SOFIA UNIVERSITY

ST. KLIMENT OHRIDSKI

FACULTY OF BIOLOGY
SOFIA UNIVERSITY

FACULTY OF BIOLOGY

SCIENTIFIC CONFERENCE
KLIMENT'S DAYS

5 NOVEMBER 2020

ABSTRACTS

Funded by:
PROJECT № 80-10-142/2020 Sofia University;
PROJECT № 3022/2020 Scientific Research Department, Sofia University

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Project № 80-10-142/2020 Sofia University;
Project № 3360/2020 Scientific Research Department, Sofia University

ISSN: 1314–4960

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SCIENTIFIC CONFERENCE
"KLIMENT'S DAYS"
5-th November 2020, Faculty of Biology
WWW.BIOFAC.INFO

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MB&BT-49	**Ivona Andonova, Radostina Stefanova, Petia Genova-Kalou, Stefka Krumova** MEASLES AND RUBELLA VIRUSES - CLASSICAL AND MODERN METHODS OF LABORATORY DIAGNOSIS
MB&BT-50	**Nora Dinova, Mihaela Belouhova, Ivaylo Yotinov, Stilyana Lincheva, Irina Schneider, Yana Topalova** THE FLUORESCENCE IN SITU HYBRIDIZATION - AN INNOVATIVE METHOD IN THE CIRCULAR SOLUTIONS FOR RESOURCES RECOVERY IN MUNICIPAL ENTERPRISE FOR WASTE TREATMENT - SOFIA

BROMUS DIANDRUS ROTH (POACEAE), NEW SPECIES FOR THE BULGARIAN FLORA

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Keywords: *Bromus, great brome, dune vegetation, native range, alien species*

Bromus diandrus is reported from Bulgaria for the first time. The species was observed on coastal sands at Irakli locality on 15.08.2020. A concise description of its morphological features and comparison with the already known representatives of Bromus sect. Genea from the country are presented. The new established locality is characterized floristically and ecologically. The origin of the studied population, in terms of its nativeness is also commented.

B&BE-2 **POSTER SESSION**

STUDY ON THE SELECTION OF PLANTS FOR SENSORY GARDENS

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The creation and maintenance of Sensory Gardens is a practice embraced by the modern society which achieves the goals set by the WHO. These gardens are detached spaces offering sensory stimulation, opportunities for educational activities, social interactions and recreation. The key concept in the design of these gardens is offering a facilitated access and taking care of the needs of visually handicapped visitors. In the University Botanic Garden – Balchik, a sensory garden has been arranged featuring a basic elements of the sensory garden. The visitors may familiarise themselves with 34 species (15 families). Best portrayed is Lamiaceae with its well-known fragrant plants rich in essential oils such as the narrow-leaved and broad-leaved species of lavender Lavandula angustifolia and Lavandula latifolia respectively, the medicinal and ritual plant with lemony scent – the common balm (Melissa officinalis) and the winter savoury (Satureja montana). From the aromatic plants, there are the fern leaf peony and Chinese peony (Paeonia tenuifolia and Paeonia lactiflora), jasmine (Jasminum officinale) etc. Interesting shapes and textures are exhibited by the medicinal plant Hylotelephium spectabile, the sticky plant with hard to the touch bristles – jasmine tobacco (Nicotiana alata), the exotic Cape gooseberry (Physalis peruviana) and the woolly foxglove Digitalis lanata. The selected examples offer sensory stimulation all year round with an emphasis on the spring and summer seasons when there is a large number of visitors to the garden. Despite the availability of time-proven techniques for the design of such spaces, the idea of a sensory garden is a developing concept which will change with time. The experience demonstrated the need for qualified staff capable of describing sufficiently well the layout where the visually handicapped visitors are located in order to facilitate their getting around in the garden. It is also necessary to be very knowledgeable about the plant collection due to the natural interest in aspects of the plants related to their use and cultural significance and not just their physical characteristics. With a view to improve the conditions for the visually handicapped visitors, a field study was carried out on the grounds of the University Botanic Garden - Sofia, with the kind collaboration of colleagues from the Special Education speciality at the Faculty of Educational Studies and the Arts at Sofia University "St. Kliment Ohridski".

ENDEMIC PLANT SPECIES IN BULGARIA: OVERVIEW ON CONSERVATION STATUS AND IN SILICO PREDICTION OF POTENTIAL ANTI-CORONAVIRUS ACTIVITY

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The Bulgarian flora includes 270 Balkan and 174 Bulgarian endemic species. Assessing the conservation status and the phytochemical composition of these plants are important aspects of the sustainable preservation, popularization and utilization of such rich natural source of compounds with unexplored biological activities. The aims of this study were to: (i) summarize conservation-relevant data on endemic plants found in Bulgaria, (ii) extract and organize data about their phytochemical profiles and (iii) compare them to naturally-occurring compounds, investigated for potential relevance to different coronaviruses. Data and methods: The NIH PubMed and PubChem systems (https://www.ncbi.nlm.nih.gov/) were used to prepare a virtual library of phytochemicals from endemic plant species found in Bulgaria. An in house virtual library of naturally-occurring compounds investigated for activity against different human and animal coronaviruses was used. Multi-conformer structure databases were prepared and the conformers were superposed to estimate shape- and chemical feature-based similarity between compounds from the two libraries. Results: Data about endemic plant species found in Bulgaria and assigned with endangered or critically endangered conservation statuses in the Red Data Book of the Republic of Bulgaria (http://e-ecodb.bas.bg/rdb/bg/vol1/), were summarized in relation to their distribution, habitats and conservation actions. Phytochemical data were collected and organized in a virtual library consisting of 100+ compounds (e.g. terpenes, flavonoids, phenolic acids, carotenoids). Similarity-based virtual screening underlined endemic plant-derived molecular scaffolds of interest for further in silico drug design studies targeting coronaviruses. Conclusions: The present study gives a brief overview on the conservation status of selected endemic plant species found in Bulgaria, summarizes data on their phytochemical composition and by application of a computer-aided approach estimates their putative pharmacological relevance in the context of modulation of coronavirus-induced diseases.

