and pests, and low economic yield, except individual strawberry varieties. New variety is needed. Wild strawberries are rich in genetic diversity, and have unique advantages in resistance to cold, drought, barren, disease and their unique aroma. However, it is difficult to transfer the excellent traits of wild strawberries into cultivars, due to the low chromosome ploidy of the Chinese wild strawberry germplasm and the poor affinity to cultivars. Here, we use an efficient and rapid primary meristem treatment technology to make strawberry germplasm innovation through Chinese wild strawberry and set up a rapid identification system for ployploidized strawberry. We successfully get tetraploidy *F. pentaphylla*, *F. nilgerrensis*, *F. vesca*, *F. mandschurica*, and octoploidy *F. moupinensis*. The flow cytometry and karyotype analysis confirmed the double of the chromosome. The morphological and functional traits showed significant difference between original individuals and now synthesized polyploidy individuals. Further studies will be conducted to compare the differences of the physiological traits and responses to biotic and abiotic stresses. Hhybridization between old strawberry and new synthesized polyploidy strawberry will be conducted. These works will provide new high-quality materials for Chinese strawberry breeding.

## **RHIZOSPHERE SOIL MICROBIAL COMMUNITIES OF INVASIVE *ALTERNANTHERA PHILOXEROIDES* CHANGED BY THE PARASITISM OF *CUSCUTA AUSTRALIS* BENEFIT THE GROWTH OF NEIGHBORING PLANT**

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**Key words:** feedback, soil microbial community, *Alternanthera philoxeroides*, next-generation sequencing, parasitic plant, invasive plant

Parasitic plants are one special group of some ecosystems. Although much is known about how parasitic plants influence growth, reproduction, physiology and metabolism of host plants, their roles as drivers of community- and ecosystem-level properties remain largely unexplored. Using the next-generation sequencing of the bacterial 16S rRNA gene and fungal rRNA internal transcribed spacers (ITS) sequences, we analyzed effects of the parasitism by *Cuscuta australis* on the structure and diversity of soil microbial communities in the rhizosphere of invasive *Alternanthera philoxeroides*. The feedback effects of the changed soil microbes on the growth of host *A. philoxeroides* and the neighboring *Trifolium repens* were also investigated. The parasitism of *C. australis* increased the diversity and changed the composition of the bacteria and the fungi community in the rhizospheric soil of *A. philoxeroides*. Such effects were higher when the soil fungi community was considered than when the soil bacterial community was considered. *A. philoxeroides* significantly produced less biomass when growing in the soil of *A. philoxeroides* parasitized than not parasitized by *C. australis*, while *T. repens* significantly produced more biomass. Although *A. philoxeroides* showed higher competitive ability to *T. repens* on both soil, the competitive ability of *A. philoxeroides* decreased when growing in the soil of *A. philoxeroides* parasited by *C. australis*. These results suggest that changes in soil microbes induced by plant parasitism can negatively affect host plant growth, strengthening the direct negative effect of plant parasitism, and positively affect neighbor plant growth.

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## ***IN VITRO* TOXICITY EVALUATION OF GO AND GONH<sub>2</sub> NANOPARTICLES WITH A549 CELLS**

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**Keywords:** nanomaterials, cytotoxicity, chemical modifications, A549 cell line

Graphene oxide (GO) and its derivatives have shown great potential in multiple biomedical applications as drug carriers, diagnostic sensors for imaging, antimicrobial agent, etc. However, GO can cause side effects, both *in vivo* and *in vitro*, as formation of reactive oxygen species, DNA damages, apoptosis, pulmonary edema and granuloma formation, inflammatory cell infiltration, thrombus formation, delayed development in offspring. To improve its biocompatibility for further biomedical applications different chemical modifications have been developed. The aim of this study is to evaluate toxicity of GO nanoparticles (NPs) and the effect of the surface chemical modification (amination GONH<sub>2</sub>) with a human adenocarcinomic epithelial cells A549. In order to verify whether the concentration is critical to the toxicity we have used five different concentrations, 0,1 µg/ml, 1 µg/ml, 10µg/ml, 20 µg/ml and 50 µg/ml. XPS analysis confirmed that the GO nanoparticles did not contain any N while the amount of N in aminated GO (GONH<sub>2</sub>) is 3%. Cell cultures experiments demonstrated a cytotoxic effect after 24h of incubation of both types of NPs especially of the aminated particle which are bigger in size. We observed that the increasing concentration of the nanoparticles influence the cells proliferation rate, cell numbers but not the spreading area of A549 cells



## THE USE OF ANTI-IDIOTYPE scFv FOR THE LOCALIZATION OF A GLOBULAR AUTOEPIOTOPE OF C1q

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**Keywords:** C1q, globular autoepitopes, scFv

Anti-C1q autoantibodies are a clinical feature of a few autoimmune disorders in humans diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), mixed connective tissue disease, Felty's syndrome, rheumatoid vasculitis, polyarthritis nodosa, polychondritis, Sjögren's syndrome, etc. It is now clear that C1q turns into an autoantigen upon immobilization and it is assumed that the process of immobilization brings about the exposure of neo-epitopes which are recognized by the anti-C1q antibodies. Yet the immunogenic stimulus that gives rise to the formation of anti-C1q antibodies is still unknown. Localizing the autoepitopes that are recognized by the polyclonal autoantibodies to C1q might shed light to the structural changes of the protein that render it autoantigenic. Recently, a major linear epitope within the N-terminal domain, designated CLR, was identified. We addressed the issue of C1q autoantigenicity by generating a scFv that is anti-idiotypic to a fraction of anti-C1q autoantibodies, isolated from Lupus Nephritis (LN) sera, which specifically recognized only the intact C1q molecule. We also studied conformational changes of the protein C1q due to the binding of anti-C1q, registered by fluorescence spectroscopy method. The anti-idiotypic scFv was found to have approximately 50 % inhibitory capacity on the recognition of immobilized C1q by the LN autoantibodies. The anti-idiotypic scFv was sequenced and *in silico* folded into a 3D structure which was superimposed on the crystalized 3D structure of the C-terminal domain of C1q thus revealing a structural homology with the globular fragments of all three types of polypeptide chains comprising C1q, designated ghA, ghB and ghC. The scFv was further revealed to inhibit the recognition of immobilized ghA by the LN autoantibodies. In conclusion the generated anti-idiotypic scFv can serve as a structural analogue to a conformational globular autoepitope of C1q and can be further subjected to *in vivo* functional analysis.

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## **TENSIOMETRIC INVESTIGATIONS OF hBEST1 AND SM MONOLAYERS**

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**Keywords:** hBest1, sphingomyelin, Langmuir–Blodgett trough

Bestrophin-1 (Best1) is aCa<sup>2+</sup>- dependent transmembrane protein, expressed in the retinal pigment epithelium, astrocytes and glia. It has been proposed that Best1 plays role in the transport of Cl<sup>-</sup> ions,  $\gamma$ -aminobutyric acid and glutamate. Mutations in the gene of human Best1 lead to pathology in retina and blindness. hBest1 transport channel also could influence some neurodegenerative conditions leading to Alzheimer's and Parkinson's disease.

We studied the effects of hBest1 interactions with sphingomyelin (SM, widely abundant lipid of neuronal cell membranes) using the advantages of Langmuir–Blodgett monolayer model systems in order to determine its binding capacity, biological activity and physiological importance of SM, comparing surface changes in response to Ca<sup>2+</sup> ions by measuring  $\pi/A$  isotherms and hysteresis curves.

In our research we used as a model system Langmuir trough to compress: monolayers of pure hBest1; monolayers of SM; and composite monolayers of hBest1+SM at the surface of 0,9% NaCl solution.

**Acknowledgment:** *This work was supported by grant DFNI - T02 / 7 from 12.12.2014 (Bulgarian National Science Fund)*

## **PROTEIN PROFILING AND IMMUNOLOGICAL CROSS-REACTIVITY OF WHEAT AND OTHER CEREALS PROTEINS**

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**Keywords:** phage display, protein profiles, immunogenicity, gluten, 2D-E

For ages now, it is known that wheat (*Triticum aestivum*) proteins are the cause of a variety of health conditions. In the recent years a progressive amount of data is being obtained in relation to the major wheat protein, gluten, being the basis for allergies and immunological responses. This is the reason for replacing wheat with alternative crops as einkorn (*T. monococcum*), kamut (*T. turanicum*) and spelt (*T. spelta*). There is evidence for the more beneficial health impact of these grains. Our study is designed to evaluate, characterize and compare the gluten fractions from the four crops. For the purpose the proteins were separated by 2-dimensional electrophoresis. The extracted wheat proteins were used as antigens in the screening of a phage-display library with the intention to develop specific antibodies. The polyclonal suspension, which was obtained after four rounds of affinity selection was used for immunoblot to test cross-reactivity to the other three cereals. Kamut and einkorn present themselves as the least cross-reactive, which suggests that if they are used in the diet, the immunological response will not be as strong as against spelt or wheat.

**Acknowledgements:** *This work was supported by fellowship award from L'Oréal-UNESCO for Women in Science's national program, Bulgaria*

## **CLONING, CHARACTERIZATION AND APPLICATION OF PROTELOMERASE FROM *AGROBACTERIUM TUMEFACIENS***

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**Keywords:** *Agrobacterium*, protelomerase, expression, linear DNA, transient expression

Heterologous expression depends on vectors of plasmid or viral origin, which exist and replicate independently or integrate in genome. In most cases these vectors are suitable for the researcher's needs but for some purposes prolonged existence either as episome or as integrated form is superfluous or unwanted. In such cases, transient expression is sufficient, but these vectors are complicated in design and/or use. An alternative approach for designing transient expression vectors is to employ linear DNA with covalently closed ends. Genetic system with linear DNA genome with covalently closed ends was described in linear bacterial chromosomes, where the covalently closed ends are produced by specialized recombinase called protelomerase. In this work we are presenting our attempts to clone and characterize protelomerase from *Agrobacterium tumefaciens* as well as to outline its use for designing linear vectors for transient expression.

*tela* gene encoding for protelomerase was isolated and the correct product was cloned. The recombinant *Tela* protelomerase was successfully expressed and purified. *Tela* activity was demonstrated on designed plasmid carrying recognized sequences. The use of protelomerase opens the possibility to design linear non-replicating vectors allowing transient expression of heterologous gene(s) at sufficient level.

## **METAL COMPLEXES OF MELOXICAM – EFFECT OF THEIR COMBINATIONS WITH DISULFIRAM OR CISPLATIN ON VIABILITY AND PROLIFERATION OF HUMAN NON-SMALL CELL LUNG CANCER CELLS**

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**Keywords:** metal complexes, non-small cell lung cancer, cisplatin, disulfiram, combined drug antitumor therapy

Lung cancer is one of the most common and lethal cancers in the world. The disease occurs more often in older people, women have lower rates than men, for smokers the risk is higher. Non-small cell lung cancer (NSCLC) represents 85-90% of all lung cancers with near 1.5 million newly diagnosed cases each year. In Bulgaria they are about 3500.

In our previous investigations we found, that selective non-steroidal anti-inflammatory agent meloxicam and its metal [Zn(II), Cu(II), Co(II), Ni(II)] complexes inhibit the in vitro growth of various cultured permanent cell lines (established from chicken hepatoma, rat sarcoma, human cervical carcinoma, glioblastomamultiforme and colon cancer).

Cisplatin is one of the most potent chemotherapy drugs widely used in current clinical oncology. Disulfiram, applied for decades in the treatment of alcoholism, has been proved to express also promising antineoplastic properties.

Combined drug antitumor therapy is a strategy to reduce toxicity as well as to decrease the risk of drug resistance.

The aim of the present study was to evaluate the influence of disulfiram, cisplatin, meloxicam, its metal [Zn(II), Cu(II), Co(II), Ni(II)] complexes and their combinations on viability and proliferation of cultured human non-small cell lung cancer cells.

As an experimental model in our investigations we used the permanent cell line A549 that was established from human NSCLC and has been reported to express the cyclooxygenase 2. Disulfiram was applied at concentrations of 0.3-100 µg/ml; concentrations of cisplatin were from 0.01-30 µg/ml; meloxicam and its metal complexes were administered at a concentration range of 2.5-500 µg/ml. The investigations were performed using MTT test – the golden standard for cytotoxicity assays, neutral red uptake cytotoxicity assay, double staining with acridine orange and propidium iodide and colony-forming method.

The results obtained revealed that: i) Disulfiram and cisplatin decreased viability and proliferation of the treated cells in a time- and concentration-dependent manner; ii) Meloxicam and its metal complexes did not reduce the percentage of viable A549 treated cells; iii) A significantly increased cytotoxic/cytostatic effect was observed when Cu(II) complex of Meloxicam was combined with Disulfiram (applied at relatively low toxic concentrations of 0.78 and 1.56 µg/ml – cell viability was > 80% as compared to the control); iv) The addition of metal complexes of meloxicam did not increase the cytotoxicity of Cisplatin.

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## GENETIC SCREENING FOR THE PRESENCE OF GENES FOR PLANTARICINS AND PEPTIDASES IN *LACTOBACILLUS PLANTARUM* STRAINS WITH ANTIMICROBIAL ACTIVITY

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**Key words:** *Lactobacillus plantarum*, peptidases, bacteriocins, plantaricins, antimicrobial activity

Lactic acid fermentation of various food products is the basis for improving of their long shelf life and safety. Proteolysis of milk proteins leads to release of oligopeptides or single amino acids with bioactive and antimicrobial properties. Bacteriocins and bacteriocin-like substances synthesized by lactic acid bacteria are among the protein metabolites that have a proven antimicrobial activity.

