ПЛОВДИВСКИ УНИВЕРСИТЕТ „ПАИСИЙ ХИЛЕНДАРСКИ”

БИОЛОГИЧЕСКИ ФАКУЛТЕТ

ВТОРА НАЦИОНАЛНА КОНФЕРЕНЦИЯ ЗА МЛАДИ УЧЕНИ

“БИОЛОГИЧЕСКИ НАУКИ ЗА ПО-ДОБРО БЪДЕЩЕ”

30 – 31·Октомври·2015, Пловдив

ПЛОВДИВСКИ УНИВЕРСИТЕТ „ПАИСИЙ ХИЛЕНДАРСКИ”

БИОЛОГИЧЕСКИ ФАКУЛТЕТ

Конференцията се осъществява с финансовата подкрепа на Фонд “Научни изследвания” при Пловдивския университет "Паисий Хилендарски" – Проект ЧФ- 34 /2015

Технологичен център към ПУ "Паисий Хилендарски", ДЗЗД "Екотехнологии'21"

„АА Медикъл България” ООД

“Биосистеми” ООД

Място на провеждане на конференцията:

Биологически Факултет на ПУ „Паисий Хилендарски“

Ул.“Тодор Самодумов“№2, Пловдив

(Стария Град, до Античен театър)

Организационен комитет:

председател: Веселин Баев

членове:

Весела Митковска
Джемал Мотен
Иван Илиев
Красимир Тодоров
Мариана Николова
Слави Тинешев
Цветелина Бацалова
Весела Янчева
Елена Апостолова
Ивелин Моллов
Марияна Гозманова
Пенка Василева
Стела Стоянова
Ценка Радукова

Научен комитет:

председател: Илия Илиев

членове:

Анелия Стоянова
Валентина Тонева
Детелина Белкинова
Иванка Димитрова
София Балтова
Балик Джамбазов
Велизар Гочев
Емилия Дамянова
Илиана Велчева
Еленка Георгиева

ISBN: XXXXXXXXXXX

ПРОГРАМА

30 октомври 2015

9:00 – 10:00 Регистрация

10:00 – 10:10 Откриване на конференцията

10:10 – 11:40 **ПЛЕНАРНА СЕСИЯ I**
(концертна зала АМТИИ до Биологически факултет)

председател: ***Илия Илиев***
секретар: ***Мариана Гозманова***

10:10 – 10:40 **акад. проф. Иван Иванов** – ИМБ-БАН, София

"Как човекът дешифрира своя геном и какво научи от него?"

10:40 – 11:10 **проф. Петя Цветкова, дбн** – ИБИР-БАН, София

"Сперматозоидът - мъжкото начало. Структурни и ултраструктурни промени при инфертилитет"

11:10 – 11:40 **чл. кор. проф. дтн Атанас Павлов** – УХТ-Пловдив, Институт по микробиология „Стефан Ангелов”, БАН, София

"Биологично активни вещества от растителни in vitro системи“

11:40 – 12:00 Почивка и подготовка за постерна сесия

12:00 – 13:00 ПЛЕНАРНА СЕСИЯ II
(концертна зала АМТИИ до Биологически факултет)

председател: ***Балик Джамбазов***
секретар: ***Елена Апостолова***

12:00 – 12:30 проф. дбн. Димитър Иванов – ИБЕИ-БАН, София

"Растително разнообразие през неозоя и флористични промени” (Cenozoic plant diversity and floristic change)

12:30 – 13:00 проф. д-р Стефан Сивков, дм - МУ, гр. Пловдив

“Human brain - Beyond limits”

13:00 – 14:00 Кафе пауза и Постерна Сесия

Секция: Молекулярна и Клетъчна Биология

14:00 – 15:00 Сесия I (Биологически факултет, 15 Аудитория)

председател: ***Веселин Баев***
секретар: ***Цветелина Бацалова***

14:00 – 14:15 **Десислава Анкова ,** Институт по биология и имунология на размножаването "Акад. Кирил Братанов" - БАН

"Immunohistochemical expression of ACE and ACE2 in human breast carcinoma“

14:15 – 14:30 **Фериха Церкова,** ПУ “Паисий Хилендарски"

"Genetic Diversity and geographic distribution of Round Goby *Neogobius melanostomus* (Pallas) as revealed by mtDNA cyt b Gene Haplotypes”

14:30 – 14:45 **Снежана Кестенджиева,** Институт по биология и имунология на размножаването Акад."Кирил Братанов", БАН

"Pluripotent state-specific markers are expressed in umbilical cord-derived mesenchymal stem cells”

14:45 – 15:00 **Елена Стоянова,** Институт по биология и имунология на размножаването ""Акад. Кирил Братанов"

"Human myeloid-like cells were obtained from reprogrammed adipose mesenchymal cells”

15:00 – 16:00 Кафе пауза и Постерна Сесия

16:00 – 18:00 **Сесия II (Биологически факултет,15 Аудитория)**

председател: ***Галина Яхубян***
секретар: ***Джемал Мотен***

16:00 – 16:15 **Деспина Пупаки, Институт по биология и имунология на размножаването, БАН,**

"Use of p63 as Myoepithelial Marker in Canine Mammary Carcinomas”

16:15 – 16:30 **Ирена Авджиева,** Софийски университет "Свети Климент Охридски"

"Function annotation enrichment assisting function prediction of plant genes”

16:30 – 16:45 **Диляна Хаджиматева,** Софийски университет "Свети Климент Охридски"

"A comparative phylogenetic analysis of hexaploid wheat evolution”

16:45 – 17:00 **Жейна Кирчева**, Медицински Факултет, Тракийски Университет

“Promoter polymorphism in IL12B and serum levels of IL-12p40 in healthy Bulgarians

17:00 – 17:30 Фирмена презентация - **A&A Медикъл България**

18:00 – 20:00 **Коктейл**

Секция: Биоразнообразие и Екология

14:00 – 15:00 **Сесия I (Биологически факултет, 14 Аудитория)**

председател: ***Илиана Велчева***
секретар: ***Ивелин Моллов***

14:00 – 14:15 **Николай Николов**, Институтът по биоразнообразие и екосистемни изследвания при БАН (ИБЕИ)

"Morphological research on ten taxa of the genus *Jurinea* Cass. from Bulgaria”

14:15 – 14:30 **Христина Христова**, Национален природонаучен музей - София

"Systematic List of Hesperioidea and Papilionoidea of Bulgaria with Application of IUCN Red List Criteria

14:30 – 14:45 **Гергана Кирова,** Медицински Университет – Пловдив

"Zinc biosorption by waste *Streptomyces fradiae* biomass: equilibrium and kinetics“

14:45 – 15:00 **Гергана Кирова,** Медицински Университет – Пловдив

"Streptomyces levoris immobilized on silica gel 60 as a novel biosorbent for copper (II) preconcentration

15:00 – 16:00 Кафе пауза и Постерна Сесия

Секция: Биотехнологии, Бионано-технологии и Приложна биология

16:00 – 17:30 Сесия I (Биологически факултет, 14 Аудитория)

председател: ***Тонка Василева***
секретар: ***Мариана Николова***

16:00 – 16:30 **Павлина Долашка,** Институт по органична химия с център по фитохимия, БАН

"Bioactive compounds isolated from garden snails”

16:30 – 16:45 **Прерна Агарвал (Prerna Agarwal)**, Медицински факултет, Тракийски Университет – Стара загора; Institute of Nuclear Medicine and Allied Sciences, India

"Aqueous Root Extract of Glycyrrhiza Glabra: An comparative study of the reaction with DPPH”

16:45 – 17:00 **Цвета Ганева,** Софийски Университет "Св. Климент Охридски"

"Freeze-embedding method for epicuticular wax isolation

17:00 – 17:15 **Христо Наков,** Пловдивски университет „Паисий Хилендарски“

Hydrolysis kinetics of synergistic prebiotic composition in vitro simulation of the GI-tract conditions

17:15 – 17:30 **Прерна Агарвал (Prerna Agarwal),** Медицински факултет, Тракийски Университет – Стара загора; Institute of Nuclear Medicine and Allied Sciences, India

"*Glycyrrhiza glabra*: "real time" oxidative status of animals

31 октомври 2015

Секция: Биоразнообразие и Екология

09:00 – 11:15 **Сесия II (Биологически факултет, 14 Аудитория)**

председател: ***Гана Гечева***
секретар: ***Весела Янчева***

9:00 – 9:30 **Димитър Попов** - СНЦ "Зелени Балкани”

"Pilot photo-identification studies of dolphins along Southern Bulgarian Black sea coast"

9:30 – 9:45 **Борислав Григоров,** СУ "Св. Климент Охридски"

"Evaluation of ecosystem services in the territory of Mala Planina by the use of contingent valuation method”

9:45 – 10:00 **Илиян Коцев,** Институт по океанология "Фритъоф Нансен" - БАН, гр. Варна,

"Landscape-based approach to identification of shallow seabed habitats. Case study: central sector of the Bulgarian Black Sea coastal zone”

10:00 – 10:15 **Павлина Петрова,** Лесотехнически университет - София,

“Influence of the composting process on nematodes in the soil substrate”

10:15 – 10:30 **Петя Иванова Димитрова-Матева,** Лесотехнически университет - София

"Eco-physiological method for investigating the effect of beech weevil (*Orchestes fagi* L.) infestation on common beech (*Fagus sylvatica* L.) trees”

10:30 – 10:45 **Симона Соколова Георгиева,** Лесотехнически университет - София,

"Effect of *Orchestes fagi* L. infestation on the pigment content of beech (*Fagus sylvatica* L.) leaves”

10:45 – 11:00 **Боян Златков**, Софийски университет "Св. Климент Охридски"

"The unknown male of *Phtheochroa unionana* (Kennel, 1900) (Insecta: Lepidoptera: Tortricidae) and supposed synonymy with other species”

11:00 - 11:15 **Петър Остоич**, Институтът по биоразнообразие и екосистемни изследвания при БАН (ИБЕИ)

"Methods, applicable to ecotoxicological biomonitoring studies with a focus on terrestrial vertebrates”

11:30 – 12:30 Кафе пауза и Постерна сесия

Секция: Генетика и селекция

09:00 – 10:00 **Сесия I (Биологически факултет,15 Аудитория)**

председател: ***Евгения Иванова***
секретар: ***Пенка Василева***

09:00 – 09:15 **Спас Джоглов,** Пловдивски Университет "Паисий Хилендарски"

"Study on biological, genetic and environmental factors for azoospermia”

09:15 - 09:30 **Вида Георгиева,** Пловдивски Университет "Паисий Хилендарски"

"Genetic characterization of selectively controlled for swarm production *Apis mellifera macedonica* (type rodopica) populations in Bulgaria”

09:30 – 09:45 **Гергана Панайотова,** Пловдивски Университет "Паисий Хилендарски"

"Biosocial characteristics of patients with paranoid schizophrenia”

09:45 – 10:00 **Роза Динчева,** Медицински колеж към Медицински университет - Пловдив

"Mutated” genofound for some common diseases, persistent multivalency with its clinical manifestations”

Секция: Медицинска биология

10:00 – 11:30 **Сесия I (Биологически факултет,15 Аудитория)**

председател: ***Еленка Георгиева***
секретар: ***Стела Стоянова***

10:00 – 10:15 **Джаспрет Патти,** Медицински университет – Пловдив

"Rosemary oil reduces electrical field stimulated tetanic muscles contractility”

10:15 – 10:30 **Людмила Велкова**, Институт по органична химия с Център по фитохимия – БАН

"Carbohydrate structures of molluscan hemocyanins from snails *Helix lucorum* and *Rapana venosa*, determined by mass spectrometry”

10:30 – 10:45 **Ирина Лазаркевич,** Институт по микробиология, БАН

"Comparative study on antibacterial activity of synthetic analogues of biologically active compounds and their combination with rhamnolipid- biosurfactant”

10:45 – 11:00 **Данаил Мартинов,** Медицински университет - Плевен

"Role of oxidative stress in male infertility”

11:00 – 11:30 **Фирмена презентация** – “qPCR Работилница”, Биосистеми ООД

Студентска сесия

11:30 – 12:45 **Сесия I (Биологически факултет, 14 Аудитория)**

председател: ***Ценка Радукова***
секретар: ***Иван Илиев***

11:30 – 11:45 **Велина Ангелова,** Пловдивски Университет "Паисий Хилендарски"

"Training on ""biology and health education"" as a basis for forming health and ecological competencies in students from grades 7-10”

11:45 – 12:00 **Сибел Азиз,** Пловдивски Университет "Паисий Хилендарски"

“Clinical laboratory tests”

12:00 – 12:15 **Екатерина Георгиева,** Медицински факултет, Тракийски Университет – Стара Загора

"Visualization of mitochondrial dysfunction in living cells, based on superoxide generation”

12:15 – 12:30 **Доника Иванова,** Медицински факултет, Тракийски Университет – Стара Загора

"Increasing the sensitivity of cancer cells to anticancer drugs by inhibiting the glycolysis”

12:30 – 12:45 **Даяна Борисова**, "Институт по Микробиология "Стефан Ангелов""- БАН, София

"Phenotypic investigation of paired Pseudomonas aeruginosa cystic fibrosis strains isolated prior- and post-inhaled tobramycin treatment”

12:45 – 13:15 Награждаване на доклади и постери
Закриване на конференцията

П л е н а р н и д о к л а д и

How Did Homo sapiens Sequence Its Own Genome and What Was the Lesson?

Ivanov I.

Institute of Molecular Biology, Bulgarian Academy of Sciences
Sofia 1113, Bulgaria

Abstract. The sequencing of human genome is one of the greatest scientific discoveries in biology in the 20th century. It is interesting that one of the authors of the DNA double helix, James Watson, was also the main manager and coordinator of the Human Genome Project (HGP). This project laid the foundations of several new branches of science like Genomics, Proteomics, Bioinformatics, etc. The HGP united more than 3000 highly qualified molecular geneticist from several developed countries in one consortium (HGP Consortium) to determine the consecutive order of 3.1 giga base pairs in our DNA. By sequencing the human genome it became clear that the real number of our genes is much lower than expected before and, surprisingly, it is much lower than the number of the protein species in our body. It has also been shown that 1% of human DNA only codes for proteins whereas the functions of the rest of it is unknown. The human genome sequencing reviled a non-random distribution of the structural genes amongst the chromosomes and identified “jungles”and “deserts” in our chromosomes. It shed much light on the evolution of Homo sapiens and revealed the genetic basis of human diversity and human genetic diseases.

Bioctive Substances from Plant In vitro Systems

Pavlov A.

University of Food Technologies, Plovdiv, 26 Maritza Blvd., 4002, Plovdiv, Bulgaria; Department of Industrial Microbiology, Laboratory of Applied Biotechnologies, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 139 Ruski Blvd., 4000 Plovdiv
E-mail for correspondence: at_pavlov@yahoo.com

Abstract. Higher plants are almost 400 000 species in the world. They are valuable producers of bioactive metabolites that are used as pharmaceuticals, agrochemicals and food additives. During the last years humans preferred to use natural products, and therefore the market for natural plant products has expanded, and this trend will continue. However, the production of plant-derived substances is limited by environmental, ecological, climatic and other conditions, which leads to shortages of some products on the market. This problem could be overcome using plant in vitro technologies, which were introduced in the late 1960s as promising methods for both production and study of plant secondary metabolites. There are several important advantages of plant in vitro technologies, especially the independence they offer of climatic and soil conditions, and the ability they provide to synthesize diverse bioactive substances (including substances produced in nature by rare and threatened plants) under controlled conditions. Nowadays several substances (such as shikonin, ginseng and paclitaxel) are being produced at an industrial scale by in vitro plant systems.

The main concomitant problems of industrial implementations of this technology are generally low yields of target products, and problems associated with cultivation in bioreactors which is the main research focus of the scientific group Bioactive substances by plant in vitro systems. The work in the area of biotechnology of plant secondary metabolites at the Laboratory of Applied Biotechnologies started thirty years ago with the pioneer work of Prof. Mladenka Ilieva on tobacco cell

suspension. Later research investigations were focused on the synthesis of specific bioactive substances using plant in vitro systems, with particular emphasis on compounds produced by rare and threatened plants, including rosmarinic acid from Lavandula vera MM plant cell suspensions, enzymes (phosphohydrolases) from Nicotiana tabacum plant cell suspensions, betalains from Beta vulgaris hairy root cultures and alkaloids from Datura stramonium and Hyoscyamus niger hairy root cultures and Pancratium maritimum shoot cultures. In addition, processes and interactions in aqueous two-phase systems for the cultivation of plant in vitro systems and polyploidy in plant in vitro systems were studied. Young colleagues had significant success in biotechnology of secondary metabolites of rare and threatened Bulgarian Salvia plants. In the last ten years, however, the main scientific attention was drawn on the development of a biotechnology for galanthamine production by Leucojum aestivum L. shoot cultures. The main achievements in development of this technology will be the topic of the current presentation.

Cenozoic Plant Diversity and Floristic Change

Ivanov D.

Institute of Biodiversity and Ecosystem Research
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., 23, 1113 Sofia, Bulgaria

Abstract. The recent biodiversity is concerned with drastic reduction, as a result of human activities – reduced and altered habitats, industrial pollution, introduction of cultivars, invasive species etc. Paleontology gives us a good opportunity to have a look how does diversity changes over the past time, in terms to receive better understanding of its recent status.

Many biologists overlook the fact that plants underpin the evolution and continued existence of all terrestrial biomes. Thus, changes in plant communities play crucial role in the development of ecosystems and animal diversity. The territory of Bulgaria with its numerous marine and non-marine basins is a key region for the investigation of Cenozoic evolution of the flora and vegetation, of the migration routes and exchange corridors of many plant species, and of the floristic bonds between Central and East Europe and Asia Minor. The present report focuses on the plant and vegetation diversity in Bulgaria in the Cenozoic. The results show that fossil record can be use to estimate changes and trends in past plan diversity. Recognition of fossil plant communities and their recent relatives is a tool for analysis of past and future ecological diversity and conclusions about vegetation and climate changes.

Human Brain - Beyond Limits

Sivkov S.

Department of Anatomy, Histology and Embryology, Medical University - Plovdiv, Bulgaria

Abstract. The human brain contains about 100 billion neurons, each interacting with 10,000 other neurons through an intricate connectivity. As a result, there may be one quadrillion connections between neurons in the brain each firing 10x in a second. Presently however this incredible network is beyond the capacity of technologies to trace it. Advances in nanotechnology, microelectronics, optics, compression and data storage, theory and synthetic biology can help to make possible studies that were impossible to conduct before. For example, scientists can enhance traditional brain scans by implantation of nanosensors, wireless fiberoptic probes to browse brain tissue and identify the neurons activated in response to various stimuli. The goal in the near future is to develop new technologies in the field of data processing, nanotechnology, optogenetics and other abstruse areas and explore the interactivity of those billions of brain cells and complex neural circuits. Acquired knowledge will create models of the organization of neural circuits and determine their effect on behavior and cognition - one of the greatest mysteries. It would also enable researchers in the biomedical science to develop more precise ways of diagnosis and treatment of disabling brain diseases like depression, schizophrenia, dementia, autism, stroke, parkinsonism.

Pilot Photo-Identification Studies of Dolphins Along Southern Bulgarian Black Sea Coast

Popov D.

Green Balkans NGO, 1 Skopie str., office 10, 4004 Plovdiv,
E-mail: dpopov@greenbalkans.org

Abstract. Amongst the techniques for studying cetaceans at the sea, photo-identification is one of the most used worldwide. This method is based on the animals' natural markings to identify them and then be able to recognize them when encountered again. The animals are photographed and catalogued individually based on natural markings criteria (e.g., pigmentation on the body, shape of the dorsal fin) and personal markings (scores, notches and scars) that identify them.

Pilot use of that technique in Bulgaria was made in 2012 aiming to produce first catalogue. Study area included coastal waters from Cape Emine to Cape Maslen nos. 19 surveys have been made in the period from June to October 2012. Totally 1008,3 km have been cruised and 102 hours and 40 minutes were spent in the sea producing 46 sightings and 10 photo-sessions. Most numerous have been sightings of Harbour porpoise (*Phocoena phocoena relicta*) – 20 cases and Bottlenose dolphin (*Tursiops truncatus ponticus*) – 19 cases, while Common dolphin (*Delphinus delphis ponticus*) has been observed only once. 87 images were selected for inclusion in the first Bulgarian catalogue – BULFINS 2012. All three Black Sea species are represented in the catalogue: Harbour Porpoise (*Phocoena phocoena relicta*) – 5 images of two individual, Common Dolphin (*Delphinus delphis ponticus*) – 6 images of three individuals and Bottlenose Dolphin (*Tursiops truncatus ponticus*) – 76 images of twenty four individuals.

Д о к л а д и

Секция: Молекулярна и Клетъчна Биология

Immunohistochemical Expression of ACE and ACE2 in Human Breast Carcinoma

Ankova D.[1], Pupaki D.[1], Metodiev D.[2], Bachurska S.[3], Rashev P.[1]

[1]. Institute of Biology and Immunology of Reproduction, BAS, Bulgaria;
[2]. Medical University Sofia, Departmen of Forensic, Medicine and Deontology;
[3]. Medical University – Plovdiv, Department "General and clinical pathology"

Abstract. Breast cancer is the most common malignant disease in women that is diagnosed accidentally. Studies of renin-angiotensin system have dramatically increased recently since it is suggested that the expression and activation of its components affect the malignant disease. The aim of the present study was to determine the immunohistochemical expression of angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2) in normal and tumor mammary gland tissues. The localization of ACE and ACE2 was performed using immunoperoxidase method on FFPE tissue sections from 33 patients with invasive ductal carcinomas. It was found that ACE and ACE2 are localized in epithelial cells of both normal and tumor tissues. The results obtained show increased expression of ACE in low differentiated tumors in comparison with normal tissue. As for ACE2 expression, stronger reaction was found in normal tissue. These novel observations suggest that ACE may be involved in the pathogenesis of breast cancer.

Genetic Diversity and Geographic Distribution of Round Goby Neogobius melanostomus (Pallas) as Revealed by mtDNA cyt b Gene Haplotypes

Tserkova F.[1,2], Gospodinov G.[2], Kirilova I.[2], Klisarova D.[1], Denev I.[2]

[1] *Institute of Fish Resources (Agricultural Academy), BG – 9000 Varna, Bulgaria*
[2] *University of Plovdiv, Department of Plant Physiology and Molecular Biology, BG – 4000 Plovdiv, Bulgaria*

Abstract. Round gobies are relatively small soft-bodied fishes, characterized by a distinctive black spot on the first dorsal fin. They are native for central Eurasia. They are euryhaline fishes and can be found both in marine and freshwater habitats including the Black Sea, Sea of Azov, Sea of Marmara and Caspian Sea, costal lakes, lagoons and river from their basins. *N. melanostomus* is an invasive species, which recently established large non-native populations in the Baltic Sea, several major Eurasian rivers, and the North American Great Lakes threatening native species and local biodiversity. Two expansion mechanisms were proposed: 1) “corridor expansion” via European rivers and human-made channels and 2) “jump dispersal” via ballast waters from international shipping. Molecular phylogeny analyses of marker genes can provide information about origin and of each non-native population and gene exchange rate with other population. Cytochrome b is considered to be the most useful marker in determining phylogenetic relationships between closely related organisms, due to its sequence variability. For this purpose, we analyzed variability in 194 Cyt b sequences isolated from of *N.(A.) melanostomus* both by us and those annotated in NCBI. Accessions KF 549989 - 549990, EU 331156 – 331236, EU 564119 – 564125, KC 814168 -814174, KC 886276 – 886278, NMU 53673 – 53677, KJ 654330 - 654332, HQ 452491 – 452492, AY 884582 – 884583 and KC 800809. Analyses revealed thirty seven haplotypes. Observed value of Haplotype Diversity, Hd: 0.7871. Polymorphic sites were 35 there was generated by DnaSP Ver. 5.10.01. The geographic distribution and frequency of polymorphic site in Europe and North America is discussed.

Pluripotent State-Specific Markers are Expressed in Umbilical Cord-Derived Mesenchymal Stem Cells

Kestendjieva S.[1], Dzerov L.[2], Nikolov A.[2], Oreshkova T.[1], Mourdjeva M.[1], Stoyanova E.[1]

[1] *Department of Molecular Immunology, Institute of Biology and Immunology of Reproduction*
[2] *Department of Obstetrics and Gynecology, University Hospital of obstetrics and gynecology, Sofia*

Abstract. Problem: Human stem cells are unspecialized cells that have two unique properties. They are able to renew themselves for long periods of time and differentiate into various specialized cell types in human body. The high proliferative potential and multilineage differentiation capacity of these cells offer new perspectives for their use in tissue regeneration, gene engineering and cell-based therapy. Stem cells are separated according to their origin - embryonic, from extraembryonic tissues and adult stem cells. Embryonic stem cells (ESC) have unlimited proliferative and differentiation potential but their production involves the destruction of live human embryos and that is a serious ethical obstacle for the progress of human ESC research. Adult stem cells isolation is an invasive procedure, and the amount of collected cells is limited. In contrast, stem cells with extraembryonic origin are derived from placenta, umbilical cord and umbilical cord blood, tissues with important role during pregnancy that are discarded after birth.

The aim of the study is to examine the expression of pluripotent state-specific proteins in mesenchymal stem cells (MSC) derived from different parts of umbilical cord.

Methods: Isolation and expansion of MSC from human umbilical cord, flow cytometry, immunofluorescence and confocal microscopy.

Results: MSC were isolated from Wharton’s jelly (WJ-MSC) and amnion (A-MSC) of the umbilical cord. The established MSC populations have plastic adherent properties, fibroblast-like morphology and more than 95% of cells are positive for mesenchymal stem cell markers (CD29, CD73, CD90, CD105). The cells were negative for CD45, a lymphocyte common antigen. Immunofluorescence staining of WJ-MSC

and A-MSC showed expression of intracellular proteins - VIMENTIN, α-ACTININ, DESMIN, AFP and GATA4. The main regulators of ESC pluripotency are the transcription factors - OCT4, SOX2, NANOG and KLF4. All these markers were expressed in WJ-MSC. In contrast, OCT4, NANOG and KLF4 but not SOX2 were detected in A-MSC. WJ-MSC were positive for the ESC surface marker antigens TRA-1-60 and SSEA4, while A-MSC expressed only SSEA4.

Conclusion: The umbilical cord is a rich, non-invasive and abundant source of MSC. Comparison of WJ-MSC and A-MSC showed different expression of ESC markers. Only WJ-MSC were positive for all tested markers, which are important in embryonic development and maintenance of pluripotency. For this reason, WJ-MSC are appropriate candidates for use in regenerative medicine.

Human Myeloid-Like Cells Were Obtained from Reprogrammed Adipose Mesenchymal Cells

Stoyanova E.[1], Oreshkova T.[1], Mourdjeva M.[1], Kyurkchiev S.[2]

[1] *Institute of Biology and Immunology of Reproduction, BAS*
[2] *University Hospital of obstetrics and gynecology, Sofia*

Abstract. Problem: Human induced pluripotent stem cells (iPSC) are in vitro generated from somatic cells by inducing the expression of pluripotent transcription factors. They are morphologically and phenotypically similar to embryonic stem cells (ESC). iPSC give broad opportunities to regenerative medicine and stem cell research. Patient- and disease-specific iPSC are an important resource for resolving human hematopoietic disorders.

The aim of the study is to characterize reprogrammed cells with markers CD34 and CD45 for evaluation of differentiation into hematopoietic progenitors.

Methods: Human fibroblasts and adipose stem cells (ASC) were targeted for production of iPSC. They were transduced with OCT4, SOX2, KLF4 and C-MYC transcription factors. The reprogrammed cells were selected according to the expression of OCT4 transgene and tumor-related antigen TRA-1-60. The positive TRA-1-60 cells from reprogrammed fibroblasts and ASC were expanded and characterized for iPSC properties. The negative TRA-1-60 cells were studied for expression of CD34 (hematopoietic progenitor cell antigen) and CD45 (common leukocyte antigen) by flow cytometry.

Results: Human fibroblasts and ASC were reprogrammed. Three weeks later, colonies with the typical morphology of human ESC and non-ESC colonies were detected. For this reason, the cells were selected according to the expression of OCT4 and TRA-1-60. Double positive cells originating from fibroblasts and ASC were defined as iPSC. The expression of CD34, but not CD45, was demonstrated in ASC-derived cells. In contrast, the obtained fibroblast-derived OCT4+/TRA-1-60- cells do not express both CD34 and CD45.

Conclusion: The results showed that 60% of ASC-derived OCT4+/TRA-1-60- cells differentiated into CD34+/CD45- myeloid-like

cells. This work demonstrated that ASC-derived OCT4+/TRA-1-60- cells are cell population suitable for generation of hematopoietic progenitors.

Acknowledgements. This study was partly supported by the grants grantsReProForce FP-7- REGPOT-2009-1, DKOF7RP02/17 /DS№D01-4787 and №BG051PO001-3.3.06-0059.

Use of p63 as Myoepithelial Marker in Canine Mammary Carcinomas

Pupaki D.[1], Ankova D.[1], Metodiev D.[2], Rashev P.[1]

[1] Institute of Biology and Immunology of Reproduction, BAS, 73 Tsarigradskoshoseblvd., 1113 Sofia
[2] Medical University - Sofia, Faculty of Medicine, Department of Forensic Medicine and Deontology

Abstract. P63 is consistently expressed in basal epithelial cells of stratified epithelia as well as in myoepithelial cells (MEC) and has been proven a specific marker for the identification of MEC in both normal mammary gland tissue and tumors. Using different immunohistochemical markers progenitor cells have been identified, that differentiate into either luminal or MEC. These cells do not express smooth muscle actin (SMA) but show the typical nuclear staining for p63. Canine mammary carcinomas represent a heterogeneous group characterized by often proliferation of MEC which has been associated with favorable prognosis since these cells rarely become malignant. The presence of cells with progenitor cell phenotype is not well characterized in canine mammary carcinomas as they are probably assigned to the myoepithelial cell population because of their p63 reactivity. The aim of present study was to assess the presence of MEC and cells showing progenitor phenotype using immunohistochemical staining for p63 and SMA on 29 canine mammary gland carcinomas. Cells that were strongly positive for SMA, but negative for p63 were found only in some cases of tubulopapillary carcinoma and in a case classified as “malignant myoepithelioma”. In tubular carcinomas, micropapillarycarcinomas and carcinomas in benign mixed tumors, cells that were positive for p63 also showed moderate staining for SMA. In invasive tubular carcinoma and comedocarcinoma no staining for p63 was observed, and SMA marked only myofibroblasts. All cells that were positive for both SMA and p63 had reduced expression of p63, while those that were negative showed strong nuclear reaction. Strong nuclear staining for p63 but negative reaction for SMA may suggest progenitor cell origin. Results emphasize the use of a combination of markers to better characterize the heterogeneity of

canine mammary carcinomas, but further studies are necessary to relate the cell types present with prognosis and outcome.