Acknowledgements: *The work is supported by the Bulgarian Ministry of Education and Science under the National Research Programme Young scientists and postdoctoral students [grant number DCM # 577/ 17.08.2018].*

B&BE-4 **POSTER SESSION**

CHARACTERIZATION OF SOIL FUNGAL COMMUNITIES IN LAVENDER AND OIL-BEARING ROSE PLANTATIONS USING DNA METABARCODNIG

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Soil fungal communities are characterized by great diversity and complex structure. As pathogens, decomposers and plant mutualists soil fungi are involved in all ecosystem processes and services. They can significantly affect plant and soil health, however the factors that influence fungal community structure and functioning are not well understood. The lack of knowledge is mainly related to hidden life mode of soil fungi, high phenotypic diversity, and difficulty for culturing, therefore culture-independent DNA-based identification methods for soil surveys are widely used. Recent metabarcoding studies using high-throughput-sequencing (HTS) allow an in-depth analysis of the entire fungal community composition in various environmental samples. In agricultural systems, the soil fungal communities are influenced by numerous factors such as local climate, soil quality and characteristics, crop type, and management practices. In the frame of the National Research Program "Healthy Foods for a Strong Bio- Economy and Quality of Life", soil fungal diversity, composition and functional structure will be used for the evaluation of ecological state and ecosystem services of selected agrosystems. Data and preliminary results on the fungal diversity and community characteristics in the rhizosphere of lavender and oil-bearing rose plantations located in the region of the Rose Valley will be presented. Ten plots from each crop managed by conventional and organic farming were selected and sampled in the period of 2019-2020. Soil fungal composition was studied by using amplicon DNA sequencing of the internal transcribed spacer 2 (ITS2) rDNA region and the diversity and community structure were analysed at genetic and higher taxonomic levels. In both crops most abundant genera were Fusarium, Cladosporium, Alternaria and Solicoccozyma. A few host-specific operational taxonomic units (OTUs) with high occurrence corresponding to the genera Chaetosphaeronema and Nothophoma, and order Chaetothyriales were present in the samples from lavender fields. The multivariate statistics (PCA) revealed clusters linked to the planted crop rather than to the management type. A brief overview of fungal diversity and selected community characteristics in the rhizosphere of lavender and rose plants will be presented.

POSTER SESSION **B&BE-5**

ECTOPARASITIC NEMATODES OF FAMILY LONGIDORIDAE (NEMATODA, DORYLAIMIDA) FROM OIL- BEARINGROSE AND LAVENDER PLANTATIONS IN THE ROSE VALLEY

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Keywords: *biodiversity, conventional farming, organic farming, Lavandula angustifolia L., Rosa damascena, Rosa raduga*

Records on longidorids associated with rose and lavender plants in Bulgaria are very few. Choleva et al. (1980) reported three Longidorus species (L. euonymus, L. profundorum, L. distinctus) and twoXiphinema species (X. diversicaudatum and X. pachtaicum) associated with oil-bearing roses in different locations in Bulgaria. However, no data on the morphology and morphometrics of recovered species have been presented. In the course of the study on biodiversity of oil-bearing roses and lavender with conventional and organic farming (National Research Program "Healthy Foods for a Strong Bio- Economy and Quality of Life"), twenty soil samples have been collected for nematological analyses in 2019 and 2020. Nematodes were isolated by Baermann funnel method (5 x 25 g of soil), fixed in formaldehyde solution (4%) after killing, dehydrated and mounted on permanent slides. Three species of family Longidoridae have been found – L. distictus, X. pachtaicum and Xiphinema sp. and characterized morphologically. All they originated from lavender fields with organic production. Xiphinema sp. which is similar to X. pachtaicum was compared with closely related species. Xiphinema pachtaicum was recovered also from a rose field with conventional production.

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

B&BE-6

POSTER SESSION

ANASSESSMENTOFDIFFERENCESBETWEENPASSERINESBIRD HOSTSBASEDONTHEIRFEATHERMITES(ACARI:ASTIGMATA: ANALGOIDEA) STUDIED IN THE SOUTHERN DOBRUDZHA (NE BULGARIA).

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The study was focused on the material of 54 feather mite species and their 48 bird hosts species. The birds were captured during the period 2005-2007 in the areas of the Biological Experimental Station "Kalimok", the Shablenska Tuzla Lake and the Durankulak Lake (Southern Dobrudzha, NE Bulgaria). The aim of this study was to assess differences between bird hosts species based on their feather mite species using a modified Zlotin's scale for faunistic similarity (from 0% – no difference to 100% different feather mite species). The difference was ranked from very high > 85% to high (85–55%), moderate (54–35%), and low < 35%. The faunistic differences between bird species was evaluated using the Jaccard Distance and cluster analysis. Dendrograms was made with the program R Core Team (2014). As a result clusters are clearly distinguished because they include bird species from one genus and/ or a family. We found that differences between bird species based on their feather mite species range from 0% to 100%. Thirteen bird species, Garrulus glandarius, Troglodytes troglodytes, Prunella modularis, Coccothraustes coccothraustes, Oriolus oriolus, Hirundo rustica, Emberiza schoeniclus, Panurus biarmicus, Muscicapa striata, Erithacus rubecula, Luscinia svecica, Turdus philomelos and Turdus merula, showed 100% difference from the others bird species on the base of feather mite species.