The *in vitro* assays determining a higher antimicrobial activity of the *Lactobacillus plantarum* strains against *Bacillus subtilis* compared to *Escherichia coli*. Good antibacterial action shows the exponential culture and cell-free supernatant of *L. plantarum* C7 grown in MRS with added potential inductor of bacteriocin synthesis - tryptone (2g/L). From all of the strains tested against *B. subtilis* the exponential culture and the cell-free supernatant of *L. plantarum* C3, cultured in MRS with KH<sub>2</sub>PO<sub>4</sub> inductor (2g/L), showed the highest antimicrobial activity. By PCR analysis of six *Lactobacillus plantarum* strains was found the presence of genes, coding the peptidases pepT and pepN as well as the genes for the synthesis of plantaricins E, F, J and K. Positive results for the presence of E, F and K genes and gene pepN were observed in all of the tested strains of *L. plantarum*: C3, C4, C5, C6, C7, C10. Only C3 from all has four of the tested plantaricin genes and both of the peptidases genes in its genome.

The results of the experiments show that all strains and their metabolites have good antibacterial potential and can be used in the production of functional foods, containing accessible bioactive peptides.

## **PROKARYOTIC ENZYME SYSTEM IN THE SERVICE OF ZOOLOGY**

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**Keywords:** CRISPR / Cas9, genes, enzyme, DNA

Excluding genes with CRISPR / Cas9 is a new approach to studying the functions of genes in different organisms.

Cas9 enzyme is isolated from the prokaryotic organisms and is used as molecular scissors for cutting of specific regions of DNA. Cas9 is a bacterial RNA-guided endonuclease that uses base pairing to recognize and cleave target DNAs with complementarity to the guide RNA. The programmable sequence specificity of Cas9 has been harnessed for genome editing and gene expression control in many organisms.

The CRISPR method is consisting of a highly effective set of molecular "scissors" - can be easy to use but is not perfect. These "scissors" can cut more than necessary by cutting DNA into unexpected and unwanted sites. In early experiments, scientists have found that side effects can occur at some sites in DNA at approximately the same frequency as the target areas. This method includes three major components: the Cas9 enzyme that cuts a portion of the DNA; single-stranded RNA sequence (a series of nucleotides in its molecule) that gives Cas9 the exact place to cut; a new template DNA to repair the cut ("repair" is a DNA double strand recovery). Using CRISPR / Cas9, double-strand fractures are induced in the genome of *Ciona intestinalis*. CRISPR / Cas9 can mutate endogenous *Ciona intestinalis* genes, a great model for clarifying molecular mechanisms for building a horizontal plan of the body. CRISPR / Cas9 is sufficiently effective and specific to generate a large number of embryos carrying mutations in the target gene of interest, which allows rapid screening of the gene in *Ciona intestinalis*.

## **IDENTIFICATION OF THE CLINICAL ISOLATES CAMPYLOBACTER JEJUNI\COLI THROUGH A MOLECULAR METHOD**

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**Key words:** *Campylobacter*, Multiplex PCR, diarrhea

Out of the most well-known agents causing campylobacteriosis, *C. jejuni* and *C. coli* are the most commonly isolated species from patients with acute diarrhea. The spread of those species in Bulgaria is unknown, because their identification is usually not practiced in the microbiology laboratories and the molecular methods regarding it are also absent. We have worked on and optimized a Multiplex PCR analysis for identifying clinical isolates *C. jejuni \ coli*. All PCR products of the analysis were divided by capillary gel- electrophoresis or standard agarose electrophoresis. Using the approbated by us molecular method we confirmed the identification of 49 isolates, 39 of which (39\49) *C. jejuni*, 3 (3/ 49) *C. coli* and 1 (1/ 49) *Campylobacter spp.*



## **ENZYME ACTIVITY OF MYCROPROPAGATED *ACHILLEA THRACICA* PLANTS**

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**Keywords:** *Achillea thracica*, mycripropagation, enzyme activity

*Achillea thracica* Velen. (*Asteraceae*) is an endangered Bulgarian endemic species. The aim of present study was to investigate the stress enzyme profiles and products of lipid peroxidation of *in vivo*, *in vitro* and *ex vitro* grown plants of *Achillea thracica* Velen. For that purpose peroxidase and catalase activity and concentrations of MDA, H<sub>2</sub>O<sub>2</sub> were measured. Sensitive plants expressed physiological changes typical for the oxidative stress reaction. Lower enzyme activity and concentration of the two metabolites were detected in *in vitro* grown plants.

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## **REGULATION OF ARTERIAL CONTRACTION BY PERIVASCULAR ADIPOSE TISSUE DERIVED MEDIATORS IN HEALTH AND DIABETES**

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**Keywords:** PVAT, Arterial contraction, Diabetes

Perivascular adipose tissue (PVAT) surrounds many blood vessels including small arteries and arterioles. PVAT is a thin sheet, which consists of several cell types – mainly adipocytes, but also endothelial cells, leukocytes, and fibroblasts. PVAT either increase, or decrease the amplitudes of force of contraction of smooth muscle layer.

The aim of our research is to study the regulatory role of PVAT mediators H<sub>2</sub>S and H<sub>2</sub>O<sub>2</sub> on the 5-hydroxytryptamine (5-HT) induced contractions of rat skeletal muscle arteries isolated from healthy and diabetic rats.

We studied the influence of an inhibitor of H<sub>2</sub>S producing enzyme cystathionine gamma lyase and of a reactive oxygen species trapping agent on the force of contraction of rat artery gracilis isolated from healthy and diabetic rats. Diabetes was induced by a single intraperitoneal injection of streptozotocin. Endothelium-denuded artery rings without or with intact PVAT were used for isometric measurements of the force of contraction induced by increasing concentrations of 5-HT.

Different agents were applied as pharmacological tools for investigation of the H<sub>2</sub>S and H<sub>2</sub>O<sub>2</sub> release from PVAT. In *a. gracilis* preparations from healthy rats, PVAT produces H<sub>2</sub>S that antagonizes the 5-HT contractions, while in diabetic animals higher concentrations of 5-HT increases the PVAT production of H<sub>2</sub>O<sub>2</sub>.

H<sub>2</sub>O<sub>2</sub> as mediator sensitizes the 5-HT-induced contraction of the studied diabetic skeletal muscle arteries. In health dominates H<sub>2</sub>S-dependent vasorelaxation. In diabetes when pro-inflammatory PVAT phenotype dominates the common effect is vasoconstriction. It, at least partly, depends on increased H<sub>2</sub>O<sub>2</sub> production.

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## **APPLICATION OF EXOGENOUS SYNTHETIC STRIGOLACTONE GR24 ON *IN VITRO* *MEDICAGO TRUNCATULA* PLANTS WITH MODIFIED AUXIN TRANSPORT IN CONDITIONS OF PHOSPHATE DEFICIENCY AND EXCESS**

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**Keywords:** *Medicago truncatula*, transgenic plants, synthetic strigolactone GR24, phenotypic analysis, GUS staining

One of the most important micronutrient required for normal development of plants is phosphorus. Both phytohormones auxin and strigolactones participate in the plant response associated with the phosphate signaling. In terms of phosphorus starvation, plants have developed mechanisms for overcoming stress, expressed in the formation of shortened and thickened cluster roots. *Medicago truncatula* plants with overexpression of *MtLAX3* gene (involved in polar auxin transport), transcriptional reporters of *MtLAX3* (expressing reporter genes GUS, for  $\beta$ -glucuronidase, and GFP, for green fluorescent protein, under control of LAX promoter) and wild type plants were *in vitro* cultivated in extreme conditions of phosphate deficiency and excess. Phenotypic characterization and morphometric measurements were performed for the plant habitus before and after treatment with synthetic strigolactone GR24. The relative transcript level of *MtLAX3*, *MtMAX2* (involved in the strigolactone signaling) and *MtMAX3* (involved in the strigolactone biosynthesis) was measured. The expression of *GUS* reporter gene fused to *MtLAX3* promoter was traced in different organs and tissues by histochemical GUS assay.

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## EFFECT OF *PSEUDOMONAS AUREOFACIENS AP9* ON DETOXIFICATION OF RIVER SEDIMENTS POLLUTED WITH PHENOL – ENZYMOLOGICAL AND FISH ANALYSIS

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**Keywords:** bioremediation, bioaugmentation, *Pseudomonas*, oxygenases, FISH

In cases of xenobiotic pollution in natural ecosystems a specific, highly specialized set of measures should be taken. One example of this is the application of bioaugmentation procedure in order to accelerate the biodegradation of the xenobiotics. It supplies the biological system with extra biodegraders with high detoxification capacity. The aim of the study was to establish the effect of bioaugmentation with *Pseudomonas aureofaciens AP9* in model bioremediation process on key enzyme activities and the bacteria from genus *Pseudomonas in-situ*. The present research is part of experiment simulating shock discharge of phenol in river sediments. The bioremediation process is stimulated trough bioaugmentation with *Pseudomonas aureofaciens AP9* (highly active biodegraders of phenol). The activity of the indigenous microflora and the effect of *P. aureofaciens* addition were estimated on the base of enzymological and genetic analysis in two lab-scale bioremediation sites. The one contained sediments from "Iskar River" with indigenous microflora. In the other one the bacterial culture was added. The studied key enzyme activities were phenol-2-monoxygenase (P2MO), catechol-1,2-dioxygenase (C12DO), catechol-2,3-dioxygenase (C23DO), protocatechuate-3,4-dioxygenase (P34DO) and succinate dehydrogenase (SDH). The applied genetic analysis was fluorescence *in-situ* hybridization (FISH) for bacteria from g. *Pseudomonas*. The model process of phenol detoxification had duration of 48 hours and it comprised the two specific phases for every xenobiotic biodegradation – initial phase of intoxication (0 h.) and subsequent phase of active biodegradation (48 h.).

The obtained results showed that at 0 h. most of the enzyme activities were significantly decreased – P2MO with 70 %, C12DO with 80 %, P34DO with 27 % and SDH with 54 %. The bioaugmentation procedure affected notably the detoxification pathway with highest activity – those with key metabolite protocatechuate. In the model process with *Pseudomonas aureofaciens AP9* the activity of P34DO was lowered with only 2 %, while in the system with only indigenous microflora the decrease was 50 %.

In the stage of active biodegradation of phenol (48 h) 77 % of the xenobiotic was eliminated by the two biological systems. The activities of enzymes from the later stages of phenol biodegradation were higher in the model system with only indigenous microorganisms compared to the one with *Ps. aureofaciens AP9* with 61 % for C12DO, 1.2 times for P34DO and 2.5 times of SDH.

According to the results from the performed model phenol detoxification the main biodegradation pathway goes through key metabolite protocatechuate. This is typical for environment with low oxygen concentration such as river sediments. The data collected in this study by enzymological and FISH analysis highlighted the supporting role of bioaugmentation in the early stages of intoxication. It also showed that after the indigenous microflora adapts towards the xenobiotic there is no stimulating effect of the external support of the microbial community.

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## **FLUORESCENT NANODIAMONDS – PENETRATION AND TOXIC EFFECT IN *BREVIBACILLUS LATEROSPORUS* BT271**

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**Keywords:** nanodiamonds, CFDA-SE, fluorescence analysis, *Brevibacillus laterosporus*, toxicity, penetration

The world of nano-materials merge more and more with the world of biological sciences offering wide range of enhanced features such as stability, penetration, adsorption, mechanical strength, biocompatibility, antibacterial activity etc. Such new materials are nanodiamonds (ND) which are known for their excellent mechanical and chemical properties but their impact on the biological systems is still poorly studied. This research is focused on detonation nanodiamonds penetration and their toxic impact on model Gram (+) bacteria. The model microorganisms were *Brevibacillus laterosporus* BT271. The nanodiamonds penetration was studied by using fluorescent nanodiamonds (1.5 % suspension) labeled with carboxyfluorescein diacetate - succinimidyl ester (CFDA-SE). Four concentrations of the dye were used – 2.5  $\mu\text{M}$ , 5  $\mu\text{M}$ , 10  $\mu\text{M}$  and 50  $\mu\text{M}$ . Five incubation periods for nanodiamonds and bacteria were applied – 0 min, 15 min, 30 min, 60 min, 120 min. Toxicity effect was estimated by visual analysis of the obtained fluorescence images. The effects are represented as part of the maximal one (100 %). Five-step scale of assessment was applied with range from 0 % (lack of fluorescence/toxicity) up to 100 % (maximal fluorescence/toxicity).

The penetration of the complexes ND-CFDA-SE with 2.5  $\mu\text{M}$  and 5  $\mu\text{M}$  CFDA-SE in *B. laterosporus* BT271 was registered after 15 min of incubation. The two types of ND complexes showed similar penetration characteristics with maximum at 30 min (50% fluorescence intensity). 100 % of fluorescence was achieved with ND-CFDA-SE with 10  $\mu\text{M}$  of the fluorescent dye at 120 min of incubation.

The toxic effect of the fluorescent nanodiamonds was estimated by the decrease of amount of the bacterial cells. Reduction of the quantity of *B. laterosporus* cells was found to some extent in all experimental variants. Most significant reduction (75 %) of *B. laterosporus* BT 271 was registered when ND-CFDA-SE 50  $\mu\text{M}$  were applied. This highlights the contribution of the dye to the overall toxicity. Lowest toxicity combined with best fluorescence was found when CFDA-SE was present in concentration of 5  $\mu\text{M}$ .

The obtained results showed that the preparation of nanodiamonds with CFDA-SE in concentration of 5  $\mu\text{M}$  has best performance regarding toxicity and fluorescent properties. Therefore this preparation could be used in further experiments with different types of nanodiamonds and different types of microorganisms.