Function Annotation Enrichment Assisting Function Prediction of Plant Genes

Avdjieva I.[1], Salse J.[2], Peychev D.[1], Hadjimateva D.[3],Vassilev D.[1,3]*

[1] *Bioinformatics group, AgroBioInstitute, 8 DraganTsankov Blvd, Sofia 1164, Bulgaria*
[2] *INRA-UBP GDEC, 5 Chemin de Beaulieu, Clermont-Ferrand 63039, France*
[3] *Faculty of Mathematics and Informatics, Sofia University “St. KlimentOhridski”, 5 James Bourchier Blvd, Sofia 1164, Bulgaria*
** - Corresponding author: i.y.stoycheva@gmail.com*

Abstract. There are currently over 100 partially or entirely sequenced plant genomes but despite progress in sequencing, the function of the majority of plant genes remains largely unknown. Experimental researches cannot cover large-scale sequencing projects and this is where function prediction methods can assist. Most of them are based on sequence similarities but such approach is not entirely reliable because similarity does not always reflect homology.

This study combines a phylogenomic approach with information from functional annotations, embedded into plant phylogenetic trees, which represent both sequence similarity and homology relationships between genes. Since functional annotations come from both manual and automatic annotations, an in-house semantic network approach was applied to improve their quality. This approach simulates the process of manual annotation and prevents generation of incorrect knowledge about gene function and domain distribution in de novo sequenced genomes.

Then, the information from functional annotations, homology relationships and evolutionary distances based on tree topology was summarized for each gene using a scoring system for all potential functions. Functions with the highest score were assigned to uncharacterized genes or used as feedback to address inaccuracies in automatically annotated genes. As a result, genes were assigned into four major groups according to the score and the origin of their predicted function. These groups also reflect the reliability of the prediction. The predicted functional information is being involved in studying evolutionary relationships between genes responsible for the regulation of C3/C4 photosynthesis and can be applied to other major plant phenotypes.

Acknowledgements. This study is supported by the National Science Fund of Bulgaria within the project "Methods for Data Analysis and Knowledge Discovery in Big Sequencing Datasets" under contract DFNI-102/7, 12th December, 2014.

A Comparative Phylogenetic Analysis of Hexaploid Wheat Evolution

Hadzhimateva D.[1], Avdjieva I.[2], Todorovska E.[2], Vassilev D.[2,1]*

[1]. Faculty of Mathematics and Informatics, Sofia University “St. KlimentOhridski”, 5 James Bourchier str., Sofia 1164, Bulgaria
[2]. AgroBioInstitute, 8 DraganTsankov str., Sofia 1164, Bulgaria
** - Corresponding author: didihadjimateva@gmail.com*

Abstract. The genome of hexaploid bread wheat (*Triticum aestivum* L.) is a result of several multiple hybridization events between three diploid progenitor species, comprising three different genomes: A, B, and D.

To track the events that have shaped the modern wheat genome, a phylogenetic-based in silico model was developed. To do this, a comparative analysis between each of the three subgenomes and its progenitor was conducted: the subgenomes A and D were compared respectively to *Triticum urartu* and *Aegilops tauschii*, while for the still disputable B subgenome predecessor the model plant *Brachypodium distachyon* was used. The phylogenetic trees for this analysis were obtained from Ensemble Plants. All analyses were done with custom-developed Python scripts.

The dataset shows almost equal gene distribution between the three subgenomes: A – 31%, B – 37,3%, and D – 31,7%. The evolutionary relationships were traced using a Species Overlap algorithm, and 54 807 groups of homologs involving wheat genes were detected, including 35 933 orthologous and 18 874 paralogous relationships. The homeologous relations between the subgenomes were included within the paralogs.

77% of the orthologous groups involved *Triticum urartu*, 79% - *Aegilops tauschii* and 64% - *Brachypodium distachyon*. The paralogs between Triticum urartu and *Triticum aestivum* were 68%, between *Aegilops tauschii* and *Triticum aestivum*, – 71%, and for the *Brachypodium distachyon* – 42%.

The comparative phylogenetic analysis allows identification of a closest homolog and probable predecessor for the majority of wheat genes. The distribution of genes, orthologs and paralogs also implies an opportunity for subsequent functional prediction and other analyses.

Acknowledgements. This study is supported by the National Science Fund of Bulgaria within the project "Methods for Data Analysis and Knowledge Discovery in Big Sequencing Datasets" under contract DFNI-102/7, 12th December, 2014.

Promoter Polymorphism in IL12B and Serum Levels of IL-12p40 in Healthy Bulgarians

Kircheva Z., Stanilova S.

Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University

Abstract. Interleukin 12 (IL-12) is an important immunoregulatory cytokine that provides a link between the innate and adaptive immune responses and promotes the development of Th1 responses. IL-12 is a proinflammatory heterodimeric cytokine, which consists of two subunits, IL-12p35 and IL-12p40, encoded by the IL12A and the IL12B genes, respectively. The IL12B gene contains several polymorphisms, including a complex polymorphism -6415CTCTAA/GC in the promoter region (IL12Bpro). In this study we assessed the relationship between the IL12Bpro polymorphism and the serum levels of IL-12p40 in healthy 18-year-old Bulgarians from the Stara Zagora region. The IL-12p40 levels of 140 subjects were determined by enzyme-linked immunosorbent assay (ELISA). Genotyping for the IL12Bpro polymorphism was performed using amplification refractory mutation system (ARMS). The allele frequencies were 0.464 for the IL12Bpro-1 allele (CTCTAA) and 0.536 for the IL12Bpro-2 allele (GC). The genotype frequences were 0.221 for IL12Bpro-11, 0.293 for IL12Bpro-22 and 0.486 for IL12Bpro-12. The obtained IL-12p40 serum levels were as follows: 83.68 ± 36.94 pg/ml for IL12Bpro-11, 85.32 ± 32.46 pg/ml for IL12Bpro-22 and 90.16 ± 43.63 pg/ml for IL12Bpro-22. A higher IL-12p40 level was found in subjects with the IL12Bpro-12 genotype compared to the other genotypes but the difference was not statistically significant ($p>0.05$, t-test). Although our data do not show a significant influence of the IL12Bpro polymorphism on the serum levels of IL-12p40 in healthy Bulgarian subjects, the possibility remains that such an effect exists for patients with certain immunomediated diseases.

Секция: Биоразнообразие и Екология

Morphological research on ten taxa of the genus Jurinea Cass. from Bulgaria

Nikolov N., Bancheva S.

Institute of Biodiversity and Ecosystem Research (IBER), Department of Plant and Fungal Diversity and Resources, Bulgarian Academy of Sciences, Acad. G. Bonchev St. bl. 23, 1113 Sofia, Bulgaria, e-mail: nikolovnik@abv.bg , bancheva@bio.bas.bg

Abstract. The genus *Jurinea* Cass. is a part of the tribe Cardueae of the Asteraceae family. It is one of the richest genera in the family. It comprises about 200 species, distributed in Western and Central Asia, Europe and Northern Africa (Susanna & Garcia-Jacas, 2007). The taxonomy of the genus is quite complicated and not well studied in the whole area of its distribution. This is the reason of the lack of an appropriate taxonomic scheme. In Bulgaria this group is still not investigated very well. As a part of a PhD thesis, a morphological survey of ten taxa – *J. tzarferdinandii* Davidov, *J.stoechadifolia* (Bieb.)DC., *J. ledebourii* Bunge, *J. glycacantha* (Sibth. &Sm.)DC., *J. mollis* ssp. *anatolica* (Boiss) Stoj. & Stefanov, *J. consanguinea* ssp. *neicevii* Kozuharov, *J. consanguinea* ssp. *consanguinea, J. consanguinea* ssp. arachnoidea (Bunge) Kozuharov, J. consanguinea ssp. bulgarica (Velen.)Kozuharov, J. albicauls ssp. kilaea (Aznav) Kozuharov, collected from different localities in Bulgaria is conducted. The species nomenclature is according to Delipavlov, D. & Cesmedziev, I. (Eds.) 2003. Key to the Plants in Bulgaria. Agrarian Univ. Acad. Press, Plovdiv.

[In Bulgarian]. and Kozhuharov, S. [Ed.], 1992. Guidebook to the higher plants in Bulgaria. Naouka & Izkoustvo, Sofia. [In Bulgarian].The number of the studied populations is 24 within the whole territory of the country. The population localities are visited more than once and the plant material is collected during three vegetation seasons. Thirty specimens from every population are examined. 14 quantitative and 31 qualitative characteristics are revealed from every specimen. A statistical analysis of the row data is conducted with Statistica7 software product. The analysis shows that J. stoechadifolia, J. tzar-ferdinandii и J. albicaulis ssp. kilaea are well delimited species due to their habitus and their habitat also. The key of the delimitation of the rest of the taxa are the characteristics of the involucral bracts, especially their shape if they are curved or not. This characteristic appears to be very hard to determine because it depends on too many factors. In conclusion, we can say that the results from the morphological analysis give a quite good information about the taxonomy of the genus Jurinea Cass., but there are still taxonomical issues which require more studies.

Systematic List of Hesperioidea and Papilionoidea of Bulgaria with Application of IUCN Red List Criteria

Hristova H.

National Museum of Natural History, Sofia, Bulgaria

Abstract. Updated systematic list of the butterflies and skippers (Papilionoidea and Hesperioidea) of Bulgaria is presented. The conservation status of all listed species is assessed at the national level applying the IUCN Red List Criteria (Version 3.1). The last published checklist of the Bulgarian Papilinoidea and Hesperioidea contains 216 species. Seven species from the last list are not included here because they were wrongly reported for Bulgaria due to misidentification or mislabeling. Since 2001 six new species of Papilionoidea and Hesperioidea have been recorded as new for the Bulgarian fauna and these are added to the new list. At present 188 species of Papilionoidea and 27 hesperiids, or 215 species in total, are known for Bulgaria. Forty species (21,3%) of the Bulgarian Papilionoidea and 9 species (33,3%) of the Bulgarian hesperiids are included in some of the three IUCN Red List categories of the threatened species.

Zinc Biosorption by Waste Streptomyces fradiae Biomass: Equilibrium and Kinetics

Kirova G.K.[1], Gochev V.K.[2], Stoytcheva M.S.[3], Velkova Z.Y.[1]

[1] *Department of Chemical Sciences, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria*

[2] *Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University “Paisii Hilendarski”, Plovdiv, Bulgaria*

[3] *Institute of Engineering, Autonomus University of Baja California, Mexicali, Mexico*

Abstract. Waste *Streptomyces fradiae* biomass from pharmaceutical industry was successfully used for zinc removal from aqueous solutions. In the present study the process parameters (initial pH of the solution, amount of biomass, initial metal concentration, stirring speed and contact time) and their influence on Zn(II) biosorption were investigated and optimized. Langmuir and Freundlich adsorption isotherms were used to describe sorption behavior between the waste biomass and the zinc ions. The biosorption process was better described by the Langmuir adsorption isotherm with 61,09 mg g-1 maximum adsorption capacity. Lagergren and Ho models were used to analyze describe the kinetic data. Ho model fited better to the experimental results.

Streptomyces levoris Immobilized on Silica Gel 60 as a Novel Biosorbent for Copper (II) Preconcentration

Kirova G.K.[1], Gochev V.K.[2], Stoytcheva M.S.[3], Velkova Z.Y.[1]

[1] *Department of Chemical Sciences, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria*

[2] *Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University “Paisii Hilendarski”, Plovdiv, Bulgaria*

[3] *Institute of Engineering, Autonomus University of Baja California, Mexicali, Mexico*

Abstract. In the present study dead *Streptomyces levoris* biomass loaded on silica gel 60 was applied as an eco-friendly solid phase extractor for copper (II) preconcentration prior to its determination by flame atomic absorption spectrometry. The influences of different parameters such as pH of the sample solution, amount of solid phase, type and concentration of eluent, flow rate of sample solution, sample volume, and interfering effect of diverse ions on the preconcentration procedure were evaluated. An enrichment factor of 25 was achieved under optimum experimental conditions. The obtained results showed that Streptomyces levoris immobilized on silica gel can be considered as a promising new biosorbent for solid phase extraction of trace amounts of copper (II).

Evaluation of Ecosystem Services in the Territory of Mala Planina by the Use of Contingent Valuation Method

Grigorov B., Assenov A.

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

Abstract. The main aim of the current research is to collect, analyse and evaluate data about ecosystem services in Mala Planina by the use of the contingent valuation method. The method is based on declared preferences, expressed by willingness to pay and intends of acting by people. It is used for evaluation of the economic value of all kinds of ecosystem services. Cameral and terrain research is done. The value of the ecosystem services is defined by fulfilling a questionnaire survey. The survey is conducted among 336 respondents in the summer of 2015. The interviewed people inhabit the territories of the four municipalities that fall within the boundaries of Mala Planina. They are the following: Sofia Municipality, Svoge Municipality, Kostinbrod Municipality and Dragoman Municipality.

Landscape-Based Approach to Identification of Shallow Seabed Habitats. Case Study: Central Sector of the Bulgarian Black Sea Coastal Zone

Kotsev I.

“Fridtjof Nansen” Institute of Oceanology – Bulgarian Academy of Sciences (IO-BAS), Department "Coastal zone dynamics"

Abstract. Despite having originated in the former USSR, the notion of the submarine landscape as a benthic geo-ecocomplex is a concept rapidly gaining popularity nowadays, especially among researchers from the Nordic countries. Pursuant to these ideas, the landscape comprises a group of interlinked, hierarchically organized subsystems, implying for the taxonomic subordination of the underwater units.

The study represents an attempt to correlate three popular broad-scale benthic habitat classification systems with such of the submarine landscape units developed by the author. The core of the investigation is a landscape ecological identification and subsequent classification of the seabed complexes occupying the shallow marine area between cape Kaliakra and cape Emine, Bulgarian Black Sea coastal zone. It incorporates a GIS-aided synthesis of the submarine landscape units on the basis of available data about the main components of the benthic environment, namely seafloor lithology and geomorphology, bathymetry, hydrodynamic regime, dominant biota etc.

The proposed hierarchic classification scheme of the seabed landscape units comprises 82 kinds, aggregated in 7 genera, 5 types and 2 classes. Subsequent step of the study is a correlation of the elaborated classification system with such of the seabed habitats, namely these set in the Council Directive 92/43/EEC (Habitats Directive), Directive 2008/56/EC (Marine Strategy Framework Directive), and Volume III (Natural Habitats) of the Bulgarian Red Data Book. The cited

correlations are exemplified in the form of GIS-compiled maps of the submarine landscape units and the associated habitat types. Main emphasis during the correlation process is put on the abiotic oceanographic characteristics of the submarine units, e.g. seafloor substrate types, intensity of the wave-induced sediment transport, hydrodynamic activity and depths of distribution.

Influence of the Composting Process on Nematodes in the Soil Substrate

Petrova P.[1], Koleva L.[1], Tsolova E.[2], Yordanova M.[1]*

[1] *University of Forestry, Sofia, Bulgaria*
[2] *Institute of Agriculture-Kyustendil, Bulgaria*
**Corresponding author: liljanamarkova@abv.bg*

Abstract. The application of compost is a beneficial and economical solution for improving physical and biochemical properties of soil. Many of the farmer’s issues relate to the different organisms involved in the composting process and the effects of compost on the soil fauna. Determining the species composition of nematodes before using compost is an assessment of the ongoing process of composting and essential for agricultural production. The aim of this work was to investigate the structure of nematode communities in various types of mature compost.

For the purposes of the study, the following types of compost were used: forest origin (KI and KII) and agricultural origin (KIII). The difference between the compost KI and KII was the size of the substrate particles.

The results in this work were obtained by field and laboratory studies. The nematode communities in the compost types have a difference in their number and species composition. The highest number was recorded in forest compost KII, followed by forest compost KI and the lowest number was in the agricultural compost KIII. The most common were species of *Aphelenchoides, Diploscapter, Monachoides, Cephalobus, Ditylenchus,* and *Diplogastrellus* and *Dorylaimus* were only found in the forest compost KI. The biodiversity was greater in the compost KII and lowest in the compost KIII. The results could be used as an estimate of the composting process and the quality of the compost.

Eco-physiological Method for Investigating the Effect of Beech Weevil (Orchestes fagi L.) Infestation on Common Beech (Fagus sylvatica L.) Trees

Dimitrova-Mateva P.[1], Georgieva S.[2], Anev S.[3], Chaneva G.[4], Tzvetkova N.[3]*

[1] *University of Forestry, Department of Economics, Sofia, Bulgaria*
[2] *University of Forestry, Sofia, Bulgaria (BSc student)*
[3] *University of Forestry, Department of Dendrology, Sofia, Bulgaria*
[4] *Sofia University “St. Kl. Ohridski”, Department of Plant physiology, Sofia, Bulgaria*
** Corresponding author: petiape@abv.bg*

Abstract. Beech weevil (*Orchestes fagi* L.) causes serious damage on the health and productivity of common beech (*Fagus sylvatica* L.). During late spring beech weevil larvae eat leaf parenchyma and serious reduce photosynthetic potential of beech trees. The young trees are most sensitive to infestation due to delicate leaves’ morphology and still weak defense system. The changes in physiological processes are sensitive indicator for stress in whole plants. Plants have a protective system which prevents them from serious disturbances in the physiological processes. At mild stress physiological activity even increases due to the compensatory effect of healthy plant parts. At high stress plant’s physiological processes are negatively affected because of disruption in biochemical cycles. The objective of this study is developing an applicable method for investigation and assessment of beech weevil damage effect on common beech leaves. The selection of a set of eco-physiological approaches is a good tool for registering of these effects as the physiological parameters can be measured at leaves with gradient of the damaged area. Thus, it can be established a tendency in the attack effect and determined the limits of the plant protective system.

Effect of Orchestes fagi L. Infestation on the Pigment Content of Beech (Fagus sylvatica L.) Leaves

Georgieva G.[1], Dimitrova-Mateva P.[2], Anev S.[3], Chaneva G.[4], Tzvetkova N.[3]*

[1] *University of Forestry, Sofia, Bulgaria, B.Sc. student*
[2] *University of Forestry, Sofia, Bulgaria*
[3] *University of Forestry, Department of Dendrology, Sofia, Bulgaria*
[4] *Department of Plant Physiology, Faculty of Biology, Sofia University “St. Kl. Ohridski”, 8 Dragan Tzankov Blvd, 1164 Sofia, Bulgaria*
**Corresponding author: gchaneva@abv.bg*

Abstract. The herbivore insect, *Orchestes fagi* L., caused serious injuries of the common beech plantations located at two different altitudes in Western Balkan mountain. It was registered a number of biochemical and physiological changes in the leaves, especially in these located at a lower altitude where the insect’s infestation was particularly pronounced.

Pigment content of beech leaves was significantly affected by the *O. fagi* attack. It was found a strong increase of chlorophyll a, chlorophyll b and carotenoid levels in the leaves of trees grown at the lower altitude. Such an enhancement can be considered as a compensatory mechanism in response to the stress resulting from the insect’s feeding. Chlorophyll A and chlorophyll b underwent similar changes – their contents increased by nearly 35% compared to the trees grown at the higher altitude. This trend was particularly expressed in the carotenoids, whose content increased by about 50%. Since carotenoids are considered as one of the most effective non-enzymatic quenchers of reactive oxygen species in the cells, that effect could be viewed as a defensive response against the oxidative stress occurring in response to herbivory.

The Unknown Male of Phtheochroa unionana (Kennel, 1900) (Insecta: Lepidoptera: Tortricidae) and Supposed Synonymy with Other Species

Zlatkov B.

Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Zoology and Anthropology

Abstract. Genus *Phtheochroa* Stephens, 1829 comprises 107 species worldwide and 53 species with Palaearctic distribution. The type material of *P. unionana* (Kennel, 1900) (two male specimens collected in Caucasus) was lost, and consequently several female specimens were assigned to this species. The females of genus *Phtheochroa* often do not demonstrate reliable taxonomical characters, but no male *unionana* was known to be collected apart from the type series and the status of the species remained doubtful. Recently collected male specimens externally similar to the females of *P. unioana* and fitting the original description were dissected and their genitalia, including the everted vesica, were studied. A detailed comparative study of these males and other syntopic males with different wing pattern showed that all belong to the same taxon which apparently is dimorphic. Most probably the two forms were described as two different species: *P. unionana* (pure white forewings) and *P. chalcantha* (Meyrick, 1912) (white forewings with two orange transverse lines), but additional study of type material is needed. If this supposed synonymy is correct, the name *unionana* Kennel, 1900 should have priority over the name *chalcantha* Meyrick, 1912.

Methods, Applicable to Ecotoxicological Biomonitoring Studies with a Focus on Terrestrial Vertebrates

Ostoich P., Nedialkova M., Mecheva R.

Institute of biodiversity and ecosystem research, BAS, Sofia, Bulgaria

Abstract. Recent years have seen an expansion in the scope and depth of ecotoxicological studies involving the environmental biomonitoring of different chemical and physical toxic agents (heavy metals, organic pollutants, ionizing radiation). The methodology applicable to such studies is a variation of the methods, applied in human diagnostics and toxicology, though some of the methods are either modified or substituted. The objective of the current work is to provide an overview of the methods which can be used in ecotoxicological biomonitoring and to assess their respective strenghts, shortcomings and applicability. Some of the methods include: morphophysiological indicators (measurement of size weight, weight of specific organs), hematological indicators, histopathological methods (analysis of organic lesions and tissue changes), cytogenetic methods (analysis of chromosomal aberrations and sister chromatid exchanges), methods from the field of molecular biology and DNA damage (comet assay, variations of the micronucleus test, alkaline elution), and determination of the radiological and other toxic burden in whole animals and organs. All the methods are reviewed critically and their strengths, weaknesses and utility are assessed in the context of ecotoxicological biomonitoring studies.

Секция: Биотехнологии, Бионано-технологии и Приложна биология

Bioactive Compounds Isolated from Garden Snails

Dolashka P.[1], Dolashki A.[1], Velkova L.[1], Stevanovic S.[2], Molin L.[3], Traldi P.[3], Beeumen J.[4], Devreese B.[4], Voelter W.[5]*

[1] Institute of Organic Chemistry, Bulgarian Academy of Sciences, G. Bonchev 9, Sofia 1113, Bulgaria;
[2] Institute for Cell Biology, Department of Immunology, University of Tübingen, Auf der Morgenstelle 15, D-72076 Tübingen, Germany;
[3] R-ISTM, Corso Stati Uniti 4, 35129 Padova, Italy;
[4] aboratory of Protein Biochemistry and Protein Engineering, Ghent University, KL Ledeganckstraat 35, 9000 Ghent, Belgium;
[5] nterfacultary Institute of Biochemistry, University of Tübingen, Hoppe-Seyler-Strasse 4, D-72076 Tübingen, Germany
**Corresponding author: pda54@abv.bg*

Abstract. The recent appearance of a growing number of resistant to conventional antibiotics, has become a serious medical problem. To overcome this resistance, the development of new compounds is encouraged. Hemolymph and mucus of *Helix lucorum* and *Helix aspersa* garden snails are a complex mixture of biochemically and pharmacologically active components.

Glycoprotein ‘hemocyanin’ and antimicrobial peptides from the hemolymph and mucus are important components of the innate immunity. Some isoforms and peptides serve as effector molecules of the defense system, providing an efficient initial effect against infectious pathogens.

The in vitro antitumor activity of *Helix hemocyanin* and its isoforms with different oligosaccharide structures was established on the bladder carcinoma permanent cell lines Cal-29, T-24. This is probably due to the specific oligosaccharide structures of hemocyanins which are exposed on the surface of the molecule.

Aqueous Root Extract of Glycyrrhiza glabra: An Comparative Study of the Reaction with DPPH

Agarwal P[1]., Karamalakova Y[2]., Adhikari M[1]., Gupta D[1]., Nikolova G[2]., R. Chawla[1], Gadjeva V[2]., Stoev S[3]., Arora R.[1], Zheleva A.[2]*

[1]Radiation Biotechnology Group, Institute of Nuclear Medicine and Allied Sciences, Brig. S.K. MazumdarMarg, Defence Research and Development Organization, Delhi-110054, India
[2]Department of Chemistry and Biochemistry, Medical Faculty,Trakia University, Armeiska 11, Stara Zagora 6000, Bulgaria
[3]Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Armeiska 11, Stara Zagora 6000, Bulgaria
**Corresponding author: rajesharoradr@gmail.com*

Abstract. As, stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) is widely used as a colorimetric reagent for determination of antioxidant and free radicals properties of natural compounds. Stable radical 1, 1-dipheny-2-picrylhydrazyl (DPPH) is widely used at in vitro models to investigate antioxidant and radical scavenging abilities of natural extracts. This work presents comparative study on DPPH radical scavenging capacity before and after UV irradiation of aqueous extract of *Glycyrrhiza glabra*, a plant species belonging to the Indian flora. DPPH scavenging activities of different extract concentrations were analyzed and compared by spectrophotometry and electron paramagnetic resonance (EPR) spectroscopy. For the EPR study - *Glycyrrhiza glabra* extract concentrations prepared from stock solution (1 mg powder dissolved in distillated water) were added to DPPH (200 mM) ethanol solution and incubated at room temperature for 2, 10 and 30 min in dark and their EPR spectra were recorded. For the spectrophotometry - 0.3 mL of each extract concentration was added to 0.1 mL/1 M Tris-HCl buffer (pH=7.9) mixed with 0.6 mL of 80 mM DPPH ethanol solution and after incubation for 20 min at room temperature its absorbance at 517

nm was measured. 9.93% and 16.79%, DPPH scavenging activities before and after UV irradiation respectively were found by spectrophotometry. By the EPR spectroscopy study statistical significant increase in DPPH radical scavenging for the *Glycyrrhiza glabra* extracts was established after UV irradiation (78.39± 0.001%) comparing to the non irradiated samples (14.02± 0.02).

Freeze-Embedding Method for Epicuticular Wax Isolation

Ganeva T.

Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8, Dragan Tsankov blvd., Sofia 1164, Bulgaria
tsveta_ganeva@yahoo.com

Abstract. Wax morphology is accepted to be important for the protective properties of the leaf’s surface and also valuable characteristic in plant systematic. In this study epicuticular waxes were mechanically isolated from *Sorbus* and *Cotoneaster* leaves and *Malus* fruits following the freeze-embedding method of Ensikat et al. (2000). Glycerol was used as soluble embedding liquid. After thawing, the waxes were transferred on artificial surface. The application of direct isolation without using solvents, but after freezing and transferring on artificial carrier material allowed wax removal of the upper epidermis separately from the lower epidermis and isolation of only pure epicuticular waxes but not intracuticular ones. Almost entire wax layers – underlying film with crystalline waxes were obtained from the surfaces of the leaves and fruits. The crystals were with well preserved shape and orientation. Consequently, our results could be useful for further studies of the waxes’ chemical composition, physical properties, and resistance to various environmental influences.

Hydrolysis Kinetics of Synergistic Prebiotic Composition in Vitro Simulation of The GI-Tract Conditions

Nakov H., Vasileva T., Iliev I.

Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University, 24, Tzar Asen, Str., Lab15, 4000 Plovdiv, Bulgaria

Abstract. Beta-Glucans are a heterogeneous group of glucose polymers, consisting of a backbone of b(1,3)-linked b-D- glucopyranosyl units with b(1,6)-linked side chains of varying distribution and length. Certain beta-glucans are found to exhibit significant physiological activities, e.g. hypoglycemic-, hypocholesterolemic- and antioncotic activities via the cellular immune system. Thus, they have drawn attentions as medicine or material therefor. The present study describes some specific characteristics of tablets, which are time-controlled to release beta-glucan and oligosaccharides at different rates in ileum of the intestinal tract in order to maintain a substantially constant concentration in the blood. The results show to synergistic compositions comprising prebiotic components selected from one or more component of a group of prebiotics consisting of modified or unmodified beta-glucan, lactulose, galactomannan and suitable partial hydrolysates thereof. The degree of polymerization of the polysaccharides and oligosaccharides and type of monosaccharides after in vitro digestive process under conditions similar to those prevailing in the GIT were determined using HPLC method. Hydrolysis kinetics of beta-glucan and oligosaccharides was studied.

Glycyrrhiza glabra: ''Real Time'' Oxidative Status of Animals

Agarwal P.[1], Arora R.[1], Adhikari M.[1], Gupta D.[1], Nikolova G.[2], Chawla R.[1], Gadjeva V.[2], Stoev S.[3], Karamalakova Y. [2], Zheleva A.[2]*

[1] Radiation Biotechnology Group, Institute of Nuclear Medicine and Allied Sciences, Brig. S.K. MazumdarMarg, Defence Research and Development Organization, Delhi-110054, India
[2] Department of Chemistry and Biochemistry, Medical FacultyTrakia University, Armeiska 11, Stara Zagora 6000, Bulgaria
[3] Faculty of Veterinary Medicine, Trakia University, Armeiska 11, Stara Zagora 6000, Bulgaria
**Corresponding author: rajesharoradr@gmail.com*

Abstract. Water root extract of *Glycyrrhiza glabra* (Licoric) plant is used in Indian traditional Hindu system of medicine (Ayurveda). Presence of biologically active components as triterpenes, saponins, flavonoids, isoflavonoids, chalcones, and glycyrrhizic acid were previously reported for this extract. Beneficial effects of *Glycyrrhiza glabra* extracts like as antiinflammatory, antiviral, antimicrobial, antioxidative, anticancer, immunomodulatory, hepatoprotective and cardio protective properties can be attributed to a number of mechanisms. Because of its excellent antioxidant activity the Licoric extract is used for in vivo treatment of peptic ulcer, hepatitis C, and pulmonary, eye and skin diseases. The purpose of the present study was by ex vivo Electron Paramagnetic Resonance (EPR) spectroscopy methods to investigate changes in “real time” biomarkers of oxidative status in organs isolated from healthy mice (12 mice) treated with *Glycyrrhiza glabra* extract (80mg/ kg/ given 3 times for 10 days). 10 days after treatment the levels of ascorbate radicals, NO radicals and ROS products, in liver, kidneys, spleen and heart homogenates of the mice were studied by direct and spin trapping EPR spectroscopy and compared to those of non treated controls (6 mice). It was established

that in almost all organs of the treated mice the levels of biomarkers tested were close to those of the untreated controls. Significantly lower levels of nitrite and ascorbate radicals were measured only in the spleens and the hearts of the treated mice compared to controls. Present EPR ex vivo study characterizes *Glycyrrhiza glabra* water extract as a good antioxidant.

Секция: Генетика и селекция

Study on Biological, Genetic and Environmental Factors for Azoospermia

Dzhoglov S., Ivanova E.