FIRST RECORD OF THE FAMILY CEROPHYTIDAE AND CEROPHYTUM ELATEROIDES (LATREILLE, 1804) (INSECTA: COLEOPTERA: ELATEROIDEA) IN BULGARIA

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The family Cerophytidae Latreille, 1834 (Coleoptera: Elateroidea) with the single European genus Cerophytum Latreille, 1806 and its species Cerophytum elateroides (Latreille, 1804) are reported with first verified record from Bulgaria. The material was collected in the Sarnena Sredna Gora Mts., central Bulgaria, with a pitfall trap. Cerophytum elateroides is a rare species everywhere in Europe, and it is an indicator of the presence of rotting wood in broad-leaved woodlands. It is a xylobiont associated with deciduous forest habitats. We found it at 1022 m a.s.l., in a mesophilous beech forest at the ridge of the mountain, with many old trees. The new locality is the most southeastern point known in the range of the species.

Acknowledgements: *This study was funded by the National Science Fund via the Project № КП-06-М21/2 Н-18-ТТЕО-010 "Study of the faunistic diversity and assessment of the condition and ecosystems services in different types of model ecosystems in the Sarnena Sredna Gora Mts."*

B&BE-8 **POSTER SESSION**

MORPHOLOGICAL STUDIES OF TWO LECITHODENDRIID SPECIES (TREMATODA: LECITHODENDRIIDAE) FROM EPTESICUS SEROTINUS (SCHREBER, 1774) (CHIROPTERA: VESPERTILIONIDAE) IN WESTERN STARA PLANINA MTS.

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The present study was based on trematodes, isolated from small intestine of road-killed Eptesicus serotinus from the region of Lakatnik Railway Station. Two lecithodendriid species: Paralecithodendrium longiforme (Bhalerao, 1926) Lotz et Font, 1983 and P. chilostomum (Mehlis, 1831) Lotz & Font, 1983 were recorded. New data about morphology of these species and the host range from Bulgaria are presented. The metric and meristic characteristics of the trematodes are compared with those from other localities of the above-mentioned species from Europe, the Middle East and North Africa.

ZOOGEOGRAPHY OF THE LUMBRICIDS AND ECOLOGICAL CHARACTERISTIC OF THEIR COENOSES FROM THE SARNENA SREDNA GORA MTS.

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The study concerns the earthworms (Oligochaeta: Lumbricidae) of Sarnena Sredna Gora Mts. A total of 381 specimens belonging to 17 species were collected in 2018–2020 from 26 sampling sites throughout the mountain. Along with the data from the literature, the species list consists of 21 species. The established lumbricids were used to develop a zoogeographical classification. The local lumbricid fauna was analyzed in the context of the modern distribution, evolutionary patterns as a consequence of the climate and continental changes, and the current conditions developed as a result of the anthropogenic activities. Factors and regularities in the vertical distribution of the species, and the influence of neighboring territories have been established. Zoogeographical analysis proved that cosmopolites prevail in Sarnena Gora. Ecological typification of the lumbricids according to their life forms showed that the surface-dwelling (epigeic) species prevail. We established some species, groups of species and quantitative biotic parameters, indicating the environmental conditions related to the determination of vertical stratification, conservation significance of habitats and the degree of anthropogenic impact. The zoogeographical and ecological analysis of the earthworms is an experiment, given the non-traditional method used to collect most of the data, e.g. pitfall traps.

Acknowledgement: *This study was funded by the National Science Fund via the Projects "Study of the faunistic diversity and assessment of the condition and ecosystems services in different types of model ecosystems in the Sarnena Sredna Gora Mts." (H-18-TTEO-010), and "SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources (STACCATO)".*

B&BE-10 **POSTER SESSION**

ECOLOGICAL CHARACTERISTICS OF THE ZOOCOENOSES AND CLASSIFICATION OF THE ANIMALS' HABITATS FROM SARNENA SREDNA GORA MTS

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During a research of the biodiversity of Sarnena Sredna Gora Mts during 2018–2020, the ecological characteristics of the habitats of several model groups of animals were studied: earthworms (Oligochaeta: Lumbricidae), earwigs (Insecta: Dermaptera), cockroaches (Blatoptera), groung beetles (Carabidae), ants (Formicidae), amphibians (Amphibia: Caudata, Anura), reptiles (Reptilia: Testudinata, Squamata: Serpentes, Sauria), birds (Aves), and small mammals (Micromammalia: Eulypotyphla, Rodentia, Lagomorpha). The results served as a basis for ecological analysis and subsequent typification of the habitats, according to the specific requirements of the animal biota. The determination of the conservation significance of the habitats was performed by analyzing the species and groups of species with conservation status or limited distribution in Bulgaria and in the studied area. The specific threats to the biodiversity and habitats in the region of Sarnena Gora were summarized and analysed, and measures for mitigation, limitation or elimination of the negative impacts were proposed, according to the Bulgarian and international nature conservation legislation.