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## **MYOGENIC DIFFERENTIATION OF MUSCLE PROGENITOR CELLS ON PDMS SUBSTRATES WITH DIFFERENT STIFFNESS**

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**Keywords:** myogenesis, satellite cells, substrate elasticity, skeletal muscle repair

Regeneration of skeletal muscles is one of the great challenges in tissue engineering because of insufficient participation of the stem cells in the muscle repair. One of the main focuses of muscle tissue engineering (MTE) is searching of an ideal biomaterial to stimulate and support regeneration of muscle. Optimization of the biomaterial characteristics may lead to significant functional improvement of severely damages in skeletal muscles. Many authors have demonstrated the important role of physicochemical properties of the substrate in the fate of most adherent cells. Adult stem cells are especially sensitive to the substrate stiffness and respond to mechanical signals by altering their adhesion, morphology, proliferation and lineage commitment. Therefore in the present work we have evaluated the effect of the substrate elasticity on myogenic differentiation of two cell types: the myogenic C2C12 cell line and primary muscle satellite cells (SCs). For this we have prepared substrates with different values of elastic modulus based on polydimethylsiloxane (PDMS). Our results showed increased myotube formation on PDMS materials for both cell types as well as increased expression levels of two myogenic factors: MyoD and Miogenin for SCs in comparison to control cover glass while cell proliferation was suppressed. Despite of the absence of clear effect of the stiffness in the studied range all tested PDMS samples were able to induce myogenic differentiation in the cells and thus could be used for the needs of skeletal MTE.

## **EFFECTS OF [(DIMETHYLPHOSPHINYLMETHYL)AMINO] (CHLOROPHENYL)-METHYLPHOSPHONIC ACID AND N-(PHOSPHONOMETHYL) GLYCINE ON ATPase ACTIVITY OF RAT LIVER MITOCHONDRIA**

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**Keywords:** aminophosphonic acids, dimethylphosphinyl-substituted phosphonates, mitochondria, mitochondrial ATPase

Aminophosphonic acids and their derivatives are widely used as antimicrobial agents, pesticides, plant growth regulators. However, along with their positive effects they can be damaging to the living systems.

The purpose of the current work was to study the effects of two aminophosphonates - a newly synthesized [(Dimethylphosphinylmethyl)amino](chlorophenyl)-methylphosphonic acid (DMCPPA) and a commercially used as a herbicide Cosmic<sup>®</sup> with an active ingredient N-(phosphonomethyl) glycine (glyphosate), on ATPase activity of rat liver mitochondria.

DMCPPA did not influence ATPase activity of both intact and 2,4-dinitrophenol-uncoupled mitochondria, suggesting that the compound does not possess uncoupling effect and is not able to pass the inner mitochondrial membrane. DMCPPA slightly reduced ATPase activity of mitochondria uncoupled by freezing/thawing. Cosmic<sup>®</sup> stimulated ATPase activity of uncoupled mitochondria and ATP hydrolysis in intact mitochondria, which was indicative for uncoupling effect and possible penetration through the mitochondrial membrane.

The difference in the effects of the two compounds may be due to supplements in the composition of Cosmic<sup>®</sup> and/or to synergistic effects of glyphosate and the supplements, as well as to different chemical structures, which determine different biological activity.

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## **PROFILING OF PLATANUS ORIENTALIS L. LEAVES BY FLUORESCENCE AND OPTICAL METHODS DURING THE COMBINED TREATMENT WITH MODERATE HIGH TEMPERATURES AND DROUGHT**

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**Keywords:** chlorophyll fluorescence, heat stress, drought stress, combined stress

Plants are subject to the combined action of multiple constantly changing environmental conditions. Plant survival, growth and reproduction are dependent on evolving adaptation mechanisms acting in concordance against the stress factors of which water deficiency and high temperatures are a trivial pair. However the magnitude of these stressors may vary from mild to acute responses. Simulating natural conditions in laboratory is crucial to understanding plant physiology in nature. Profiling of 2-year old *Platanus orientalis* trees, grown in laboratory conditions, was conducted on 3 leaves per plant. Chlorophyll fluorescence and optical methods were used during gradual application of and recovery from moderate high temperatures and drought done separately and in combination. Chlorophyll a fluorescence is considered fast, non-destructive and highly sensitive method giving detailed information about the light dependent photosynthetic reactions. It allows studying stress dynamics. The difference in stress induced changes in the photosynthetic machinery with respect to the different physiological age of leaves were determined.

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## **COMPARISON OF THE ANTIOXIDANT POTENTIAL OF MEDICINAL PLANTS FROM THE RHODOPE MOUNTAINS WITH COMMERCIALY PURCHASED PLANTS**

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**Keywords:** secondary metabolites, phenolics, antioxidant potential, medicinal plants

Phenolic compounds are secondary metabolites of plants with different activities such as protection against pathogens and predators, mechanical support, attraction of pollinating animals and seed distributors, and protection against ultraviolet radiation. Phenols also perform various functions in the human body - antioxidant protection, anti-viral, antibacterial, anti-tumor and anti-inflammatory activity. The aim of this study was to examine the phenolic compounds and antioxidant activity in the following medicinal plants: yarrow (*Achillea millefolium*), chamomile (*Matricaria recutita*), thyme (*Thymus vulgaris*), peppermint (*Mentha piperita*), green tea (*Camellia sinensis*). Extracts were prepared from herbs grown in Rhodope Mountains in the period of flowering, and commercially purchased ones. From the experimentally obtained results, we analyzed the correlation between total phenol content and antioxidant activity. It can be concluded that endogenous and exogenous factors may influence the concentration of phenolics. These studies could be applied to study the optimal conditions for growing different herbs and their potential beneficial effects on health

## **DYNAMIC LIGHT SCATTERING AND ELECTROKINETIC STUDY OF *BRYOPHYLLUM DAIGREMONTIANUM* THYLAKOIDS**

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**Keywords:** thylakoid membranes, DLS, zeta potential, *Bryophyllum daigremontianum*

*Bryophyllum daigremontianum* (*Kalanchoe daigremontianum*) is a succulent plant native to Madagascar. *Bryophyllum* is used medicinally in tropical Africa, South America, India, China and Australia.

We report an experimental investigation of electrokinetic properties and dynamic light scattering (DLS) for thylakoid membranes from *Bryophyllum daigremontianum* plant and plantlets.

There were no data about evaluating size and polydispersity as well as zeta potential of thylakoids from *B. daigremontianum* plantlets by the method of DLS and particle microelectrophoresis. In order to study the physicochemical properties of thylakoids from *B. daigremontianum* plant we used *Vicia faba* L. thylakoids for comparison as a classical model of higher plants.

As seen by the zeta potential measurements (laser Doppler velocimetry), only a few fractions of thylakoids from *B. daigremontianum* and *Vicia faba* had zeta potential values higher than  $\pm 30$  mV line for stable dispersion (Malvern, Instruments Ltd., 2005; Müller, 1996). Having these values allowed them to disperse at smaller sizes, increasing the exposed surface area and possibly allowing for more interaction with buffer solution.

Thylakoids from *B. daigremontianum* plantlets possessed an approximately 24 % increase in size compared to the particle size values from *V. faba* thylakoids, suspended in low ionic strength media.

Thylakoids from *B. daigremontianum* plantlets showed a similar fractions of 3,431  $\mu\text{m}$  with comparison to fraction of *Vicia faba* (3,39  $\mu\text{m}$ ) thylakoid membranes in the buffer solution of 25 mM Hepes (KOH), 5 mM  $\text{MgCl}_2$ , pH 7.50.

By the values of particle size analyses, thylakoids from *B. daigremontianum* plantlets suspended in 50 mM Mes (KOH) buffer, pH 6.0 or 50 mM Tricine (KOH), pH 7.50 possessed a similar size values of 3,707  $\mu\text{m}$  vs. 3,74  $\mu\text{m}$ .

Significant differences in surface properties of thylakoids from *B. daigremontianum* plant and plantlets were observed. Low ionic strength media (25 mM Hepes (KOH), 5 mM  $\text{MgCl}_2$ , pH 7.50) incubation led to a significant decrease of negative charges on the outer surface of thylakoid membranes. There were no changes in electrophoretic mobility (EPM), zeta potential ( $\zeta$ ) and surface electrical charge ( $\sigma$ ) of thylakoids in buffer solution at pH 6.0.

The surface charge effects and possible aggregation of thylakoids from *B. daigremontianum* plant and plantlets in buffered solution were discussed.

## **STUDY OF THE FUNCTIONING AND THE ACTIVITY OF THE BIOLOGICAL SYSTEM IN THE TECHNOLOGY FOR BIOGAS PRODUCTION OF WWTP "KUBRATOVO"**

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**Keywords:** biogas, anaerobic digestion, functional control, enzymes, fluorescence

The technologies for biogas production through the process of methanogenesis are widely distributed in Bulgaria and abroad. Several types of organic waste such as food biowaste, manure, silage, sludge, etc. could be recycled in these technologies. As well as that this kind of wastes serve as a valuable resource for the production of biogas and soil amendments.

The focus of this study has been on the functioning and the activity of the syntrophic and synergetic biological system performing the process of methanogenesis and on the necessity of implementation of new indicators for functional control in the technologies for biogas production. A comparison of the process of anaerobic digestion in WWTP "Kubratovo" (part of "Sofyiska voda" AD) in two different situations has been made – the first sampling was in the summer season in July 2017 and the second one was in the autumn season – in October 2017. The technology for biogas production in WWTP "Kubratovo" has been chosen for this study as properly working based on detailed SWOT and PEST analyses (described in a previous article) of eleven biogas installations in Bulgaria. WWTP "Kubratovo" operates four mesophilic digesters which use the sludge formed in the process of wastewater treatment as a substrate. The analyses have included key technological (dry matter, retention time, biogas yield, content of the methane in the biogas), chemical (pH, organic dry matter, chemical oxygen demand, ammonium and phosphorus ions) and enzymological parameters (aerobic and anaerobic dehydrogenase activities, phosphatase activity) of the process of methanogenesis. As well as that it has been made a fluorescent control directed to the seeking of correlations with the other analyzed parameters and to the development of a fluorescent bio-indicative system for control of the technologies for biogas production.

The results of this study have proved that the biological system in the digesters is working properly and that there is an effectively performing process of biogas production in WWTP "Kubratovo".

We express gratitude to the management team and the operators of WWTP "Kubratovo" for providing the samples from the anaerobic digesters for this study and for sharing data about the technological parameters of the process. This work has been financed by "Sofyiska voda" AD /project: Living water – river, dam, biodiversity, life/ and Scientific fund of Sofia University "St. Kliment Ohridski" /project: Functional control of the methanogenesis in technologies for biogas production/.

Focusing the control on the functioning and the activity of the biological system in the anaerobic digesters could increase the effectiveness and the efficiency of these technologies and improve the total performance of the process.

## **THE POSITIVE EFFECT OF RESVERATROL ON MOTILITY AND DNA FRAGMENTATION OF HUMAN SPERMATOZOA INDUCED BY CRYOPRESERVATION**

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**Keywords:** Resveratrol, human spermatozoa, cryopreservation

Freezing and thawing procedures of human spermatozoa trigger the excessive production of reaction oxygen species (ROS) that cause damage to spermatozoa. Resveratrol, a natural polyphenol, produced from several plants is being envisioned as a valuable antioxidant that prevents DNA damage. Furthermore, it could be regarded as a useful medium supplement for efficient cryopreservation of human spermatozoa.

The aim of this study was to analyze the effect of resveratrol supplementation on the motility and DNA fragmentation of human spermatozoa.

Semen was collected from 26 normozoospermic patients and divided into three aliquots prior to cryopreservation. The first aliquot was analyzed before the cryopreservation. The second (control) aliquot was mixed with SpermFreeze solution (Vitrolife). The last aliquot was mixed with the same solution and resveratrol (0.01 mMol). All samples were cryopreserved in nitrogen vapour after ten minutes incubation. After one week samples were thawed and sperm motion was measured by Mackler's camera and Olympus microscope. Using the Sperm Chromatin Structure Assay (SCSA), the number of DNA fragmented sperm, expressed as DNA fragmentation index (DFI) was evaluated in each sample.

The addition of 0.01 mM resveratrol into cryopreservation medium have lead to better motility in 81% of the studied patients. The number of progressive spermatozoa was higher with  $10 \pm 3\%$ . The impact of resveratrol on DNA fragmentation of investigated spermatozoa was relatively small and led to insignificant change in the DFI ( $3 \pm 0.5$ ). However, in 63% of the patients the addition of resveratrol had a positive effect and resulted in lower DFI in comparison with the control semen samples.

In conclusion, the supplementation of 0.01 mM resveratrol significantly improves the post-thawed human spermatozoa progressive motility and decreases the level of DNA fragmentation.

## **EFFECT ON REPLICATION CYCLE OF HHV-2 OF EXTRACTS FROM *STACHYS THRACICA* DAV. AND *TEUCRIUM CHAMAEDRYS* L.**

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**Keywords:** Human herpes virus type 2, HHV-2, *Theucrium Chamaedrys* L., *Stachys Thracica* Dav.

HHV-2 is a main causative agent of genital herpes, one of the most common, persistent and highly infectious sexually transmitted viral infection. The therapy is effective, but longer usage leads to a selection of drug-resistant strains, as well as the drug toxicity that hamper the treatment. That's why development of new antivirals is important question in virology field. Special attention is focused on compounds with natural origin (Plant extracts).

*Teucrium chamaedrys* L., known as germander, is a perennial herb, spread to Europe and South West Asia. It is used in folk medicine to treat dyspepsia, anorexia, nasal catarrh, chronic bronchitis, gout, rheumatoid arthritis, fever and uterine infections.

*Stachys Thracica* Dav. is Balkan endemic plant, with small areas in Bulgaria (in Strandzha mountain and on the Southern Black Sea coast) and Turkey. In folk medicine, the plant is known to be used for wound healing, treating of neuralgia, cephalalgia and other conditions associated with disorders of the central and peripheral nervous system.

The antiviral activity of four extracts from *Teucrium chamaedrys* L. and three extracts derived from *Stachys thracica* Dav were examined and important phytopharmacological characteristics have been determined. One extract derived from *Teucrium chamaedrys* L and one obtained from *Stachys thracica* Dav. show a decrease in the quantity of extracellular virions-decreasing titer of treated virus with approximately 1.5lg at 30 minutes of onset of the contact. The effect of the extracts on the replication of HHV-2, strain Ba was determined. One extract obtained from *Teucrium chamaedrys* L. influence viral cytopathic effect reaching ~66% inhibition of viral cytopathic effect.