Plovdiv University“Paisii Hilendarski”, Biological Faculty, Department of Developmental Biology, Section of Genetics

Abstract. The absence of sperm in the seminal fluid is known as azoospermia. This medical condition is in a direct association with male’s infertility. The problems in the sperm production or the presence of any obstruction in sperm delivery could be the most common cause of azoospermia. Azoospermia is defined as the complete lack of sperm in the ejaculate and may be observed in up to 20% of male infertility. There are two types of azoospermia – non-obstructive and obstructive.

A complete medical history, physical examination, tests of selected hormones and tests for male fertility (including sperm analysis) are used for the diagnosis of azoospermia. By the usage of the medical history and physical examination of the patient, any childhood illnesses, any disorders or any family history with reproductive problems (such as presence of dilated veins or varicocele) and secondary sex characteristics could be analyzed.

The number of 1314 men, with fertility problems, in age between 18 and 48 years, were included in this investigation. The data obtained in the study sowed that azoospermia occurred in 5.25% of the studied group (69 male individuals).

There was fount that 42% of males with azoospermra have been smokers, 5.8% of them have been addicted to drugs, 15.94% have took medicaments (such as anabols (4.35%), antidepressants, omnadren, concor, andrositol, tertensif), 4.35% – have had radiation therapy, 4.35% have had a diagnosis “varicocele”. Some of studied males have had overweight or high blood pressure. The volume of the ejaculate was less

than 2 ml (which showed testicular insufficiency or functional reduction in the sexual glands) in 37.68% of the studied man and more than 7 ml (which showed hyperplasia of semen vesicles) in 2.89% of them. The Klinefelter syndrome was cytogenetically diagnosed in 2.9% of the studied male individuals.

Future investigations including other methods (such as detection of microdeletions in the Y-chromosome) could help to specify better the reasons for disrupted spermatogenesis and azoospermia.

Genetic Characterization of Selectively Controlled for Swarm Production Apis mellifera Macedonica (Type Rodopica) Populations in Bulgaria

Georgieva V.[1], Petrov P.[2], Petkov N.[3], Ivanova E.[1]

[1]*Plovdiv University“Paisii Hilendarski”, Biological Faculty, Department of Developmental Biology, Section of Genetics*
[2]*Agrarian University – Plovdiv*
[3]*National Bee Breeding Association*

Abstract. The genetic variability in selectively controlled in Bulgaria local honey bee populations, representing *Apis mellifera macedonica* subspecies (type rodopica), has been studied by usage of alloenzymic analysis of six enzymic systems (MDH-1, ME, EST-3, ALP, PGM and HK) corresponding to 6 loci. Totally 324 worker bee individuals from 9 different local populations belonging to selective bases of National Bee Breeding Association for swarm production were included in this investigation. All of the studied loci were found to be polymorphic in most of the populations with the exception of EST-3 locus which was established to be fixed in two of investigated populations. Polymorphism with three alleles was ascertained for MDH, ME, ALP and PGM loci and with four alleles – for EST-3 and HK loci. The most common alleles in all of the investigated populations were ME 100, EST-3 100, PGM 100 and HK 100. Two private alleles (frequency < 0.05) were found for two of the studied populations. The calculated level of polymorphism was between 88.33% and 100%. The observed and expected heterozygosities were found to range from 0.186 to 0.301, and from 0.205 to 0.305, respectively. The calculated mean Fst level was 0.028. Allele frequencies of all studied loci were used to estimate Nei’s (1972) genetic distance, which was established to range between 0.001 and 0.028 among the selectively controlled populations studied.

Biosocial Characteristics of Patients with Paranoid Schizophrenia

Panayotova G.[1], Chengeliyska V.[2], IvanovaE.[1]

[1]Plovdiv University“Paisii Hilendarski”, Biological Faculty, Department of Developmental Biology, Section of Genetics
[2]State Psychiatric Hospital “Dr. Georgi Kisyov”, Radnevo

Abstract. Schizophrenia is knows as a complex disorder combining both genetic and environmental factors. Different genes have been tested as candidates for association with schizophrenia and different environmental factors have been examined in many studies on schizophrenia epidemiology. Specific environmental factors, such as nonspecific stress, mental and physical abuse, maternal diet during pregnancy, drug use, living in an urban setting, migration, seasonal effects on birth and exposure to infections, have been discussed as possible risk for schizophrenia.

The present preliminary study is focused on sexual, age and ABO blood group system differentiation in different cognitive levels and emotional styles with paranoid schizophrenia.

Totally 72 male and female patients in age between 23 and 64 years with diagnosis of paranoid schizophrenia have been included in the study. There was found presence of hereditary factors in 56.76% of the female and 34.29% of the male patients included in the study. The ABO blood system belonging (A – 40.28%, O – 36.1%, B – 15.28%, AB – 8.33%) has been discussed in connection with other studied factors. Concerning educational level – 5.56% were found to be with an elementary level, 16.67% – with primary, 55.56% – with secondary and 18.06% – with a higher educational level. About 7% of the studied paranoid schizophrenia patients were with a bent for music and poetry and with an aptitude for painting. Evidences for aggressive behaviour have been found in 11.45% of male and in 2.7% of female individuals.

Suicide behaviour has been ascertained in 2.86% of male and in 29.73% of female individuals included in this study. A presence of environmental risk factors (such as a pregnancy, loss of blood, cranial trauma, scare or accident) has been established in 25.71% of male and in 10.81% of female patients studied.

Future comparative investigations including genetic markers and psychogenetic approaches should be used in complex, in order to characterize better the reasons for paranoid schizophrenia and possible relations with cognitive and emotional peculiarities.

"Mutated" Genofound for Some Common Diseases , Persistent Multivalency with Its Clinical Manifestations

Dincheva R.

Day center for children with disabilities “St. Anna” Plovdiv

Abstract. Mutations in genes affecting generational change except in the substrate itself of the gene, but also in its clinical expression. Diverse manifestation of clinical symptoms brings us into a new unknown world, into a new kind of manifest generation is not uniform, not homogeneous and which the world has yet to start research. The correct genetic diagnosis of a child with a genetic problem in development the first step in therapeutics work.

Секция: Медицинска биология

Rosemary Oil Reduces Electrical Field Stimulated Tetanic Muscles Contractility

Patti J.[1], Stefanova I. [1], Draganova-Filipova M.[2], Zagorchev P.[1]

[1]*Department of Medical Physics & Biophysics,*
[2]*Department of Medical Biology, Medical University – Plovdiv*

Abstract. Rosemary oil (RO) is an essential oil extracted from Rosemary officinalis. It consists of more than 50 compounds with different biological activities. Empirically it is used in therapy for many years. RO is analyzed in vivo and in vitro as many of its effects - antimicrobial, antitumor, antioxidant etc. are known. We cannot find suggested reports concerning the RO effect on contractility of striated muscles. The aim of this study was to investigate the influence of RO on maximal tetanic contraction provoked by electrical field stimulation (EFS). We use abdominal transversal muscles strips isolated from guinea pigs. The tetanic muscles force, was provoked by means of repeated multipulse EFS, square-wave pulses of supramaximal intensity (60V) and 0.5 ms in duration were applied at frequency of 50 Hz for 3s followed by 7s pause. The concentration effect curve of the action of RO (1, 5.10-6M ¸1, 5.10-3M) on this type electrical field stimulated - muscle activity was plotted on a statistical graph.During our observation the effect of RO on contractile properties of abdominal muscles and calculated half maximal effective concentration (EC50). Our study revealed that myorelaxant activity of the essential oil in this concentration is time-dependent and after application in interval of 25 minutes the contractile ability is decreased to 5,5% within comparison with the initial one. Our investigation is a first report on an action of RO on the guinea pig's striated muscles strips.

Carbohydrate Structures of Molluscan Hemocyanins from Snails Helix lucorum and Rapana venosa, Determined by Mass Spectrometry

Velkova L.[1], Dolashka-Angelova P.[1], Devreese B.[2], Van Beeumen J.[2]*

1Institute of Organic Chemistry, Bulgarian Academy of Sciences, G. Bonchev 9, Sofia 1113, Bulgaria.
2Laboratory of Protein Biochemistry and Biomolecular Engineering, Ghent University, KL Ledeganckstraat 35, 9000 Ghent, Belgium.

Abstract. Hemocyanins (Hcs) are copper-containing respiratory glycoproteins with quaternary structure, freely dissolved in the hemolymph of several arthropods and molluscs. The carbohydrate structure of molluscan Hcs has recently received particular interest due to their immunostimulatory properties, antitumor and antiviral activity. This is mainly related to their high carbohydrate content and specific monosaccharide composition. We have determined and analysed oligosaccharide structures of molluscan hemocyanins, isolated from the hemolymph of Bulgarian garden snails Helix lucorum and of marine snail Rapana venosa. The oligosaccharide structures of the structural subunits of Rapana venosa hemocyanin (RvH) and the isoform βc-Helix lucorum hemocyanin (βc-HlH) were studied by sequence analysis of N-glycans using MALDI-TOF-MS and tandem mass spectrometry on a Q-Trap mass system, after enzymatical liberation of the N-glycans from the polypeptides. Our study revealed a highly heterogeneous mixture of N-glycans with compositions Hex3-7HexNAc2-5MeHex0-4Pent0-1Fuc0-1 and Hex0-9HexNAc2-4MeHex0-1Pent0-2Fuc0-3, in the isoform β-HlH and subunits of RvH, respectively. Identified glycans of β-HlH have predominantly monoantennary and diantennary structure with complex type and hybrid type. As in most molluscan hemocyanins, the glycans of β-HlH contain mainly a terminal MeHex residue, in some cases even two, three and four. Several carbohydrate chains in the HlH we analyzed

are core-fucosylated, and also possess a high degree of methylation. Identified glycans in RvH have mainly complex- and high mannose-type structures. A novel type of N-glycan, with an internal fucose residue connecting one GalNAc(β1-2) and one hexuronic acid, was detected in RvH. The glycosylation sites occupancies were subsequently elucidated by precursor ion scanning of the intact glycopeptides from RvH and β-HlH using a Nano-ESI mass spectrometry and Q-trap-LC/MS system. The oligosaccharides found in HlH and RvH reveal a complex N-glycan pattern combining typical structural features of different higher organisms (mammals, plants, insects, nematodes, trematodes). Moreover, they are a potential source of novel N-glycans that are important for the stimulation of the immune response in humans and/or for the production of antibodies used in diagnosis and therapy.

Comparative Study on Antibacterial Activity of Synthetic Analogues of Biologically Active Compounds and Their Combination with Rhamnolipid-Biosurfactant

Lazarkevich I., Sotirova A., Avramova T., Galabova D.

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

Abstract. The resistance of bacteria to antibiotics is a serious public problem with increasing significance. The development of novel pharmaceuticals with wide spectrum of activity for application in human and veterinary medicine or for plant protection is necessary. The biological activity of two groups of synthetic analogues of naturally occurring compounds with antimicrobial properties was studied. The esters of thiosulfonic acid are similar to allicin - the antibiotic substance of Allium sativa. Compounds with 1,4-naphthoquinone structure are derivatives of lawsone, contained in the leaves of henna (Lawsonia inermis). The antimicrobial potential of these substances was assessed against model strains from different genera, able to cause damages to industrial manufacture, agriculture and human health. The results revealed that the naphthoquinone derivatives demonstrated relatively lower antibacterial activity compared to thiosulfonate esters and had not bactericidal effect even at the highest tested concentrations. The possibility for potentiating of the antibacterial activity of thiosulfonates and 1,4 - naphthoquinones by rhamnolipid – biosurfactant was alsostudied. It was suggested that the biosurfactant provokedchanges in the cell membrane organization that facilitate the access of antimicrobials into bacterial cell. The presence of rhamnolipid - biosurfactant decreased significantly the minimal bactericidal concentration of thiosulfonates, but affects only the minimal inhibitory concentration of naphthoquinones and bactericidal effect was not registered again. The enhancement of antibacterial activity of inhibitors in

the presence of biosurfactant significantly increased the therapeutic potential of these compounds. The development of combined preparations enables to modify the effect of the antimicrobial agents thus contributing to overcome the drug resistance.

Role of Oxidative Stress in Male Infertility

Martinov D.[1,2], Alexandrova A. [3], Ayvazova N. [1], Ilieva L.[1], Alexandrova R. [4], Konova E.[1,2]

1Clinical Institute for Reproductive Medicine Pleven
2Medical University Pleven
3Institute of Neurobiology Bulgarian Academy of Sciences, Sofia
4Institute of Experimental Morphology and Anthropology with Muzeum, Sofia

Abstract. Summarized data of the Department of Reproductive Health and Research at WHO (2010) show that global reproductive problems affect an increasing proportion of the population. Pathological effects of free radicals in male reproductive tract is associated with DNA fragmentation, apoptosis, and lipid peroxidation. Organisms have developed an antioxidant protection. It is a complex of enzymatic and non-enzymatic activities, measured as total antioxidant capacity (TAC). The purpose of this work was to compare the levels of TAC and integrity of sperm DNA (DFI) in seminal plasma of fertile and infertile men. The studied 48 men were divided into two groups: first group - fertile men (n = 17) and second group - infertile men (n = 31). The seminal analysis was made according WHO criteria from 2010. The assessment of total antioxidant capacity was performed by Koracevic’s method. The integrity of sperm DNA was determined with Sperm DNA Integrity Test (SDI). A negative correlation between the levels of TAC and DFI was established. Our results had confirmed the existence of relationship between oxidative stress and DNA fragmentation established by other authors. DNA damage of the male germ cells was associated with poor performance in fertilization in vitro, pre-implantation embryonic defects and high incidence of abortion.

Студентска сесия

Training on "Biology and Health Education" as a Basis for Forming Health and Ecological Competencies in Students from Grades 7-10

AngelovaV.[1] , Kitova R[1], Panayotova M.[2],. Vakleva Zl.[2]

[1] Plovdiv University "P. Hilendarski", Faculty of Biology, BSc Biology and Physics
[2] Plovdiv University "P. Hilendarski", Faculty of Biology, Dept. Botany and MOB

Abstract. Thematic area "Nutrition, Health, Environment" brings together some topics of the curriculum of Biology and health education from grades 7-10. This article analyzes the relationship between knowledge, skills and attitudes (as an expected result from training students on these topics) and the possibilities of forming health and ecological competencies in adolescents to protect their own health.

Clinical Laboratory Tests

Aziz S.

University of Plovdiv, Faculty of Biology – BSc - student

Abstract. Introduction: In the clinical laboratory are made various studies: reporting of complete blood count (Hematology), biochemical analysis, researching of hormones and tumor markers.

Target: The purpose of these studies is to give a clean idea to people for their health condition. This is achieved through specialized and highly specialized medical diagnostic tests.

Methods: The clinical laboratory has built a good informational system that allows reporting of results from different studies. It is using modern equipment thanks to which achieves reliable results.

Results: During the practical training in the clinical laboratory many people have entered. The results that were obtained, some of them corresponded to the normal values, while others exceed the reference values. Most often this occurs in people suffering from diabetes or by researching of hormones and tumor markers. About 70 % of the people don’t keep a diet. By researching turns out that 45 % of the patients receive “Syndrome”.

Conclusion: Various laboratory tests that take place allow better practical training. They give a clear idea about the condition of patients. At any time are held even cyto-analysis.

Visualizationo of Mitochondrial Dysfunction in Living Cells, Based on Superoxide Generation

*Georgieva E.,[1] Ivanova D.,[1] Zhelev Z.,[1,2] Aoki I.,[3] Bakalova R.,[3,4]**

[1]Medical Faculty, Trakia University, 11 Armejska Str., Stara Zagora 6000, Bulgaria;
[2]Institute of Biophysics & Biomedical Engineering, Bulgarian Academy of Sciences, 21 Acad. G. Bonchev Str., Sofia 1114, Bulgaria;
[3]Molecular Imaging Center, National Institute of Radiological Science, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan;
[4]Medical Faculty, Sofia University, 1 Koziak Str., Sofia 1507, Bulgaria
**Corresponding author: bakalova@nirs.go.jp*

Abstract. Here we report that superoxide radical is involved in the enhancement of T1-weighted magnetic resonance imaging (MRI) contrast in living cells in the absence and presence of nitroxides as contrast substances. The nitroxide-enhanced T1-weighted MRI allows a direct visualization of overproduction of superoxide in cells and model chemical systems. It is an appropriate technique for detection of mitochondrial dysfunction, resp. for detection of metabolic activity in living cells and tissues. The study demonstrates that superoxide increased, but hydrogen peroxide decreased the intensity of T1-weighted MRI contrast. Thus, the proposed methodology allows also a detection of the balance between superoxide and hydrogen peroxide in situ and in vivo – a very important approach for clarifying of the fine molecular mechanisms in the regulation of carcinogenesis, neurodegeneration, inflammation, etc. This report gives a direction for discovering of new opportunities for MRI diagnostics – for detection of metabolic activity, accompanied by overproduction of superoxide (and/or nitric oxide). The real-time detection of mitochondrial dysfunction might be also a key for discovering of the molecular mechanism of functional MRI in its commonly accepted definition as a method for detection of neurovascular coupling.

Increasing the Sensitivity of Cancer Cells to Anticancer Drugs by Inhibiting the Glycolysis

*Ivanova D.,[1] Georgieva E.,[1] Lazarova D,[2] Bakalova R[2,3] Aoki I,[3] Zhelev Z.,[1,4]**

[1]Medical Faculty, Trakia University, 11 Armejdka Str., Stara Zagora 6000, Bulgaria
[2]Medical Faculty, Sofia University “St. Kliment Ohridski”, 1 Koziak Str., Sofia 1407, Bulgaria
[3]Molecular Imaging Center, National Institute of Radiological Sciences (NIRS), 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan
[4]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev. Str., Sofia 1113, Bulgaria

Abstract. One of the most prominent specific metabolic alterations in cancer cells is the increase in aerobic glycolysis and the dependency on glycolytic pathway for ATP synthesis, known as “Warburg effect”. The inhibition of the glycolysis can kill the malignant cells preferentially or at least to sensitize these cells to conventional chemotherapy and radiotherapy.

The aim of the present study was to investigate the possibility to sensitize cancer cells to anticancer drugs, using 2-Deoxy-D-Glucose (2-DDG; a well-known inhibitor of glycolysis and redox-modulator), as well as to find combinations with synergistic cytotoxic effects. The study covers fifteen anticancer drugs – conventional and new generation.

The experiments were performed on cancer cells (Jurkat), treated by drug or 2-DDG – separately or in combination with all anticancer drugs, within 24-72 hours. Cell viability was analyzed using trypan blue staining and Countess™ Automated Cell Counter (Invitrogen) at very precise standardization of the measurements.

At 24-hours of treatment, most of the combinations (drug + 2-DDG) were characterized by enhanced inhibition of cell proliferation than drug only, but the cytotoxic effects were mostly additives. Very well expressed synergistic cytotoxic effects were found after 48-hours treatment of

cancer cells with 2-DDG in combination with: Palbociclib, Everolimus, Lonafarnib, Bortezomib, Barasertib. This synergism significantly increased after 72-hours incubation and was detected at almost all combinations.

The study shows that combining anticancer drugs with 2-DDG gives a possibility to enhance enormously the anticancer effect, at very low concentration of the drug. This fact allows development of therapeutic approach with minimal side-effects on non-cancer cells and tissue.

Phenotypic Investigation of Paired Pseudomonas Aeruginosa Cystic Fibrosis Strains Isolated Prior- and Post-Inhaled Tobramycin Treatment

Borisova D [1]., Strateva T [2]., Paunova-Krasteva Ts [1]., Stoitsova S [1].

[1] *Department of General Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Department of Microbiology, Medical University of Sofia, Bulgaria*

Abstract. Cystic fibrosis (CF) is a hereditary disease accompanied by extensive secretion of viscous mucins in the digestive and respiratory tracts. Bacterial respiratory tract infections, very often caused by Pseudomonas aeruginosa, are the leading cause for exacerbations and early death of patients. Tobramycin solution for inhalation is indicated as chronic suppressive treatment in CF patients aged six years and older chronically infected by P. aeruginosa. In spite of the initial success of the treatment however some part of the bacterial population remains persistent in the lungs. The comparison of paired strains isolated from the same patient prior- and post-antibiotic treatment would elucidate some adaptive mechanisms related with the bacterial persistence in cystic fibrosis, which determined the objectives of the present study. We performed a comparative phenotypic investigation on a panel of 6 couples of paired strains, each couple containing a strain isolated prior- and a strain isolated post-inhaled tobramycin treatment from the same patient. We characterized the strains’ growth parameters, tobramycin sensitivity, biofilm formation, and motility (swimming, swarming, and twitching). Strain-to-strain differences were registered with most of the tests. As a distinct trend demonstrated in the examined pairs, the changes in growth parameters should be underlined, and especially – the elongation of the lag phase of the post-treatment strains.

П о с т е р и – 3 0 . 1 0 . 2 0 1 5

Секция: Молекулярна и Клетъчна Биология

P1

Expression of Levansucrase Gene from Leuconostoc mesenteroides Lm17 in Lactobacillus plantarum

Salim A., Vasileva T., Bivolarski V., Iliev I.

Department of Biochemistry and Microbiology, Plovdiv University, Tsar Assen St. 24 Plovdiv 4000, Bulgaria

Abstract. *Leuconostoc mesenteroides* Lm 17 produces two types of glycosyltransferases – GTF with molecular weight, corresponding to 180 kDa and FTFs with molecular weights of 120 kDa and 86 kDa. The following enzymes produce different prebiotic oligosaccharides. The production of the enzymes in a complex make difficult their biochemical characterization and study the structures of the synthesized products. The aim of the present study is to isolate, clone and express the gene encoding levansucrase (120 kDa) in order to characterize its biochemical properties, related to its industrial application for production of novel functional oligosaccharides and polymer with therapeutic potential.

The gene encoding FTF with molecular mass of 120 kDa from *Leuconostoc mesenteroides* Lm 17 was amplified and cloned in *E. coli* BL21 cells. In the presence of sucrose or raffinose the studied recombinant levansucrase synthesizes a polymer identified by 13C-NMR as levan. The next stage of the experiments is to transfer and overexpress the cloned gene *in Lactobacillus plantarum* using sakacin P-based expression system for controlled gene-expression. The recombinant enzyme is used to study its transferase capacity for production of fructooligosaccharides with prebiotic potential.

P2

Serological Immunoassay for Hepatitis E Detection Based on Open Reading Frame 2 Recombinant Proteins Genotype 3 Expressed in N. benthamiana Using pEAQ- HT Vector

Varadinov N., Mazalovska M., Zahmanova G.

Department of Plant Physiology and Molecular Biology, University of Plovdiv, Bulgaria
E-mail: gerganaz@uni-plovdiv.bg

Abstract. Hepatitis E virus (HEV) is transmitted mainly through faecal-oral route and it is found worldwide. There have been reports of other routes of transmission and potential zoonotic spread of the virus. It is thought that 50% of acute hepatitis in adults it is due to Hepatitis E virus with death rate of 1-2%, and surprisingly 20% in pregnant women. HEV infected mammals have been classified into four genotypes: 1, 2, 3, and 4. Serological immunoassay for detection of HEV infection is often based only on recombinant ORF2 peptides of HEV genotype 1 and 2. In some cases with genetically proven HEV gt 3 infection these serological assays failed to detect anti-HEV ORF2 antibodies. In this work, we demonstrated the transient expression, purification and characterization of HEV ORF2 capsid protein in plant tissue using pEAQ-HT vector. The recombinant protein can be used as a diagnostic antigen for detection of HEV gt 3 infections, the most prevalent in industrialized countries.

We describe a high-yield, low cost and simple method for the production of HEV ORF2 protein in *N. benthamiana* and usefulness of this protein for the serological detection of HEV infection.

Acknowledgements. The authors want to thank Prof. George Lomonossoff for his contribution. The investigations presented were funded by the Bulgarian Science Fund project DMU03/33, ДКОФ7РП-02/117.

P3

Dark Drops of Prompt Chlorophyll Fluorescence as a Novel Approach for Evaluation of the Photosynthetic Machinery State

Dimitrova S.[1], Dankov K.[1], Paunov M.[1], Goltsev V.[1], Velikova V.[2], Tsonev T.[2], Kalaji H.[3], Strasser R.[4]

[1] Department of Biophysics and Radiobiology, Faculty of Biology, St. Kl. Ohridski University of Sofia, 8 DraganTzankov Blvd., Sofia, 1164, Bulgaria
[2] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bontchev Str., bl. 21,1113 Sofia, Bulgaria
[3] Department of Plant Physiology, Warsaw University of Life Sciences SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland
[4] Bioenergetics Laboratory, University of Geneva, CH-1254, Jussy-Geneva, Switzerland

Abstract. Analyzing the changes that occur in the photosynthetic machinery of plants is one of the most informative approaches for evaluation of the plant physiological state, their stress reactions, productivity and the adaptive mechanisms that develop in order to protect the plant in a changing environment. An informative method for detection and analyzing of the photosynthetic process is measuring the chlorophyll a fluorescence emitted from leaves. Illumination of a plant sample induces a rise in the chlorophyll a fluorescence that draws characteristic induction curves which carry broad spectrum of data about every step of the photosynthetic process.

Chlorophyll a fluorescence gives two types of signals – prompt (PF) and delayed chlorophyll fluorescence (DF). The Multifunctional Plant Efficiency Analyzer (MPEA), developed by Hansatech is constructed to measure both simultaneously in only one measurement, but as the initial steps of their kinetics overlap they cannot be recorded together while the sample is illuminated. Thus the apparatus is set to switch between light (recording of PF) and dark (recording of DF decay kinetics) conditions at specific periods of time. When the light is switched off during the dark

period oxidation processes of the Photosystem II (PSII) reaction center occur which lowers the level of chlorophyll fluorescence. Thus when the light is put on again, the PF signal continues from a lower level. This phenomenon is called Dark Drops (DD) of chlorophyll fluorescence and it can be a valuable source of information.

The aim of our study is to develop a new approach to collect and analyze the information from the Dark Drops and use it to investigate the changes in the photosynthetic process in leave samples of the plant *Phaseolus vulgaris* in normal conditions and in conditions of heat stress.

P4

Effect of Water Stress on Photosynthetic Light Phase in Leaves of Two Ecotypes of Platanus orientalis l. Plants

*Paunov M.[1], Dankov K.[1], Dimitrova S.[1], Velikova V.[2], Tsonev T.[2], Strasser R.[3], Kalaji H.4, Goltsev V.[1],**

[1] Department Biophysics and Radiobiology, University of Sofia “St KlimentOhridski”, 8, Dr. Tzankov Blvd., Sofia 1164, Bulgaria
[2] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bontchev Str., bl. 21, 1113 Sofia, Bilgaria
[3] Bioenergetics Laboratory, University of Geneva, CH-1254 Jussy/Geneva, Switzerland
[4] Departmentof Plant Physiology, Warsaw University of Life Sciences WULS—SGGW, 159 Nowoursynowska Str., 02-776 Warsaw, Poland
**Corresponding author e-mail: goltsev@biofac.uni-sofia.bg; fax: +359 2 865 66 41*

Abstract. *Platanus orientalis* is an endangered species that is almost extinct in the natural ecosystems of Western Europe and, because of its hydrophilic habitat, may also be strongly affected by increasing water limitations in Eastern Europe. The effects of drought stress were studied and compared in young plane trees of two ecotypes from Bulgarian and Italian regions. The dynamics of drying and following re-watering was monitored in vivo by changes in activity of photosynthetic light phase reactions in leaves of 4-5 month old seedlings subject to controlled moderate water limiting regime for 12 days and subsequent recovery by controlled gradual irrigation for 10 days.

The physiological state of photosynthetic machinery was estimated by analysis of signals of prompt and delayed chlorophyll a fluorescence measured in attached plane leaves by the Multifunctional Plant Efficiency Analyzer (Hansatech Instruments Ltd., UK). Prompt chlorophyll a fluorescence directly correlates to the redox state of the electron carriers in photosystem II at light conditions, while delayed fluorescence indicates rate constants of direct and back electron transport reactions within the same structure. The fluorometer allows simultaneous measurement of both prompt and delayed chlorophyll fluorescence signals that provide

complementary information concerning the reactions in the photosynthetic light stage.

We show that both donor and acceptor sides of photosystem II as well as intersystem electron transport are inhibited during drought stress. Moreover, the intersystem electron carriers appear to be the most sensitive part, indicated by strongly reduced size of their pool in the thylakoid membrane. The stress reaction of Bulgarian ecotype is expressed clearly. However, fluorescent parameters undergo partial recovery during re-watering. On the other hand, parameters in Italian ecotype exhibit weak changes during drought stress but the effect proceeds throughout the whole monitoring period.

P5

Investigation of the Photosynthetic Characteristics of the Fern Sicksonia antarctica In Vivo Upon Illumination with Different Intensity and Under Conditions of Heat Stress

Dankov K., Dimitrova S., Paunov M., Goltsev V.

Department of Biophysics and Radiobiology, Faculty of Biology, St. Kl. Ohridski University of Sofia, 8 DraganTzankov Blvd., Sofia, 1164, Bulgaria
Corresponding author: k.dankov@gmail.com

Abstract. Investigation of the signals of prompt chlorophyll a fluorescence (PF), delayed chlorophyll a fluorescence (DF) as well as modulated light reflection (MR820) by the Multifunctional Plant Efficiency Analyzer (MPEA, developed by Hansatech) allows gathering of immense information about plants physiology state in different conditions. It is a very sensitive tool for analyzes of the behavior of plants biochemical and physiological reactions to stress, to establish the boundaries of their stress tolerance and adaptive mechanisms.

Ferns are one of the least studied plant objects despite their potential application as nutrition, in medicine and many others. In our study we created a photosynthetic profile of the fern *Dicksonia antarctica* upon illumination with different intensity and under conditions of heat stress by measuring the signals of PF, DF and MR820. Also we calculated important parameters that give very detailed information about the photosynthetic processes that occur during conditions of stress.

P6

Development of Different Microscopic Methods for Detection of Cell Changes and Intra-Cellular Interactions

Sainova I., Ilieva I., Valkova I., Nikolov B., Nikolova E.