FAILED HIBERNATION OF THE GALL-MAKER SIGNIFICANTLY REDUCES PARASITOID AND INQUILINE DENSITY IN SOME GALL COMMUNITIES OF DIPLOLEPIS ROSAE (L.) (HYMENOPTERA: CYNIPIDAE) IN BULGARIA

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Gall communities of the Rose Bedeguar wasp, Diplolepis rosae (L.), were analyzed in 12 mountainous localities in Bulgaria. At least 139 and 349 mature galls induced on Rosa canina were collected during the autumn of 2018 from the Western Rhodope Mountains and Stara Planina Mountains, respectively. The gall material was stored in laboratory at room temperature for a minimum of one year. Reared inhabitants were counted and fixed weekly. Later identification revealed 19 different species represented in bedeguar gall community - Diplolepis rosae and Periclistus brandtii (Cynipidae), Orthopelma mediator and Exeristes roborator (Ichneumonidae), Oomyzus galerucivorus and Stepanovia sp. n. (Eulophidae), Aximopsis collina, Eurytoma caninae and E. rosae (Eurytomidae), Eupelmus barai, E. confusus, E. fulvipes, E. urozonus and E. vesicularis (Eupelmidae), Pteromalus bedeguaris and Caenacis inflexa (Pteromalidae) and Glyphomerus stigma, Torymus bedeguaris and T. rubi (Torymidae). Most of these species are common in rose bedeguar galls. Among the sampled localities D. rosae was represented with maximum of 5 % in one sample from Western Stara Planina Mts. Furthermore, the emerged material from six localities was free of D. rosae adults. The overall abundance of every particular species varied among samples, but the dominant role of G. stigma and P. bedeguaris was undoubted. However, all parasitoids and the inquiline were in extremely low density in all samples. In this work we discuss the possible reasons for this community changing and present detailed information for the community composition. Also, two species are recorded for the first time from Bulgaria – E. caninae and E. fulvipes. One association with the rose bedeguar galls is new – the eulophid O. galerucivorus was not previously known as member of this community. Unknown species belonging to genus Stepanovia was reared from two localities – one in Stara Planina Mts. and one in the Rhodopes.

B&BE-12

POSTER SESSION

IMPORTANCE OF CITIZEN SCIENCE FOR THE ZOOLOGICAL RESEARCHES IN BULGARIA: AMPHIBIANS AND REPTILES IN BULGARIA GROUP AT THE SOCIAL PLATFORM FACEBOOK

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The using of non-professional contributors in zoological researches is a widespread practice in many scientific projects, monitoring schemes, etc. worldwide. In Bulgaria, one of the main sources for communication with such contributors in the recent years are the Facebook-based groups, dedicated to different taxon of organisms. The present study provides details on the functioning, scientific and social benefits of one of them: The amphibians and reptiles in Bulgaria. At present the group members number more than 6300. The significant amount of contributions resulted in the obtaining of new herpetological information, e.g. new locations of species, some of which were later published as scientific papers. Along with the scientific importance, the group proved as an educational source for people interested in herpetology and also had an impact on the attitude of people, concerning amphibians and reptiles, which in many cases can be a significant problem for their conservation.

POSTER SESSION **B&BE-13**

CONTRIBUTION TO THE RESEARCH OF THE AVIFAUNA IN THE LITTORAL ZONE OF THE BULGARIAN BLACK SEA REGION

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The current research was conducted in the spring, summer and autumn of 2020. These studies build on previous research by the current team in this area. Additional data have been collected on the distribution biology and behavior of some bird species. Field research has been carried out in key places along the Black Sea coast such as Durankulak – Krapetz Sea Coast, Kaliakra and Emine peninsulas, Strandja seacoast. Most of the data are confirmatory and will be used for statistical analysis in the long term. New locations of nesting bird species have been established. A free description of the behavior of the observed individuals is given, which will serve for analysis of their adaptive strategies. New nesting locations of breading birds were found such as colonies of Sand martin (Riparia riparia), Kentish plover (Charadrius alexandrinus), European shag (Phalacrocorax aristotelis) and others have been discovered on the southern Black Sea coast. Their specific hunting strategies are described. observations were made on the autumn migration of birds in the littoral zone and the way in which the different species are used.