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**COMPARATIVE ANALYSIS OF THE EFFECTIVENESS OF L-ARGININE AND Na-NITROPRUSSIDE ON CHOLINESTERASE ACTIVITY IN VERTEBRATE (ACETYLCHOLINESTERASE: AChE AND BUTYRYLCHOLINESTERASE: BChE) AND INVERTEBRATES (ACETYLCHLININGERASE: AChE) ANIMALS. THE ROLE OF THESE ENDOGENOUS AND EXOGENOUS SOURCES OF NITRIC OXIDE (NO) AS ANTIDOTES AND CHOLINESTERASE REACTIVATORS IN INTOXICATION WITH ANTICHOLINESTERASE AGENTS AND OTHER POISONOUS PRODUCTS**

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**Keywords:** L-Arginine, Na-Nitroprusside, Acetylcholinesterase, Butyrylcholinesterase, antidote, reactivator, Invertebrates and Vertebrates, comparative analysis

It was found that L-Arginine at concentrations of 1,0-50 mM activates significantly both enzymes (AChE and BChE) in vertebrates. This effect is tissue and species specific. As specific inhibitors of cholinesterases, are used substances from carbamate orders. In this case, L-Arginine plays the role of antidote against anticholinesterases and as a cholinesterase reactivator. Deserves attention a considerable stimulation on BChE, which leads to rapid hydrolysis of extra-synaptic acetylcholine, various other esters, narcotic agents and many others compaunds.

The role of L-Arginine on the activity of AChE in invertebrate animals is a powerful stimulator and reactivator in case of intoxication.

The mechanisms of influence of Na-Nitroprusside (0.01-10 mM) and various constituents of this preparation on AChE in invertebrates were analyzed by various model experiments.

It is believed that L-Arginine, in combination with other ingredients, may be an effective antidote for cholinesterase intoxication and an enzyme activity reactivator under ecotoxicological conditions

## **MOLECULAR BIOLOGICAL AND SEROLOGICAL STUDY OF CIRCULATING MEASLES VIRUSES IN BULGARIA IN 2017**

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**Keywords:** measles virus, RT-PCR, ELISA IgM/IgG

Measles is an acute illness caused by Measles virus (genus *Morbillivirus*, family *Paramyxoviridae*). The disease is transmitted via airborne respiratory droplets, or by direct contact with nasal and throat secretions of infected individuals.

The **aim** of this study was to determine of measles viruses circulation in Bulgaria in 2017.

The present study included 121 Bulgarian patients most of them (90/121, 74.38%) children aged between 0 and 14 years reported as possible measles cases for the period January 2017 - September 2017 from 11 regions of the country. Serum, urine and nasal swab samples from these persons were collected as clinical materials. The serological - indirect EIA test for detection of the specific IgM/IgG antibodies and molecular methods - extraction and detection of viral RNA were used.

From all serologically tested by ELISA serum samples in 59/121 (48.76%) were presented positive results for the specific measles IgM antibodies and in 73/121 (60.33%) - for the specific measles IgG, respectively. Viral RNA was extracted directly from the 41 urine and 66 nasal swab samples. A positive result was obtained in 29/41 (70.73%) of the urine samples and in 59/66 (83.33%) of the nasal swab samples. Three of laboratory confirmed samples (with positive results from the both methods – ELISA and One-Step RT-PCR) were successful sequenced and genotyped, as an evidence of a local or an imported measles infection. During the study period a circulation of one measles genotypes – B3 was confirmed.

## **ISOLATION OF MESOPHILIC IRON-OXIDIZING BACTERIA FROM A PYROMETALLURGICAL FINAL SLAG**

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**Keywords:** iron-oxidizing bacteria, chemolithotrophic bacteria, slag, *Acidithiobacillus*

Pyrometallurgical final slag from the copper smelting industry has the potential to be a valuable resource of different heavy metals. Metal recovery from sulphide minerals and slags is based on the activity of different chemolithotrophic bacteria mainly *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, which convert insoluble metal sulfides into soluble metal sulfates. The purpose of the research is the isolation and determination of different chemolithotrophic bacteria inhabiting pyrometallurgical copper final slag. Differential scheme, based on the growth requirements (pH, temperature, electron donor) was applied to samples of crushed copper slag. Six novel strains of obligately chemolithotrophic iron-oxidizing bacteria have been isolated from the collected samples. Conducted physiological and morphological analyses showed that these strains are related to mesophilic *Acidithiobacillus* species. Additionally, their taxonomic status was confirmed by PCR analysis.



## INFLUENCE OF COPPER IONS ON THE ACTIVITY OF *ACIDITHIOBACILLUS FERROOXIDANS* JCM 3863 BIOFILM IN AN AIRLIFT BIOREACTOR

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**Keywords:** *Acidithiobacillus ferrooxidans* JCM 3863, biofilm, copper ions, airlift bioreactor

*Acidithiobacillus ferrooxidans* is a genus of extremophilic bacteria used in various biotechnological processes, based on the ability of these bacteria to oxidize ferrous to ferric ions. This process plays an important role in biohydrometallurgy, environmental biotechnology, waste-gas treatment in particular, as well as in eliminating solid waste from industries containing heavy and toxic metals. Many achievements in the field of ecology and recycling of rare metals are related to studying the mechanisms of resistance of *Acidithiobacillus ferrooxidans* to heavy metals such as copper, chromium, nickel. The presence of copper in almost all of the industrial processes requires a study of the behavior and adaptation of the bacteria to the higher concentrations of the metal. The purpose of the experiment is to observe the influence of high concentrations of copper ions on the activity of *Acidithiobacillus ferrooxidans* JCM 3863 biofilm, cultivated in an airlift bioreactor. The research describes the vital role these resistance mechanisms have on the survival of acidophilic bacteria, as well as on the intensity of the ferrous ions biooxidation. The presence of copper ions in the media leads to adaptation processes and changes in the physiology of *At. ferrooxidans*. The formation of additional biofilm structures on the inner surface of the bioreactor leads to a significant increase in the rate of the ferrous ions biooxidation process. The high concentrations of copper ions, however, have minor influence on the growth of the biofilm – they slow down the process of precipitation of jarosite, which stabilizes the bacterial biofilm structure.

## **COMPARATIVE ANALYSIS OF EFFECTS OF LACTONES AND POLYPHENOLS FROM INULA OCLUS-CRISTI ON NORMAL AND CANCER CELLS**

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Plant extracts are widely used in traditional medicine for centuries. Experimental research findings indicate the anti-tumor effect of certain components of extracts, from some herbs from genus *Inula*.

Our previous investigations revealed different effects of total extracts from *Inula oculus-cristi* on cancer and non-cancer cells. Recent efforts of our group are to determine those of the extracts' components, responsible for observed differences.

The aim of the present study was to establish the effects of extracts with high concentration of lactones or polyphenols (phenolic acids and flavonoids). As a model systems we used non-cancer MDCKII cell line and cancer A549 cell line.

Cytotoxicity was measured spectrophotometrically by crystal violet assay and the IC<sub>50</sub> (inhibition concentration) for both extracts on both cell lines was determined. Brightfield microscopy was used to observe changes in cell morphology.

Our results indicate stronger impacts of studied extracts on cancer cells. For non-cancer cells IC<sub>50</sub> was higher and the observed morphological changes after treatment were milder, in comparison to cancer cells. The extract enriched with lactones showed stronger cytotoxic effect.

## **THE EFFECT OF POLYPHENOLS ON ACTIN CYTOSKELETON OF EPITHELIAL CELLS**

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**Keywords:** actin cytoskeleton, phenolic acids, flavonoid glycosides, *Inula oculus-christy*

Actin cytoskeleton is essential for various functional activities of cells, such as migration, adhesion and others, that are fundamental for the survival and proliferation of cancer cells. The regulation of these parameters is an approach in the development of new anti-cancer strategies for treatment and is of pharmacological interest. Besides cytostatic treatment, in order to prevent metastasis, cell migration should be suppressed.

We compared the activity of extract from *Inula oculus-christy*, enriched with phenolic acids and another in combination with flavonoid glycosides on actin cytoskeleton.

The present study aimed to explore how extracts affect actin cytoskeleton in cancer (A549) and non-cancer (MDCK) cells. We performed TRITC-phalloidin staining to visualize polymeric actin (F actin).

We found that both extracts affect differently cancer and non cancer cells.

## **THE INDIVIDUAL CAPACITY FOR REPAIR OF DNA DAMAGE MAY BE AN INDEPENDENT FACTOR DETERMINING THE POTENTIAL USES OF HUMAN STEM CELL LINES**

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**Keywords:** DNA repair, individual repair capacity, risk, in vitro culturing, aging

A single type of stem cells (haematopoietic stem cells) is currently routinely used in clinical medicine for etiological treatment of blood disorders and metabolic disease. Several other types of preparations are undergoing trials for potential use in clinical applications. The methodologies for derivation, maintenance and expansion of stem cells and targeted differentiation of multipotent and pluripotent stem cells have seen great development in the past several years. A number of safety concerns related to the use of cell and tissue preparations have recently arisen with regards to the potential for changes that may occur in in vitro propagated stem cells and cell-derived products and the harmful effects that may ensue for the recipient. Individual DNA repair capacity (IRC) is a major factor determining the scope of genomic changes in the course of normal and pathological ageing. IRC may vary significantly between different individuals. The effects of these differences, however, may only become apparent with age or under significant genotoxic stress. To date, the number of studies about the role of subtle variations of IRC in the maintenance in vitro of stem cell lines is quite limited. We hereby review the available data about the role of IRC in different types of stem cells and contemplate the potential adverse effects that may result from use of cell preparations with lower-than-average capacity for repair of genotoxic damage and maintenance of genomic integrity.

## **UTILIZATION OF EXTRACELLULAR PROTEINS FROM CUMULUS-OOCYTE COMPLEX FOR SELECTION OF HUMAN SPERMATOZOA**

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**Keywords:** Cumulus complex, sperm selection, hyaluronidase

Cumulus matrix is one of the natural selective barriers in front of the spermatozoa before fertilization. Providing natural ligands from cumulus matrix to human spermatozoa ensures better selection conditions closest to "natural" ones. Nowadays, there are selective tests, based on this hypothesis, such as hyaluronan binding assay, that are already routinely applied in many IVF clinics. However, the development of new methods for functional selection of spermatozoa based on the presence of specific membrane receptors is needed for IVF success improvement.

In this study a strategy was developed in order to develop a new test for selection of human spermatozoa, based on their adhesion abilities onto protein layer of extracellular ligands. The ultimate step was the choice of enzyme for disintegration of cumulus complex among four candidates: trypsin, collagenase I, hyaluronidase and accutase.

Twenty two semen samples from fertile men with normozoospermia and twenty two aggregates from cumulus cells from donors were used. Sperm was prepared by centrifugation and swim-up. The aggregates of cumulus cells were treated separately with trypsin (Genaxxon, Cat. No. C4287), hyaluronidase (Origio, Cat. No. ART-4007-A), collagenase I (Genaxxon, Cat. No. C4255) or accutase (PAA, Cat. No. L11-007) until their disintegration. The isolated cumulus matrix fragments were coated onto plates (VWR 734-2324) as drops and dried on room temperature. The effectiveness of each enzyme treatment was assessed by counting the number of the attached spermatozoa on the protein layer.

The percentage of attached spermatozoa was significantly higher in the samples treated with hyaluronidase ( $0.86 \pm 0.12$  cells/ $\mu^2$ ) in comparison with those treated with trypsin ( $0.05 \pm 0.02$  cells/ $\mu^2$ ), collagenase I ( $0.39 \pm 0.06$  cells/ $\mu^2$ ) and accutase ( $0.64 \pm 0.24$  cells/ $\mu^2$ )

The obtained results indicate that hyaluronidase is the most suitable enzyme among the investigated group for treatment of cumulus complex. This leads to an effective isolation of cumulus matrix fragments that can be coated as a layer for the adhesion and selection of human spermatozoa.

## **HOW DOES RESVERATROL TUNE THE MEMBRANE FLUIDITY AND DOMAIN ORGANIZATION AS A FUNCTION OF LIPID FATTY ACID UNSATURATION**

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**Keywords:** resveratrol, lipid raft, docosahexaenoic acid, oleic acid, lipid ordering, TEMPO, DPH

Resveratrol (RV) possesses great number of beneficial biological effects (anti-oxidant, anti-inflammatory and anti-carcinogenic properties) but the mechanism of its action remains unclear. It is believed that these pleiotropic effects are the result from the interaction of the lipid bilayer with RV. Our interest is directed to lipid rafts, particular membrane microdomains, enriched in sphingolipids, cholesterol and specialized proteins. Lipid rafts take part in many cellular processes. Three component vesicles composed of palmitoyl- docosahexaenoyl phosphatidylcholine (PDPC) or palmitoyl-oleoyl phosphatidylcholine (POPC), egg sphingomyelin (eggSM) and cholesterol (Chol) were used to mimic the cellular rafts. POPC and PDPC were chosen to probe the degree of fatty acid unsaturation at *sn*-2 position of phosphatidylcholine. Both DPH fluorescent anisotropy and DPH-Tempo quenching method were used to monitor RV effect on the fluidity and the raft formation. We found that RV exhibits complex behavior depending on the SM/Ch ratio, degree of fatty acid unsaturation as well as RV/lipid ratio. RV is able to increase the raft fraction in monounsaturated PC ternary mixture at 2/1 SM/Ch ratio and to decrease it at 1/1 SM/Ch. In polyunsaturated PC ternary mixtures, the general trend of RV is to inhibit raft formation. Lipid/RV ratio is essential for the observed phenomena. Since RV changes physicochemical features of lipid bilayer in different ways it might be suggested that one of the roles of this compound is to tune the domain organization and fluidity in the membranes.

## **SOLID- STATE FERMENTATION OF *L.BULGARICUS* STRAINS INTO NON-MILK MEDIA**

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**Keywords:** non-milk media, *L.bulgaricus*

The interest in fermented non-milk foods is constantly increasing .This is scientifically based on the healthy claims of their consumption.