Institute of Experimental Morphology, Pathology and Anthropology with Museum to Bulgarian Academy of Sciences – “Acad. G. Bonchev” Street 1113 Sofia, Bulgaria

Abstract. Different microscopic techniques for determination of changes, underlining the cell fate during cell differentiation and maturation and/or damages, are applied and tested. Cell organelles as nucleus, endocytosis vesicles, membrane structures, are important for basic cell functions. The used techniques give a possibility to be revealed changes in appropriate in vitro-cultivation not only of cells in early stages of maturation, but also in terminal phases of differentiation. These methods facilitate the identification of small, but significant irregularities or morphological abnormalities. In this connection, the main goal is directed to search cell structures, participating in the control of cell growth, proliferation and differentiation. Light, scanning and transmission electron microscopy (SEM, TEM) are applied for evaluation of changes in incubated in various conditions different cell types in various phases of maturation and differentiation (early progenitors – mouse embryonic 3T3 fibroblasts, epithelial stem cells from human cornea and oral mucosa, and terminally differentiated cells – human spermatozoa). The light-microscopy observations show myeloid-like characteristics, but also fusion between separated cells or mineral depositions in them, respectively. This could be explained with the influence of the cryo-protector Dymethylsulfoxide (DMSO) on the physical and chemical characteristics of the cell structures, and reveals a possibility for application of this and other organic detergents in different tissue- and cell-engineering manipulations. Also, differences in the light microscopy preparations in stem/progenitor cells from human cornea and oral mucosa are established, and a possibility for application of cell progenitors from both different sources in derivation of concrete mature cell lineage is suggested. The noted features characterize cell types,

received by differentiation of early cell progenitors. Other changes, observed on electronograms, are essential for terminally-differentiated cells. They affect all intra-cellular organelles, and could indicate the exact location of damaged cell components (chromatin density, lack of acrosome in the spermatozoa head, but also structural dislocations of its neck and tail, presence of lesions or cytoplasmic droplets on the cell surface in cases of injuries). Typical in this connection are the reorganizations, observed in the process of spermatozoa formation. The techniques, presented in the current study, might be useful for identification of unknown cell changes and inter-cellular interactions at different levels. The combination of different techniques for microscopic examination allows a decrease of a subjective error inherent, appearing in the application of conventional methods for investigation.

P7

Attenuation of Cellular Oxidative Stress by Natural Products and Plant Extracts after Chemotherapeutic Exposure

(Review)

Sainova I., Pavlova V., Alexieva B., Valkova I., Ilieva I.,Nikolova E.

Institute of Experimental Morphology, Pathology and Anthropology with Museum to Bulgarian Academy of Sciences – “Acad. G. Bonchev” Street 1113 Sofia, Bulgaria

Abstract. Some natural foods are proved to be useful in prevention of the side effects, caused by the chemotherapeutic agents, both in vitro and in vivo. Many of the compounds, containing in them, are also approved as potent anti-malignacy agents. As one of the main mechanisms of the action of anti-oxidant molecules is by protection against chemically-induces oxidative stress, by cascade mechanisms, involving modification of autophagy and apoptosis. Also, the role of regulatory pathways, underlining the differentiation of immune cells and immune system, is proved. In this way, the combination treatment with both chemotherapeutic agent plus natural product(s), is proposed to allow a stronger toxicity to malignant cells, rather than to reduce the side effects. Among the main molecules in these processes, is established the reduced form of tri-peptide Glutathione (GSH), Glutathione-S-transferase (GST), Superoxide dismutase (SOD), Catalase (CAT) and beta-galactosidase. The mechanisms of cell protection probably involve the reduction of intra-cellular oxidative stress, maintaining GSH availability, but also increased expression and activity of GST enzyme.

P8

Primary Cell Cultures As Experimental Models in Breast Cancer Research and Oncopharmacology

Zhivkova T.[1], Dyakova L.[2], Dinev D.[1], Gavrilova-Valcheva I.[3], Abudalleh A.[1], Gavrilov I.[3], Alexandrova R.[1]

[1] Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. “GeorgiBonchev” Str, Block 25, Sofia 1113, Bulgaria;
[2] Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. “GeorgiBonchev” Str, Block 23, Sofia 1113, Bulgaria;
[3] National Spezialized Hospital for Active Treatment in Oncology, 6 “Plovdivsko pole” Str., Sofia, Bulgaria

Abstract. Primary cell cultures from cancer samples have been suggested to be suitable models for tumor biology investigations as well as to evaluate in vitro the effectiveness of antitumor drugs and treatment strategies. These experimental systems are expected to represent better the morphological and functional characteristics of the original cancer cells in patients and to be of benefit for the so called individualized medicine.

The aim of our study was to establish primary cultures from human breast cancer samples and to determine some of their biological features.

Samples from eight invasive ductal carcinomas in patients aged from 38 to 60 years (50,75 +/- 2,42) were obtained. Primary cultures were established by cultivating cell suspensions (obtained by EDTA/Trypsin treatment) and/or tumor explants. Growth curves were prepared to follow the ability of cells to grow and proliferate in time-dependant manner. The cell sensitivity to the cytotoxc/cytostatic effects of Zn/Ag and Zn/Au complexes with Schiff bases was determined by MTT test.

Acknowledgements. Supported by Fund “Scientific Research”, Bulgaria, Grant № DFNI Б 02/30 from 12.12.2014 and a bilateral project between Bulgarian Academy of Sciences and Romanian Academy.

P9

Differential effect of a novel plant cystatin on the initial adhesion of normal and cancer breast cells

Hristova K.[1], Keremidarska M.[1], Rakleova G.[2], Tchorbadjieva M.[2], Krasteva N.[1*]*

[1] *Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
[2] *Department of Biochemistry, Faculty of Biology, Sofia University*
***** *Corresponding author: kameliahristova@abv.bg*

Abstract. Cystatins are cysteine protease inhibitors that belong to the superfamily of the protease inhibitors. They are present in mammals, birds, fish, insects, as well as in plants and some protozoa. Previously, a novel extracellular phytocystatin was identified in *D. glomerata* L. embryogenic suspension cultures, and the protein was cloned and expressed in *E. coli*. Recent studies have shown that plant protease inhibitors, including phytocystatins, can block invasion or metastasis of different cancers. In the present study we have investigated the putative anticancer effect of the recombinant cystatin DgECPI by evaluating its effect on the adhesive behavior of a breast cancer cell line – MDA-MB-231 and normal mammary epithelial cells, MCF-10A. Interestingly, DgECPI contains the cell adhesion motif RGD, typical for the extracellular matrix proteins but not for plant cystatins, suggesting the ability of this phytocystatin to influence adhesion of cancer cells and therefore cancer metastasis. A mutant of the cystatin, DgECPI (R6H) containing the tripeptide HGD instead of RGD was investigated also. Our results showed that both phytocystatins exerted differential effect on cellular adhesion of normal and cancer cells, whereupon the cancer cells were much more responsive. DgECPI, when adsorbed on a substrate, improved significantly the attachment of breast cancer cells, and also induced cell aggregation which is a sign typical for normal mammary cells. As expected, cancer cell adhesion was completely abolished when the mutant DgECPI (R6H) was used. On the other hand, both plant cystatins only slightly reduced adhesion of normal mammary cells. These

preliminary results open the possibility of considering the plant cystatinDgECPI for anti-cancer strategies.

P10

Next-generation sequencing and Assembly of the Chloroplast Genome of Haberlea rhodopensis

Ivanova Z., Daskalova E., Yahubyan G., Apostolova E., Zahmanova G., Baev V.*

Department of Plant Physiology and Molecular Biology, University of Plovdiv, TzarAssen 24, Plovdiv, Bulgaria
**Corresponding author: vebaev@plantgene.eu*

Abstract. Complete chloroplast genome sequences provide valuable information about plant evolution, development, molecular ecology and are the foundation for comparative genomic studies and phylogenetic reconstruction analysis. *Haberlea rhodopensis*, a member of the *Gesneriaceae* family, is a resurrection species endemic for Bulgaria and Greece. The unique feature of this plant is the resistance to drought stress and desiccation but also the ability to withstand low temperatures and freezing. Chloroplast DNA (cpDNA) was isolated from intact chloroplasts and subjected to Next-Generation sequencing using Illumina (Solexa) technology (HiSeq 2000). For two biological replicates, 8,365,536, and 7,129,508 100bp pair-end read sequences were generated in total. Here, we present the bioinformatics approach and methodology that we developed for reconstructing the chloroplast genome of *H. rhodopensis*.

P11

Detection of Cytomegalovirus (CMV) DNA by PCR in Patients with Unknown Inflammatory Eye Diseases

Grozdanov P., Nikolova I., Galabov A.*

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
**Corresponding author: grozdanov@microbio.bas.bg*

Abstract. Cytomegalovirus (CMV) is well known as pathogenic agent of intraocular inflammatory diseases. Light microscopy and immunohistochemical studies have limitations in the identification of this virus, but detection and typing viral DNA with Polymerase Chain Reaction (PCR) offers a rapid, highly specific, and easily interpretable means of identifying CMV in patients with ophthalmic lesions. Two patients (34-year-old male and 48-year-old male) who developed retinitis with unknown causative agent were studied for presence of CMV DNA. We used PCR kit for the qualitative detection of Cytomegalovirus (CMV 500/800 IC) provided for us by “Sacace Biotechnologies”. The target of the PCR reaction was the “Major Immediate-Early” (MIE) gene. Positive and negative controls were used to avoid false results. Our PCR analysis showed the presence of CMV-DNA within the samples.

P12

viR-SCAN – A Tool for Detection of Viroid- and Virus-Derived Small RNAs Using Plant Next-Generation Sequencing Datasets

Dimitrov D., Gozmanova M., Ivanova D., Daskalova E., Minkov I., Yahubyan G., Baev V.*

Department of Plant Physiology and Molecular Biology, University of Plovdiv, TzarAssen 24, Plovdiv, Bulgaria
**Corresponding author: vebaev@plantgene.eu*

Abstract. Small RNAs (sRNA), including microRNAs (miRNA) and small interfering RNAs (siRNA) are produced abundantly in plants and animals. They are critical players in defense against virus and viroid infections and in gene expression regulation. On the other side the accumulation in plant tissues of virus- and viroid-derived small RNAs (v-sRNAs) is usually associated with ongoing pathogen infection. Analysis of plant sRNA libraries and their profiles can indicate if plant sustains viral-induced silencing process, even more - it may allow strain differentiation, and de novo assembly of virus/viroid genomes.

We have developed a stand-alone tool called viR-SCAN, based on an algorithm for search of v-sRNAs in plant sRNA libraries. The tool allows characterization of identified v-sRNAs (size, sequence, strand polarity etc.) and mapping of (+) or/and (-) v-sRNAs on the reference genome with visualization of their hotspots and distribution profiles. The present version of viR-SCAN allows users to identify 45 viroids and 1724 plant viruses. It also includes a module for endogenous plants miRNA detection in samples and miRNAs differential expression upon viral infection.

P13

Effect of Ammonium Vanadate on Viability and Growth of Human MDA-MB-231 Triple Negative Breast Cancer Cells

Dyakova L.[1], Zhivkova T.[2], Dinev D.[2], Abudalleh A.[2], Alexandrova R.[2]

[1] *Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. “GeorgiBonchev” Str, Block 23, Sofia 1113, Bulgaria;*
[2] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. “GeorgiBonchev” Str, Block 25, Sofia 1113, Bulgaria*

Abstract. Breast cancer is the most common neoplasm in females and one of the leading causes of tumor death for women worldwide. Among the known breast cancer subtypes the triple negative breast cancer is one of the most challenging to treat because the cells lack the receptors for the available targeted therapies. Ammonium vanadate (NH_4VO_3) has been reported to possess antineoplastic properties against various animal tumor models but its anticancer activity is not fully clarified yet. The aim of our study was to examine the effect of NH_4VO_3 on viability and proliferation of cultured MDA-MB-231 triple negative breast cancer cells. The investigations were performed using methods with different cellular/molecular targets and mechanisms of action: thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake cytotoxicity assay, crystal violet staining, double staining with acridine orange and propidium iodide and colony-forming method. The compound was applied at a concentration range of 0.5 to 20 μg/ml. The results obtained by short-term (24, 48 and 72 h, with monolayer cultures) and long-term (16 days, with 3D colonies) experiments reveal that ammonium vanadate decreases viability and proliferation of the treated cells in a time- and concentration-dependent manner.

Acknowledgements. Supported by Fund “Scientific Research”, Bulgarian Ministry of Education and Science, Grant № DFNI Б 02/30 from 12.12.2014.

P14

ARDRA Analysis of Acidithiobacillus ferrooxidans Isolates Inhabiting Different Natural and Industrial Sites

Ilieva R., Groudeva V., Iliev M.

Sofia University “St. KlimentOhridski”, Faculty of Biology, Sofia, Bulgaria
Ralitsa_Ilieva@abv.bg

Abstract. Various strains of *A. ferrooxidans* have been isolated from natural sources like rocks, ores, mine waters and from industrial sides as ore concentrates and pulps The aim of the current study was to assess the applicability of ARDRA analysis for the characterization of *A. ferrooxidans* isolates. The mentioned habitats are extremely diverse and vary in their phisico-chemical conditions (presence of particular sulfide minerals and their ratio, pH, temperature, and the content of toxic compounds in the liquid phase). 18 *A. ferrooxidans* isolates were subjected to ARDRA analysis in total. Taxonomical status of the isolates were previous determined by classical microbiological scheme. Amplified ribosomal DNA restriction enzyme analysis was conducted to obtain ARDRA profiles of all *A. ferrooxidans* isolates as well of *A. thiooxidans* and *L. ferrooxidans* strains. PCR products were digested in two reactions separately using restriction endonucleases, Alu I and HaeIII. The obtained fingerprint profiles proved ARDRA as a useful technique for characterization of *A. ferrooxidans* isolates, regardless of the described genetic variability of the species. The method could be used for discrimination among *A. ferrooxidans, A. thiooxidans* and *L. ferrooxidans* in mix samples. The correlation between ARDRA profiles of *A. ferrooxidans* and the source was not observed.

Секция: Биоразнообразие и Екология

P15

Ecological Classification of the Amphibian and Reptilian Fauna in the City of Plovdiv

Mollov I.A., Velcheva I.G.

University of Plovdiv “Paisii Hilendarski”, Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tzar Assen Str., Plovdiv, Bulgaria

Abstract. The current study attempts to classify the amphibian and reptilian fauna in urban environment, characterized by: ecological plasticity and habitat preferences; temperature regime; humidity and level of synanthropy. Totally seven amphibian species (*Bufo bufo, Bufo viridis, Hyla arborea, Pelobates syriacus, Rana dalmatina* and *Pelophylax ridibundus*) and eight species of reptiles (*Mediodactylus kotschyi, Lacerta virdis, Lacerta trilineata, Podarcis tauricus, Emys orbicularis, Natrix Natrix, N. tessellsta* and *Dolichophis caspius*) are analyzed and classified in ecological groups according to the above-mentioned characteristics.

P16

Heavy metal effects on the Lysosomal Membrane Stability and Respiratory Rate in Chinese Pond Mussel (Anodonta woodiana) under Ex situ Exposure

Yancheva V.[1], Mollov I.[1], Velcheva I.[1],*
Georgieva E.[2], Stoyanova S.[2]

1 University of Plovdiv, Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tsar Assen Str., Plovdiv, Bulgaria
2 University of Plovdiv, Faculty of Biology, Department of Developmental Biology, 24 Tsar Assen Str., Plovdiv, Bulgaria
**Corresponding author: veselayancheva@yahoo.com*

Abstract. The Chinese pond mussel (*Anodonta woodiana*) is a unionid mussel species that has recently been reported as being invasive worldwide. As a filter feeder, it is known to accumulate heavy metals, making the species useful for biomonitoring. However, the effects on some physiological functions of this species, after acute exposure to heavy metals, is still poorly studied. The current study aimed to investigate the lysosomal membrane stability in heamocytes of Chinese pond mussel (*Anodonta woodiana*) by applying the neutral red retention assay (NRRA), as well as changes in the respiratory rate under acute metal exposure. The mussels were treated with different concentrations of Ni and Pb in laboratory conditions for 72nd h. These metals are considered as priority substances according to Directive 2013/39/EU of the European parliament and the Council. The metal concentrations were prepared as 75, 50 and 25% of the maximum permissible levels set by law. After the 72nd h exposure to Ni and Pb the lysosomes retained the dye between 30 to 60 minutes in the mussels exposed to the higher concentrations. We registered a negative, statistically significant correlation between the metal concentrations and

the average time the lysosomes retained the dye after the 72nd hour. The respiratory rate was measured at the 72nd hour and it increased in a dose-dependent manner. We can conclude that the acute metal exposure, including all metal concentrations below the allowable concentrations, lead to destabilization of the lysosomal membrane stability and changes in the respiratory rate of the Chinese pond mussel, thus altered physiological functions.

Acknowledgments. This paper is supported by the NPD - Plovdiv University “Paisii Hilendarski” under Grant No NI15-BF-003 "Integrated biological approaches for monitoring priority substances in water".

P17

First Finds of Cosmospora magnusiana and Pseudocosmospora eutypae (Nectriaceae, Hypocreales) in Bulgaria

Stoykov D.Y.

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

Abstract. The genus *Pseudocosmospora* C. Herrera & P. Chaverri is reported as new to Bulgarian mycota. *Cosmospora magnusiana* is recorded for the first time from Bulgaria on old stromata of *Diatrypella quercina* in Sofia region. Brief descriptions and original illustrations of the examined specimens are included. Comparison between the new finds and the closest, recently recorded in Bulgaria, *Dialonectria episphaeria* (Nectriaceae) on stromata of *Diatrype stigma* in Balkan and Rila Mts is presented.

P18

Several New Records of Lichenized Fungi (Ascomycota) from Bulgaria

Stoykov D.Y.

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

Absract. New finds and recently recorded lichenized fungi - established in Bulgaria on bark of trees are reported. The genus *Graphina* Müll. Arg., presented with *Graphina cf. ruiziana,* is discovered for the first time from our counrty in Rila Mts. The morphologic features of the fungi are illustrated with colour photographs made under Boeco Binocular light microscopes with the help of Canon PS A460 and PS A1400 HD digital cameras. Measurements of the main characters of the specimens studied were taken in water with the help of software Carnoy 2.0.

P19

Determination of the Conservation Values of the Platanus orientalis L. population in the protected area "Chinarite", Village Belastitsa, Minicipality Rhodopi

Radoukova T.I., Todorov K.T., Stojanov P.S., Nikolov N.A.

University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Botany and MOB, Plovdiv, Bulgaria

Abstract. On the ground of “National methodological guidance for determination, management and monitoring of forests of high conservation significance’’ (2005), an observation was done about the condition and conservation values of the determine as a protected area plane-tree forest, in the land of Village Belastitsa, Minicipality Rhodopi.

According to the twelve indicators, the status of *P. orientalis* population is good; as the low seed renovation capacity of the forest should be considered, because it would endanger its future condition and growth.

P20

New Information on the Distribution of Some Limno-terrestrial Tardigrades (Tardigrada: Eutardigrada et Heterotardigrada) in Bulgaria

Yankova M., Georgiev D.

University of Plovdiv, Faculty of Biology, Department of Ecology and Environmental Conservation, Tzar Assen Str. 24, BG-4000 Plovdiv, Bulgaria, email: yankovva.m@gmail.com, diliangeorgiev@abv.bg

Abstract. The study aims to provide information on the distribution of limno-terrestrial tardigrades in Bulgaria. In the period between February 2015 and July 2015 134 moss and lichen samples were collected from Upper Thracian Lowland, Sredna gora, Rhodopes and Strandhza Mountains. 103 of them were positive for tardigrades, 29 specimens were indentified to species-level and 412 to genus-level. 63 specimens were not indentified due to poor preservation. First data on limno-terrestrial tardigrades for Sredna Gora and Strandzha Mts., and Stara Zagora city was provided. At least 4 species of *Milnesium* were registered. Our preliminary results stress that the Bulgarian tardigrade fauna is poorly known and needs more detailed future studies.

P21

Opportunities to Use Pea - Wheat Mixes in Organic Farming

Ivanov G.

Institute of Plant Genetic Resources, Sadovo, Bulgaria
E-mail: grigori_ivanov71@abv.bg

Abstract. This article studied the changes in the growth rate of the individual components in eight pea - wheat mixes grown in conditions of organic farming : 4 wheat varieties - Sadovo 1, Geia 1, Guinness, Farmer and 4 varieties of winter peas - Mir, Vesela, №11, L12AB, at different ratio between them - 50:50 and 30:70 %. The selection of varieties is made based on previous studies of their complex characteristics – ripening, yield, chemistry. Setting up and raising the experimental mixture of seeds has been made in a medium free of organic and mineral fertilizers. We have studied the changes in green mass yield and precocity of the blend and the biochemistry of surface biomass. According to the results obtained at early ripening the mixture is Sadovo 1 – Mir 30:70 %. The most productive is the mixture Geia -1 – Vesela 50-50 %. By biochemical assessment carried out under the following parameters : crude protein , crude fiber, ash elements are it was found that raising the blend under conditions of organic farming reduces the crude protein content and increase that of raw fiber in the above-ground biomass.

P22

Study of Influence of Sludge WWTP in the Paper Industry on Growth and Development of Corn

Krasteva-Malinova D.

University of Plovdiv “Paisii Hilendarski”, Faculty of Biology, Department of Ecology and Environmental Conservation. 24 Tsar Assen Str., BG-4000 Plovdiv, Bulgaria

Abstrct. Sludge from urban wastewater treatment plants is characterized by the presence of a number of harmful components including and some heavy metals. For use in agriculture is necessary to meet the requirements of the Ordinance their application.

The purpose of this study was to explore the possibility of growing corn using sewage sludge from the paper industry.

Up was two years (2013 and 2014) Polish experience culture corn variety "" Knezha 613 "" in conditions of fertilizing with sewage sludge wastewater from pulp and paper industry, two standards - 1000 and 1500 kg./dka. Experience is displayed on alluvial soil near Stamboliyski.

It traces indicators - germination stage 3-4 leaf, stage 5-6 leaf, height and thickness of stem, number and length of the cob.

The results indicate the dependence of the amount of the deposited precipitate. Growth and development of plants grown at fertilization with 1500 kg./dka. sludge show much higher values of biometric identifiers from control.

P23

Evaluation of the Efficacy of Malachite Green, Sodium Chloride and Formalin for Control of Ichthyophthiriasis in Whitefish (Sander lucioperca L.)

Gevezova-Kazakova M., Zaykov A., Hubenova T., Atanasova R., Yankova M.

Institute of Fisheries and Aquaculture Plovdiv, Plovdiv, Bulgaria

Abstract. This study investigated the efficacy of malachite green, sodium chloride, and formalin for control of ichthyophthiriasis in pikeperch fingerlings (*Sander lucioperca*) with an average weight of 0,587 g ± 1,16. Malachite green was used as positive control at concentration of 100 mg.m-3 and 30 min exposure, as early as 4 days after the beginning of the experiment, the number of the parasite is considerably reduced, and on day 8 of the experiment the fish are completely freed from it .

Treated with sodium chloride (salt) in a therapeutic dose of 0.5 gm-3 for a period of 8 days at stopped-flow showed that the fish are clinically healthy and no visible side effects were registered during the experimental period.

The use of formaldehyde at a concentration of 200 ml.m-3 and an exposure of 30 min is less effective. Formaldehyde treatment decreased the number of the parasite, but did not lead to its complete removal. Also the use of formalin holds risks for the survivability of the fish.

Therefore salt is more environmentally friendly alternative for the control of *I. multifiliis*, after the ban on the use of malachite green.

P24

On the Daily Activity of the Stone Marten (Martes foina Erxl.) in Forest Habitats in Bulgaria

Dudin G., Georgiev D.

University of Plovdiv, Faculty of Bilogy, Department of Ecology and Environmental Conservation, Tzar Assen Str. 24, BG-4000 Plovdiv, Bulgaria

Abstract. The study was done on a kind of a small predator by digital photo cameras in two remote geographic areas. There have been 634 photos of stone marten (*Martes foina*), of which 518 in Sinite Kamani Nature Park and 118 in State Hunting Department "Chepino". By analyzing the daily activity of the species is recorded night or twilight activity between 17 and 7:00 hours (morning, evening or night). In the time interval between 07-17 during the day, the activity of the type is not registered.

P25

Two Rare Pyrenocarpous Lichens from Bulgaria Deposited in BP

Shivarov V.[1], Lőkös L.[2]

[1] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin St., 1113 Sofia, Bulgaria*
[2] *Department of Botany, Hungarian Natural History Museum, 1476 Budapest, Pf. 222, Hungary*

Abstract. One of the largest collections of lichen-forming fungi from Bulgaria is deposited in the lichen herbarium of the Hungarian Natural History Museum (BP). The specimens were collected by the prominent Hungarian lichenologist, Dr Ödön Szatala mainly in 1929 and 1954. Most of the specimens have already been identified and published, but some collections remained unidentified. *Thelidium pertusatii* (Garov.) Jatta, recently recognized among the unidentified specimens of the former Bulgarian collections, was regarded as endemic for the southern Alps up to now. *Verrucaria bulgarica* Szatala is known only from few localities worldwide. A detailed description, illustrations and comments based on the type material of *V. bulgarica* are provided. Old collections can give remarkable contributions to the biodiversity of lichen-forming fungi of certain area, and also can increase our knowledge on the variability of a less studied species. Moreover they can even hold important information for changes in the environment for several decades.

P26

Identification of Bacillus Strains Isolated from Rock Paintings in Magura Cave, Bulgaria

Mitova M.M., Angelova R.G., Iliev M.V.,*
Voynova V.V., Groudeva V.I.

Sofia University “St. Kliment Ohridski”, Faculty of Biology, 1164 Sofia, Bulgaria
** Corresponding author: mmitowa@abv.bg*

Abstract. Magoura Cave (Bulgaria) is one of the caves with paleolithic paintings. An assessment of the composition of bacterial communities that have colonized this cave represents a first step in understanding and potentially controlling their proliferation in order to protect the paintings. Members of the *Bacillus* genus are generally found in caves and represent a wide range of physiological abilities, allowing the organisms to grow in every environment. These bacteria compete desirably with other organisms within the environment due to its capability to form extremely resistant spores and produce metabolites that have antagonistic effects on other microorganisms. Many *Bacillus* species are of remarkable importance because of the potential of Bacillus species to synthesize a wide variety of metabolites which can take part in deterioration of rock paintings.

The aim of this study was connected with the identification of Bacillus strains isolated from different samples of Paintings gallery. The identification of the isolates was achieved by the methods of classical and molecular taxonomy

The results showed that the strains are belonged to the species of *B. lutimorbus, B. licheniformus, B. macercen, B. validus, B. lautus B. amyloliticus, B. polymyxa, B. insolutus* and *B. circulans*. A better understanding of the diversity of the genus *Bacillus* present on art

objects is important for the development of effective conservation and restoration strategies of the unique rock paintings.

Acknowledgements. The study is supported by National Science Fund of Ministry of Education and Science, Bulgaria (Project № ДДВУ/73/2010).

P27

Chlorophyll content in Fontinalis antipyretica Hedw. under ex-situ heavy metal influence Chlorophyll content in Fontinalis antipyretica Hedw. under ex-situ heavy metal influence

Hristeva Y., Gecheva G, Gribacheva N.

University of Plovdiv “Paisii Hilendarski”, Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tzar Assen Str., Plovdiv, Bulgaria

Abstract. This study presented ex situ experiment in order to provide information about potential effects that heavy metals (Pb, Ni and Cd) may have on the pigments ratio (chlorophyll a and b) in aquatic moss species. For the purpose of the study was selected species Fontinalis antipyretica Hedw. due to its proven role as a biomonitor and morphological characteristics of its leaves.

Acknowledgments. This paper is supported by the NPD - Plovdiv University “Paisii Hilendarski” under Grant No NI15-BF-003 "Integrated biological approaches for monitoring priority substances in water".

P28

The Thylakoid Membranes from Arabidopsis thaliana and Pisum sativum Exhibit Strikingly Different Thermodynamic Behavior

Petrova N.[1], Todinova S.[1], Schröder W.[2], Taneva S.[1], Krumova S.[1]

[1] *Institute of Biophysics and Biomedical Engineering, BAS, 1113 Sofia*
[2] *Umeå University, Biological Chemistry, SE-901 87 Umeå, Sweden*

Abstract. Differential scanning calorimetry (DSC) is a suitable approach for the determination of the thermal stability of biological samples as complex as the chloroplast thylakoid membrane where the membrane embedded protein complexes account for 80% of its area. The method allows discriminating the thermal stability of the photosynthetic complexes in their native microenvironment. Here we test whether DSC can detect changes in the protein stability related to differences in the membrane’s structural organization between two plant species - *Arabidopsis thaliana* and *Pisum sativum*. The thermodynamic features of thylakoid membranes in stacked (highly ordered macroorganization of the photosynthetic complexes) and unstacked (randomized lateral organization) condition are compared. We demonstrate that the number, location and enthalpy of the endothermic transitions differ between the two species. Our data suggest that the components of photosystem II supercomplexes and the disorganization of their ordered arrangement contribute to the most prominent transition in *Arabidopsis thaliana* thermogram (centered at -62º C), while these events are well discernible as separate transitions in *Pisum sativum*. The striking contrast between the thermograms of *Pisum sativum* and *Arabidopsis thaliana* thylakoid membranes suggest differences in the microenvironment (different protein-protein and/or lipid-protein

interactions) of the protein complexes in the two species that affect their thermal stability.

P29

Published Data and New Records to the Fauna of Eupelmidae (Insecta: Hymenoptera) in Bulgaria

Antov M., Stojanova A.

University of Plovdiv, Faculty of Biology, Department of Zoology, 24 Tsar Assen Str., 4000 Plovdiv, e-mail: miroslavantov@mail.bg, stanelia@pu.acad.bg

Abstract. This study presents information about published data on eupelmid fauna of Bulgaria and new records of 16 species distributed in the country. Some of them are reared from Cynipidae (Hymenoptera) galls on *Quercus, Rosa, Hypecoum*, as well as from Cecidomyiidae (Diptera) developing in stems of *Eryngium campestre*. Other species are reared from pods of *Astragalus glycyphyllos* and seeds of *Dianthus giganteus*. New host relationships are established. As a result of the study 4 species and 1 genus are new to the fauna of Bulgaria.

P30

Preliminary Data of Fungus Gnats Studies (Diptera: Family Bolitophilidae, Family Keroplatidae, Family Mycetophilidae) in Bulgaria, Greece, Turkey

Pavlova A.

University of Plovdiv, Faculty of Biology, Department of Zoology, 24 Tsar Assen Str., 4000 Plovdiv, e-mail: miroslavantov@mail.bg, stanelia@pu.acad.bg

Abstract. The presented data is a result of author’s studies over the years 2010-2015. New localities and fungus gnats (Diptera: Bolitophilidae, Keroplatidae, Mycetophilidae) new species to the fauna of Bulgaria, Greece and Turkey have been reported. Two species new to the science from genus Docosia, have been determined and descriptions have yet to be done.

P31

Comparative Study on Levels of Ni in Water and Musculature of "Zebra" Mussel (Dreissena polymorpha) from Ovcharitsa Dam, Stara Zagora Region, Bulgaria

Valkova E.