B&BE-14 **POSTER SESSION**

COMPONENTS AND SEASONALITY OF WOLF DIET IN OSOGOVO MOUNTAIN IN 2018-2020

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In order to understand the wolf (Canis lupus L.) diet in Osogovo mountain, 112 scats were collected using the transect method. The study period was between April and November during three consecutive years (n = 26 for 2018, n = 25 for 2019 and = 61 for 2020). For the scat analyses the classical method of Kruuk and Parish (1981) was used. Additionally, data (n = 64) from a study in 2004 (between June and November) from the same region, was also used for comparison. The obtained results showed that during our study the wild boar (Sus scrofa L) prevailed in the food spectrum of the wolf. The percentage distribution of wild boar in the wolf scats over the three years was distributed as follows: 60.87% (2018), 84.62% (2019) and 73.33% (2020). The second important element in the wolf's diet was the domestic horse (Equus ferus caballus L.) – 26.09% (2018), 11.54% (2019) and 1.67% (2020). Contrary to the data from 2004, the roe deer (Capreolus capreolus L.) had a smaller share in the wolf diet during our study – 71.9% (2004) vs. 10.87% (2018), 3.87% (2019) and 25% (2020). Despite the fact that a camera trap analysis indicates a higher population density of roe deer (3,08 - 3.22 ind./km2) compared to the wild boar (0.82-0.90 ind./km2) in Osogovo, a significant decrease of roe deer components in the wolf's diet preferences was noted, compared to 2004. The increase in the percentage of higher-biomass species in the wolf's diet over the years may be due to the increasing number of wolves in the region – from 4 individuals in 2004 to at least 3 packs of up to 8 individuals per pack. Increased numbers of individuals in the packs is generally associated with larger prey selection. Most of the scats were collected in the spring and autumn, while only two scats were collected in summer for each of the years 2018 and 2019, and 6 for 2020. The wild boar also showed the highest significance in the wolf diet seasonally. For the spring it was at the average of 70.99% (37.5% in 2018; 100% in 2019 and 75.49% in 2020), followed by the domestic horse – average of 21.46% (62.50% in 2018; 0% in 2019 and 1.89% in 2020). In the spring the roe deer was present only in 2020 (22.64%). In summer, the wild boar formed 55.56% of the wolf diet, while the domestic horse was present only in 2018 (50% of the diet) and the roe deer – only in 2020 (33.33%). In the autumn, the wild boar formed 88.89% of the wolf diet (77.78% in 2018; 100% in 2019), while the horse and roe deer were present only in 2018 (3.70% and 18.52% respectively).

POSTER SESSION **B&BE-15**

TESTING OF CAMERA TRAP INSTALLATION FOR ARBOREAL MAMMALS – PINE MARTEN AS AN EXAMPLE

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The camera trap technology was first designed to record animals on the ground surface. Yet, a lot of species that can be registered might have also arboreal or semi-arboreal way of life. All the studies in Bulgaria so far were also based on surveys on the ground surface. That is why a camera trap installation for arboreal mammals was designed in our current study, aiming identification of martens on the species and individual level, the pine marten in particular. We also used the stone marten as an accompanying species, which also climbs trees, but is not a typical arboreal species. To the best of our knowledge, this is the first such attempt in Bulgaria. Individual recognition in these two species is possible, as pine marten and stone marten have a specific shape and location of the breast spot in each individual. The installation consists of camera traps (Ltl Acorn 5210A, Bolymedia, Scout Guard - Boly Media SG2060-K, Moultrie A-Series MCG-13201 and Moultrie M40I models) mounted on suitable trees. A parallel board and a platform are mounted against the traps, on which the animal can stand and be photographed in such a way as to provide for their identification. Each of the upper boards has a measuring line on the side, facing the camera trap, and on the back – a plastic box with a bait attached. Two types of bait were tested – the first is a mixture of blood and stale meat soaked towel, and the second – Premium Lockmittel Marder by Hagopur. The height of the scientific installation is between 38 and 222 cm, and the distance between the camera traps and the boards varies from 55 to 150 cm. There is a big difference in the two values for the distance between the camera traps and the recognition installation, as 5 different models of camera traps were used. Each model has different resolution and visualization of the captured frames. The distance between the two boards varied from 40 to 50 cm and is in accordance with the body length of the species used as an example. The flash on the Acorn model was covered with 3 layers of aluminum foil to prevent over-exposure of the photos, as the flash on this model is very strong at close range. The scientific installation was set up in 10 locations in each of two mountains - Vitosha and Western Rhodopes. The project is ongoing since May and the results are expected at the end of 2020.

B&BE-16 **POSTER SESSION**

CETACEAN STUDIES IN BULGARIAN WATERS OF THE BLACK SEA IN 2020, USING VANTAGE POINT SURVEYS AND OPPORTUNISTIC SIGHTINGS

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Observations were carried out from a vantage point on Cape Emine, between June and September 2020. The survey covers 28 field days and 224 hours. All of the three species, occurring in the Black sea, were observed: 812 animals in 385 sightings. The highest number of individuals recorded, was in June: 351 animals in 140 sightings. High rates were also observed in July and August. Vantage point surveys were also conducted from Cape Kaliakra. On 29-30.07 and 14.08.2020, observations were carried out, in the waters north and south of the town of Tsarevo, using platforms of opportunity. The sightings data were collected onboard a small fishing vessel. The highest number of individuals observed, was on 30.07.2020: dozens of Black sea short-beaked common dolphins (Delphinus delphis ponticus) and Black sea bottlenose dolphins (Tursiops truncatus ponticus). Observations and recordings, on the feeding and social behaviour of the cetaceans, were conducted. More than 3,000 high-resolution photographs were obtained, which are suitable for photo-identification of specific individuals. Data and photographs of cetaceans (with exact coordinates) were also collected, onboard a military vessel. Confirming the results of previous studies, the servey indicates, that Cape Emine is the most suitable observation point for all three cetacean species in the Bulgarian Black Sea. Platforms of opportunity are a reliable method of gathering information on the distribution and behavior of cetaceans, whether or not a marine mammal expert is on board.