A number of fermented functional foods on the milk base in the presence of *L. bulgaricus* have been developed.

In the present study, the influence of carrot and tomato juice , gluten-free flours and plant proteins as a components of non-milk media on the growth of 3 selected *L.bulgaricus* strains with high technological and probiotic potential was studied. The non-milk media inoculated with selected *L.bulgaricus* strains were incubated at different temperatures for the solid state fermentation. The change in pH, acidity, the number of lactic acid bacteria were measured. The survival of *L.bulgaricus* strains during freeze-dried process was studied.

## **INTEGRAL CONTROL OF WASTEWATER TREATMENT WITH TECHNOLOGICAL SCHEME DENITRIFICATION-NITRIFICATION-BIOLOGICAL PHOSPHORUS REMOVAL IN WWTP "KUBRATOVO"**

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**Keywords:** activated sludge, wastewater, DNBPR, biodegradation, purification efficiency

Control is a set of actions related to monitoring, condition assessment and recording deviations from the desired course of the processes, if available. The high quality of the purification processes is unthinkable without the use of means of control. The importance of decoding regulatory mechanisms in the biodegradation process is undeniable, because this would make it possible to choose the optimal solution in case of a risk situation or a technological problem.

The purpose of the study was to assess the integrated control of the wastewater treatment processes at the WWTP "Kubratovo", with the contemporary scheme of operation of denitrification-nitrification-biological phosphorus removal during the winter months January and February of 2017. At this time of the year the temperatures are lower, which implies a slower performance of biological processes and respectively difficult wastewater purification. The wastewater was tested by means of chemical (COD, BOD<sub>5</sub>, ammonia, nitrates, nitrites, and phosphates) and technological indicators (effectiveness of purification) at three critical control points of the wastewater treatment process - influent wastewater: at the entrance of the WWTP, effluent water from primary clarification and effluent water at the exit of the WWTP. Activated sludge from the denitrification zone of one of the biobasins was tested for indicative for the purification processes groups of microorganisms (the amount of aerobic and anaerobic heterotrophs at 30°C, denitrifying and nitrifying microorganisms). The activated sludge was also observed by microscopic analysis and the following indicators have been evaluated - the quantity of protozoan and metazoan fauna, structure, form and size of flocs.

It was determined from the research that low temperatures did not adversely affect the effectiveness of purification according to the indicators COD (92 %), BOD<sub>5</sub> (93 %), ammonia (56 %), phosphates (14 %). However, there is an accumulation of oxidized forms of nitrogen (nitrates and nitrites) in the wastewater treatment system. The activated sludge was well colonized ( $\geq 10^6$  individuals/l) and had a large number of taxonomic units ( $> 10$  taxa of indicatory fauna). All this affirms that the purification process was within the provided limits and the set of methods and control indicators were appropriately selected.

**Acknowledgments:** *The present study was financially supported by the Project: "Alive Water," a joint project of the Department of General and Applied Hydrobiology of the Faculty of Biology - Sofia University "St. Kliment Ohridski" and "Softyska Voda" AD, operated by Veolia.*



## **AGE EFFECTS ON THE STRESS RESPONSE OF TWO ECOTYPES OF PLATAN (*PLATANUS ORIENTALIS*) SUBJECTED TO ELEVATED TEMPERATURE**

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**Keywords:** age effects, *Platanus orientalis*, chlorophyll fluorescence, temperature stress

When subjected to unfavorable changes in the environment, plants develop different adaptive mechanisms in order to achieve the highest productivity possible for the circumstances. In nature there are different factors that influence the way plants react to stimuli. Age has some of the most significant impacts on plants ability to overcome a stressful condition. Thus it is important to trace the differences in the response to environmental changes in plants of different age.

The photosynthetic machinery is very sensitive to changes in plants physiological state. The best noninvasive *in vivo* method for detection of the changes in the photosynthetic characteristics is measuring the chlorophyll a fluorescence.

In our study we follow the differences of the stress response of *Platanus orientalis* plants from two ecotypes (Bulgarian and Italian) between leaves of different age when subjected to elevated temperatures (41°C) for 4 hours in 3 days, the next 3 days recovery is following. The chlorophyll fluorescence data showed significant differences in the plants reaction to the temperature stress depending on the age of the leaves.

**Acknowledgement:** *We are grateful to Bulgarian National Science Fund (Project № DFNI B02/8) for financial support.*

## **EFFECTS OF GOLD-CONTAINING NANOPARTICLES ON METABOLIC ACTIVITY AND PLASMA MEMBRANE OF EUKARIOTIC CELLS**

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**Keywords:** gold-containing polymer nanoparticles, A549, HepG2

Synthetic systems, that could transport biologically active molecules through cell membranes, must meet a number of requirements: low cytotoxicity, stability, not affecting metabolic activity, cell structure, morphology, integrity and connectivity of the cells.

Nanoparticles, made of cationic polymers, have good biocompatibility, versatility, and controllable molecular size. Cationic polymers are widely used as carrier of DNA molecules displaying enhanced binding and recognition. The effectiveness of cell uptake and delivery of these materials principally depends on DNA and polymer concentrations, their size and structure.

The aim of this study is to explore the effects of gold-containing nanoparticles on metabolic activity and plasma membrane integrity of two human cells lines- A549 and HepG2.

We used nanoparticles of poly(vinyl butyl trimethyl ammonium chloride) (Homo) and block copolymer of poly(vinyl butyl trimethyl ammonium chloride) and oligoethylene glycol metacrylate (Block), containing gold. The working concentrations of nanoparticles contain 0.2 µg/ml DNA.

We examined the metabolic activity of treated cells by using MTT assay. The effect of nanoparticles on plasma membrane was studied by staining the treated cells with Trypan blue.

## **IMPACT OF STATE EPIDEMIOLOGICAL CONTROL MECHANISMS OVER THE INCIDENCE OF VARICELLA IN THE ORGANIZED GROUPS OF CHILDREN IN SOFIA FOR 2014-2016 PERIOD**

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**Keywords:** Varicella, epidemiology, kindergartens

Varicella is currently the most common infectious disease under epidemiological surveillance in Bulgaria (Analysis of Infectious Diseases, NCIPD 2014-2016). On a national scale, the number of cases has steadily increased over the studied period. For the prevalence of the disease of great importance are the childcare facilities in which the transmission of the infection is often carried out (85-87% of all cases), and where anti-epidemic measures have been taken to limit it.

The study was conducted on the basis of epidemiological and statistical data on the disease incidence derived from RHIS (Regional Health Inspectorate Sofia) from the infected population in Sofia.

Data show region specificity in the disease incidence with more cases in the heavily populated districts with fewer stand-alone kindergartens. This leads to the conclusion that the density of the groups in child care facilities is the main factor contributing to the spread of the infection.

The control over the facilities by the state institutions shows influence over the epidemics of the disease. Where the varicella epidemic progresses in the country as a whole – in the city of Sofia it is damped and rise with more than 26% of the number of inspections is observed.

## **OPTIMIZATION OF GROWTH MEDIUM FOR HIGH CELL DENSITY CULTIVATION OF RHIZOSPHERIC *PSEUDOMONAS* STRAINS**

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**Keywords:** *Pseudomonas*, high cell density cultivation, PGPR

*Pseudomonas* species are one of the major constituents of rhizobacteria in soil. In this work are studied five strains of genus *Pseudomonas* which were previously identified as *Pseudomonas putida Or2*, *Pseudomonas putida Or5*, *Pseudomonas putida 1046*, *Pseudomonas chlororaphis 1S4* and *Pseudomonas stutzeri Rsb 22*. The main objective was to develop new broth media model for the cultivation of the selected *Pseudomonas* strains to achieve high cell biomass and productivity. Many industrial fermentation processes require rapid growth to a high cell density. Batch cultivation processes are considered to be the most efficient way of achieving high-cell density cultures but depend on suitable substrates. For investigation of the growth characteristics of all strains were used three types of broth media. To observe the growth profiles of the selected strains they were cultivated at  $29\pm 1^{\circ}\text{C}$  and 250 rpm. Samples were analyzed for CFU/ml, assimilation of glucose, and total protein concentration in the culture broth. pH was measured without correction. All of the tested *Pseudomonas* strains assimilate glucose as carbon source. During the 24h of batch cultivation the strains assimilate almost 60–70% of the initial glucose concentration. The results showed that nutrient media P1 was not suitable for cultivation of selected *Pseudomonas* strains because they did not assimilate the disaccharide sucrose. The dynamics of the bacterial growth observed typical curve for nutrient media P2 and P3 for all of the tested strains. For *Ps. putida 1046*, *Ps. putida Or2* and *Ps. chlororaphis 1S4* was detected better growth characteristics at nutrient media P3.

## **MEASUREMENT OF SERUM PROGESTERONE LEVELS ON THE DAY OF EMBRYO TRANSFER IS A USEFUL TOOL IN PREDICTION OF SUCCESSFUL PREGNANCY**

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**Keywords:** Progesterone, predictor, pregnancy, single frozen embryo transfer

**Study question:** Does progesterone levels measured on the day of single frozen embryo transfer (SFET) in natural cycle could be used as a predictor for IVF outcome?

**Summary answer:** Serum progesterone measured on the day of SFET is a valuable predictor for successful pregnancy. Concentrations below 14.4 ng/ml are associated with worse pregnancy outcomes.

Progesterone plays a key role in the establishment of endometrial receptivity and embryo implantation. There are many reports in the literature suggesting specific cut-off values for ovulatory progesterone in natural cycles as a criterion for ovulation. Also, it is well known that elevated progesterone levels on the day of hCG administration in stimulated cycles is associated with significantly decreased pregnancy rates. Furthermore, low progesterone concentrations after embryo implantation correlate with higher risk of miscarriage or ectopic pregnancy. However, knowledge on the prognostic significance of serum progesterone on the day of embryo transfer in natural cycle is still scarce.

A single center prospective study of single frozen embryo transfer (SFET) was performed during the period October 2015 – November 2016 with 252 women undergoing ICSI cycles. In all cases a blastocyst stage ET was done five days after LH surge in normal menstrual cycle. Inclusion criteria were women with age < 42 yrs, basal FSH ≤ 12 mIU/l, regular menstrual cycle and BMI: 18–28 kg/m<sup>2</sup>. Progesterone concentrations were measured on the day of SFET and pregnancy test (bhCG) was done 14 days later. Receiver Operator Characteristic curve (ROC) was used to evaluate the predictive value of serum progesterone for discriminating women with successful pregnancy and to delineate the optimal cut-off value. All statistical analyses were carried out using SPSS-21 software.

Successful implantation registered by bhCG test occurred in 44.8% of the patients. There were no significant differences in age, FSH, BMI and quality of the transferred embryos between pregnant and nonpregnant women. Mean serum progesterone levels were significantly lower in women with negative bhCG-test compared with those with positive pregnancy test (20.08 ng/ml ± 9.44 vs. 27.9 ng/ml ± 10.52 respectively (p < 0.034)). At cut-off value of 14.40 ng/ml, progesterone had 88% sensitivity and 75% specificity. The area under the ROC curve for progesterone was 0.67 (95% Confidence Interval, 0.52-0.89).

In conclusion, these findings indicate that serum progesterone measured on the day of embryo transfer could be used for prediction of successful pregnancy.

## **STUDY THE EFFECT OF ERUFOSINE ON APOPTOSIS AND MIGRATION OF CANCER CELLS**

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**Keywords:** Erufosine, apoptosis, migration, breast cancer cells

We investigated the anticancer activity of erufosine (erucylphospho-N,N,N,-trimethylpropylammonium, ErPC3) on the triple negative breast cancer cell line MDA-MB 231 in terms of induction of apoptosis and inhibition of cell migration. The induced cell death was revealed by using acridine orange/ethidium bromide (AO/EtBr) live cell staining and flow cytometry assay. The effect on cell migration was evaluated by wound-healing assay. Our data pointed out that the treatment with Erufosine caused an increase of early- and late-stage apoptotic cells and polyploid cells, which are sign for mitotic catastrophe. The prolonged treatment with Erufosine did cells more sensitive and the apoptosis was visible at lower drug concentration. In the same time the anti-tumor agent caused slower closure of "wound" comparing to the untreated control cells.

Taken together, our results strongly suggest that erufosine have dose- and time-dependent inhibitory effects on a cell death and migration and may be effective as anti-tumor agent against breast cancer.

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## **CANINE SEMINAL PLASMA PROTEINS**

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**Keywords:** canine, proteins, progressiveness, vigour

In the recent years the seminal plasma proteins are a subject of a significant interest. The data show that the proteins of the seminal plasma have a variety of actions and important function regarding significant processes. Some seminal plasma proteins are responsible for fertilization, acrosome reaction, capacitation, reactions of the female immune system and other prevent the oxidative stress or protect the sperm cells during cryopreservation. This research observe certain proteins present in canine seminal plasma. Using CASA assay the present study demonstrated that the presence of a certain seminal plasma proteins are involved in enhancing sperm vigour and progressiveness in *in vitro* capacitation conditions. The influence of the proteins contained in seminal plasma are still not fully understood, and are also a subject of future researches, regarding the fertilization ability of spermatozoa.

## **BIOCHEMICAL ANALYSIS OF THE INTERACTION OF HUMAN C1q WITH PHOSPHATIDYLSERINE**

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**Keywords:** C1q, phosphatidylserine, globular heads

C1q, the recognition molecule of classical complement pathway, specifically targets phosphatidylserine (PS) from the membranes of early apoptotic cells via its globular domains. The interaction of C1q with PS is being intensively studied with respect to the contribution of each of the globular fragments ghA, ghB and ghC in forming the PS-binding site of gC1q. The importance of this interaction is stressed by the suggestion that impairment of apoptotic clearance is associated with development of autoimmune disorders like SLE. Given the contribution of all globular fragments is known, it will be possible to analyse the possible overlapping between C1q autoepitope(s) and C1q binding site for PS. We developed a model ELISA system with immobilized DOPS (Dioleoyl phosphatidylserine) to study the interaction of C1q with PS. We registered a dose-dependent binding of C1q. We also found that the interaction of C1q with PS is Ca<sup>2+</sup> dependent. Using this model ELISA system we registered dose-dependent binding of immobilized DOPS with ghA and ghB of C1q. Given that ghA and ghB expose autoepitopes for the anti-C1q autoantibodies our data suggests that C1q via its globular domain can be a mediator between apoptosis and autoimmunity.