Thrakia University, Faculty of Agriculture – Stara Zagora,
E-mail: Elica_Valkova@abv.bg

Abstract. Ni is considered an essential for microorganisms, plants and animals (including aquatic) as is compilation part of numerous transport, regulatory and enzymatic proteins. This element is often found in aquatic ecosystems due to its widespread use in households and industry. The aim of this study is comparative research the levels of the heavy metal nickel (Ni) in water and musculature of ""Zebra"" mussel (*Dreissena polymorpha*) from Ovcharitsa Dam, Stara Zagora, Bulgaria. The individual samples of water and musculature were collected in August and November 2012 and January and March 2013. International standards of ISO and BSS for sample preparation of water and musculature of mussels analyze were used. Concentration of Ni in the analyzed samples was determined by atomic adsorption spectrometry. The Ovcharitsa Dam is located in areas with a high degree of anthropogenic load. The concentrations of nickel, registered in Ovcharitsa Dam during the study period were significantly lower than the norms stipulated in the Directive 2008/105/EO and Directive 2013/39/EO from of the European legislation. In the existing standards of Bulgarian and European legislation lack clearly defined limits on the levels of this metal in the body of mussels. In this case as a landmark was used the average value for the study period. High concentrations exceeding the X

average is observed on the heavy metal nickel in the musculature samples of mussel “Zebra” delivered during January and March 2013, as the highest levels are reported in the month of March 2013 (1.37 mg / kg). The established values of the element nickel were significantly lower in the water of the studied waterbody compared with the musculature of *Dreissena polymorpha.* Since the levels of this metal found in water shows the current condition of the hydroecosystem, more reliable biological indicator is the organism (the musculature) of investigated mussel kind.Accumulating the highest levels of these elements in time, these mollusks could be used as an excellent indicator of pollution with the heavy metals.

P32

Study of Bird Migration Using a Metrological Radar

Michev B., Zehtindjiev P.

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

Abstract. The bird migration over Bulgarian Black see cost is a subject of long time scientific discussion in respect to phenology, dynamics and number of birds passing thought this migratory flyway known as Via Pontica. Many studies documented large front of migration of soaring birds in autumn along Bulgarian Black see coast, but the dynamics and distribution of birds has never been investigated by objective methods such as radar. Results from first study in Bulgaria of bird migration with the help of C-band Dopler Radar -Meteor 250 with carrier frequency 5.5 GHz situated at the city of Varna are presented. Data were kindly supplied by BULATSA (Bulgarian Air Trafic Services Authority – Sofia) and proceeded with Dokter et al. (2010) algorithm of data analyzes. The results of the weather radar were verified by the visual observations and fix beam radar BirdSckan1. The first results show that the method is reliable tool of objectively reporting on a number of basic parameters of bird migration in NE Bulgaria. Data processing continues with a view to cover the whole territory of the country.

P33

GIS Models of the Environmental Factors and the Sustainable Territorial Development Constraints in Three Nature Protection Areas in the City of Plovdiv

Dimitrov D.[1], Minchev N.[1], Ikonomov L.[2], Karaivanov N.[3], Stoyanova A.[4], Mitkovska V.[4], Pavlova A.[4], Mollov I.[5], Filipova-Marinova M.[6], Dimitrov D.[7]*

[1] *Technical University-Varna, Department of Ecology and Environmental Protection, Varna, Bulgaria*
[2] *Institute for Ecological Modernisation, Varna, Bulgaria*
[3] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4] *University of Plovdiv, Faculty of Biology, Department of Zoology, Plovdiv, Bulgaria*
[5] *University of Plovdiv, Faculty of Biology, Department of Ecology and Environmental Conservation, Plovdiv, Bulgaria*
[6] *Reginal Museum of Natural History - Varna, Bulgaria*
[7] *National Museum of Natural History - BAS, Sofia, Bulgaria*
** Corresponding author: d.angelov@tu-varna.bg*

Abstract. The aim of this study was to define and to analyze the environmental and territorially-determined factors and constraints influencing the sustainable development and the sustainable management of three protected areas located in the city of Plovdiv. The study sites are natural protection areas of unique nature due their mixed natural and anthropogenic functions. Being integral parts of the city they are balancing the requirements of specialized nature protection with their typical urban recreational role as urban parks. In order to meet the requirements of all these overlapping and sometimes controversial functions, the present study analyses the territorial development factors and constraints through detailed geo-informational models of the territory and its biotic, abiotic, natural and anthropogenic structures. These

models were developed as part of the work on the management plans of the three protected sites. The biotic and abiotic factors were investigated through field surveys where all major plant and animal groups were studied. All important species and habitats, all specific threats and conservation problems together with all anthropogenic structures were mapped as parts of the GIS model which then served as a basis for the functional zoning of the three protected territories.

P34

Mt Falakron, Mt Slavianka and Limestone Parts of Mt North Pirin – Natural Wealth on the Balkan Peninsula

Karakiev T., Asimov A.

Sofia Iniversity „St Kl. Ohridtski”,
E-mail: karakiev@abv.bg, asenasenov71@yahoo.com

Abstract. Mt Falakron (Bozdag, 2232 m), Mt Slavianka (Orvilos, 2212) and Mt Pirin (2914) are situated on Greece-Bulgarian border area on dawn stream of rivers Struma and Mesta, on 30-70 km distance each other and 50-130 km distance by Aegean sea. Climate is changes by Transitional Mediterranean on south slopes of Falakron and Slavianka to continental on North Pirin. Marble limestone base rock is reason for very rich plant diversity and a lot of endemic and protected plants (some of which are local or regional endemic): *Abies borisii-regis* Mattf., *Dianthus drenowskyanus* Rech. f., *Echium russicum* J. F. Gmel., *Erysimum drenowskyi* Degen, *Fritillaria drenovskyi* Degen et Stoj., *Haberlea rhodopensis* Friv., *Lathraea rhodopea* Dingl., *Oxytropis urumovii* Jav., *Oxytropis kozhuharovii* D. Pavlova, D. Dimitrov, M. Nikolova, *Papaver degenii* (Urum. & Jav.) Kuzmanov, *Petkovia orphanidea* (Boiss.) Stef., *Pulsatilla slaviankae* (Zimm.) Jordanov & Kuzmanov, *Saxifraga ferdinandi-coburgi* Kellerer & Sűnd., S. stribrnyi (Velen.) Podpera, *Sideritis scardica* Griseb., *Viola delphinantha* Boiss., *Viola perinensis* Becker etc. We can set these three marble mountains like natural museums concentrating very rich and endemic flora on so limited area.

P35

Seasonal Dynamics of the Intensity of Migration of Birds of Prey (Orders: Falconiformes, Accipitriformes) in the Region of Balgarevo Village, Municipality of Kavarna

Karaivanov P.N.[1], Karaivanov N.P.[2]

[1] Sofia University “St. Kliment Ohridski”, Sofia

[2] Institute of Biodiversity and Ecosystem Research, BA S

Abstract. This study presents data on the migration of 16 bird species, observed in the Northern Bulgaria, Municipality of Kavarna. The territory is located in the eastern part of the migration route Via pontica and is in close proximity to the Black Sea coast. The observations were conducted from a stationary observation point with GPS coordinates N: 43°26'29,75'' and E:28°24’48.76'', by the method of observation of daily migrants described by Michev et al. (2010) and approved by the Ministry of Environment and Water. The aim of the study was to track the number and dynamics of migration of birds of prey in the observed area, in the spring and autumn. The study was carried out during the period 2004-2009 and includes 6 autumn and 5 spring migration seasons. The spring season includes the period from March to May, and the autumn one – from August to November.

A total amount of 7205 specimens was observed. The numbers are significantly higher during the autumn migrating season. During the spring migration season the numbers of the migrants varied between 51 and 260 specimens, while in autumn the range shifts to 519 – 1709 specimens. The highest numbers were registered in autumn, 2009. Among the species observed the following appeared in the highest numbers: Lesser Spotted Eagle (*Aquila pomarina*), Marsh Harrier (*Circus*

aeruginosus), Common Buzzard (*Buteo buteo*), Honey Buzzard (*Pernis apivorus*), Sparrowhawk (*Accipiter nisus*), Krestel (*Falco tinnunculus*), Red-footed Falcon (*Falco vespertinus*). The obtained results are compared to those from other studies of bird migration.

P36

Malacofauna (Gastropoda and Bivalvia) of the “Pomoriysko ezero” Protected Area: Preliminary Results

Kalcheva S.[1], Georgiev D.[2], Georgiev D.[3]

1 Department of „Biology and Aquaculture“, Trakia University, Faculty of Agriculture, Stara Zagora 6014, Bulgaria, e-mail: silviya_kalcheva@abv.bg
2 Department of Ecology and Environmental Conservation, University of Plovdiv, Tzar Assen Str. 24, BG-4000 Plovdiv, Bulgaria, email: diliangeorgiev@abv.bg
3 Department of „Biology and Aquaculture“, Trakia University, Faculty of Agriculture, Stara Zagora 6014, Bulgaria; e-mail: mihaylov@uni-sz.bg

Abstract. The “Pomoriysko ezero” Lake is a hipersaline lagoon of natural origin, situated next to the town of Pomorie. The hydrobiological studies of the lake have 80 years of history. The malacology studies on the terrestrial, freshwater, and brackish molluscs in the adjacent territory of the lake, the molluscs in the lagoons and in the majority of the Bulgarian Black Sea coast are as a whole too scarce.

For the purpose of the study some qualitative and quantitative samples of the coast, fresh water and benthos were collected from the lake "Pomoriysko ezero". A total of 26 marine and brackish species from 23 families and two classes - Bivalvia (16 species) Gastropoda (10 species); 3 freshwater species from 3 families (Physidae; Vertiginidae; Planorbidae), from the class Gastropoda; and 12 terrestrial species from same class, representatives of four families (Hygromiidae; Helicidae; Enidae; Orculidae).

P37

Comparative Study of the Influence of Climatic Conditions on the Biological and Agricultural Indicators of Indigenous and American Varieties of Burley Tobacco in Terms of Bulgaria

Dyulgerski Y.[1], Radoukova T.[2]

[1] *Institute of Tobacco and Tobacco Products (ITTP), Markovo, Bulgaria*
[2] *Plovdiv University “Paisii Hilendarski”, Faculty of Biology, Plovdiv, Bulgaria*

Abstract. The comparative study and correlation between climatic parameters: average daily temperature and rainfall amount and the most important biological and agricultural indicators by two indigenous and two introduced from the United States, varieties of Burley tobacco, was done. The results of all tested variants in all the years of the study show a strong influence of the amount of rainfall compared to the temperature sum. Of all the investigated indicators, the length of vegetative period is influenced mostly by the weather conditions, especially the amount of precipitation. On the length of vegetative period, the amount of rainfall and temperature sum operates mixed. Rainfall amount show a significant positive correlation with the size of the yield and the percentage of first-class and a negative one with the percentage of third-class. Temperature sum does not significantly impact the agricultural indicators of varieties of Burley tobacco. From the four studied varieties, least affected by environmental conditions is Burley 1344 variety and therefore it is most suitable for cultivation. Burley 1317 variety also has good results and appears as a relatively suitable for cultivation. Variety Burley 21 is more sensitive to environmental conditions, therefore, its cultivation is associated with a greater risk. Tennessee 86 variety is presented as the most sensitive to environmental conditions, making it unsuitable for

growing conditions in Bulgaria. The results from the study show the superiority of the indigenous over the introduced varieties of Burley tobacco with respect to their sensitivity to weather conditions.

P38

Changes in the Antioxidant Stress Markers in the Beech Leaves after Orchestes fagi Infestation

Chaneva G.[1], Dimitrova-Mateva P.[2], Anev S.[3], Georgieva S.[4], Tzvetkova N.[3]

[1] Department of Plant Physiology, Faculty of Biology, Sofia University “St. Kl. Ohridski” Dragan Tzankov Blvd, 1164 Sofia, Bulgaria

[2] University of Forestry, Blvd.”Kl.Ohridski” 10, Sofia, Bulgaria

[3] University of Forestry, Department of Dendrology, Sofia, Bulgaria

[4] University of Forestry, Sofia, Bulgaria - B.Sc. student

**Corresponding author: gchaneva@abv.bg*

Abstract. The present study is focused on the influence of leaf-mining insect *Orchestes fagi* L. on the leaves of common beech (*Fagus sylvatica* L.). It was investigated the changes in antioxidative stress markers – malone dialdehyde (MDA), hydrogen peroxide (H2O2) and free proline in the beech trees located at two different altitudes - 680 m asl and 1400 m asl, at the Petrohan Training and Experimental Forest Range. The level of infestation, as well as the parameters that were studied, were highly dependent on the trees’ location. The conditions at low altitude were favorable for the rapid development of *O. fagi* which resulted in significant changes in the leaves’ growth and physiology. The heavily damaged leaves at lower altitude contained a higher amount of MDA - 35% more that the leaves grown at higher altitude. However, the increased level of lipid peroxidation was not accompanied by the corresponding enhancement of H_2O_2. The damage caused by *O. fagi* invasion was most clearly manifested by the increase of free proline content – it was more than two times higher than the tree leaves grown at the higher location. In general, the herbivory resulted in serious

biochemical changes occurring in the leaves, indicating a shift in the oxidative status of the beech trees.

P39

Effect of Beech Weevil (Orchestes fagi L.) Infestation on Water Regime of Common Beech (Fagus sylvatica L.) Leaves

Anev S.[1], Georgieva S.[2], Dimitrova-Mateva P.[3], Chaneva G.[4], Tzvetkova N.[1]*

[1] *University of Forestry, B.Sc. student*
[2] *University of Forestry, Department of Dendrology*
[3] *University of Forestry*
[4] *Sofia University, Department of Plant physiology*
** Corresponding author: svetoslav.anev@gmail.com*

Abstract. Transpiration rate and water use efficiency of infested leaves are sensitive indicators for stress level of plants, because negative impact of impaired water regime affects the overall condition of the plant. This study aimed to establish the effect of beech weevil (*Orchestes fagi* L.) infestation on water regime of common beech (*Fagus sylvatica* L.) leaves. The eco-physiological measurements on different affected leaves was conducted during the period of infestation by larvae in first instar. The results showed that the transpiration rate increased strongly with increase of infestation and as a consequence water use efficiency decreased simultaneously. Impaired water regime can worsen whole plant sustainability and productivity.

P40

Content of Heavy Metals in the Flesh of the Different Marine Fish Species

Stoyanova St., Sirakov I., Velichkova K., Staykov Y.

Department of Biology and aquaculture,
Agricultural Faculty, Trakia University, Stara Zagora, Bulgaria

Abstract. Fish is one of the most important and complete food for people. Different species are characterized by a certain nutritional value to humans. The accumulation of heavy metals in their body a potential danger to human health. The purpose of this study was to assess the chemical composition of meat and concentrations of cadmium (Cd), lead (Pb), nickel (Ni) and zinc (Zn) in different species fishs: horse mackerel (*Trachurus declivis*), Mackerel (*Scomber scombrus*), Sprat (*Sprattus sprattus*) and chernokop (*Pommatomus saltatrix*) in muscles were determined. The concentration of heavy metals were measured by atomic absorption spectrophotometry after digestion of the samples using heating digester.

P41

Influence of Heavy Metals (Cd, Ni and Pb) on the Growth and Morphology of Microalgae Scenedesmus incrassatulus Bohl. (Chlorophyta, Chlorococcales)

Basheva D., Stoyanov P., Russinova S.,
Andonova Ts., Teneva I., Belkinova D.

Plovdiv University, Faculty of Biology, Plovdiv, Bulgaria

Abstract. Pollution of aquatic ecosystems with heavy metals leads to decrease of biodiversity and bioaccumulation of toxicants in the food chain. Species of the genus *Scenedesmus* a sensitive indicators of environmental changes have been used for evaluation of risk factors for contamination of aquatic ecosystems. The microalga *Scenedesmus incrassatulus* has the ability to remove chromium and cadmium from the growth medium. This study presents the influence of different concentrations of Cd2+ , Ni2+ and Pb2+ on growth, development and morphology of *Scenedesmus incrassatulus.* The effect of three heavy metals occurs at different time points (24-48-72 hours) of treatment and has a different impact on the studied parameters.

P42

Study on the Biodiversity in the Area of Dedovo Village (Rodopi Municipality, Plovdiv District) in Order to Develop a Conceptual Project for Construction of the Eco-trail

Todorov K., Petrova S., Nikolov B., Raynova G., Toteva H., Peltekova M., Vaseva V., Petrova D., Yorgova A., Stoyanova M., Molla F., Karagyozova-Dilkova D.*

Faculty on Biology, University of Plovdiv “Paisii Hilendarski”, 24 Tzar Assen Str., 4000, Plovdiv, Bulgaria

Abstract. We aimed to study the biodiversity in the area of Dedovo Village, Rodopi Municipality, Plovdiv District, in the light of its potential for a eco-trail construction and developing ecotourism. Plant and animal diversity in the region, and the presence of typical rocky forms were examined through numerous field studies on the method of linear transect in the period 2014-2015.

We found that in the region of Dedovo Village meet coniferous, deciduous and mixed forests of fir (*Abies alba* Mill.), spruce (*Picea abies* (L.) H. Karsten), white (*Pinus sylvestris* L.) and black pine (*Pinus nigra* L.), beech (*Fagus sylvatica* L.), birch (*Betula pendula* Roth.), aspen (*Populus tremula* L.), linden (*Tilia sp.*) and others. Subforest consist of ferns (Class Pteridopsida), juniper (*Juniperus communis* L.), blackberry (*Rubus fruticosus* L.), raspberry (*Rubus idaeus* L.), dog rose (*Rosa canina* L.), red (*Vaccinium vitis-idaea* L.) and bilberry (*Vaccinium myrtillus* L.), wild strawberry (*Fragaria vesca* L.) and others. From herbaceous representatives were described about 70 plant species typical of the region, including 1 rare (*Erodium hoefftianum* CA Meyer,

included in the Red List of Bulgarian higher plants and in the IUCN Red list with category - NT), 5 Balkan endemics (*Silene fabarioides* Hausskn., *Genista rumelica* Velen., *Digitalis viridiflora* Lindley, *Fritillaria pontica* Wahlenb., included in the Bulgarian Law on Biological Diversity, the Red List of Bulgarian vascular plants and in the IUCN Red List with category – LC, *Rorippa lippizensis* (Wulfen) Reichenb) and 30 species, included in the Bulgarian Law on Medicinal Plants.

Animal diversity is also high, including hare (*Lepus europaeus* Pallas), wild boar (*Sus scrofa* L.), roe deer (*Capreolus capreolus* L.), fox (*Vulpes vulpes* L.), wolf (*Canis lupus* L.), squirrel (*Sciurus vulgaris* L.), hedgehog (*Erinaceus concolor* Martin) and others. There are found also different kinds of birds like Lesser Spotted Eagle (*Aquila pomarina* Brehm), cuckoo (*Cuculus canorus* L.), woodpeckers (family Picidae), Falcons (family Falconidae), owls (Family Strigidae) and others. Amphibians are presented by salamander (*Salamandra salamandra* L.), a large frog (*Pelophylax ridibundus*), etc .; reptiles – by several snakes, lizards, land and marsh turtles.

Data obtained confirm that the studied area is characterized by preserved natural environment, rich variety of plant and animal species and therefore offers many opportunities for building an eco-trail.

P43

Influence of Heavy Metals (Cd, Ni and Pb) on the Growth and Morphology of Microalgae Scenedesmus incrassatulus Bohl. (Chlorophyta, Chlorococcales)

Basheva D., Stoyanov P., Russinova S.,
Andonova Ts., Teneva I., Belkinova D.

Plovdiv University, Faculty of Biology, Plovdiv, Bulgaria

Abstract. Pollution of aquatic ecosystems with heavy metals leads to decrease of biodiversity and bioaccumulation of toxicants in the food chain. Species of the genus *Scenedesmus* a sensitive indicators of environmental changes have been used for evaluation of risk factors for contamination of aquatic ecosystems. The microalga *Scenedesmus incrassatulus* has the ability to remove chromium and cadmium from the growth medium. This study presents the influence of different concentrations of Cd2+ , Ni2+ and Pb2+ on growth, development and morphology of *Scenedesmus incrassatulus.* The effect of three heavy metals occurs at different time points (24-48-72 hours) of treatment and has a different impact on the studied parameters.

P44

Directed Search of Bacteria of Genus Salmonella and Pseudomonas from Irrigation Water

Bankova V.[1], Pencheva D.[1], Genova-Kalou P.[2], Stoyanova I.[1]

[1] *BB-NCIPD, 1504 Sofia, 26 Yanko Sakazov blvd., Bulgaria*

[2] *National Centre of Infectious and Parasitic Diseases, 1504 Sofia, 26 Yanko Sakazov blvd., Bulgaria*

Abstract. This work is focused on isolation of stains of the genus *Salmonella* and *Pseudomonas* from agricultural water. This is a good opportunity for future tests of their resistance to the action of hybrid materials with included silver nano-particles in the structure.

In this work agricultural waters are analyzed from different areas of Bulgaria. 29 stains were isolated from them. Detection of stains of the genus *Salmonella* and *Pseudomonas* is made by using selective and differential cultural media: deoxycholate-citrate-lactose agar (DCLA) for *Salmonella sp.* and cetrimid agar (CA) for *Pseudomonas sp.* Morphological analysis is made of all isolated stains and then they are tested for oxydase and catalase activity. Negative oxydase test of stains from DCLA and positive oxydase test of stains from CA are signs that these stains meet requirements of belonging to two genera and they can be identified biochemically. Choosed stains are tested biochemically through convectional biochemical analysis for identification of bacteria of the Enterobacteria family and identification of Gram-negative, aerobic, non-fermentative microorganisms. The results are deciphered by software “PIB”.

This work has data for physic-chemical characterizations of the tested water samples.

Morphological analysis has all 29 stains. Bacteria from genus *Salmonella* have many biochemical differences because of that the attention was focused on lactose-negative colonies with black centre.

22 stains are tested for oxydase and catalase activity and 11 of them are tested biochemically.

There aren’t detected bacteria of *Salmonella* genus among three isolates from DCLA. Only Citrobacter younger was identified.

There are three isolates belonging to *Pseudomonas sp.* among the 8 isolates from CA.

In this research it was demonstrated that in irrigation water can be found pathogenic micro-organisms nevertheless wide-ranging physic-chemical values. The pathogenic microorganisms can endanger plant development and health of human. The future tests of their resistance to the action of silver nano-particles in the structure of novel hybrid materials are important as a manner for “fighting” with their presence in different kind of water.

P45

Dinamics in Chlorophyll Content as a Biomarker of Tree Tolerance to Urban Environment (Plovdiv, Bulgaria)

Kadirova D., Petrova S., Todorova K., Dakova M., Mehmed E., Denev I., Stratiev M., Georgiev G., Delchev A., Stamenov S., Firkova L., Gesheva N., Velcheva I.

Faculty on Biology, University of Plovdiv “Paisii Hilendarski”, 24 Tzar Assen Str., 4000, Plovdiv, Bulgaria

Abstract. As polluted air is a stress factor that contributes to the decline of urban trees we aimed to investigate the impact of anthropogenic activity on chlorophyll content of four tree species (*Acer heldreichii, Tilia tomentosa, Fraxinus excelsior* and *Pinus nigra*). Seedlings were purchased from a certified greenery and planted by us at four selected sites in the city of Plovdiv (Bulgaria) during spring of 2015. Leaf samples were taken monthly and photosynthetic pigments content was measured immediately after sampling. Results of this preliminary study confirmed that pigment levels in plants varied between species, locations and seasons. Although an extension to the above work is necessary to quantify possible differences between the levels at which chlorophyll components are affected, it is clear that chlorophyll can be a very useful indicator of stress level. Because of the non-specificity in pigment reaction to different type of anthropogenic impact, we recommended to apply a combination among chlorophyll concentration and another parameters (morphological, biochemical, physiological) for the targets of biomonitoring.

P46

Induction of Systemic Acquired Resistance (SAR) in Pepper to X. vesicatoria Pathotype P after Treatment with Avirulent Strains Pathotype T of X. vesicatoria and Pseudomonas syringae pv. tomato

Bogatzevska N., Stoyanova M.

Institute of soil science, agrotechnologies and plant protection “Nikola Pushkarov”

Abstract. Bacterial spot caused by *Xanthomonas vesicatoria* is an economically important disease of pepper which is the reason for yearly losses in vegetable production. Control of the pathogen is hard and is mainly relied on the extensive use of copper-based chemicals. In the present study we induced systemic resistance in pepper plants from the susceptible cultivar Yellow kapiya towards the pepper pathotype of X. vesicatoria. We used avirulent strains of tomato pathotype of X. vesicatoria race T1, T2, and T3 and *Pseudomonas syringae* pv. tomato race R0 and R1 to achieve this effect. Resistance is expressed as hypersensitive reaction in leaves after which the plants develop without symptoms.

P47

Integration of Passive and Active Phytomonitoring in Assessing the Air Quality in Urban Areas (Plovdiv, Bulgaria)

Kadirova D., Petrova S., Todorova K., Dakova M., Mehmed E., Denev I., Stratiev M., Georgiev G., Delchev A., Stamenov S., Firkova L., Gesheva N., Velcheva I.

Faculty on Biology, University of Plovdiv “Paisii Hilendarski”, 24 Tzar Assen Str., 4000, Plovdiv, Bulgaria

Abstract. Air pollution is one of the most serious environmental problems in urban centers, including in the city of Plovdiv, affecting population, economy, causing the loss of agricultural production, death of flora and fauna. As an alternative and supplement to instrumental monitoring methods are developed and tested some methods for biological monitoring (biomonitoring) based on analysis of chemical elements content in living organisms (Steinnes et al., 1994; Aničić et al., 2009a, b; Thöni et al., 2011). According Hankard et al. (2004) the biological monitoring can generally be defined as a ""taking into account the response of living organisms to changes in their environment, including changes in the chemical composition of the biota such as the accumulation of contaminants, as well as changes in physiological status of the plants.

Depending on the methodology used the biomonitoring can be divided to:

1) Passive - sampling is performed in situ in the study area;

2) Active - can be accomplished in various ways. One option is to gather material from relatively unaffected area which to be transferred in the study area in the form of live transplants or collectors with dried

material. Another possibility is growing standardized cultures under controlled conditions, which can then be moved in the study area (Fränzle, 2006).

Specific location and topography of Plovdiv city, on the one hand, and the registered high level of air pollution in it, on the other, are a prerequisite for the application of methods of passive and active biomonitoring. However, since now in Plovdiv have been conducted only single phytomonitoring researches and they have concerned individual point sources (Dushkova & Ninova, 1977, 1981, 1982; Dimitrova, 2000; Dimitrova & Yurukova, 2005; Hristeva et al., 2011; Petrova et al. , 2013; Petrova et al, 2014 a, b).

Assessment of air quality in various suburbs and residential areas of the city, the identification of air pollutants and their origin is a key issue for the development of municipal strategies and plans for action to improve the environmental conditions in the city. Identifying the types of specificity in the response of different plant species and their tolerance to air pollution is critical for forecasting the green system of the city of Plovdiv.

P48

Antiviral Activity of Sukomycin Against Potato Virus Y and Tomato Mosaic Virus

Petrov N.[1], Tishkov S.[2]

[1] *Institute of soil science, agrotechnologies and plant protection “ Nikola Pushkarov ”, Department of Phytopathology*

[2] *Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of General and Industrial Microbiology, e- mail: m_niki@abv.bg*

Abstract. Potato Virus Y (PVY) and Tomato Mosaic Virus (ToMV) are one of the most important plant viruses that strongly influence the quality and quantity of vegetable production and cause substantial losses to farmers. The most conventional and common method of pest and disease control is through the use of pesticides. Unfortunately, most of them are synthetic compounds without antiviral activities and possess inherent toxicities that endanger the health of the farm operators, consumers and the environment. In order to carry out a control of viral infections in plants and to reduce the loss of production it is necessary the search for alternative and environmentally friendly methods for control. Sukomycin is a complex of substances with antimicrobial and antiviral activities produced from *Streptomyces hygroscopicus* isolated from soil. This natural complex reduces significantly symptoms and DAS-ELISA values of Potato virus Y and Tomato mosaic virus in tobacco plants.

P49

Antiviral Activity of Plant Extract from Tanacetum Vulgare Against Cucumber Mosaic Virus and Potato Virus Y

Petrov N., Stoyanova M., Valkova M.

Institute of soil science, agrotechnologies and plant protection “Nikola Pushkarov”, e-mail: m_niki@abv.bg

Abstract. Cucumber mosaic virus (CMV) and Potato virus Y (PVY) has been described as top five important viruses infecting vegetable species worldwide. They cause severe damages of the fruits and cultivated plants. There is currently no available effective pesticide to control these viral diseases. Higher plants contain a wide spectrum of secondary metabolites such as phenolics, flavonoids, quinones, tannins, essential oils, alkaloids, saponins, sterols and others. Extracts prepared from different plants have been reported to have a variety of properties including antifungal, antiviral and antibacterial properties against pathogens. *Tanacetum vulgare* (Tansy) is native to Europe, Asia, and North Africa. It has many horticultural and pharmacological qualities. *T. vulgare* is principally used in traditional Asian and North African medicine as an antihelminthic, antispasmodic, stimulant to abdominal viscera, tonic, antidiabetic, diuretic and is antihypertensive. In our research we established antiviral effect of methanol extract from *T. vulgare* against CMV and PVY in tomato plants.

P50

Genotoxicity Biomonitoring of Anthropogenic Pollution in Rice Fields Using the Micronucleus Test in Field Mouse (Apodemus agrarius Pallas, 1771)

Dimitrov H.[1], Mitkovska V.[1], Koleva P.[1], Chassovnikarova Ts.[1,2]

[1] Plovdiv University, Faculty of Biology, Department of Zoology, 4 Tzar Assen Str., Plovdiv 4000, Bulgaria

[2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel blvd., Sofia 1000, Bulgaria

Abstract. Zoomonitoring of small mammal populations, exposed to potential mutagens, can provide an early detection system for the initiation of cell dysregulation. The field mouse (*Apodemus agrarius*) is an appropriate zoomonitor species suitable for genotoxicological research, especially due to its widely distribution, r-type reproductive strategy, relatively small home range, high trophic chain position and metabolic rate. The present study was carried out in differently polluted areas. The field mice were collected in the rice fields located near Plovdiv (South Bulgaria) and in background region of Strandzha Natural Park (Southeastern Bulgaria). The rice fields are exposed to different anthropogenic pollutants (heavy metals and PAHs), due to the nearby located highway and the use of various fertilizers in agricultural practices. The results showed that anthropogenic pollution in rice fields induces DNA and chromosomal lesions in field mouse’s cells, which was well demonstrated by the micronucleus assay. The mean frequency of micronuclei in the individuals from the rice fields was significantly higher compared to the mean frequency of the individuals from the background region of Strandzha Natural Park. This proves the existence of geno- and cytotoxic effect in the region of the paddies. The micronucleus assay

showed no gender differences. The statistically significant differences in mean frequencies of micronuclei in field mice both from the impact and from the background area demonstrated the good genomic sensitivity of the species against anthropogenic pollution. The obtained results confirm the importance of Apodemus agrarius as a zoomonitor species for biomonitoring studies in the species characteristics habitats – wetlands.