POSTER SESSION **B&BE-17**

CHARACTERIZATION OF THREE NEWLY- ISOLATED, BROAD HOSTRANGEORPOLYVALENT,XANTHOMONASSP.EFFECTIVE BACTERIOPHAGES

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For effective phage biocontrol of plant diseases, caused by more than one phytopathogenic bacteria, such as bacterial spot on pepper and tomato, phage cocktails are needed. Such cocktails, containing phages with different host range, as control strategy in fighting crop diseases, is a rapidly evolving field in agro sciences. As alternative to such cocktails, phage pesticides, containing polyvalent phages, might be applied. In this study we present, for the first time, the isolation and initial characterization of three polyvalent bacteriophages (SfXv124t/1, SfXv124t/2 and SfXv124t/3), effective against three phytopathogenic bacteria causing bacterial spot on tomato and pepper: Xanthomonas vesicatoria, Xanthomonas euvesicatoria and Xanthomonas gardneri. Bacteriophage isolation was done by applying double agar overlay plaque assay (DAOPA). The initial characterization of the phage isolates includes: 1/ host range analysis of the three isolates, 2/ determination of the types of plaque morphology and measuring the plaques diameter, formed by the phages on solid agar plate by DAOPA and 3/ determination of the virions morphology by using TEM. Phage strains were isolated from tomato rhizosphere soil, using Xanthomonas vesicatoria, a strain isolated from tomato, as specific host. According to TEM and DAOPA results, there might be a Siphoviridae phage (SfXv124t/1) and a Podoviridae (SfXv124t/3) phage, among the isolates. The phage host range determination was carried out using 23 phytopathogenic strains belonging to the genus Xanthomonas and Pseudomonas, as specific hosts. The results showed that phages SfXv124t/2 and SfXv124t/3 are capable to lyse two phytopathogenic bacteria: X. euvesicatoria and X. vesicatoria as opposed to SfXv124t/1 lysing three phytopathogenic bacteria: the two aforementioned and X. gardneri. None of the tested phages has lytic activity against Pseudomonas syringae pv. tomato strains. The broad range of phages effectiveness showed that the newly isolated bacteriophages are polyvalent, hence - a good alternative to chemical pesticides as they are capable to destroy the all three causative agents of bacterial spot disease on tomato and pepper.

Acknowledgement: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 /17.08.2018".*

B&BE-18 **POSTER SESSION**

GENETIC VARIATION FOR GRAIN PROTEIN CONTENT IN A DIVERSE BREAD WHEAT POPULATION

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Wheat is the main grain crop in the temperate climate zone, providing about 20 % of calories and 25 % of proteins in human diet. Given the growing consumers' awareness and demands for healthier food, programs are recently focusing not only on increasing productivity, but also on higher nutritional quality of derived products. Both nutritional value and end-use quality of wheat is largely determined by the grain protein content (GPC). In this study, we evaluated the genetic variation for GPC using a diverse panel of 264 bread wheat accessions consisting of elite cultivars, advanced breeding lines and double haploid lines originating from 28 countries from five continents. GPC was determined in seeds from 3 crop seasons using the Kjeldahl method. A considerable amount of variation in the protein content of wheat grains was established within this population. GPC values ranged from 5.54 to 18.42 % over the genotypes and the three harvest years, with average of 11.29 %, CV=17 %. ANOVA showed that this variation was induced by the environmental factors, but was also attributed to genetic differences. The mean square for genotype (G), harvest year (Y), and G x Y was highly significant suggesting the differential performance of genotypes in different crop seasons. The highest average GPC was registered in the wheat pools originating from Croatia (13.61 %) and Hungary (13.43 %), and the lowest – in the genotypes from France (9.97 %). The representatives of Bulgaria showed intermediate average GPC value (10.96 %). Three accessions (two from Russia and one from Croatia) showed consistent high GPC ranking in all years. The information on the extent of wheat diversity obtained in this investigation will be helpful for generating novel variation for GPC, understanding the genetic basis of grain quality traits, and, in turn, for developing appropriate science-based breeding strategies to achieve further improvement in wheat end-use quality.

Acknowledgements: *This study was supported by the Bulgarian NSF under contract KP-06-N31/17.*

POSTER SESSION **B&BE-19**

GENETIC VARIATION AND POPULATION STRUCTURE ANALYSIS OF BULGARIAN BREAD WHEAT GERMPLASM AS REVEALED BY SNP MARKERS

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A collection of 184 Bulgarian wheat (Triticum aestivum L.) accessions, including landraces, old traditional cultivars and modern releases was genotyped with 24 145 single nucleotide polymorphism (SNP) markers. Data analyses were performed after reducing the number of SNPs to 19 707. The SNPs distribution on the 21 wheat chromosomes ranged from 159 on chromosome 4D to 1733 on chromosome 2B. The statistically expected level of heterozygosity in the population, expressed by the Wright's "fixation index" F (the proportion by which heterozygosity is deviated relative to the heterozygosity in a randomly mating population with the same allele frequencies) was 0.17. This means that the pool of Bulgarian wheats contains 17 % fewer heterozygotes than would be expected in a population with the same allele frequencies experiencing random mating and the other conditions set out in the assumptions of Hardy–Weinberg. The percentage of heterozygosity within the population was 62.4, and the percentage of homozygosity – 37.6, $\chi 2 = 0.09$. Population structure analysis by the K means clustering algorithm in package R elucidated clear clustering of the wheat genotypes. The number of subgroupings was highest at K=5, denoting 5 recognizable groups. The majority of landraces formed a distinct cluster, whereas the old and modern cultivars were structured according to the releasing breeding center/geographic region and formed sub-populations. When applying principal component analysis (PCA) to the data, the 184 genotypes formed 184-dimensional space and similar subgroupings were identified. In PCA, the two principal components explained 12.2 % of the total variation (PC1 8.6 % and PC2 3.6 %). Information on the amount of genetic variation and population structure in a crop is fundamental for its improvement and for diversity preservation.