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## **THE ROLE OF EXONUCLEASE VII FROM *E. COLI* IN PROCESSING DNA GYRASE-DNA COMPLEXES**

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**Keywords:** *E. coli*, DNA topoisomerases, DNA gyrase, exonuclease VII, quinolone antibiotics, supercoiling, cleavage

DNA in the cell can exist in many different topological forms and the interconversions between them are catalyzed by topoisomerases. Due to their essentiality, they have become very successful targets for antibiotics. The action of the quinolone drugs, such as ciprofloxacin, involves the stabilisation of a DNA-protein complex formed between the target enzyme (DNA gyrase) and bacterial DNA. This event ultimately leads to cell death but the mechanism is still unclear. In this study, the role of Exonuclease VII (ExoVII) from *E. coli* in processing DNA gyrase-DNA complexes was examined. Genetic evidence shows that mutants in both subunits of the enzyme are hypersensitive to quinolone antibiotics which suggests the involvement of the enzyme in processing these complexes. Different types of experiments were set in various conditions to see if the enzyme has any effect on the supercoiling and cleavage reactions. In addition to that, we purified our own exonuclease VII and proved its catalytic activity by using lambda DNA-based assays. Eventually, we demonstrated that the addition of ExoVII (commercial or "homemade") to the gyrase cleavage reaction had little effect on it but further work is required to explore this.

## **DETECTION OF ENZYME ACTIVITIES OF STRAINS FROM GENUS *BACILLUS* AND GENUS *LACTOBACILLUS* AND STUDY OF THEIR EFFECTS ON THE *OCIMUM BASILICUM*, *IN VIVO***

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**Key words:** *Bacillus*, *Lactobacillus*, *Ocimum basilicum*, *in vivo*, API ZYM™

Some of *Lactobacillus* strains form biofilm on different parts of plants, synthesize biologically active substances and have antagonistic effect against phytopathogenic microorganisms in the soil. Strains of genus *Bacillus* have proven phosphate solubilizing and antimicrobial activity. Accordingly, strains of both genera favor the growth and development of plant species and have a high ecological, economic and agricultural importance. In the current work, four newly isolated strains – two from genus *Bacillus* (PB1 and PD1) (unpublished data) and two from genus *Lactobacillus* (ML1 and ML2) (unpublished data) - are tested for their enzyme activities by semi-quantitative micromethod API ZYM™. The same strains were tested for their effect on the growth and development of the *Ocimum basilicum* plant, *in vivo*.

The results of the study with API ZYM™ indicate the presence of leucine and valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase for strains of genus *Lactobacillus*. For PB1 strain, enzymes like alkaline phosphatase, esterase, esterase lipase and naphthol-AS-BI-phosphohydrolase are detected, while for the PD1, the present enzymes are esterase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase.

For all studied strains, tests on *Ocimum basilicum* show an increase in the number of branches (ML1 –30,8%; ML2 –34,6%; PB1 –15,4%; PD1-19,2%) in test plants compared to control ones. Results also show an increase in the number of flowers and buttons for plants treated with strains ML1 (26,7%) and ML2 (15%), an elevation of stems for ML1 (4,8%), ML2 (6,1%) and PD1 (12,9%), increased fresh biomass for ML2 (15,9%), PB1 (5,7%) and PD1 (36,6%) and dry biomass for ML1 (5,4%) and ML2 (6,9%).

From the data obtained it can be concluded that the strains tested from genus *Bacillus* and genus *Lactobacillus* have the potential to be applied as components of biological fertilizers aiming to increase the bioproduction.

## **PROTEINS FIBROIN AND SERICIN IN SILKWORMS "BOMBYX MORI"**

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**Keywords:** Bombyx mori, silkworms, fibroin, sericin

The silkworm *Bombyx mori*, part of genus *Bombyx*, family *Bombycidae*, is one of the most valuable and well-known species from Order *Lepidoptera*, having been used by man since ancient times for the production of silk. Nowadays, this species is still of particular interest for many researchers of different fields. The two main constituent proteins of silk – fibroin and sericin, account for 70-80% and 20-30%, respectively, of silk composition. Fibroin builds the structural center of silk, whereas sericin is the gum coating the fibers and allowing them to stick to each other. The two proteins have remarkable physicochemical and biochemical properties, which determine their various biomedical and pharmaceutical applications reported in recent years.

## **PHARMACOKINETIC RELEASE OF ERUFOSINE FROM NEW CRYOGELS BASED ON POLYCAPROLACTONE/POLY (ETHYLENE OXIDE) POLYMERS**

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**Keywords:** Erufosine, PCL/PEO biodegradable cryogels, drug release, Graffi myeloid tumor

The aim of the study was to test novel macro-porous polycaprolactone (PCL)/poly (ethylene oxide) PEO cryogels as potential carries of new anti-tumor agent Erufosine (EPC3) under controlled conditions of polymer degradation. Alkylphosphocholine EPC3 represent promising anti-neoplastic drug that induce cell death in tumor cells by primary interaction with the cell membrane. Light microscopy was used to detect the cytotoxic effect of EPC3-loaded scaffolds. To study the kinetics of EPC3 release from the co-polymers was used chromatographic separation followed by tandem-mass spectroscopy. Finally, in *in vivo* conditions, PCL/PEO carriers were tested for effect on biometric parameters such as tumor growth, lethality and mean survival time.

The microscopic observations showed cell degradation after 4 h of incubation duo to the high release of EPC3 from the scaffold. In vitro tests demonstrated release of EPC3 at therapeutic doses (5.6 mg/ml) after 6 h, leading to cell death. Intratumorally implanted PCL/PEO/EPC3 exhibited a temporary positive effect on the biometric parameters of tumor growth - increased survival, prolonged average survival time and decreased lethality rate. Even better results were achieved when PCL/PEO/EPC3 was implanted into the operative field after surgical tumor extirpation.

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## **ANALISYS OF THE COMPOSITION OF LACTIC ACID MICROBIOTA OF THE SELECTED PROBIOTIC PRODUCTS**

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**Keywords:** probiotic products, lactic acid bacteria, phenotypic characterization,  
16S-23S rDNA

One of the approaches to health care and disease control is the use of bacterial and other nutritional supplements that have a beneficial effect on the human gastrointestinal tract and prevent the entry of pathogens.

The probiotics, which are the subject of this study, are selected after an end-user study of their consumption and include products for both children and adults.

Our studies on the composition of the ten selected probiotics indicated the inclusion of different types of lactic acid bacteria. The analyzes performed established the content of 1-2 to 7 different types of lactic acid bacteria: (*L. bulgaricus*, *L. acidophilus*, *S. thermophilus*, *L. lactis*, *L. casei*, *E. faecium*, *B. coagulans*, *Bifidobacterium breve*, *Bifidobacterium longum*, *B. bifidum*, *L. helveticus*, *L. reuteri*).

The quantitative microbiological analysis for the presence of lactobacilli, lactococci and enterococci in the samples showed a large difference (from two to six orders of magnitude) between the experimentally determined live bacteria concentrations and those specified in the technical characteristics.

The species identification of the isolated strains was determined by phenotypic characterization including morphological and physiological methods as well as genotyping.

The molecular typing of the strains was based on the polymorphism at the length of the 16S-23S rDNA intergenic regions and the number of ribosomal operons.

## ANTIBACTERIAL EFFECT OF THIN TiO<sub>2</sub>:Ag:Cu FILM

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**Keywords:** nanomaterials, thin films, method, bacteria

Infections caused by medical devices are a significant social and economic problem. Rapidly developing resistance to traditionally used antibiotics makes the search for new antimicrobial materials a serious contemporary challenge. Recently, the use of nanoparticles and nanomaterials for biomedical research, such as Cu, TiO<sub>2</sub>, Ag, etc., has gained more attention due to their unique properties. Nanomaterials are chemicals or materials with a minimum one dimension of 1 to 100 nm. The thin TiO<sub>2</sub>:Ag:Cu films are made by magnetron sputtering, under different deposition conditions and with different technological characteristics. They have been tested on several bacteria with different cell wall structure: *Pseudomonas putida*, *Salmonella enterica*, *Bacillus cereus*, *Staphylococcus epidermidis*, *E. coli*, arranged in order of sensitivity to the film. Inhibition in dynamics is determined by the Koch method and corresponding measurements of the optical density. Both methods used showed a similar growth curve.

The stability of the films will be the challenge in their medical practice applications

## **CYTOTOXICITY OF VIPOXIN AND ITS COMPONENTS ON RPE CELLS**

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**Keywords:** cytotoxicity, phospholipase A2, vipoxin, retinal pigment epithelium

Vipoxin is the main heterodimeric neurotoxic component, isolated from the venom of *Vipera ammodytes meridionalis*, which is composed of a basic and toxic secreted PLA<sub>2</sub> enzyme subunit (sPLA<sub>2</sub>, GIIA Asp49, designated as vipoxin basic component, VBC) and an acidic, non-catalytic and non-toxic subunit - vipoxin acidic component (VAC).

Secreted Phospholipases A<sub>2</sub> ( EC.3.1.1.4) catalyze the hydrolysis of the acyl ester bond at the sn-2 position of membrane phospholipids in a Ca<sup>2+</sup> - dependent manner, liberating free fatty acids and lysophospholipids as products (secondary messengers with a great impact on cell signaling). sPLA<sub>2</sub>s play important physiological roles in remodeling of cell membranes, cell proliferation, survival and gene expression. The toxic members of the GII group possess various pharmacological activities provoking neurotoxicity, myotoxicity, cardiotoxicity, nephrotoxicity, hepatotoxicity, platelet aggregation, inflammation, temporary blindness, etc., as well as pathological conditions as rheumatism, osteoarthritis, asthma, and psoriasis, which mechanism is still under debate.

The aim of this study is to determine the cytotoxicity of vipoxin and its components on two epithelial cell lines - RPE-1 and ARPE-19, used as model systems in order to elucidate RPE response to neurotoxic sPLA<sub>2</sub>, manifested by temporary blindness.

Retinal pigment epithelium (RPE) is a layer of single cells outside the neurosensory retina with indispensable and significant roles in light absorption, epithelial transport in retina, visual cycle of the photoreceptors, phagocytosis and secretion.

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## **INFLUENCE OF SOIL ORGANIC MATTER ON THE WATER-SOLUBLE FORMS OF RADIONUCLIDES IN DIFFERENT SOIL TYPES**

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**Keywords:** radioactive isotopes, soil organic matter, mobile forms, gamma-spectrometry

Studies of on the mobile forms of radioactive isotopes, released in different soils after nuclear accident are of significant importance to predict their transfer to the vegetation and further migration within the food chains. This study presents the influence of soil organic matter on the water-soluble forms of  $^{241}\text{Am}$ ,  $^{60}\text{Co}$ ,  $^{137}\text{Cs}$ , U and  $^{234}\text{Th}$  in six different soils in Bulgaria. The investigation aimed to examine the migration ability of the radionuclides in soils with removed organic substances and in untreated soils, stored at 18 °C up to 3 weeks after radioactive contamination. The experiment was carried out by Chromic cambisol, Eutric fluvisol, two Calcaric chernozem soils, Gleyic fluvisol and Vertisol, taken from the surface soil layer 0-10 cm. The initial soil samples did not contain radionuclides and were contaminated by radioactive solution in the laboratory. The soil organic matter was removed from aliquot of the samples by treatment with solution of NaClO and heating at 96 °C. The radioactivity of solid samples and leachates was measured by gamma-spectrometry. The results showed that the retention of Uranium in the soils is not influenced by organic matter content in the soil with low clay content, while removing organic matter from the soil with high clay led to increase of uranium retention. Thorium was found to be retained better by the soils, containing organic matter, compared to those with removed organics. The retention of Am in the soils is not influenced by the organic matter content in all the studied soils. The retention of  $^{137}\text{Cs}$  in the soils with high cation exchange capacity (CEC) is not influenced by the organic matter content, while removing organic matter from the soils with low CEC caused decrease of its retention. Further studies in this field would clarify the impact of organic matter content in soils with different characteristics on the retention and immobilization of radionuclides in case of radioactive contamination.



## **A QUANTIFICATION OF DISPERSING WATER BUGS (HEMIPTERA, HETEROPTERA: NEPOMORPHA) IN SREBARNA NATURE RESERVE (BULGARIA)**

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**Keywords:** Corixidae, light trap, UV, sex-ratio, wetland

True water bugs (Nepomorpha) are important prey for fish, amphibians and birds. On the other hand, water bugs are top predators on a wide range of smaller invertebrate organisms. As predators they help in disease vectors' control by feeding on the larvae of *Anopheles*, *Culex*, *Aedes* mosquitoes and *Biomphalaria* snails. Despite the significant role of water bugs in aquatic food webs, studies on the population dynamics of this insect group at a landscape scale and related effects on the rest of the aquatic community are scarce. Many water bugs are active flyers. However, there is lack of data about the dispersal activity of most of the species. We used a common light trap with an ultraviolet light bulb, close to a wetland (Srebarna Lake, Bulgaria) to quantify water bugs dispersal. We evaluated the sex ratio of the dispersing water bugs and studied the influence of weather variables (air temperature, atmospheric pressure, wind velocity) on the water bugs' flight activity.