P51

Ex situ and In situ Conservation of Centaurea pseudaxillaris (Asteraceae) by Means of Plant Biotechnology

*Traykova B., Bancheva S., Gorgorov R., Delcheva M., Stanilova M.**

Institute of Biodiversity and Ecosystem Research, Department of Plant and Fungi Diversity and Resources; 23, Acad. G. Bonchev Str., 1113 Sofia, Bulgaria

**Corresponding author: marina.stanilova@gmail.com*

Abstract. *Centaurea pseudaxillaris* Stef. & T. Georgiev (Asteraceae) is a Bulgarian endemic plant species with limited area comprising two populations located in the floristic regions Thracian Lowland and Toundzha Hilly Country. It is critically endangered, protected by the Biodiversity Act. To preserve the species both ex situ and in situ measures have been applied. Seeds germinated in vitro after 70 days of incubation on basal MS medium. Leaf explants isolated from the seedlings formed callus and leaf rosettes on MS medium supplemented with 1 mg/l BAP. The rosettes rooted spontaneously on the basal medium. Callus formed also on root segments and was fast growing on medium containing 0.5 mg/l BAP and 0.2 mg/l 2,4-D. Root-derived calli sub-cultured on MS free of plant growth regulators developed only roots while those transferred to media with 0.5 mg/l BAP or 1 mg/l 2iP regenerated whole plantlets. They were potted in soil substrate and ex vitro adapted using phytotron. The well-developed roots and the gradual humidity decrease were of crucial importance for the success of this step. Plants were further adapted to greenhouse and finally acclimatized to the field plot. The ex situ collection consisted of 14 plants which bloomed and gave seeds during their first year. After the wintering they

grew bigger and two years later, with the help of volunteers, 9 of them were transferred to their population of origin, in order to strengthen it. Two months later the monitoring confirmed the survival of the plants which were all in very good condition.

P52

The Rare Species Lathyrus transsilvanicus in Bulgaria – Contribution to the Pollen Morphology and Seed Characters

Tosheva A.[1], Marinov Y.[2], Pachedjieva K.[1]

[1] Sofia University “St. Kliment Ohridski”, Faculty of Biology, 8 Dragan Tzankov, blvd., Sofia 1164, Bulgaria

[2] Regional Natural History Museum of Plovdiv, 34 Christo Danov, str., Plovdiv 4000, Bulgaria

Abstract. In the Bulgarian flora the genus *Lathyrus* (Fabaceae) is represented by 30 species. One of the representatives of the section Orobus - *Lathyrus transsilvanicus* (Spreng.) Fritsch was reported for the Bulgarian flora in 1903 from Neichev in Central Stara planina Mts. but has not been found since 1905 and now the species is considered as “Regionally Extinct”. In 2007 the authors found a small group of *Lathyrus transsilvanicus* individuals near the locality mentioned by Neichev and reassessment according to the criteria of the IUCN Red List Categories was made in 2013. According to the reassessment L. transsilvanicis should be considered as “Critically Endangered”. The population of the species is located in Central Balkan National Park and is protected by the Biodiversity Act. The species is included in the Red List of the Bulgarian vascular plants and the Red Data book of the Republic of Bulgaria, Volume 1.

The aim of the present study is an investigation of the pollen morphology and the seed characters and testa texture. This was made by LO microscope and SEM (with light and scanning electron microscope). The study presents results for the pollen morphology of

Lathyrus transsilvanicus for the first time from a population of the species in Bulgaria.

The pollen grains are 3-zonocolporate of subprolate type, medium in size, elliptical in equatorial view and circular in polar view. The ornamentation is perforate-foveolate in mesocolpium and the polar area has scattered perforations. The seeds are oblong, medium in size, with brown, monochrome and ornamented testa. The hilum is usually oblong, 1/3 of the seed circumference. The SEM pattern of the testa established is papillae in the hilar region and multi-reticular pattern in the midseed area.

P53

Comet Assay Assesment of a DNA damage in Rice Fields Population of Field Mouse (Apodemus agrarius Pallas, 1771)

Mitkovska V.[1], Chassovnikarova Ts.[1,2], Krasteva M.[1], Dimitrov H.[1]

[1] Plovdiv University, Faculty of Biology, Department of Zoology, 4 Tzar Assen Str., Plovdiv 4000, Bulgaria

[2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel blvd., Sofia 1000, Bulgaria

Abstract. The wetlands, especially the rice fields, have global importance in providing food through agricultural activities. That‘s why the efforts should be geared toward identifying appropriate biomonitoring organisms as a risk assessment criteria. The field mouse (*Apodemus agrarius*) is an appropriate zoomonitor species suitable for genotoxicological research, especially due to its widely distribution, r-type reproductive strategy, relatively small home range, high trophic chain position and metabolic rate. Over the recent years, the comet assay has become an important tool for assessing DNA damage in exposed populations. The present study investigated the overall mutagenic potential in rice fields located close to the industrial area of Plovdiv city, and along a highway nearby. The Strandzha Natural Park in Southeastern Bulgaria was chosen as a background region. An increase in the comet assay parameters in mice from the impact region compared to the control probes was observed. The results showed no gender differences in the species genomic response in vivo to the genotoxic agents in the environment. Comet assay parameters (tail length, % tail DNA, tail moment, Olive tail moment) of the mice from the rice fields

were statistically significant higher than those of individuals from the background area (P <0.0001). The latter proves the existence of genotoxic effect in the region of rice paddies. Comets of class 1 (up to 20% tail DNA) and class 2 (up to 40% tail DNA) prevailed in the individuals inhabiting the rice fields, while comets of class 0 and class 1 were most common among individuals from the background area. The obtained results confirm the importance of Apodemus agrarius as a zoomonitor for biomonitoring studies in the species characteristics habitats – wetlands.

P54

Interesting Boletales from Bulgaria and the Adjacent Countries

Assyov B.

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

Abstract. Five noteworthy boletes are presented on materials from Balkan collections, namely *Baorangia emilei, Boletus poikilochromus, Lanmaoa fragrans, Rubroboletus lupinus* and *R. pulchrotinctus.* Three species, *B. poikilochromus, L. fragrans* and *R. pulchrotinctus,* are new records for the mycota of Bulgaria and the presence of *R. lupinus* in this country is confirmed. *Baorangia emilei* is studied on specimens from Greece and Serbia. Scanning electron microscopy of the spores of all species is included. *Baorangia emilei, L. fragrans, R. lupinus* and *R. pulchrotinctus* are studied for the first time by the means of SEM-analysis, confirming the assumption that basidiospores in those species are smooth.

P55

First Records of Xerocomus chrysonemus and X. silwoodensis (Boletales) in Southeastern Europe

Assyov B.

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

Abstract. The recently separated and still little-known species *Xerocomus chrysonemus* and *X. silwoodensis* are described and illustrated on the basis on their first collections in the Balkan Peninsula from Bulgarian localities. Comparison with the closely related *X. ferrugineus* and *X. subtomentosus* is included. The habitats in the new stations of *X. chrysonemus* are thermophilous woodlands of deciduous oaks (*Quercus cerris, Q. frainetto* or *Q. pubescens*). *Xerocomus silwoodensis* is found so far in one locality in Bulgaria in a stand of *Populus tremula* with understory of *Carpinus betulus* and *Coryllus avellana*. The new collections expand the knowledge on the distribution and the variability of those noteworthy boletes, suggesting that they extent of occurrence in Europe is larger than currently known.

П о с т е р и – 3 1 . 1 0 . 2 0 1 5

Секция: Биотехнологии, Бионано-технологии и Приложна биология

P56

The Effect of Extraction Time on the Antioxidant Activity of Fresh Bulgarian Melissa officinalis L.

Mihaylova D.[1], Popova A.[2], Alexieva I.[2]*

[1] Dep. Of Biotechnology, University of Food Technologies, Plovdiv, Bulgaria
[2] Dep. Of Catering and Tourism, University of Food Technologies, Plovdiv, Bulgaria
**Corresponding author: e-mail: popova_aneta@abv.bg*

Abstract. The antioxidant capacity of aqueous extracts of fresh leaves of lemon balm (*Melissa officinalis* L.), used as a medical plant and culinary spice, was studied. The aim of the present study was to measure the effect of extraction time on total phenolic compounds (TPC) and antioxidant activity (ABTS - , DPPH- and FRAP - assays). The obtained results demonstrated highest levels of TPC (5.25 GAE/g fresh plant weight), ABTS (72.04 μMTE/g FW), DPPH (81.55 μMTE/g FW) and FRAP (116.58 μMTE/g FW) at 30 min of thermal treatment. A high correlation between antioxidant capacities of fresh leaves of M. officinalis and their total phenolic contents (R=0.97-0.99) was established.

P57

GS-MS Based Metabolite Profile of Different Trademarks of Fenugreek

Hasan Y[1]., Vrancheva R.[1], Ivanov I.[2], Dincheva I.[3], Badjakov I.[3], Pavlov A.[1,4]*

[1] *Department of Analytical Chemistry, University of Food Technologies, Plovdiv, 26 Maritza Blvd., 4002, Plovdiv, Bulgaria;*
[2] *Department of Organic Chemistry, University of Food Technologies, Plovdiv, 26 Maritza Blvd., 4002, Plovdiv, Bulgaria;*
[3] *AgroBioInstitute, Agricultural Academy, 8 Dr. Tsankov Blvd, 1000, Sofia, Bulgaria;*
[4] *Department of Industrial Microbiology, Laboratory of Applied Biotechnologies, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 139 Ruski Blvd., 4000 Plovdiv*
**Corresponding author: e-mail: radka_vrancheva@yahoo.com*

Abstract. GS-MS based metabolite profile of fenugreek (*Trigonella foenum-graecum* L.) of three different trademarks was established. In polar fractions totally 40 primary metabolites (carbohydrates, organic and amino acids) and 9 phenolic acids (with caffeic and 4-hydroxybenzoic acids as major) were identified. The dominant metabolites in unpolar fraction were palmitic, linoleic, oleic and stearic acids. Two untypical triterpenic acids (oleanolic and ursolic acid) for family *Fabaceae* were identified in one of the analyzed sample. The presence of these triterpenoids in the sample of fenugreek indicates for probably adulteration, during the experiment.

P58

Study of the Energy Potential of Vinasse

*Velichkova P. *, Lalov I.*

[1] *Department of Biotechnology, UCTM, Kl. Ohridsky 8, 1756 Sofia, Bulgaria*
**Corresponding author: e-mail: poli_1989@mail.bg*

Abstract. The biochemical methane potential of one typical for Bulgaria organic waste – vinasse was studied. For this purpose, the BMP test was carried out in batch mode at mesophilic temperature (33°C). A biochemical methane potential (BMP) assay provides a measure of the anaerobic digestibility of a given substrate. The use of BMPs provides a relatively inexpensive and repeatable method to make relative comparisons of the anaerobic digestibility and potential biogas production between various substrates. Biochemical Methane Potentials can be used to determine the amount of organic carbon in a given material that can be anaerobically converted to methane and to evaluate potential biogas production efficiency of the anaerobic process on a given material. The information provided by BMPs is valuable when evaluating potential anaerobic substrates and for optimizing the design and operation of an anaerobic digester. This paper describes the BMP assay procedure developed and used in our laboratory for quantifying both biogas production and methane content. In overall, our results suggest that tested substrate can be treated anaerobically and is potential source for the production of methane.

P59

Sequence Analyses of Genes Coding Key Enzymes Responsible for Degradation of Phenolic Compounds by Aspergillus fumigatus strain AL3

Litova K., Manasiev J., Gerginova M., Peneva N., Alexieva Z.*

[1] *Department of General Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev str., bl. 26, 1113 Sofia, Bulgaria*
**Corresponding author: katya_litova@abv.bg*

Abstract. Two genes coding proteins with phenol hydroxylase and catechol 1,2-dioxygenase enzyme activities which take part in the degradation pathway of phenolic compounds in Antarctic fungal strain *A. fumigatus* AL 3 have been identified. The primers applied in the PCR analyses for both genes were designed on the basis of the identical genes sequences of A. fumigatus strain Af293. For the detection of phenol hydroxylase and catechol 1,2-dioxygenase genes were created and used two sets each consisted of 3 pairs of primers. The obtained oligonucleotide DNA fragments were sequenced by Sanger method. The overlapped three fragments for each one of the investigated genes were combined. The sequence obtained for phenol hydroxylase gene (1398 bp without introns) was organized into 3 exons and 2 introns. The length of the partial catechol 1,2-dioxygenase gene count without introns was 642 bp. It included 4 exons and 3 introns. The basic logical alignment search confirmed that the phenol hydroxylase gene had 99% nucleotide identity with the corresponding gene of *A. fumigatus* Af293. The same percent of identity of the catechol 1,2-dioxygenase genes of *A. fumigatus* Af293 and *A. fumigatus* AL 3 was established. The translation of the obtained DNA sequences revealed a protein with phenol hydroxylase activity consisting of 213 amino acids as well as a protein with catechol 1,2–dioxigenase activity consisting of 465 amino acids. The proximity of the studied proteins with closely related enzymes with similar functions were demonstrated on the created distant tree’s cladograms.

P60

Passive Treatment of Mine Waters Followed by Electricity Generation

Nicolov I., Spasova I., Georgiev P., Nicolova M., Groudev S.*

[1] *University of Mining and Geology "Saint Ivan Rilski", Sofia 1421, Bulgaria*
**Corresponding author: e-mail: mnikolova@mgu.bg*

Abstract. Acid mine waters polluted with heavy metals (Cu, Zn, Cd, Fe, Mn) and sulphate were subjected to a passive treatment by a permeable reactive multibarrier. The multibarrier was a plastic cylindrical column 80 cm high, with an internal diameter of 30 cm, and a volume of 56.5 L. The column was filled with a mixture of limestone (crushed to minus 10 mm particle size) and organic matter consisting of spent mushroom compost, fresh leaf compost, animal manure and saw dust. These components contained their own viable indigenous microflora consisting of different anaerobic microorganisms, mainly of sulphate-reducing bacteria and other interconnected microorganisms. Apart from the natural microflora of the organic substrates, inoculum containing selected electrochemically active microorganisms was also added to the multibarrier effluents, together with a nutrient solution containing biologically essential elements. The contnuous-flow circulation of such solutions from the inlet and outlet of a membrane-less microbial fuel cell resulted in an efficient electricity generation with a power density within the range of 1045 - 1085 mW/m^2. The microbial fuel cell was a plexiglas column 50 cm high, with an internal diameter of 10 cm, with corrigated slab graphite anode and cathode electrodes located in the bottom and in the top sections of the column, respectively. The electroded had round form. with diameter of 8 cm each. The optimal conditions for such treatment were the relatively high contents of biodegradable organic substrates in the solutions (within 5000 - 7000 Chemical Oxygen Demand/L) and pH and temperature of about the neutral point and 32 - 35 degrees Celsius, respectively. The aeration rate in the cathode section was 50 mL O_2/min.

P61

Formation of Artificial Sheaths of Leptothrix sp. Under Laboratory Conditions

Angelova R.[1,2], Iliev M.[1], Slavov L.[2], Koutzarova T.[2], Closset R.[3], Nedkov I.[2], Groudeva V.[1]*

[1] *Department of General and Industrial Microbiology, Sofia University, Bulgaria*
[2] *Laboratory Microwave Magnetics, IE, BAS, Sofia, Bulgaria*
[3] *LSIC, Chemistry Department B6, University of Liege, Belgium*
[*] *Corresponding author: raly_angelova@abv.bg*

Abstract. The object of this research are the neutrophilic sheath forming iron bacteria from genus *Leptothrix* isolated from natural stream located in Vitosha Mountain. These bacteria facilitate the iron mineralization and formation the insoluble ferric oxides/(oxy)hydroxides after Fe^{2+}-oxidation at neutral pH. The aim of this investigation is establishing the conditions for the formation of tubular structures (sheaths) typical for these bacteria. For this goal cultivation on an elective growth medium and the characterization of the structures formed by optical microscopy and SEM, SEM/EDX, TEM and XRD were performed.

The formation of the sheaths was observed at 20^{o}C using a flask-shaking technique. It started after 7 days of the cultivation.

Light micrograph images and SEM unveiled that the average size of the sheaths is around 7 – 10 μm, and the average diameter is up to 1 μm. SEM/EDX revealed the elemental composition of the tubular structures. The XRD data showed single-phase composition of the iron oxides on the sheaths obtained. TEM micrographs unveiled the shape of the biogenic nanoparticles.

The bacterial mediated formation of iron containing tubular structures and their magnetic properties are of great interest for application in the nanotechnologies and different biomedical and bioengineering applications.

Acknowledgements. This work was supported by the Bulgarian National Science Fund of Ministry of Education and Science under project T02-17/2014.

P62

Selection of Electrochemically Active Microorganisms by Continuous-Flow Treatment of Rich-In-Organics Water in Microbial Fuel Cells

Ilieva R.[1], Groudeva V.[1], Iliev M.[1], Groudev S. [2]*

[1] Department of General and Industrial microbiology, Sofia University “St. Kliment Ohridski”, Sofia, Bulgaria;
[2] Department of Engineering Geoecology, University of Mining and Geology “St. Ivan Rilski”, Sofia, Bulgaria
**Corresponding author: bacto@abv.bg*

Abstracts. Effluents from a large-scale permeable multibarrier, used as a component of a passive system for treatment of metal-polluted mine waters, were characterized by neutral pH, low electrochemical potential, practical absence of dissolved oxygen and toxic heavy metals. At the same time, these effluents contained high concentrations of biodegradable organic compounds and different anaerobic heterotrophic bacteria. These waters were tested in a microbial fuel cell to establish if they are suitable for electricity generation and can be used as a source for isolation and selection of electrochemically active microorganisms. The tests revealed that the effluents contained microorganisms (bacteria) which generated electricity by transfer of electrons from the dissolved organic substrates to the anode electrode located in the anoxic section of the microbial fuel cell by two different mechanisms - by direct contact of the relevant microbial biofilm formed on the anode surface or by production of mediators dissolved in the waters and involved in the electron transfer from the bacteria to the anode. The first mechanism was used by some Fe^{3+}-reducing bacteria (mainly related to the genera *Geobacter* and *Shewanella*), while the second mechanism was used mainly by sulphate-reducing bacteria producing hydrogen sulphide as mediator. These two physiological groups of bacteria were able to survive together in the presence of both ferric and sulphate ions in the waters tested by the microbial fuel cells. Some fermenting bacteria were also present in the relevant mixed populations. The step-wise selection

of electrochemically more active microorganisms was carried out under continuous-flow conditions and was an efficient but a relatively slow process.

Acknowledgements. The authors acknowledge the financial support of the Bulgarian National Science Fund for this study performed in connection with the project ДФНИ ТО2/12/2014

P63

Changes in the Microflora Composition of Polluted Waters during Their Treatment in a Permeable Multibarrier and a Microbial Fuel Cell

Iliev M.[1], Groudeva V.[1], Ilieva R.[1], Groudev S.[2], Spasova I.[2], Georgiev P.[2], Nicolova M.[2]*

[1] *Department of General and Industrial microbiology, Sofia University “St. Kliment Ohridski”, Sofia, Bulgaria;*
[2] *Department of Engineering Geoecology, University of Mining and Geology “St. Ivan Rilski”, Sofia, Bulgaria*
**Corresponding author: bacto@abv.bg*

Abstract. Acid drainage waters polluted with some toxic heavy metals (mainly Cu, Zn, Cd, Fe, Mn) were efficiently treated by means of a permeable reactive multibarrier intended for microbial dissimilatory sulphate reduction, biosorption and chemical neutralization. The multibarriers effluents were enriched in biodegradable organic compounds and were used for electricity generation by means of a membrane-less microbial fuel cell. The treatment under continuous-flow conditions was connected with the gradual selection of electrochemically active bacteria related to three principal physiological groups - sulphate-reducing (mainly of the genera *Desulfovibria*, *Desulfobacter*, *Desulforomonas*), iron-reducing (mainly of the genera Geobacter and Shewanella) and fermenting (mainly of the genera *Clostridium*, *Bacillus* and *Alteromonas*). A considerable portion of these bacteria were members of the biofilm formed on the anode electrode of the microbial fuel cell. However, the transfer of electrons from the bacteria to the anode was performed mainly by hydrogen sulphide generated during the process of microbial dissimilatory sulphate reduction. It must be noted that the iron-reducing bacteria were not only able to use ferric hydroxide as an electron acceptor in the process of anaerobic respiration but also to transfer electrons directly to the anode.

At the sometime, most of the other microorganisms present in the multibarrier effluents (related to the identified 21 taxonomic genera) were gradually eliminated during the treatment in the microbial fuel cell. The maximum power generation achieved by the system used in this study was 1085 mW/m^2.

Acknowledgements. The authors acknowledge the financial support of the Bulgarian National Science Fund for this study performed in connection with the project ДФНИ ТО2/12/2014

P64

Plant Growth Regulatory Metabolites of Fungal Strain Rhizopus Arrhizus KB-2

Evstatieva Y., Ilieva A., Nikolova D., Tropcheva R., Savov V.*

[1] *Department of Biotechnology, Faculty of Biology, Sofia University “St. Kliment Ohridski”, Sofia, Bulgaria*
**Corresponding author: yana_stefanova@mail.bg*

Abstract. The genus *Rhizopus* is a well recognized filamentous fungi, inhabiting soil, rotting fruits, vegetables, food, etc. Species of this genus are known as a postharvest deterioration microorganisms, causing serious problems in successful storage and marketing of agricultural production. However the species *Rhizopus arrhizus* is a technologically important producer of organic acids and hydrolytic enzymes and it is widely applied in biotechnology.

The aim of this study is to estimate the capability of the strain *Rhizopus arrhizus* KB-2 to produce metabolites with plant growth regulatory activity. Morphological characteristics, pH variation and hydrolytic enzyme activity of this strain were defined during the submerged batch cultivation. The presence of some plant regulation metabolites in the cultural liquid, such as trans-zeatin, thidiazuron, gibberellic acid, abscisic acid and indole-3-propionic acid, were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. High amounts of trans-zeatin, abscisic acid (ABA) and indole-3-acetic acid (IBA) were recorded.

In order to study the effect of this complex of regulatory metabolites, different dilutions of cell free supernatant were applied in the test for germination of *Lactuca sativa* seeds in vitro.

P65

Antifungal Activity and Enzymological Characteristics of Lactobacillus bulgaricus GLB44 (PROVIOTIC®)

Tropcheva R. [1], Evstatieva Y.[1], Nikolova D.[1], Saeva-Kondratenko S.[2], Petkov P.[3], Petkov K.[3]*

[1] Department of Biotechnology, Faculty of Biology, Sofia University St. Kliment Ohridski, 8, Dragan Tsankov Blvd., 1164, Sofia, Bulgaria;
[2] Genesis Laboratories LTD, Sofia, Bulgaria
[3] Proviotic LTD, Sofia, Bulgaria
**Corresponding author: tropcheva12@mail.bg*

Abstract. Moulds and yeasts are the main spoilage microorganisms, responsible for significant economic losses and several healthy risks in human food chain. The antimicrobial activity is an important criterion for the selection of bio-protective lactic acid bacteria (LAB). A limited data exists on the antifungal activity of Bulgarian LAB and their enzyme profile. With this aim, the activity of the commercially available probiotic Proviotic®, containing the strain *Lactobacillus bulgaricus* GLB44, against yeasts and moulds, and the presence of key enzymes, were studied.

Lactobacillus bulgaricus GLB44 (property of Genesis Laboratories LTD) was screened for antifungal activity against five mould species - *Aspergillus flavus*, *Aspergillus niger*, *Fusarium graminearum*, *Trichoderma viride* and *Penicillium claviforme* and three yeast species - *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Rhodotorulla sp*., using agar diffusion method. The enzyme profile of the *L. bulgaricus* GLB44 was determined using API ZYM miniaturized test (BioMerieux, France), following the manufacturer’s instructions.

Lactobacillus bulgaricus GLB44 possess a high amino-peptidase, acid-phosphatase and β-galactosidase enzymatic activity and a complete lack of the associated with the colon carcinogenesis β-glucuronidase activity.

The in vitro tests revealed a broad spectrum of antifungal activity. *L. bulgaricus* GLB44 completely (100%) suppress the growth of *Aspergillus flavus*, *Fusarium graminearum*, *Trichoderma viride*, *Penicillium*

claviforme. With regard to *Aspergillus niger*, a lower inhibitory activity (66.6%), was observed. Proviotic® demonstrated a stronger inhibition against *Saccharomyces cerevisiae*, compared to the used as a control commercial antibiotic Fungostatin.

The demostrated strong proteolytic activity of Proviotic® makes the *Lactobacillus bulgaricus* GLB44 interesting for use in the production of antihypertensive and immuno-modulatory products and also in the manufacture of different dairy products.

The antifungal activity of Proviotic® is a promising advantage, suggesting its potential applications in different food technologies as a bio-preservative agent and a health promoting products against fungi.

P66

Effect of Nitrogen Source on the Growth and Biochemical Composition of a New Bulgarian Isolate of Scenedesmus sp.

Vasileva I., Marinova G., Gigova L.*

[1] *Institute of Plant Physiology and Genetics,BAS,Acad.G.Bonchev Str., bldg. 21,1113,Sofia, Bulgaria*
**Corresponding author: ivanina_vasileva1@abv.bg*

Abstract. The influence of different nitrogen sources (ammonium nitrate, urea and ammonium nitrate + urea) and different concentration of the medium, containing both nitrogen sources on the growth and the content of pigment, protein, carbohydrate and lipid of a newly isolated green alga Scenedesmus sp. BGP was studied. Even though in the recipe of Setlik (1967) modified by Georgiev et al.(1978) (routinely used in the lab), the two nitrogen sources are present, the experiments show that the use of each one of them separately gives better yield of algal biomass for the whole cultivation period. The best growth was observed in urea-containing medium, where the dry weight reached 9.0 g/l. Dilution of the standard medium (2, 4 and 8 fold) has a positive impact on the growth, unlike a 2-times-concentrated medium. The total amount of pigments (chlorophyll a+b and carotenoids) correlates with the change of growth in the different media. As a whole, the biochemical composition of Scenedesmus sp. BGP is dominated by carbohydrates, followed by proteins and lipids. The biomass of the alga, grown in urea- or ammonium nitrate-containing medium, is characterized by stable qualitative content over the entire period of cultivation (about 29% proteins, 41% carbohydrates and 24% lipids).

These results show that the medium with urea is most suitable for cultivation of Scenedesmus sp. BGP, because as a nitrogen source, urea is more efficient in terms of productivity and is less expensive.

P67

Characterisation of a Newly Isolated Thermophilic Bacillus Strain and of the Produced Exo-Inulinase

*Gavrailov S., Ivanova V.**

[1] *University of Food Technologies – Plovdiv, Department of Microbiology, 26 Maritsa blvd, 4002 Plovdiv, Bulgaria*
**Corresponding author: vn.ivanova@abv.bg*

Abstract. A strain of *Bacillus sp.* SG10, isolated from thermal water samples from the region of Velingrad, Bulgaria, showed good capacity to produce extracellular inulinase. The enzyme was synthesized in the presence of monosaccharides and inulin, as carbon sources. The best results were obtained with yeast extract and other sources of organic nitrogen, comparatively to the mineral nitrogen. Optimal growth temperature was 60°C. No growth was established at 40°C and 70°C. It also showed rapid growth in wide pH range from 6.5 to 9.5 with optimum at pH 8.0-8.5. The optimum pH of the crude enzyme for inulin hydrolysis was found at pH 7.0 and the optimum temperature at 60°C. The enzyme showed capacity to hydrolyse sucrose, raffinose and inulin from which it liberated only fructose units, therefore showing an exo-action mechanism. Acting on inulins from several sources, the enzyme showed hydrolysis of the polissaccharide from chicory, dahlia and Jerusalem artichoke tubers.

P68

Biosorption Capacity of Treated Biomass of Antarctic Strain Cryptococcus laurentii AL65

Rusinova-Videva S.[1,3], Nachkova S.[2], Mladenov R.[3], Stoyanov P.[3]*

[1] Laboratory of Applied Biotechnologies, Department of Applied Microbiology Institute of microbiology-BAS, 139 Ruski Blvd., 4000 Plovdiv, Bulgaria
[2] Department of Analytical Chemistry and Computer Chemistry, Plovdiv University “Paisii Hilendarski”, 24 Tzar Assen Str., 4000 Plovdiv, Bulgaria
[3] Department of Botany, Plovdiv University “Paisii Hilendarski”,24 Tzar Assen Str., 4000 Plovdiv, Bulgaria
**Corresponding author: jrusinova@abv.bg*

Abstract. The problem of environmental pollution by industrial production wastewater, contaminated with heavy metals, has been regarded as a major threat to human health in recent years. The traditional physical-chemical purification methods are inapplicable and expensive in some cases. Therefore the unconventional methods for polluted industrial water purification are of great interest. Organic sources that act as biosorption of target metals are sought. The study of certain types of fungi shows good potential for purification of aqueous solutions of Pd, Cd and Cu.

In the present work psychrophilic yeast strain *C. laurentii* was used in order to study its biosorption capabilities. The accumulation of biomass was traced in dynamics in the context of bioreactor cultivation (400rpm, 1.25L/L/min, 22ºC). It was pretreated and incubated in the presence of a multi-element standard solution (Multi IV, Merck). To determine the concentration of metal ions in the solution, ICP-OES was used. The effect of pH on sorption capacity was studied. The results showed that at the optimal pH5 found, three groups of elements are observed: Ga, In, Cr, Fe, Bi and Al with a relatively high value of adsorption; average for Cu, Ag and Pb; and low for Mn, Ni and Co.

P69

In vitro culture development and polyphenolics production of Artemisia alba Turra

*Koleva P.[1], Wolfram E.[2], Raynova Y.[1], Evstatieva L.[3], Danova K.[1]**

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, BAS, Sofia, Bulgaria*
[2] *Zurich University of Applied Sciences (ZHAW), Institute of Biothechnology, Wadenswil, Switzerland*
[3] *Institute of Biodiversity and Ecosystem Research, BAS, Sofia, Bulgaria*
**Corresponding author: k_danova@abv.bg*

Abstract. *Artemisia alba* Turra is a fragrant shrub, distributed in Southern Europe, traditionally applied as and digestive in the form of decoction. Research work has shown the anti-inflammatory and spasmolytic activity of extracts of the plant as well as the antimicrobial activity of its essential oil. In vitro cultures of the plant have been previously established with the purpose of investigation of secondary metabolites in controlled laboratory conditions. The terpenoid profile of the plant in vitro has been studied, leading to the development of two distinctive systems for the yield of essential oils with either monoterpenoid or sesquiterpenoid domination.