Acknowledgements: *This study was supported by the Bulgarian NSF under contract KP-06-N31/17.*

B&BE-20 **POSTER SESSION**

THE CHALLENGE OF LABORATORY WORK IN THE FACE OF THE CONSTRAINTS IMPOSED BY COVID-19

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Abstract In the conditions of restrictions imposed by COVID-19 in the 2019-2020 school year, an alternative to the laboratory classes at school could be the home science experiments. The home experiment is a simple self-conducted experiment that students perform at home without the immediate guidance of the teacher in the course of the experiment. This article presents a model for performing home experiments of interdisciplinary nature in the conditions of distance e-learning. The experiments were in the school subject biology and health education in regular classes with students from 9th grade in SPGE "John Atanasov", Sofia. Students perform experimental tasks at home independently, using the available equipment. One of the advantages of this type of experiment is that when setting tasks, the teacher does not need to take into account the different speed at which students perform them. This allows all students to be given the same task. A special value of this type of activity is that it motivates students and increases their interest in the studied subjects.

POSTER SESSION

B&BE-21

ATTRACTING TALENTED BULGARIAN YOUNG BIOLOGISTS TO SCIENCE BY SMALL LEARNING AND RESEARCH SCHOOL COMMUNITY

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This report presents a new approach at the educational process in Bulgaria which aimed to attract high school students to highest academic level of science. Small learning and research community was established at school in the frame of a joint program between Bulgarian Academy of Sciences and the Ministry of Education and Science. Students' knowledge and skills were enriched by creating new contacts with researchers working in various fields of biology. Bulgarian leading scientists and young post-doctoral researchers carried out science class activities with the small school community. High school students improved their personal grades by learning about the failed experiments and innovation success. Students presented their own projects and received achievement awards at different competitions as Chemistry Festival, World Biodiversity Day, Students' research session of Bulgarian Academy of Sciences, etc.

B&BE-22 **POSTER SESSION**

ASSESSMENT OF BODY COMPOSITION BY BIOELECTRIC IMPEDANCE ANALYZER IN 7-18-YEAR-OLD CHILDREN AND ADOLESCENTS FROM SOFIA (BULGARIA)

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The present study is based on a cross-sectional anthropometric investigation of the physical development of children and adolescents carried out between 2014 and 2018 in Sofia, Bulgaria. A total of 1568 schoolchildren (723 boys and 845 girls, between 7 and 18 years old) have been measured using standard anthropometric methods. Data on height (m), body weight (kg), waist and hip circumference (cm) has been obtained as a basis for further bioelectric impedance analysis of the body composition with the medical device "ABC-01 MEDASS". The individual values of active (R) and reactive resistance (Xc), as well as phase angle (φ, arctg φ = Xc/R; at 50 kHz and 800 μA) have been also obtained. These measurements have been taken as the basis for the assessment of body composition, and are presented herein. The body composition has been characterized by both the absolute and relative value of its components: fat mass (kg) normalized by height, fat-free mass (kg), total body water, extracellular, and intracellular water (kg), skeletal muscle mass (kg), active cell mass (kg), basic metabolic rate (BMR, kcal/day), specific BMR (kcal/sqm per day). Age-gender growth curves have been constructed to assess the deviations from the norms for physical development, and the current physiological status of the schoolchildren from Sofia.

POSTER SESSION

B&BE-23

PONTECHIUM MACULATUM (L.) BÖHLE & HILGER (ECHIUM RUSSICUM J.F. GMEL.) (BORAGONACEAE) ON THE ZEMENSKA MOUNTAIN, WESTERN BULGARIA

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Pontechium maculatum has a high conservation value: Red list of Bulgarian vascular plants (Vulnerable), Annex IV of the Directive 92/43 / EEC and Biological Diversity Act (2002) (Annex 2,3).
Field surveys were carried out in the period 2006-2020. The transect method was used.
As a result of of field research and a study of the other habitats of the species in Bulgaria, it was found that the population of *Pontechium maculatum* on the Zemenska Mountain is the largest in Bulgaria.
The direct census identified about 4,000 generative individuals and 500 vegetative individuals on an area of 45 ha, in several fragments of a branch population that spans the northern part of the mountain. Two major fragments were identified - in the area of Peak Silni vrah (Fragment 1) and in the area of the Peak Glamen (Fragment 2)
Fragment 1: 42°28'13"N 22°41'30"E: 3000 generative individuals
Fragment 2: 42°29'12"N 22°41'03"E: 1000 generative individuals
There are also quite a few individuals found in the area close to Sv. Petar saddle and Peak Banderata.
The future prospects for its existence are positive, as at this stage there are no immediate threats.

B&BE-24 **POSTER SESSION**

ANTHROPOLOGICAL CHARACTERISTICS OF THE BULGARIAN POPULATION BY INTEGRATING DERMATOGLIPHIC AND ODONTOLOGICAL DATA

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Key words: *integration, dermatoglyphical traits, odontological traits, Principal component*

Contemporary Bulgarian population from eight regions of Central Western Bulgaria was studied. Dermatoglyphical and odontological features of diagnostic and taxonomic significance have been studied. Dermatoglyphics of 1,600 individuals (800 men and 800 women) were investigated. Five most significant dermatoglyphical traits - Delta index (DL10), Cummins index (Ic), axial traradii (t), hypotenar images (Hy), additionel axial triradii (AIT) were analyzed. The processing and analysis were done according to the method of Cummins, Midlo (1961). Odontological scars were taken from 800 individuals (male and female). The traits which were analyzed are diastem, 4M2, tuberculum Karabelli, furrow 2 med (II), crawding, spatular form of upper medial incisors, kneeling fold, distal ridge, TAMI. The processing and analysis were done according to the method of Zubov (1968). Based on the results obtained, an integration analysis was carried out using the PC (Principal component) method. Correlation between the traits in both sexes was established, and also correlation of the traits with geographic location.