## **NEW DATA ON RANGE EXPANSION OF ROUND GOBY *NEOGOBIUS MELANOSTOMUS* (PISCES: GOBIDAE) IN THE BULGARIAN TRIBUTARIES OF LOWER DANUBE RIVER BASIN**

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**Key words:** *Neogobius melanostomus* , range expansion, Danube tributaries, Bulgaria

The round goby *Neogobius melanostomus* (Pallas, 1814) is fish species native to the Ponto Caspian region and invasive for most of Europe, performing rapid expansions upstream of the Danube River. The aim of the current study is to investigate the distribution of the species in Bulgarian tributaries of Danube River. The surveyed included a total of 27 sites from 12 rivers. Fish presents were detected by electrofishing.

A clear tendency for expansion of the species range has been established in Iskar, Vit and Yantra Rivers. Since 2012 the round goby was has expanded its range upstream with 9 km in Vit River, 28 km in Iskar River and 43 km in Yantra River. For the first time the species was found in the rivers Osam and Rosica. Large obstructions, such as HPPs and dams seem to be the main limiting factor of round goby's upstream expansion.

## **SYNTAXONOMICAL STUDY OF THE FOREST VEGETATION IN ETROPOLSKA STARA PLANINA MOUNTAINS**

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**Keywords:** syntaxonomy, forest phytocoenoses, Braun-Blanquet (1964).

The current study presents the results from syntaxonomic analysis of forest communities on the territories of Etropolska Stara Planina Mountains and the most western part of Zlatishko –Tetevenska Stara Planina Mts. The region is within the boundaries of Central Balkan Range which is characterized by very rich biodiversity. The three vegetation is represented from well-developed beech forest belt occupying the largest area. *Carpinus betulus*, *Quercus dalechampii* and *Betula pendula* are other dominant species which form main forest communities in the studied area.

The study was carried out in the period June - October 2017 according to the floristic methodology of Braun – Blanquet (1964). A total of 57 sample plots between 80 – 300 m<sup>2</sup> were made. The cluster analysis was performed by the SYNTAXA program (Podani 2002). The leading work for the determination of syntaxonomic schema was the work of Mucina & al. (2016).

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## **FLORISTIC STRUCTURE OF THE COMMUNITIES OF *GENISTA LYDIA* COMPLEX IN BULGARIA**

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**Keywords:** *Genista lydia*, phytocoenological releves, floristic structure, biological spectrum

The communities of *Genista lydia* complex (incl. *Genista rumelica* Velen.) are a part of the sub-endemic Balkan-Anatolian vegetation. They are distributed in Europe exclusively in North-East Greece and South Bulgaria. Their habitat - F3.1d Balkan-Anatolian genistoid scrub, was assessed as "Vulnerable" in the Red List of European Habitats (Janssen & al. 2016), but their floristic features were practically not known until now. The recent study provides detailed information based on 129 phytocoenological releves of the communities of *Genista lydia* complex in Bulgaria, on their floristic and phytogeographical structure and biological spectrum. About 506 vascular plant taxa belonging to 239 genera and 56 plant families were established to participate in their floristic structure. The phytogeographical relationships are strongest with the East Sub-Mediterranean areas. The hemicryptophytes prevail in the biological spectrum of the plant life forms, but also the participation of therophytes and cryptophytes is comparatively high. The main regions of their distribution in Bulgaria are East Rhodope, Thracian Plain, southern slopes of East Balkan Range Mts., valley of Mesta River etc., where the phytocoenological material has been collected.

## **ALGAL PROBLEMS OF SEA BASS (*Dicentrarchus labrax*) FARMING LAND PONDS IN TURKEY**

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**Keywords:** Algal problems, Sea bass farming, land ponds, Turkey

In this study, algal problems of sea bass (*Dicentrarchus labrax*) farming land ponds were investigated in a fish farm in Muğla, Turkey. For this purpose, water samples were collected from 4 different ponds, main entrance and main drainage to identify the algae composition. Samples were collected on May 2017 from the fish farm. The water temperature varies from 7°C to 8°C in winter, depending on the weather temperature and dissolved oxygen is around 10 mg/L. The pH changes from 7.0 to 7.5 and salinity vary between 20‰ and 28‰ of artesian well water. A total of 21 taxa, belonging to Bacillariophyta (14), Chlorophyta (3), Cyanobacteria (1), Euglenozoa (1) and Miozoa (2) were recorded. In terms of species numbers the diatoms were found the richest group in all ponds. According to density *Fragilaria crotonensis*, *Navicula cryptocephala* and *Nitzshia panduriformis* of diatoms, *Coelastrum microporum* of green algae and *Anabaena sphaerica* of blue-green algae were recorded in high numbers than other recorded species. The aim of our study is to determine which phytoplankton species cause problems in sea bass fish farms in land ponds and to take attention on this subject. It is known that excessive algal blooms lead to massive fish deaths in the aquaculture ponds. For this reason, monitoring and controlling the algae composition and density with water quality analyzes is required periodically in fish farms.

## **THE EFFECTS OF HARPOON FISHING ON SUSTAINABLE FISHERIES**

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**Keywords:** Harpoon fishing, sustainable fisheries, negative effects

Harpoon fishing is a hunting for sport purposes and it has an economical value over the World. This hunting method is done by diving. The hunter has to hold his/her breath during this method. Since oxygen intake of the diver underwater won't be fair and equal, using diving tube is unethical. Therefore, in many countries, such as Turkey, using diving tube during the harpoon fishing is forbidden. The reason of this law is that it leads to an excessive fishing and damages on various kinds of fish. The aim of this study is to investigate the negative effects of hunting with harpoon on some fish species. In many countries harpoon fishing is limited by laws. However, since the laws can't be controlled underwater, most of the laws are believed to be disobeyed by the divers. In order to keep the circle of fish life safe, every fish has to mate at least once in their lifetime. Otherwise, population of fish that can't mate will decrease and their kinds will be faced with the threat of extinction. Therefore, the laws must be applied by the relevant authorities. By applying the laws, it is believed that the risk of extinction that is done by harpoon fishing will be decreased.

## COMPARISON BETWEEN TWO VARIANTS OF MICROBIAL PREPARATION "COMPOST PLUS" ON GARDEN WASTE COMPOSTING

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**Keywords:** aerobic dehydrogenase activity, bioaugmentation, composting, effectiveness of organics biodegradation, microbial preparation

Composting is one of the environmental biotechnologies used for biowaste utilization. In national plan, the application of composting is waiting to increase during the next years in point of view to meeting the targets for reduction of the deposited waste to 35% until 2020 year. The long period for organic waste biodegradation and for compost stabilization is one of the main disadvantages of this biotechnology. Microbial preparations are often used as a solution for accelerating the process. The present study was accomplished in a pilot plant at the Faculty of Biology of Sofia University where the process of garden waste composting was carried out for a period of 299 days. Two variants of lyophilized preparation „Compost Plus“ were studied as inoculation materials during the process and they were added at start-up of the process. The first of them (microbial preparation „Compost Plus“) was prepared according to the requirements of the producer company adding water for rehydration of microorganisms. The second variant was a modification of the preparation „Compost Plus“ (modified preparation) and was prepared in the same way but to the water was added glucose (1%) for faster revitalization of the lyophilized microorganisms. The purpose of this study was to determine how the modification of the preparation affects on: 1/ the microbial activity measured by the total aerobic dehydrogenase activity and 2/ the organics biodegradation effectiveness in comparison to preparation „Compost Plus“. The humidity and temperature of the environment and in the composters were analyzed with the enzyme activity and organics concentration during the process.

It was found that during the process, the aerobic dehydrogenase activity was higher between 1.2 and 3.3 times when using preparation "Compost Plus" compared to the modified preparation. The used modified preparation as inoculation material at the beginning of the process doesn't have a stimulating effect on the microbial activity measured by the aerobic dehydrogenase activity. A positive effect was observed on the effectiveness of the organics biodegradation, which was higher with 57% for the modified preparation at the end of the process. The organic content in the compost dropped to 64% for preparation "Compost Plus" at 299 day and up to 54% for the modified preparation, which is close to the requirements for stable compost (between 10% and 50% organic content of total dry matter according to European legislation). In conclusion, it can be summarized that the modification of the preparation "Compost Plus" with addition of glucose no influence on the early phase of active biodegradation but has a positive effect on the final phase of composting (stabilizing the compost).

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## **PENETRATION OF NON-PSAMMOPHYTES ON THE DUNES SOUTH OF KAMCHIA RIVER MOUTH**

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**Keywords:** coastal habitats, invasion, psammophytic flora

Growing tourism along the Black Sea coast caused negative effect on the psammophytic plants. They suffer from direct destruction or limitation of available space. Another threat is the penetration of non-psammophytic plants on the dunes. In order to reveal how far one of the largest dune complexes at the Bulgarian Black Sea Coast is affected by weeds and ruderals, species from neighboring areas and aliens, we studied the Kamchia dune system during 2013-2015. Vegetation was sampled at 162 plots along 12 transects systematically situated to cover all habitat diversity. A total 207 plant species were registered. They were referred to five groups as follows: psammophytes – 38, weeds and ruderals – 36, plants typical for grasslands and shrublands – 94, plants typical for forests – 18 and alien (including invasive) species – 21. The grey dunes contain the highest percentage of non-psammophytic plants which decrease toward yellow and embryonic dunes. The region south of Kamchia river mouth is one of the best dune complexes and deserves further effective protection.



## **SPECIES COMPOSITION AND STRUCTURE OF THE ZOOPLANKTON COMMUNITY AND TROPHIC STATE OF SMALL NEGOVAN LAKE**

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**Keywords:** sand pit lake, zooplankton, trophic status

The current study presents recent data of the zooplankton community of a small sand pit lake /Lesnovska River drainage/ near Negovan Village. Our objective is to assess the trophic state of the lake after a restoration project aiming habitat management and protection as a priority wetland along the migratory route "Via Aristotelis" in the vicinity of Negovan Village. The study was carried out between November 2016 and June 2017. Quantitative zooplankton samples were collected by filtering water through Apstein plankton net from three points in the littoral zone of the lake. The planktonic component of the collected samples belongs to different taxa rank. We observed species belonging to four phyla, 21 families and subfamilies and 35 genera. Out of the 74 taxa, 54 were identified to species and subspecies level, and twelve – to genus. Most of the species (e.g. *Brachionus angularis*, *Keratella tecta* and *Hexarthra mira*) may be regarded as indicators of eutrophication. The crustacean plankton was represented mainly by species with small body size such as *Chydorus sphaericus* and *Bosmina longirostris*. The RCC index was used in sand pit lake for first time to assess the trophic state by features of zooplankton taxonomic structure. Determination of trophic levels was made by RCC Index, based on the quantitative proportion between the main zooplankton groups: Rotifera, Cladocera and Copepoda. The values of the RCC index varied between 2 and 83 as a result of highly dynamic environmental conditions.

## **EFFECTS OF GAMMA-IRRADIATION TREATMENT WITH FUNGICIDE DOSE ON THE MORPHOLOGY AND THERMAL DECOMPOSITION OF LEATHERS**

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**Keywords:** gamma-irradiation, disinfection, leathers, morphology, thermal stability

Biological attack of fungi is a serious problem in the preservation and long-term keeping of natural materials (wood, paper, leather, textiles, *etc.*) when stored in improper conditions. There are several advantages of radiation disinfection, compared to the traditional chemical treatment: higher effectiveness, reliability, lack of toxic residues, applicability on large amount of objects *etc.* However there are not enough data on the side-effects of gamma irradiation on leather items, especially at fungicide radiation doses. The aim of the present study is to evaluate the influence of gamma-irradiation treatment with 15 kGy on the morphology and thermal decomposition of three leather types: calf leather, calf suede and pig skin. Each sample was gamma-irradiated with 15 kGy, using radiation facility BULGAMMA, based on JS-850 60Co type gamma irradiator of Sopharma JSC, Bulgaria. The general morphology of the non irradiated and irradiated leather samples was revealed by means of scanning electron microscopy (SEM). A scanning electron microscope Lyra 3 XMU (Tescan) was employed. Prior to the measurements, the samples were covered with a thin film of carbon. Analysis of the non-irradiated leathers was performed by SEM-EDX in order to obtain information on the elemental composition of the samples and the tanning methods. The thermal properties of the samples were studied by thermogravimetry (TG) in pure argon, using Perkin-Elmer TGS-2. The obtained results showed that gamma-irradiation treatment of calf leather, calf suede and pig skin with fungicide doses can be successfully applied for disinfection and preservation without causing significant changes in their structure and morphology.

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## **USE OF SOIL DEHYDROGENASE ACTIVITY AND POLLUTION INDUCED COMMUNITY TOLERANCE TO DETECT AZOXYSTROBIN ADVERSE EFFECTS ON SOIL MICROORGANISMS**

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**Keywords:** azoxystrobin, dehydrogenase, soil microorganisms, EC50

Azoxystrobin (Az) is a fungicide, which is worldwide used for crop protection in agriculture. Az is effective against Ascomycota, Deuteromycota, Basidiomycota and Oomycota, inhibiting their mitochondrial respiration and spore germination. Indirect adverse effects of Az is recorded also on soil bacteria. Soil dehydrogenase activity (Dha) and pollution-induced community tolerance (PICT) concept were used to study the effects of Az on soil microbial communities (SMC). Az was added in increasing concentrations to agricultural soils (loamy sand and loamy clay) in a mesocosm experiment. The effects of Az on microbial activity were studied for three months. Az activity on Dha was soil type dependent, inhibiting and stimulating it in sandy and clay soils, respectively. Dha was reduced during the whole incubation period of sandy soils, and the level of reduction varied from 2% to 55%, with mean value of 30%. In Az amended clay soils, Dha was 15% (on average) higher than that of the control for the first two months of incubation, followed by a rapid decrease (40% of the control, on average) at the end of the experiment. SMCs were highly resistant to Az during the first day (sandy soil) and first month (clay soil) of soil incubation, when the EC50 was calculated to be 270 mg Az/kg soil (sandy and clay soils). The EC50 during the rest of the incubation time varied in the range of 50 - 75 mg Az/kg soil (sandy and clay soil). The study shows ecotoxicological effect of Az on soil microbial activity, and the manner of its changes in a context of soil properties and the duration of exposure time to the toxic agent.