Here effect of auxins and cytokinins on the morphogenetic response and polyphenolic productivity of the plant in vitro were studied. Leaves responded with callusogenesis upon auxin treatment (2,4-dichlorophenoxyacetic acid, 2,4-D and α-naphtylacetic acid, NAA), and with callusogenesis and indirect shoot formation to combination of the both auxins with benzyl adenine (BA). The individual application of BA did not lead to any morphogenesis, but to necrosis of the explants. Stem segments responded with callusogenesis upon 2,4-D and combination of 2,4-D and BA, callusogenesis and rooting upon NAA, auxillary shoots and callus formation upon NAA and BA, as well as individual BA treatments. Root segments responded with callusogenesis to 2,4-D,

combination of 2,4-D and BA, and with rooting to NAA and NAA and BA combination. BA alone did not induce marked morphogenic response of the root explants. Further on, root cuttings were chosen as the starting explant for the development of conventional root, callus and suspension cultures, due to the lower levels of lipid peroxidation (expressed as malondyaldehyde content) as compared with shoots in vitro. Four lines of *A. alba* suspensions were developed based on medium composition and to the light regime. Relations between morphogenetic response and polyphenolics production of the plant are discussed.

Acknowledgements. Swiss National Science Foundation in the framework of the Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZ0_142989; DO2-1153)

P70

Enterococcus faecium Strain Used as an Adjunct Culture in the Starter for Kashkaval Cheese Plays Important Role to Proteolytic Processes and Release of Bioactive Peptides During Ripening

*Gotova I., Dimitrov Zh. **

LB-Bulgaricum Plc. R&D Center, Sofia, Bulgaria
**Corresponding author: jechkoelby@yahoo.com*

Abstract. In the present study the potential of preliminary selected strain *Enterococcus faecium* used as an adjunct culture in starter for kashkaval cheese was evaluated. The strain *Enterococcus faecium* MH5 was selected among 17 isolates on the basis of its high level of proteolytic and peptidase activities. The active proteolytic system of *E. faecium* MH5 leads to high level of transformation of caseins to end products. A high degree of transformation of β-casein and αs1-casein in the initial stage of ripening (up to 15 d) and during storage up to 90 d was determined by Urea-Polyacrylamide gel electrophoresis (exceptionally high degree of degradation – 91% and 83% for αs1-casein and β-casein). The results reveal that the new starter combination, including strain *Enterococcus faecium* MH5 as a nonstarter culture, can be successfully used to enhance proteolysis, shorten the ripening period and improve the quality of hard cheeses. Kashkaval produced using *Enterococcus faecium* MH5 in the starter contains significantly more free amino acids and increased concentration of low molecular weight peptides. The inhibitory activity of low molecular weight peptides against angiotensin-converting enzyme was significantly higher than those of common kashkaval cheese. The aim of the present work was to study the possible use of *Enterococcus faecium* strain as an approach towards

enhancing the proteolytic processes during Kashkaval cheese ripening, acceleration of the ripening process and improving the quality of the end product.

P71

Presence of Heavy Metals (Pb, Cd and Hg) in Danube Herbivorous Fish Species - Carp and Grass Carp

Ahad V.[1], Bliznakov A.[1], Mineva E.[2], Tenev T.[2], Satchanska G.[1]

[1] Dept. Natural Sciences, New Bulgarian University, Montevideo 21, 1618 Sofia, Bulgaria
[2] Bulgarian Food Safety Agency, Central Laboratory of Veterinary Sanitary Expertise and control, Iskarsko shousse 5, 1526 Sofia, Bulgaria
**Corresponding author: zlatna81@mail.bg*

Abstract. Object of Research: Carp (*Cyprinus carpio*) and grass carp (*Ctenopharingodon idella*) are the most often distributed herbivorous fresh water fishes in Danube river.

Materials and methods: Both species were collected in January 2015 in Vidin section of the Danube river and the lead (Pb), cadmium (Cd) and mercury (Hg) content in muscle tissues was analyzed. Heavy metal concentrations were evaluated via Electrothermal Atomic Absorbtion Spectrophotometry with vapor atomization (ETAAS) and Atomic Absorbtion Spectrophotometry (AAS). Data obtained were compared with the Maximum residual limits (MRL) of fish contaminants described in the EC Regulation № 1881/2006.

Results: Our analysis showed concentration 0.0062 $mg.kg^{-1}$ of Pb (MPS 0.3) for *Cyprinus carpio* and 0.019 $mg.kg^{-1}$ Pb (MPS 0.3) - for *Ctenopharingodon idella*. Hg and Cd concentrations also demonstrated no contamination of both fish species. Hg concentration in *Cyprinus carpio* was registered under the limit - 0.0004 $mg.kg^{-1}$ as well as in *Ctenopharingodon idella* - 0.0009 $mg.kg^{-1}$ (Maximal Residual Limit (MRL) = 0.05 $mg.kg^{-1}$). The results received are in accordance with the Regulation № 1881/2006 as well.

Conclusions: The concentrations of Pb, Cd and Hg in herbivorous Danube fish carp and grass do not exceed the Maximum residual limits and both species are safe for consumption.

Acknowledgements. This analysis was funded by Grant № 972/2015 of the Central Fund for Strategic Development (CFSR), New Bulgarian University.

P72

Kinetic Data of Beta-Galactosidase from Lactobacillus plantarum S26 in Present of Lactulose

*Zeleva P., Bivolarski V., Vasileva T., Iliev I.**

[1] *Department of Biochemistry and Microbiology, Plovdiv University, 24, Tzar Asen, Str, Lab 15, 4000 Plovdiv, Bulgaria,*
**Corresponding author: ilievini@abv.bg*

Abstract. Beta-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) is an enzyme that catalyzes two basic reactions, hydrolysis of the milk lactose and structurally related galactosides (lactulose) and swiching over to transglycosilation, resulting, for example, in a mixture of galactooligosaccharides (GalOS), when lactose or lactulose are the starting material for the latter reaction. GalOS are complex mixtures consisting of numerous different oligosaccharides with different degree of polymerization and glycosidic linkage. Galacto-oligosaccharides (GalOS) together with fructo-oligosaccharides are amongst the most important and best-studied groups of prebiotic oligosaccharides. In this work, a detailed characterization of products obtained by transgalactosylation reactions of the prebiotic lactulose, by using beta-galactosidase of *Lactobacillus plantarum* S26 strain is reported. Galactose, fructose an oligosaccharides were detected and quantified by HPLC analysis. The effect of lactulose concentration on the initial velocity of the enzyme reaction were also compared and Michaelis-Menten constant were calculated at 62.9 mmol of lactulose.

P73

Identification of a Lactic Acid Bacterial Flora within the Honey Intestinal Tract of Apis mellifera from Different Regions of Bulgaria

Rabadjiev Y.[1], Christova P.[1], Iliev I.[2], Ivanova I.[1]*

[1] Department of General and Industrial Microbiology, Faculty of Biology, Sofia University, 1000 Sofia, Bulgaria
[2] Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University, 4000 Plovdiv, Bulgaria
**Corresponding author: yavor.rabadjiev@gmail.com*

Abstract. Fructophilic lactic acid bacteria (FLAB) are specific group lactic acid bacteria (LAB) characterized and described only recently. They prefer fructose as growth substrate and inhabit only fructose-rich niches such as intestinal tract of bees that are high-fructose-consuming insects and important pollinators in nature, but reported to be decreasing in the wild. In the present study, we analyzed LAB microflora in the intestinal tract of bees *Apis melifera* on the territory of Bulgaria. Sixteen isolates were collected from the honey stomach of the honeybee Apis mellifera. Poor growth was recorded when strains were incubated anaerobically in the presence of D-glucose as sole carbon source. All of isolated strain showed fructophilic characteristics fermenting fructose faster than glucose. The isolates were Gram-stained and tested for catalase reaction. The 16S rRNA genes from extracted DNA of bacterial colonies were amplified with polymerase chain reaction using universal primers 27F and 1492R and were sequenced. The 10 isolated strains yielded five distinct 16S sequences of *Lactobacillus plantarum*, *L. pentosus*, *L. iwatensis*, *L. kunkeei*, *Weissella Confusa*. DNA of sequenced strains were amplified with specific primers in order to confirm the genus of the samples LBMA-1

(CTCAAAACTAAACAAAGTTC) and R16-1 (CTTGTACACACCGCCCGTCA). These strains can be good candidates for potential application as probiotics in honeybees and also as natural food preservatives, which, in turn, may be useful in the food industry.

Acknowledgements. This work was supported by the Sofia University Fund of Science under project № 168/17.04.2015.

P74

Influence of The Culture Conditions and the Composition of Culture Medium on the Antimicrobial Properties of Bacillus subtilis Strain 46/H1 Against Some Filamentous Fungi

Tumbarski Y., Petkov E., Cholakov R., Yanakieva V., Denkova Z.*

Department of Microbiology, University of Food Technologies
26, Maritsa Blvd., Plovdiv, Bulgaria,
**Corresponding authors: tumbarski@abv.bg*

Antibacterial and antifungal activities are characteristic features of many representatives of the genus *Bacillus*, in particular of the non-pathogenic species Bacillus subtilis. By using the classical agar-well diffusion method, we investigated the influence of the culture conditions and the composition of the culture medium on the antimicrobial activity of *Bacillus subtilis* strain 46/H1 against the filamentous fungi *Fusarium oxysporum* and *Aspergillus flavus*. The fungal suspensions were preliminarily inoculated in the agar media, whereas the cell-free supernatant, the cell biomass and the culture liquid of *B. subtilis* 46/H1 were pipetted into the wells. The influence of the cultural conditions on the inhibitory activity of *B. subtilis* 46/H1 was determined by cultivation in agar media with pH ranging between 5.0 and 8.0, at temperature 25^{o}C and 30^{o}C. The influence of the composition of culture medium on the inhibitory activity of *B. subtilis* 46/H1 was determined by changing the carbon and nitrogen sources. After 72 hours of incubation, the antimicrobial effect was determined by measuring the diameter of the zones of inhibition around the wells. The antimicrobial activity of *B. subtilis* 46/H1 against both fungal species was higher at temperature of cultivation 30^{o}C. The change of the carbon and nitrogen sources in the standard culture medium led to an increase in the inhibitory effect, which was stronger against the fungus *Fusarium oxysporum* and almost insignificant against the second test-microorganism - *Aspergillus flavus*. In addition, the antifungal activity against *Fusarium oxysporum* was

higher after changing the nitrogen source than after the change of the carbon source.

Key words: *Bacillus subtilis*, *Fusarium oxysporum*, *Aspergillus flavus*, antimicrobial activity, bacteriocins

P75

Phenolic profile and antioxidant activity of methanolic extract of CARDUUS ACICULARIS BERTOL.

Zhelev I., Dimitrova-Dyulgerova I., Mladenov R.

Department of Botany and methods of biology teaching, Faculty of Biology, Plovdiv University”Paisii Hilendarski”, Plovdiv, 24 Tzar Assen str., 4000 Bulgaria

Phenolic acid and flavonoid profiles of Carduus acicularis were investigated for the first time. Eleven phenolic acids and eight flavonoids were identified and quantified in the inflorescences, by high performance liquid chromatography. The main phenolic compounds were found to be: sinapic acid (930.41 ± 21.72 μg/g dw), chlorogenic acid (582.66 ± 13.60 μg/g dw), rutin (545.65 ± 12.82 μg/g dw), apigenin (478.75 ± 11.38 μg/g dw), luteolin (288.46 ± 6.86 μg/g dw) and myricetin (276.32 ± 5.21 μg/g dw). The antioxidant activity of methanolic extract of inflorescences has been investigated, employing four different established testing systems: scavenging activity on 2,2-diphenil-1-picrylhydrazyl (DPPH), 2,2’-azinobis-(3-ethyl-benzothiazoline-6-sulfonate (ABTS) radical cation decolorization assay, ferric reducing antioxidant power (FRAP) and copper reduction antioxidant assays (FRAP). The highest antioxidant activity values were measured by the ABTS assay, among all performed methods.

P76

Enzymatic Hydrolysis of Polysaccharides from leaves of Plantago major L.

Lukova P.[1], Karcheva D.[1], Nikolova M.[2]*, Bivolarski V.[2], Mladenov R.[1], Iliev I.[2]*

[1]Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 120 Bratja Bukcston Blvd.,
[2] Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski",Tzar Assen" str. 24, Plovdiv, Bulgaria
**Corresponding authors: paolina.lukova@yahoo.com, mariana.nikolova@mail.bg*

Plantago major L. leaves are used for centuries in the traditional medicine for treating infectious diseases related to the respiratory, urinary and digestive tracts. Some researchers reported that hot water extracts of *Plantago major* possess a broad-spectrum of anticancer, antiviral and atioxidant activities, as well as activities which modulate cell-mediated immunity. Their beneficial properties may be due to the significant content of polysaccharides. The polysaccharides that have been isolated from the leaves of *Plantago major* L. have different structure – pectic substances, arabinogalactan, galactan, glucomannan.

This study describes the polysaccharides isolated from the leaves of *Plantago major* L. by water and acid extraction and their subsequent enzymatic hydrolysis. The hydrolysis reactions are performed with two enzymes - hemicellulase and mannanase. Products of the enzymatic hydrolysis are different monosaccharides, uronic acids and oligosaccharides with different degree of polymerization (DP). DP of the oligosaccharides and the type of monosaccharides were determined using HPLC method. Hydrolysis kinetics of the isolated polysaccharides from *Plantago major* L. leaves was studied.

Acknowledgements. The authors acknowledge the financial support of Medical University of Plovdiv for this study performed in connection with project CDP-03/2015.

P77

Comparative analysis of the inhibiting enzyme activity of bilberries (Vaccinium myrtillus) extracts

Karcheva D.[1], Lukova P.[1], Nikolova M.[2]*, Mladenov R.[1], Iliev I.[2]*

[1]Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 120 Bratja Bukcston Blvd.,
[2] Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski",Tzar Assen" str. 24, Plovdiv, Bulgaria
**Corresponding authors: diana395@abv.bg, mariana.nikolova@mail.bg,*

This study explores the impact of polyphenols extracted from fruits of the *Vaccinium myrtillus* type on enzymes α-amilase, α-glucosidase, β-glucosidase and amyloglucosidase. The tested extracts of berries of the type *Vaccinium myrtillus* originate from the following Bulgarian regions: Stara Planina (Beklemeto pass), Rila (Ibar area), Rhodopes (areas Yundola and Cigov Chark).

From the berries, two types of extractions were derived and concentrated – acetone and aqueous – and their quantitative contents of total polyphenols has been determined. The minimum inhibiting concentration of the polyphenol composition was defined in the tested extracts concerning enzymes from carbohydrate exchange which are material for diabetes mellitus type 2 (α-amilase, α-glucosidase, β-glucosidase and amyloglucosidase).

Acknowledgements. The authors acknowledge the financial support of Medical University of Plovdiv for this study performed in connection with project CDP-05/2015.

P78

DIETARY FIBERS – Definitions, Classifications and Analytical methods for the physiological assessment of the content in foods

Gyurova D., Enikova R.*

National Center of Public Health and Analyses, Acad. Ivan Evst. Geshov 15 blvd, Sofia, Bulgaria, tel. +3592/8056258,
**Corresponding authors: d.k.gyurova@gmail.com*

The aim of this study is to present the most contemporary interpretation of the term “dietary fiber” as an ingredient of carbohydrates and its importance in human nutrition.

The authors emphasize the “evolution” of the definition for dietary fibers in the years, as well as different approaches in the methods for their determination.

The knowledge of the composition of foods rich of dietary fibers and oligosaccharides; and the harmonization of analytical methods together are a prerequisite for their correct identification and determination in the process of completion of the database for chemical composition of Bulgarian foods as well as in exchange of analytical data at regional and international level.

P79

Conributions to the Bulgarian tables for food chemical composition

Gyurova D.

National Center of Public Health and Analyses, Acad. Ivan Evst. Geshov 15 blvd, Sofia, Bulgaria, tel. +3592/8056258, d.k.gyurova@gmail.com

Standardised approach to describe and document food composition data is needed. The traditional tables for chemical composition of Bulgarian foods can no longer fully meet the information needs of physicians, nutritionists, chemists, food technologists, biologists, and specialists in agricultural sciences; especially the free movement of foodstuffs and their diversity becomes overwhelming. National Center of Public Health and Analyses is responsible for Bulgarian food composition tables and is a general contractor as undertaken in EuroFIR Project. The good collaboration with leadership of the EuroFIR Project contributed to the launching of a new modern Bulgarian food composition database.

P80

Bioactive Compounds Isolated from Garden Snails

Dolashka P.[1], Dolashki A.[1], Velkova L.[1], Stevanovic S.[2], Molin L.[3], Traldi P.[3], Beeumen J.[4], Devreese B.[4], Voelter W.[5]*

[1] Institute of Organic Chemistry, Bulgarian Academy of Sciences, G. Bonchev 9, Sofia 1113, Bulgaria;
[2] Institute for Cell Biology, Department of Immunology, University of Tübingen, Auf der Morgenstelle 15, D-72076 Tvöbingen, Germany;
[3] R-ISTM, Corso Stati Uniti 4, 35129 Padova, Italy;
[4] aboratory of Protein Biochemistry and Protein Engineering, Ghent University, KL Ledeganckstraat 35, 9000 Ghent, Belgium;
[5] nterfacultary Institute of Biochemistry, University of Tvöbingen, Hoppe-Seyler-Strasse 4, D-72076 Tvöbingen, Germany
**Corresponding author: pda54@abv.bg*

Abstract. The recent appearance of a growing number of resistant to conventional antibiotics, has become a serious medical problem. To overcome this resistance, the development of new compounds is encouraged. Hemolymph and mucus of *Helix lucorum* and *Helix aspersa* garden snails are a complex mixture of biochemically and pharmacologically active components.

Glycoprotein ‘hemocyanin’ and antimicrobial peptides from the hemolymph and mucus are important components of the innate immunity. Some isoforms and peptides serve as effector molecules of the defense system, providing an efficient initial effect against infectious pathogens.

The in vitro antitumor activity of Helix hemocyanin and its isoforms with different oligosaccharide structures was established on the bladder carcinoma permanent cell lines Cal-29, T-24. This is probably due to the specific oligosaccharide structures of hemocyanins which are exposed on the surface of the molecule.

Секция: Генетика и селекция

P81

Establishment of the Main Elements Variation of Productivity on Variety Potok

Bonchev B.[1], Valcheva D.[2], Mokreva T.[3]

[1]*Institute of Plant Genetic Resources, “Konstantin Malkov”, Druzhba 2, 4122 Sadovo, Bulgaria*
[2]*Institute of Agriculture Karnobat, Industrialna 1, 8400 Karnobat, Bulgaria*
[3] *Agricultural University, Mendeleev 2, 4000 Plovdiv, Bulgaria*

Abstract. Purpose of the study was to establish the variation of the basic elements of productivity in variety Potok and broadcast morphological markers to identify variety.

Use two-factor dispersion analysis and descriptive statistics of ANOVA program SPSS 19 for analysis of variance, variation coefficient also an indicator of accuracy. It was found that the density of the spike as a complex tag incorporating the length of the spike and number of grains in the spike varied over the years of the study, but within a year, manifests itself as a stable trait. The density of the spike can be used as an additional marker for identification of the variety with another generally accepted marker - the length of the spike. From the analysis of variance was established other marker, that characterize genotype variety Potok, and was found the number of productive spikes per plant.

P82

Study on Variability, Heritability, Genetic Advance and Associations Among Characters in Emmer Wheat Genotypes (Triticum dicoccon Schrank)

Kyosev B., Desheva G.

Institute of Plant Genetic Resources, “Konstantin Malkov”, Druzhba 2, 4122 Sadovo, Bulgaria

Abstract. Thirty eight emmer wheat genotypes grown in IPGR-Sadovo, Bulgaria during 2012-2014 were evaluated to estimates of variability, heritability and genetic advance in the randomized block design in three replications. Eight agronomic traits were included in the study. The phenotypic coefficient of variation (PCV) were higher than genotypic coefficient of variation (GCV) for all the traits. High PCV and GCV were observed in trait grain weight per spike (PCV=30.36%, GCV=24.93%). High genetic advance combined with high heritability showed characters: spike length, grain weight per spike and thousand grain weight. The highly significant and positive phenotypic correlation was found between grain yield per plant and following components: plant height, grain weight per spike, number of grains per spike and thousand grain weight. The grain weight per spike and plant height had strongest direct effect on grain yield per plant. The number of grains per spike via grain weight per spike and thousand grains weight via grain weight per spike had the highest positive indirect effect on the grain yield per plant.

P83

Title Study of Ecotypes and Varieties of Species Festuca pratensis Huds. and Festuca arundinacea Schreber.

Stamatova M.

Institute of Plant Genetic Resources, “Konstantin Malkov”, Druzhba 2, 4122 Sadovo, Bulgaria

Abstract. The aim of this study is the genetic basis broadening in the collection of forage grasses with original germplasm – natural populations of Festuca pratensis and Festuca arundinacea. This genetic diversity should be preserved both by ex situ and in situ methods and further used for breeding improvement work on the basis of variability evaluation of economic traits. In the field collection is determined the variation of each ecotype using 11 bio-morphological traits by evaluation of 30 individual plants per ecotype. In comparison with control varieties are proved different classes of variation both between individual ecotypes and inter-population variation. The most significant variation is detected by traits: habitus, inter-stage periods incl. early ear emergence, number of culms per plant, plant high. Significant statistical correlations between the most important couples of morphological and forage quality characters were established to develop forage and turf varieties models. The results of present study are the basis for consideration the natural meadows and pastures as an important source of genetic diversity in the breeding improvement of forage grasses. Traditional maintenance of natural grassland habitats is a promising strategy for protection of valuable forage grass diversity.

P84

Elements Relevant to Productivity and Quality of Introduced Rice Varieties

Tosheva S., Sabeva M.

Institute of Plant Genetic Resources, “Konstantin Malkov”, Druzhba 2, 4122 Sadovo, Bulgaria

Abstract. Turkish and Italian rice varieties, adopted in the production in Bulgaria are studied. It was made a comparative characteristic by significant characters for rice producers: vegetation period, paddy yield, panicle length, panicle weight, total grain numbers per panicle, weight of 1000 grains. Raw protein and lysine content in the grain was determined. The yield and its structural elements vary in a greater degree in the studied varieties compared to the qualitative characters. It was established statistically proven positive correlation relationships between the total grain number in the panicle and the paddy yield, panicle weight, number of empty grains and there is a negative relationship with the raw protein content. Positive relationships also exist between the lysine content in the grain and raw protein content, between lysine content in the protein and biological value of the protein as well as between lysine in protein and the biological value. Varieties which might be included in the breeding programmes as sources of valuable economical characters were determined.

P85

Genetic Characterization of Selectively Controlled for Swarm Production Apis mellifera Macedonica (Type Rodopica) Populations in Bulgaria

Georgieva V.[1], Petrov P.[2], Petkov N.[3], Ivanova E.[1]

[1]Plovdiv University“Paisii Hilendarski”, Biological Faculty, Department of Developmental Biology, Section of Genetics
[2]Agrarian University – Plovdiv
[3]National Bee Breeding Association

Abstract. The genetic variability in selectively controlled in Bulgaria local honey bee populations, representing *Apis mellifera macedonica* subspecies (type rodopica), has been studied by usage of alloenzymic analysis of six enzymic systems (MDH-1, ME, EST-3, ALP, PGM and HK) corresponding to 6 loci. Totally 324 worker bee individuals from 9 different local populations belonging to selective bases of National Bee Breeding Association for swarm production were included in this investigation. All of the studied loci were found to be polymorphic in most of the populations with the exception of EST-3 locus which was established to be fixed in two of investigated populations. Polymorphism with three alleles was ascertained for MDH, ME, ALP and PGM loci and with four alleles – for EST-3 and HK loci. The most common alleles in all of the investigated populations were ME 100, EST-3 100, PGM 100 and HK 100. Two private alleles (frequency < 0.05) were found for two of the studied populations. The calculated level of polymorphism was between 88.33% and 100%. The observed and expected heterozygosities were found to range from 0.186 to 0.301, and from 0.205 to 0.305, respectively. The calculated mean Fst level was 0.028. Allele frequencies of all studied loci were used to estimate Nei’s (1972) genetic

distance, which was established to range between 0.001 and 0.028 among the selectively controlled populations studied.

P86

"Mutated" Genofound for Some Common Diseases , Persistent Multivalency with Its Clinical Manifestations

Dincheva R.

Day center for children with disabilities “St. Anna” Plovdiv

Abstract. Mutations in genes affecting generational change except in the substrate itself of the gene, but also in its clinical expression. Diverse manifestation of clinical symptoms brings us into a new unknown world, into a new kind of manifest generation is not uniform, not homogeneous and which the world has yet to start research. The correct genetic diagnosis of a child with a genetic problem in development the first step in therapeutics work.

Секция: Медицинска биология

P87

Classification of Side Effects of Anabolic Steroid Abuse

Ivanova St.[1], Ivanov K.[1], Ivanova St.[1], Papanov St.[1], Petkova Ek.[2]

[1]Medical University Plovdiv, Faculty of Pharmacy, Department Pharmacognosy and Pharmaceutical Chemistry, Plovdiv, Bulgaria
[2]Medical University Plovdiv, Medical College , Plovdiv, Bulgaria

Abstract. Sociocultural standards of beauty for males emphasize strength and muscularity. Many professional athletes and amateurs use anabolic-androgenic steroids (AAS) to obtain a well-trained, athletic, and healthy looking body. The health consequences of steroid abuse are very serious. This abuse is observed not only in men but also for women athletes because bodybuilding and power sports became also and a female sport. Many female bodybuilders and other professional athletes began to use steroids. Worrying is that there are fashion trend to use steroids among people who engage in sports. Very often these people have only a crude knowledge about the pharmacological databases and the serious side effects regarding these drugs. In this paper we have made a detailed classification of the side effects of AAS and we have summarized the long term consequences of this problem. We have divided the AAS side effects in seven big groups: cardiovascular, neuropsychiatric side effects, endocrine, infertility, hepatotoxicity, feminization and masculanization. Very often some of these side effects are irreversible. The proportion benefit – risk shows that any kind of abuse with AAS drugs is extremely dangerous. We have made a comprehensive review of clinical trials and literature data on this issue. Steroid abuse disrupts the normal production of hormones in the body, causing both reversible and irreversible changes. Changes that can be reversed include reduced sperm production and shrinking of the testicles

(testicular atrophy). Irreversible changes include male-pattern baldness and breast development (gynecomastia) in men, masculinization in women.

Abbreviation: anabolic-androgenic steroids (AAS)

P88

Attenuation of Cellular Oxidative Stress by Natural Products and Plant Extracts after Chemotherapeutic Exposure

Sainova I., Pavlova V., Alexieva B., Valkova I., Ilieva I., Nikolova E.

Institute of Experimental Morphology, Pathology and Anthropology with Museum to Bulgarian Academy of Sciences – “Acad. G. Bonchev” Street 1113 Sofia, Bulgaria

Abstract. Some natural foods are proved to be useful in prevention of the side effects, caused by the chemotherapeutic agents, both in vitro and in vivo. Many of the compounds, containing in them, are also approved as potent anti-malignacy agents. As one of the main mechanisms of the action of anti-oxidant molecules is by protection against chemically-induces oxidative stress, by cascade mechanisms, involving modification of autophagy and apoptosis. The role of regulatory pathways, underlining the differentiation of immune cells and immune system, is proved. In this way, the combination treatment with both chemotherapeutic agent plus natural product(s), is proposed to be able to allow a stronger toxicity to malignant cells, rather than to reduce the side drug effects. Among the main molecules in these processes, is established the reduced for of tri-peptide Glutathione (GSH), Glutathione-transferase (GST), Superoxide dismutase (SOD) and beta-galactosidase. The mechanisms of cell protection probably involve the reduction of intra-cellular oxidative stress, maintaining GSH availability, but also increased expression and activity of GST enzyme.

P89

Serological Parvovirus B19 Screening of Patients with Rheumatoid Arthritis and Ankylosing Spondylitis

Ivanova S.[1], Golkocheva-Markova E.[2], Ivanova M.[3], Gekova I.[3], Stoilov N.[3], Stoilov R.[3], Teoharov P.[2]

[1]NRL of Measles, Mumps and Rubella, Virology Department, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
[2]NRL Viral Hepatitis, Virology Department, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
[3]Clinic of Rheumatology, University Hospital for Active Treatment “St. Ivan Rilski, Sofia, Bulgaria

Abstract. Parvovirus B19 is single-stranded DNA virus and can cause general flu-like symptoms or asymptomatic infection with rash and, more commonly in adults, arthritis, especially in the fingers and hands but also in the wrists and ankles. The present study aimed to investigate the involvement of parvovirus B19 in the development of rheumatoid arthritis and ankylosing spondylitis by demonstrating the presence of specific antiviral antibodies in patient’s serum samples. Serological methods (indirect ELISA test and Immunoblot test) to demonstrate the specific parvovirus B19 IgM/IgG antibodies were used. A total 36 serum samples, collected in 2015 from patients with rheumatoid arthritis (n=24) and ankylosing spondylitis (n=12) were tested. The average age of patients studied was 50 ± 13.2 years. Positive results for B19-IgM antibodies which are marker for acute B19 infection in 2/36 (5.55%) serum samples were detected. These two sera were from patients with diagnosis rheumatoid arthritis. Protective B19-IgG antibodies evidence of past infection in 17/36 (47.22%) patients were found. Thirteen of them were diagnosed with rheumatoid arthritis and four with ankylosing spondylitis. Two of the tested serum samples from patients with rheumatoid arthritis

were positive for both investigated B19 markers. Rheumatoid diseases are one of the oldest problems of mankind, which is widespread today. This study is the basis for future more detailed tests aimed enrichment of diagnostic range on these diseases and inclusion of parvovirus B19 assays.

P90

Prevalence of Markers for Hepatitis B Virus Infection Among Patients with Ankylosing Spondylitis and Rheumatoid Arthritis (Prevalence of Hepatitis B Virus Among Patients with AS and RA)

Golkocheva-Markova E.[1], Ivanova St.[2], Ivanova M.[3], Gekova I.[3], Stoilov N.[3], Kostadinova A.[4], Alexiev I.[4], Stoilov R.[3], Teoharov P.[1]

1NRL Viral Hepatitis, Virology Department, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
2NRL of Measles, Mumps and Rubella, Virology Department, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
3Clinic of Rheumatology, University Hospital for Active Treatment “St. Ivan Rilski, Sofia, Bulgaria
4NRL of HIV, Virology Department, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

Abstract. Bulgaria is a country with the intermediate endemicity for hepatitis B virus (HBV) with predominance in adults between 40 and 49 years. Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are musculoskeletal conditions that cause severe long term pain and disability which prevalence increased with ageing. Reactivation of HBV is not rare event in patients receiving immunosuppressive therapy, but the prevalence of HBV markers among such patients is still unclear. The aim of the present work is to study distribution of two main HBV markers – HBV surface antigen (HbsAg), as marker for the present infection, and antibodies against HBV core antigen (anti-HBc), as a marker for past infection, among patients with AS and RA. The screening for HBV markers was done by commercial enzyme-linked assays for detection of HBsAg and anti-HBc in blood samples, for 14 sera samples concentration of HBV DNA were measured by quantitative real-time

PCR. The study included 23 patients with AS and 24 – with RA. Two (8%) of the patients with AS were positive for HBsAg and 9 (39%) - were anti-HBc positive. From patients with RA 2 (8%) were HBsAg positive and 7 (29%) – were anti-HBc positive. The presence of low HBV DNA concentration (between 182 copies/ml and <116 copies/ml) were established in 2 patients with AS. In conclusion the prevalence of markers for past HBV infection was high among patients with AS and RA, which is reason for more strict screening of patients before immunosuppressive therapy.

P91

Omega-3 Fatty Acids and Alzheimer's Diseases-Benefits from Placebo-controlles Clinical Trials

Hadzhieva B.[1], Ivanova St.[2], Obreshkova D.[3]

[1]Medical University – Plovdiv, Bulgaria, Medical college, Department Assistant Pharmacist,
[2]Medical University – Plovdiv, Bulgaria, Faculty of Pharmacy, Department of Pharmakognosy and Pharmaceutical Chemistry,
[3]Medical University – Sofia, Bulgaria, Faculty of Pharmacy, Department of Pharmaceutical Chemistry
**Corresponding author: yara@abv.bg*

Abstract. Some clinical trials of prophylactic and therapeutic effects of ω-3 (eicosapentaenoic acid and docosahexaenoic acid) and ω-6 fatty acid have been conducted. Their mechanism of action is uncertain, although there are several effects of their taking - reducing effect on serum triglycerides, decreasing risk of arrhythmias, anti-atherogenic and antithrombotic effects, inhibiting inflammation and improving the condition in autoimmune disorders, including rheumatoid arthritis, prostatitis, ulcerative colitis. Omega-6 and omega-3 fatty acids are structural components of neuronal membranes and influence the cellular function and therefore appear as a pool for lipid-derived messengers. Taking supplements containing omega fatty acids protects against cell inflammation in the brain, which would have positive results for people suffering from Alzheimer's disease. We have screened 50 studies and 83 published sources and found 5 studies with published results which pertain to our survey. Supplementation of eicosapentaenoic acid and docosahexaenoic acid in patients with Alzheimer’s desease can compasate previously found deficiency. The evidence from double-blind, placebo-controlled clinical trials has shown benefits of supplementation with omega-3 PUFAs only for patients with very mild cognitive impairment.

P92

Study on Aminopeptidase A Activity in Normal Mouse Mammary Gland and in Murine In Vivo Model of Ehrlich carcinoma Solid Form

Petrova V.[1], Pavlova V.[1], Iliev I.[1], Ivanov I.[2], Tasheva D.[3], Dimitrova M.[1]

[1]IEMPAM-BAS, “Akad. G. Bonchev” Str., block 25, 1113 Sofia, Bulgaria
[2]Department of Medical Chemistry and Biochemistry, Medical University of Sofia, 2 Zdrave Str., 1431 Sofia, Bulgaria
[3]Univesity of Sofia, Faculty of Chemistry and Pharmacy, 1 J. Bourchier Blvd., 1164 Sofia, Bulgaria

Abstract. Aminopeptidase A (APA) is a membrane-bound zinc metallopeptidase that has a widespread tissue distribution and is involved in many diverse biological processes. Apart from its role in renin-angiotensin system recently APA has been linked to different tumor disorders like breast cancer and other types of malignancies. The alterations of the enzyme activity levels have been proposed as an auxiliary diagnostic/prognostic marker for breast carcinoma in human. In the present work we study APA activity in normal mouse mammary gland and in an in vivo mouse model of Ehrlich carcinoma (solid form). The enzyme activity levels were evaluated biochemically by using the standard p-nitroaniline based substrate. APA distribution in cryosections of normal mouse mammary gland and in Ehrlich carcinoma was determined histochemically by applying a fluorogenic enzyme substrate recently synthesized by us. We showed that APA activity was high in the normal mouse mammary glands whereas in murine solid Erlich carcinoma the enzyme activity proved to be considerably lower. These results support the proposed diagnostic value of the enzyme for mammary gland carcinoma and are in favour of the application of Ehrlich carcinoma model in human breast cancer research.

P93

Serum NAD(P)H Fluorescence vs. Serum Proteome Calorimetry for IgM Multiple Myeloma Discrimination

Danailova A.[1], Dzhonova D.[2], Todinova S.[1], Gartcheva L.[3], Taneva S.[1], KrumovaS. [1]*

[1]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, 1113 Sofia, Bulgaria
[2]Department of Clinical Research, Medical Faculty, Bern University, Murtenstrasse 50, 3008 Bern, Switzerland
[3]National Specialized Hospital for Active Treating of Haematological Diseases, Sofia 1756, Bulgaria

Abstract. Multiple myeloma (MM) is hematological disease with increasing incidence. Due to the development of novel tools for diagnosis and monitoring of the effect of treatment there is a significant improvement in patients’ survival but there is still no cure and the nature of the disease remains elusive. Our previous works have demonstrated the potential of differential scanning calorimetry (DSC) to classify the different MM types into specific calorimetric groups and to distinguish them from healthy controls. Here we elaborate on another biophysical technique based on the fluorescent properties of NAD(P)H claimed to be useful for metabolism dysfunction detection in cancer sera. A group of patients diagnosed with MM with secretion of immunoglobulin M (IgM) paraprotein that are well characterized on basic clinical criteria was studied. Our data reveal that about half of the studied population could be distinguished on the basis of NAD(P)H fluorescence and demonstrate that the ability of NAD(P)H fluorescence approach to detect the malignant state is far lower than that of DSC and therefore cannot be recommended as a disease marker, at least for the case of IgM MM.

P94

The Effect of ACE-inhibitor Therapy on Serum Level of Angiotensin II

Hristova M. [1], Penev M.[1], Miteva L.[2]*

[1]*Department of Internal Medicine, Medical Faculty, Trakia University, Stara Zagora, Bulgaria*
[2]*Department of Molecular biology, immunology and medical genetics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria*

Abstract. Introduction: The central role of angiotensin II in the pathogenesis of cardiovascular diseases has necessitated the development of pharmacological agents which target the renin-angiotensin-aldosterone system. Angiotensin-converting enzyme (ACE) inhibitors block the conversion of angiotensin I into angiotensin II and have renoprotective and cardioprotective effects in addition to their antihypertensive activity. Blood pressure reduction is achieved with monotherapy with an ACE inhibitor in 35-70% of hypertensive patients.An estimated 20–40% of patients do not respond to and/or tolerate ACE inhibitors. The purpose of this study was to investigate the resistance to ACE-inhibitor therapy is caused by ACE-independent generation of angiotensin II

Materials and Methods: Serum levels of angiotensin II (Ang II) were studied in 30 patients with stage 1 essential arterial hypertension (AH) and 10 healthy controls by commercially available enzyme-linked immunosorbent assay. Hypertensive patients were being treated with lisinopril and only 63.3% were with controlled hypertension.

Results: Serum levels of angiotensin II were significantly lower in the patients than in the healthy controls (18.7±1.86 pg/ml vs. 20.6±244 pg/ml; p=0.013). Besides, there was no statistically significant difference between the group with good blood pressure control and with uncontrolled hypertension (18.87±1.89 pg/ml vs. 18.4 ±1.86 pg/ml; p=0.53). Previously, we examined the serum chymase levels in the study

group and found a significantly elevated in the AH patients compared to the healthy controls and similar quantity of serum chymase in controlled and uncontrolled hypertension. In our current study, a strong positive relationship between chymase and Ang II levels in uncontrolled hypertension (r=0.6518; p=0.03) was observed, in contrast to patients with good blood pressure control (r=0.1113; p=0.65). Conclusion: Our study demonstrates a decrease of angiotensin II serum level in patients with stage 1 essential arterial hypertension treated with an ACE-inhibitor. In addition, a strong positive correlation between chymase and angiotensin II levels in patients with uncontrolled hypertension suggests a role of chymase in the formation of Ang II in case of resistance to ACE-inhibitor therapy.

P95

Effect of the Antitumor Agent Miltefosine on the Thermodynamic Behaviour of MCF-7 and MDA-MB-231 Breast Cancer Cell Lines

Pehlivanova V., Todinova S., Nikolova B., Tsoneva I., Krumova S., Taneva S.

Institute of Biophysics and Biomedical Engineering, BAS, Sofia

Abstract. Miltefosine (hexadecylphosphocholine) is a synthetic alkilphospholipid (ALP) that exhibits substantial antiproliferative activity and is used predominantly for treatment of skin metastases and breast cancer. Unlike conventional anticancer agents which act predominantly at the DNA level, ALPs act on the cellular membrane, because of their similarity to the endogenous phospholipids. At low doses, they act on the phospholipid turnover; cell signaling and survival pathways. Here we utilize differential scanning calorimetry (DSC) to obtain new information about the cell-drug interactions from a thermodynamic point of view. The calorimetric properties of two breast cancer cell lines – MCF-7 and MDA-MB-231 that possess low and high metastatic potential, respectively are compared. Our DSC data reveal that MDA-MB-231 cells are far more susceptible to the drug as compared to MCF-7 cell line, in correspondence with the viability test. The reduction and shifting of the thermal transitions in the low-temperature range of the thermogram of MDA-MB-231 cells suggest cytoskeleton reorganization upon the drug application. The results substantiate the DSC approach as a fast and low cost assay for cell status monitoring and contribute towards elucidating the mechanisms of miltefosine interaction with different cell components.

P96

Morphological and Tripeptidyl Peptidase I Activity Changes in the Rat Cerebral Cortex and Thalamus After Acute Hypoxia

Petrova E.[1], Ivanov I.[2], Pavlova V.[1], Dimitrova S.[1], Kadiysky D.[1], Dimitrova M.[1]

[1]Department of Experimental Morphology, Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences (IEMPAM – BAS)
[2]Faculty of Biology, Sofia University “St. Kl. Ohridsky”

Abstract. Hypoxia is one of the major pathological events that may cause substantial brain damage. The hippocampus, cerebellum and striatum are known to be the most vulnerable to hypoxic stress. The insufficient oxygen supply generates a series of biochemical events with cellular and functional consequences. Tripeptidyl peptidase I (TPPI) activity is crucial for the neuronal functions. Recent studies in fish suggest a possible involvement of TPPI in response to hypoxia. Similar studies are not performed in mammals. In this work, the effect of sodium nitrite-induced acute hypoxic shock on the rat brain morphology and TPPI activity in the cerebral cortex and thalamus was studied at different post-treatment periods. Morphological changes were assessed in tissue sections using silver-copper impregnation for neurodegeneration. TPPI activity was localized by enzyme histochemistry. Morphological signs of tissue damage were observed in both studied brain areas. Neuronal shrinkage and enlargement of pericellular spaces became visible as early as an hour post-treatment. At later stages, blood vessels packed with erythrocytes (hyperemia) were observed. Dilatation of the smallest capillaries and impairment of neuronal processes were also demonstrated in the cortex and thalamus. The enzyme histochemistry revealed a temporary TPPI deficiency in all the neuronal types. The

enzyme activity was decreased from the 1st hour post-treatment in both studied brain areas. The TPPI deficiency was more dramatic in cortical neurons of cerebral cortex where the enzyme activity remained low up to the 5th day following administration. These findings indicate a possible involvement of the enzyme in the tissue response to the hypoxic shock.

P97

An Exploration of the Association between BMI and Number of Normal Motile Sperm with Putative Fertility Potential

Mateev B., Mateeva M.

BORA Medical Centre for Assisted Reproduction

Abstract. Overweight and obesity is still a serious problem, especially in industrialized countries. This retrospective study was designed and created to explore the association between body mass index (BMI) and male fertility in general, comparing the number of motile sperm-according to WHO lower reference value for sperm motility, as reference marker for achieving a successful pregnancy. Semen samples were obtained from 198 male partners of couples undergoing fertility investigations in BORA medical centre in Plovdiv. BMI assessing and the semen analysis of the probes was performed by one person, well trained according to the WHO criteria and guideline for examination of human semen, with the aim to avoid on this way sampling-criteria and methodological errors. Men were classified into groups according to their BMI: A 18.5–24.99 Normal range (n=47); B 25–29.99 Overweight/Pr-obese/(n=92); C 30 -34,99 Obese class I (n=44); D ≥35,0 kg/m^2 Obese class II and III(n=15). After analyzing and evaluation the data collected, we arrived at the conclusion that there is significant percentage change (indicating negative aspect) in the ratio between fertile and infertile men with increasing the BMI values in the groups of/with: normal range, overweight and obese.

P98

Anti-C1q autoantibodies against the globular domain of the C1qB-chain correlated with Lupus nephritis activity

Radanova M.[1], Nikolova S.[1], Vasilev V.[2], Ikonomov V.[1], Kishore U[3], Ivanova D.[1]*

[1]*Medical University - Varna, Varna, Bulgaria*
[2]*Medical University - Sofia, Sofia, Bulgaria*
[3]*Brunel University, Uxbridge Campus, London, UK*

Abstract. Introduction: Lupus nephritis (LN) is one of the most severe manifestations of systemic lupus erythematosus (SLE). Presence of anti-C1q autoantibodies correlates with active LN and is associated with proliferative lupus nephritis, specifically class IV. These autoantibodies bind to neo-epitopes within the collagen region of C1q. Recently we have found that anti-C1q autoantibodies from LN sera, recognizing epitopes within the globular domain (gC1q) of C1q (anti-gC1q antibodies). We established that anti-gC1q antibodies against B-chain inhibited the interactions of C1q with IgG and CRP. In the present study, our aim was to investigate the relationship between serum anti-ghB C1q antibodies levels and some renal pathological characteristics in LN as well as to evaluate whether these antibodies have a potential to be measured as a non-invasive marker for assessment of disease activity. Materials and methods: Anti-C1q autoantibodies, complement proteins - C1q, C4 and C3, IgG and IgM CICs levels were screened by ELISA in 46 LN patients. Complement hemolytic activity was detected by CH50 test. The presence of ANA and anti-ds DNA antibodies were detected by double immunodiffusion assays and indirect immunofluorescence. Sera from 196 normal donors served as controls. Results: 30.43% (n=14) of patients were seropositive for anti-C1q autoantibodies and seven of them were seropositive for anti-ghB C1q autoantibodies. Correlations between levels of C1q, C4, and C3 as markers for complement activity and anti-C1q or anti-ghB C1q autoantibodies were not significant. We found

significant correlations between IgG CICs and anti-C1q antibodies (r=0.586; p=0.001) as well as between IgG CICs and anti-ghB C1q antibodies (r=0.323; p=0.048). Presence of anti-C1q or anti-ghB C1q antibodies significantly correlated with activity of LN as determined by SLEDAI (r=0.425; p=0.008 and r=0.372; p=0.048). The both anti-C1q and anti-ghB C1q antibodies significantly correlated with the severity of nephritis, which manifested as proteinuria g/24 h (r=0.454 p=0.007 and r =0.371 p=0.031) and low serum albumin (r=-0.377, p=0.031 and r=-0.401, p=0.021). Anti-C1q but not anti-ghB C1q antibodies correlated with anti-ds DNA antibodies (r=0.482; p=0.043). Conclusion: Anti-ghB C1q have properties to be potential useful marker in making predictions of renal histopathology in LN and assessment of disease activity.

P99

Antibodies Recognizing the Globular Domain of C1q - View on Association between Lupus Nephritis Manifestation and Anti-gC1q Autoantibodies

Radanova M.[1], Vasilev V.[2], Kishore U.[3], Ikonomov V.[1], Ivanova D.[1]*

[1] *Medical University - Varna, Varna, Bulgaria*
[2] *Medical University - Sofia, Sofia, Bulgaria*
[3] *Brunel University, Uxbridge Campus, London, UK*

Abstract. Lupus nephritis is a serious complication of the systemic lupus erythematosus (SLE). Anti-C1q antibodies correlate with the occurrence and high clinical activity of lupus nephritis, especially proliferative lupus nephritis. The anti-C1q autoantibodies bind epitopes within collagen region of C1q (CLR). Recently we have found that autoantibodies against C1q are also directed against the gC1q domain (gC1q antibodies) in lupus nephritis patients. We also found that anti-B-gC1q autoantibodies, markedly inhibited C1q interaction with IgG as well as CRP. In the present study, our aim was to evaluate the potential pathological consequences of presence of anti-gC1q antibodies in lupus nephritis. The recombinant globular head region of the three chains of C1q -A, -B and -C were expressed in E. coli BL21 and purified. Anti-C1q, anti-gC1q autoantibodies, complement proteins - C1q, C4, C3 and IgG-, IgM-CICs levels were screened by ELISA in 53 LN patients. The presence of ANA and anti-ds DNA antibodies were detected by double immunodiffusion assays and indirect immunofluorescence. Sera from 196 normal donors served as controls. All lupus nephritis patients were divided in four groups – positive for anti-C1q antibodies but not for anti-gC1q antibodies (12); positive for anti-B-gC1q antibodies (8); positive for anti-A and/or anti-C-gC1q antibodies (10) and negative for anti-C1q antibodies (23). We compared serum C1q, C4 and C3 levels in these groups. We found that patients positive for anti-B-gC1q antibodies presented with significantly lower serum C4 levels than patients positive

for anti-A and anti-C-gC1q antibodies (120.00 μg/ml vs. 161.00 μg/ml, p=0.014) and with significantly lower levels of C3 than patients positive for anti-A and anti-C-gC1q antibodies and patients without anti-C1q antibodies (634 μg/ml vs. 1013 μg/ml, p=0.005; 875 μg/ml, p=0.018). The levels of C3 in patients seropositive for anti-B-gC1q antibodies were also lower than the C3 levels in positive for anti-C1q antibodies patients (634 μg/ml vs. 851 μg/ml, p=0.068). For anti-C1q and anti-B-gC1q antibodies there were significant correlations to IgG CICs (r=0.371, p=0.001 and r=0.431, p=0.003. The levels of IgM CICs decreased in accordance with the increase of only anti-C1q antibodies (r=- 0.238, p=0.051). Lower levels of C1q, C4 and C3 in the serum of the patients with anti-B-gC1q autoantibodies could be an indication for a possibility the binding of these anti-gC1q autoantibodies with C1q to trigger mechanical stress and a structural change within the CLR domain of C1q, compatible with C1r-C1s complement activation in fluid phase.

P100

Carbohydrate structure of the βc–HlH structural subunit of Helix lucorum hemocyanin

Velkova L.[1], Dolashka-Angelova P.[1], Dolashki A.[1], Devreese B.[2], Van Beeumen J.[2]

[1] *Institute of Organic Chemistry, Bulgarian Academy of Sciences, G. Bonchev 9, Sofia 1113, Bulgaria*
[2] *Laboratory of Protein Biochemistry and Biomolecular Engineering, Ghent University, 9000 Ghent, Belgium.*

Abstract. Hemocyanins (Hcs) are copper-containing respiratory glycoproteins with quaternary structure localized in the hemolymph of several arthropods and molluscs. Molluscan Hcs usually have are powerful immunogens, probably due to their high carbohydrate content and specific monosaccharide composition.
We analyzed the oligosaccharides and the carbohydrate linkage sites of the structural subunit βc-Helix lucorum hemocyanin (βc-HlH) isolated form hemolymph of garden snail Helix lucorum using tandem mass spectrometry.
Monosaccharide sequencing and determination of the configurations of N-glycans released from βc-HlH after PNGase F treatment was performed by Q-Trap mass spectrometry. The carbohydrate structures of βc-HlH contained various larger N-glycans with a number of methylated sugars, which could be identified as 3-O-methyl-mannose and 3-O-methylgalactose, in addition Fuc and/or Xyl residues were found in polysaccharides. In total 26 glycans, were identified as a highly heterogeneous mixture with compositions Hex3-7HexNAc2-5 MeHex0-4 Pent0-1Fuc 0-1. The primary structures of N-glycans from high mannose, complex- and hybrid type have been determined.

Acknowledgements. This work was supported by Bulgarian Ministry of Education, projects DHRC/01/6 and Youth and Science, DMU 03/26,

Bulgaria and co-financed by FWO - VS.025.12N, Belgium and DFG-STE 1819/5-1/2012 – Germany

P101

Mass Spectrometry Analysis of Immunoglobulin before and After Treatment with Iron Ions

Velikova R.[1], Dolashka-Angelova P.[1], Dolashki A.[1], Vasilev T.[2] Pashov A.[2]

[1] *Institute of Organic Chemistry, Bulgarian Academy of Sciences, G. Bonchev 9, Sofia 1113, Bulgaria*
[2] *The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, G. Bonchev 9, Sofia 1113, Bulgaria*

Abstract. The immune system is capable of producing a large repertoire of antibodies. The polyspecificity of a fraction of IgG antibodies can be increased in vitro by transient exposure to protein destabilizing agents (low or high pH, high salt concentration or chaotropic agents). We have compared the native and Fe2+, pH – treated IVIg by mass spectrometry. To discriminate between antigen-binding in antibodies with natural and induced polyspevificity, both have been treated with a dissociating agent and Fe2+ ions. After dissociation of the natural and induced polyspecificity antibodies in pH 3.0 buffer. Both fractions were analyses by MALDI-TOF-TOF. Several "blocking molecules"– peptides or oligosachharides were identified by their MS analyses. The results shows that treatment the intensity of few ions increase and some new ions are observed after dialysis of IVIg against buffer with pH 3. Same new ions are observed after treatment of IVIg with Fe2+ ions. We have identified prolin, serin, tryptophane and tyrosine containing peptides after treatment of IVIg with Fe2+ ions, they are binding peptides to IVIg and they are involved in the antibodies polyreactivity.

Acknowledgements. This work was supported by Fund Research, Bulgaria. These rezalts are part of the program of the progect: Antibodies with induced polyreactivity – role in immune homeostasis and therapeutic.

P102

Effect of Allopurinol on Oxidative Stress in Obesity and Liver Content of Free Fatty Acids

Bratoeva K., Radanova M., Merdzhanova A.

Medical University-Varna, Bulgaria

Abstract. Introduction: Oxidative stress appears as the key feature associated with dysfunction in adipose tissue and a major factor in the mechanisms of altered lipid metabolism in obesity. Cellular response of adipocytes in the conditions of oxidative stress results in maintaining systemic pro-inflammatory state, insulin resistance and increased accumulation of very long-chain saturated fatty acids (VLCSFAs) to the liver, which are lipotoxic and lead to further injury. Therefore, the therapeutic purposes of lowering the production of ROS, may have beneficial effects on obesity and its associated complications.

The aim of the study was to determine the influence of Allopurinol (xanthine oxidase inhibitors) on oxidative stress in adipose tissue and liver saturated fatty acids content in a model of fructose-induced obesity.

Materials and Methods: We used a model of high-fructose diet (HFD) in male rats Wistar (16 weeks, 35% glucose-fructose corn syrup), divided into three groups: control; HFD; HFD and Allopurinol administration (150 mg/kg in drinking water for 16 week). Analysis of fatty acid was performed by Gas Chromatograph with MS detector. Serum levels of TNF-a and glucose; weight, markers of oxidative stress- MDA (malondialdehyde), glutathione (GSH) and glutathione peroxidase (Gpx) in the retroperitoneal tissue were investigated.

Results: The results showed significantly elevated VLCSFAs, retroperitoneal tissue/body weight ratio, MDA, Gpx, TNF-a, glucose levels in serum and decreased levels of glutathione in HFD rats compared to the control group. In the group treated with Allopurinol the retroperitoneal tissue/ body weight ratio, the levels of MDA, Gpx, VLCSFAs, TNF-a and glucose levels in serum were significantly reduced while glutathione levels were elevated in comparison with HFD rats.

Conclusion: The inhibition of xanthine oxidase by Allopurinol prevents the development of oxidative and inflammatory changes in adipose tissue. This effect probably improves insulin sensitivity, reduce VLCSFAs levels and thereby prevent the further lipotoxic liver damage.

Студентска сесия

P103

Fossil Molluscs (Mollusca: Gastropoda, Bivalvia) from the Region of the City of Haskovo (Eastern Rhodopes)

Toteva H., Georgiev D., Petrova S.

University of Plovdiv “Paisii Hilendarski”, Faculty on Biology, 24 Tzar Assen Str., Plovdiv, Bulgaria

Abstract: Subject of this study are fossils of Eocene marine molluscs found in the vicinity of the city of Haskovo by a teacher on geography. He has collected material for many years, and then donated them to a local museum, and finally it was transferred to the Natural History Museum - Plovdiv. Fossils were kindly provided to the Department of Ecology and Environmental Conservation, University of Plovdiv, for identification, cataloging and describing and later they will be included in the permanent museum exhibition. Total number of 61 preserved and fragmented fossilized shells were studied. We identified 44 gastropods belonging to 18 species from 11 genera and 17 bivalves belonging to 6 genera.

P104

Eco-Trails as a Tool for Environmental Protection and Integration of Methodological Models for Environmental Education in the Natural Environment

Peltekova M., Petrova S, Nikolov B., Raynova G., Toteva H., Vaseva V., Petrova D., Yorgova A., Stoyanova M., Molla F., Todorov K., Karagyozova-Dilkova D.

Faculty on Biology, University of Plovdiv “Paisii Hilendarski”, 24 Tzar Assen Str., 4000, Plovdiv, Bulgaria

Abstract. Interest in ecotourism occurs due to a combination of increasing demand for authentic tourist experiences and increasing interest in environmental protection. For the successful development of ecotourism many mountain hiking trails have been created and supported, named eco-trails. Definition of "eco-trail" in Bulgarian literature is given by Petrov & Kisselkova (1999): "this is the path (route that can be traveled on foot) in the strict sense, which includes highly attractive resources and has a very strong presence of biotic component". Specific innovative elements in this proposal for the eco-trail construction are birdhouses, houses for hedgehogs, bird feeders and hotels for insects, their installation and maintenance periods. They will be prepared by the scholars and students using all natural materials that do not pollute the environment. Similar facilities are popular in many European countries, but in Bulgaria they are still not widespread.

These actions will provide opportunities for scholars and students:

-To fully interact with the natural environment;

- Carry out direct morphological and ethological observations on organisms, communities and habitats;

- To participate in events on recovery of vulnerable natural resources.

A&A Медикъл България

140

7 София, ул. „Голо бърдо” 8
Тел: 02/962-3982, 02/962-5707 Факс: 02/962-5386
e-mail: aamedicalbulgaria@gmail.com www.aamedical-bg.com

„АА Медикъл България” ООД, гр. София е организация с предмет на дейност – търговска дейност с диагностикуми и консумативи за клиничната и научноизследователската практика, медицинска и лабораторна апаратура нова употреба, с осигурено гаранционно и извънгаранционно обслужване - създадена през октомври 2006 година.

Нашата цел е да работим в здравната сфера за издигане на нивото на медицинското обслужване чрез предлагане ва висококачествени продукти на водещи световни фирми в областта на ин-витро диагностиката и фармацията.

Организацията работи по бизнес програма, в която се залагат инвестиции за увеличаване обема на услугите, постигане на нови параметри. Изпълняват се задачи по подобряване качеството на услугите с цел все по-пълно задоволяване на нормативните изисквания и удовлетворяване потребностите на клиентите. Качеството и постигнатите параметри на предлаганите от „АА Медикъл България” ООД услуги са познати и намират клиенти в цялата страна.

Стратегията на „АА Медикъл България” – ООД е съобразена с пазарните тенденции и икономическите условия в страната. Винаги е търсено оптимално съотношение между високо качество на предлаганите услуги и приемлива цена с цел най-пълно удовлетворяване на изискванията на клиентите и на нормативните изисквания.

Организацията има съобразена със съвременните изисквания организационно-функционална структура, която създава предпоставки за ефективност на системата за управление на качеството и реализиране на документираната политика и планираните цели.

“АА Медикъл България” ООД е разработила, документирала, внедрила, поддържа и непрекъснато подобрява система за управление на качеството /СУК/, съгласно изискванията на ISO 9001:2008, като основен елемент от общата управленческа стратегия на организацията с цел постигане удовлетвореност на клиентите и спазване на нормативните изисквания.

От началото на 2007 година, фирма „АА Медикъл България” ООД е официален представител на фирма Био-Рад за България, а от месец октомври 2007 година – и официален дистрибутор на продуктите на фирмата за ин-витро диагностика за хуманитарни и ветеринарни цели и свързаната с това апаратура.

„АА Медикъл България” ООД разполага с квалифициран търговски и технически персонал, които чрез своя висок професионализъм и коректност, работят за удовлетворяването и на най-високите изисквания на нашите клиенти.

Дружеството разполага с необходимите ресурси - собствениците са с опит от над 30 години в международната търговия, високо квалифициран персонал, в т.ч. 2 броя сервизни инженери, 5 броя продуктови специалисти и 1 складов специалист, складова база за съхранение на медицинските изделия, транспортни средства за превозване на продуктите и цялостна гъвкава система за възможно най-добро изпълнение поръчките на нашите клиентите.

Основни клиенти са Министерство на Здравеопазването – Кръвна банка, Българска Агенция за Безопасност на Храните; Военномедицинска Академия; „Специализирана болница за активно лечение по детски болести” ЕАД – гр. София; „МБАЛ – Търговище” АД, гр. Търговище; „МБАЛ – Русе” АД, гр. Русе; Национален център по трансфузионна хематология, гр. София; МБАЛ „Св. Пантелеймон” АД, гр. Ямбол, Лаборатории ЦИБАЛАБ, Лаборатории БОДИМЕД, Лаборатории РАМУС. Успоредно с работата по участието в обществените поръчки, имаме разработена бизнес стратегия за увеличаване на директните продажби с клиенти от цялата страна.

Биосистеми ООД
Гр. София, бул. „ Ботевградско шосе” № 247

С Биосистеми ООД и лабораторията ни – Проген, работим в най-бързо развиващата се област на биологичните и медицински науки – молекулни технологии за идентификация, диагностика, опазване на околната среда и биоразообразието, контрол на качеството. Доставяме апаратура, реактиви и консумативи.

Провеждаме теоретични и практически курсове.

www.biosystems.bg / http://progene.my.contact.bg

ИНДЕКС НА ПОСТЕРИ

P1 - P14 Молекулярна и клетъчна биология
P15 - P55 Биоразнообразие, екология и консервационна биология
P56 – P80 Биотехнологии, Бионанотехнологии и Приложна биология
P81 – P86 Генетика и селекция
P87 - P102 Медицинска биология
P102 – P104 Студентска секция