## **CIRCULAR ECONOMY AS AN OPPORTUNITY TO IMPROVE MUNICIPAL WASTE MANAGEMENT IN THE REGION OF PERNIK**

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**Key words:** circular economy, environment, natural resources, waste management

Term circular economy has attracted attention in recent years. Circular economy is regenerative by design and aims to keep products and materials at their highest level of utility and value. In the last years, economic models have been implemented to improve human well-being on the one hand and save the environment on the other hand.

Waste is undoubtedly an environmental problem. It contributes to the ecological pressure on the planet. The product once discarded becomes waste and a whole set of impacts has been involved in its processing. Waste management plays a key role for achieving of recourse efficiency and includes collection, transport, storage, treatment including recycling and recovery of the municipal waste and provision of the necessary technical infrastructure to perform these activities. The main priority in waste management is the construction of a system of facilities providing environmentally friendly treatment of the domestic waste generated on the territory of the region.

The aim of the study is to analyze the waste management system in the region of Pernik, to differentiate the introduced and functioning elements of the circular economy, to identify critical moments in improvement of waste management system. The following methods were used for development of the study: analysis of statistical sources data for determination of tendency, derived by population to domestic waste generation; data comparison with aim to reveal trends related to waste generation; SWOT analysis of the of the existing waste collection system in the region of Pernik; determination of critical moments in the waste management system in the region. Using the TQM approach (total quality management) and planning the critical control points by location, by time, and by indicator.

The results reveal that towns and villages included in the system for organized waste collection comprise 154 out of 170 total number of towns and villages, situated on the territory of the region of Pernik. 99.3% of the population in the region has been covered by the system of organized waste collection. The separate waste collection system has been introduced in the regional center and one municipal center. Critical problems remain: non-inclusion of peripheral villages of the Waste Management System, lack of separate waste collection containers, presence of unregulated landfills.

The food waste is the largest part of the municipal waste - on average 27%. The next fraction is the „other“ fraction. Garden and plastic waste takes third place and there is considerable season variation in garden waste. The total quantity of paper waste is about 8%. The rest part of the examined fractions are as follows in descending order of aggregates, glass, textile, wood, metals, rubber, leather and hazardous. The most part of the waste generated is biodegradable - food waste from households and green waste. The energy and material resources of these wastes can be used more rationally by applying EU financial instruments.

In 2015 a new facility and installation were put into operation on the territory of the municipality of Pernik for the environmentally friendly treatment of municipal waste - „Regional landfill for non-hazardous waste – Pernik. The technological line has been developed for treatment of municipal waste generated in the area in accordance with environmental protection requirements. The regional landfill for non-hazardous waste in the town of Pernik has been in operation for a short period of time, which does not allow being drawn explicit conclusions regarding the waste management in the region

The waste management system in the region of Pernik is well organized and it is a basis for further development and implementation of the circular economy principles.

## EFFECT OF STABILIZED COMPOST AND MICROBIAL PREPARATION „COMPOST PLUS” ON GARDEN WASTE COMPOSTING

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**Keywords:** aerobic biodegradation of garden waste, aerobic dehydrogenase activity, compost, inoculation materials, microbiological preparation

Composting as a biotechnological process and as a process of controlled aerobic biodegradation of biowaste plays important role in waste management and helps to reduce negative impacts on the environment. The quantity of landfilled waste is reduced and part of them is transformed into new raw material for agriculture (compost). One of the most important factors for start-up of environmental biotechnologies, along with the operational parameters of the technologies (oxygen, humidity, C:N, temperature, pH), are the applied inoculation materials and in particular the quantity and activity of the microorganisms in them. The aim of the study is: i/ to compare two types of inoculation materials (microbial preparation "Compost Plus" and stabilized compost) on the basis of microbiological and enzymological parameters and ii/ to evaluate the effect of the preparation "Compost Plus" on the garden waste composting in comparison to the stabilized compost.

In the first part of the study, data about quantity of key groups of microorganisms (aerobic heterotrophs, cellulose-degrading microorganisms, actinomycetes and fungi) and aerobic dehydrogenase activity were compared in the two types of inoculation materials. It was ascertained that the total microbial number, the amount of aerobic heterotrophs, fungi and actinomycetes was higher in the stabilized compost. However the amount of cellulose-degrading microorganisms and total aerobic dehydrogenase activity were higher in the commercial preparation. In the second part of the study, the effect of the used inoculation materials was analyzed during composting process with other parameters as temperature, humidity and volatile solids. The garden waste composting process was investigated for a period of 299 days in pilot plant localized in Faculty of Biology to the Sofia University. The effect of microbial preparation "Compost Plus" on the aerobic dehydrogenase activity and on effectiveness of organics biodegradation was determined. It is found that the commercial preparation during the process has a positive effect on the total aerobic dehydrogenase activity compared to the stabilized compost. The enzyme activity for microbial preparation was higher (between 19 and 165%) from the beginning of the process to 223 day. The effect of the microbial preparation on organics biodegradation was positive and was higher (between 3 and 13%) in comparison to stabilized compost. The obtained results showed the positive effect of microbial preparation "Compost Plus" as inoculation material for garden waste composting.

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## **ALGAL PROBLEMS OF SEA BASS (*Dicentrarchus labrax*) FARMING LAND PONDS IN TURKEY**

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**Keywords:** Algal problems, Sea bass farming, land ponds, Turkey

In this study, algal problems of sea bass (*Dicentrarchus labrax*) farming land ponds were investigated in a fish farm in Muğla, Turkey. For this purpose, water samples were collected from 4 different ponds, main entrance and main drainage to identify the algae composition. Samples were collected on May 2017 from the fish farm. The water temperature varies from 7°C to 8°C in winter, depending on the weather temperature and dissolved oxygen is around 10 mg/L. The pH changes from 7.0 to 7.5 and salinity vary between 20‰ and 28‰ of artesian well water. A total of 21 taxa, belonging to Bacillariophyta (14), Chlorophyta (3), Cyanobacteria (1), Euglenozoa (1) and Miozoa (2) were recorded. In terms of species numbers the diatoms were found the richest group in all ponds. According to density *Fragilaria crotonensis*, *Navicula cryptocephala* and *Nitzshia panduriformis* of diatoms, *Coelastrum microporum* of green algae and *Anabaena sphaerica* of blue-green algae were recorded in high numbers than other recorded species. The aim of our study is to determine which phytoplankton species cause problems in sea bass fish farms in land ponds and to take attention on this subject. It is known that excessive algal blooms lead to massive fish deaths in the aquaculture ponds. For this reason, monitoring and controlling the algae composition and density with water quality analyzes is required periodically in fish farms.

## **PERSONAL ECOLOGICAL FOOTPRINT AS A SUSTAINABLE DEVELOPMENT MEASURE OF THE SOUTH-WEST REGION OF BULGARIA**

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*Keywords: sustainable development, personal ecological footprint, global hectares*

The Ecological Footprint (EF) is a resource accounting tool that measures how much biologically productive land and sea is used by a given population or activity, and compares this to how much land and sea is available by measuring the sum of these areas, wherever they physically occur on the planet. A quiz based on a methodology known as Footprint 2.0 was used for calculating the EF of 103 randomly chosen responders. The EF was expressed as global hectares as a measurement unit. Furthermore, more detailed analysis was conducted based on the age, sex, education and type of settlement of the participants. The average EF accounts on 46.6 global hectares which equals to 2.6 planets. Results show that the responders who live in older suburbs and those with higher and secondary education have higher EF which average values range from 2.3 to 2.6 planets. The lowest EF (up to 2 planets) is calculated for the group of respondents at the age of 50 and more years. In general, the main reasons identified for the higher EF are: the use of old cars with worse environmental properties, inhabiting homes with less green design features, using less biodegradable and non-toxic cleaning products, relative low proportion of recycled materials. On the other hand, habits that lead to EF decrease in Bulgaria are primary: using energy efficient appliances and compact fluorescent bulbs, minimizing shower time and toilet flushing, washing cars rarely, running clothes and dish washers only when full.

## INVESTIGATION OF DENDROFLORA IN THE CITY OF SEVLIEVO

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**Keywords:** urban vegetation, tree species, life-form, geo-element

Sevlievo is a city with a rich urban biodiversity. It's had also a very long history since the First Bulgarian State, later Kingdom because it was built Hotalich - a medieval city-fortress, 4 km northwest of the current city of Sevlievo. A study of urban vegetation was carried out in 2017 to establish the diversity, abundance and space distribution of tree species. This specific study is very popular and innovative now in Bulgaria. As a result, we establish that the most common tree species are *Tilia cordata* Mill., *Robinia pseudoacacia* L. and *Betula alba* L. The average age of the inventory tree species is 60 years. This is the period after the World War 2, where all the cities in Bulgaria were afforested and ennobled for it's inhabitants. The study of the species diversity is important for the management of the municipalities as a sustainable ecological system.

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## **SPATIAL STRUCTURE OF THE GREEN SYSTEM IN THE CITY OF SEVLIEVO**

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**Keywords:** green city, sustainable development, urban and green management, GIS

Sevlievo is a sustainable city (Rusev, 2009) with a successful economic, social and environmental strategy. On the territory of the municipality there are 8 protected areas and 12 protected zones. This is the first city in Bulgaria due to its green innovative management had created a park on the territory of abandoned military barracks. Sevlievo is one of the richest cities in Northern Bulgaria after Veliko Turnovo. In this study we mapped and assessed the spatial structure of urban vegetation using ArcGIS 10.1 software. For this purpose, the data from the city's inventory was used, which was held this year. The Urban ecosystem research is of a paramount importance for the sustainable management and development of the green system in Sevlievo city.

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## **PHYSIOLOGICAL CHARACTERISTICS OF THE NATURAL REGENERATION IN DECIDUOUS FORESTS**

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**Keywords:** *Carpinus betulus*, *Fagus sylvatica*, pigments, phenols, flavonoids, total antioxidant activity

A comparative study between two deciduous species – *Fagus sylvatica* L. and *Carpinus betulus* L., depending on their location and exposure, was carried out. The experimental trees were grown at three different altitudes, at the Petrohan Experimental Forest Range. We found a significant increase of chlorophyll levels in the trees grown at the forest's patches. That tendency was more pronounced in the leaves of hornbeam - the content of chlorophyll *a* was about 60% enhanced and that one of chlorophyll *b* - 19% enhanced.

The common beech saplings grown at the forest's patches at the highest altitude (1400 m asl) showed 30% increase of total phenolic compounds, and the changes of total antioxidant activity followed closely that trend. However, in the hornbeam leaves were noticed the opposite changes - the phenolic amounts in the leaves of the open expositions were greatly reduced, as well as the total antioxidant activity. The full sun exposure caused only a slight increase of the flavonoids in the leaves of both species, independently of the altitude.

The changes observed in the antioxidant state of the leaves may also be associated with the abundance of herbivore insects that could provoke serious damages in the leaves, especially at the lower altitudes.

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## **EFFECT OF SALT STRESS ON CHLOROPHYLL A FLUORESCENCE OF TWO *LYCIUM* SPECIES GROWN *EX VITRO* IN HYDROPONIC**

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**Keywords:** *Lycium barbarum*, *Lycium chinense*, chlorophyll a fluorescence

A hydroponic experiment was carried out to investigate the effect of salinity on fluorescence characteristics in *Lycium barbarum* L. and *Lycium chinense* Mill. grown *ex vitro* in Hoagland solution added with 50 mM, 100 mM and 200 mM NaCl. Both plants showed a very slight inhibition of PS II activity as revealed by the quantum yield of the primary photochemical reaction ( $\phi_{P_0}$ ). Intersystem electron transport ( $\phi_{E_0}$ ) was not inhibited with increasing salinity levels, whereas reduction of the end electron acceptors ( $\phi_{R_0}$ ) were inhibited to a greater extent in *Lycium barbarum* than in *Lycium chinense*. The performance index of photosynthesis ( $PI_{ABS}$ ) was stimulated in both plants with increasing concentration of NaCl in nutrient solution, while the other index -  $PI_{Total}$  was reduced only in *Lycium barbarum* after treatment with 50 and 100 mM NaCl. The index  $DI_0/RC$  decreased to a greater extent in *Lycium chinense* than in *Lycium barbarum* with increasing salinity levels. Our results showed that the higher tolerance of *Lycium chinense* to salt stress was accompanied with higher resistance of the overall photosynthetic performance, including both PS II and PS I.

## **SEASONAL VARIATIONS IN THE STRUCTURE AND THE FUNCTIONAL PERFORMANCE OF PHOTOSYNTHETIC APPARATUS OF *PETASITES HYBRIDUS***

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**Keywords:** *Petasites hybridus*, fatty acid composition, histological analysis, seasonal variations, thermoluminescence

Species belonging to *Petasites* genus are perennial herbs with thick rhizomes and large leaves developing after flowering in spring. The main medicinal plant in *Petasites* genus used in European phytotherapy is *P. hybridus* (common butterbur). To date, most of the drugs originate from *in situ* collections. Leaves play key roles in plant function and long-term adaptation to the environment. The histological analysis showed morphological changes in spongy mesophyll of autumn leaves. Photosynthesis is a fundamental physiological process determining plant productivity. For that reason we investigated seasonal variations in structural - functional characteristics of photosynthetic apparatus of *P. hybridus* from May 2015 to October 2017. Plant material was collected from population growing at region of Devil's bridge in the vicinity of the village of Kokalyane, Sofia Capital Municipality. During our studies, using a highly sensitive thermoluminescence technique, we observed some peculiarities of PSII redox reactions that can indicate specific adaptive characteristics of the photosynthetic system in dependence of growth conditions. The analyses of fatty acid composition of total lipophilic extracts, performed using thin-layer and gas chromatographic techniques, revealed a substantial quantity of unsaturated very-long fatty acids.