POSTER SESSION

B&BE-25

SURVEY ON THE TAXONOMY AND BIOACOUSTICS OF THE BULGARIAN SPITTLEBUGS (HEMIPTERA: CERCOPOIDEA)

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The superfamily Cercopoidea (spittlebugs) contains two families in Europe – Cercopidae and Aphrophoridae. Currently there are 18 species – 4 cercopids and 12 aphrophorids known from Bulgaria. Four of them have been recorded without any specific data about localities in Bulgaria and their presence needs confirmation. The present study provides an annotated species list of Cercopoidea based on the literature and new distribution records.
Vibration sound production transmitted mainly by the substrate is known for many spittlebugs. One of the main biological function of these signals in males is the attracting of the females (calling signals). Our study on the acoustical signals of Cercopoidea is the first for Bulgaria.
The calling sounds of 5 species – Cercopis intermedia (Kirschbaum, 1868) (Cercopidae), Aphrophora alni (Fallén, 1805), Lepyronia coleoptrata (Linnaeus, 1758), Neophilaenus infumatus (Haupt, 1917) and Philaenus spumarius (Linnaeus, 1758) (Aphrophoridae) – are recorded as oscillograms and sonograms are analyzed. New data contribute to the initial modern revision of the the Bulgarian Cercopoidea fauna including detailed distribution maps and bioacoustical records.

E&SD-1 **POSTER SESSION**

SİVAS KANGAL THERMAL THERMAL SPRING AND ENVIRONMENTAL FUNCTIONS

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Fish Thermal Spring, is Turkey's located 13 km from the city of Sivas Kangal district and 90 km. In the pools built on the thermal spring water from underground, the water with a pH of approximately 7.2 is isothermal and its temperature varies between 35 C-36 C throughout the year. Fish found in the thermal; They are poikilothermic creatures whose hematological parameters are observed to change as a result of environmental factors such as bacteria, parasites, water temperature, oxygen content, pH, and they also change with the effect of climatic-seasonal changes. These fish with different sizes are called doctor fish. Patients who come to Balıklı Thermal Spa for the treatment of their diseases from many parts of the world recover with the treatment of doctor fish and the spa water. Doctor Fish, one gram of selenium per liter and softens the skin, as soon as the patients enter the water, they examine the patient and start the treatment with rapid and interesting movements. The hot spring water; It has been found to be beneficial in psoriasis, rheumatic diseases, neurological, orthopedic and traumatological sequelae, gynecological problems, skin diseases, kidney stones (drinking) and psychosomatic disorders. The thermal spring water and fish, which are an important factor in the treatment of diseases, have been examined by taking into account the environmental factors. Keywords: Fish Thermal Spa, Healing Water, Fish Treatment.

SUBSTRATE AND SALINITY INFLUENCE ON SEED GERMINATION AND SEEDLING GROWTH OF CHELIDONIUM MAJUS L.

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Abiotic stress such as salinity affects plant growth, development and physiological and biochemical processes such as photosynthesis, protein synthesis and lipid metabolism. As a result, salinity can suppress germination and severely limit plant growth and productivity. Chelidonium majus L. is a medicinal perennial species which contains alkaloids such as chelidonine, chelerythrine, sanguinarine, protopine and coptisine. The species is cultivated in some countries in Central and Eastern Europe. More and more land is affected by salinity worldwide, which has turned into a serious problem for crop growth and production. Plants' abilities and strategies to cope with salinity vary among each separate species. The object of the study was to investigate Ch. majus's sensitivity, seed germination ability and seedling growth on substrates supplemented with different concentrations of NaCl and the influence of the type of the substrate itself on the toxic effects of salinity. The substrates used in the study were filter paper moistened with destilled water and water agar both supplemented with 50, 100 or 150 mM NaCl. NaCl didn't have a strong impact on seed germination. But the type of the substrate influenced the germination percentage and the effect of NaCl when the seeds germinated on agar. Therefore, seed germination on filter paper was from 85.00% to 95.00%, which differed greatly from the seed germination on agar which was from 20.00% to 62.50% for the different concentrations of NaCl. The influence of NaCl on the primary root growth depended to a great extent on the type of the substrate. The reducing impact of NaCl on root length was present on agar, whereas it was absent on filter paper. This, however, was not established in relation to the hypocotyl growth. NaCl reduced their length in all applied concentrations and on both substrates. The reducing effect of NaCl on hypocotyl growth rose with the increase in the concentation. The results showed that the type of substrate influenced seed germination more than NaCl in the investigated concentrations. However, as far as the length of the primary roots and hypocotyls was concerned, NaCl suppressed their growth in both substrates, especially of roots, where its reducing effect was strengthened by the substrate.

Acknowledgements: *This research was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Project КП-06-M26/4 from 01.12.2018).*

E&SD-3 **POSTER SESSION**

EFFECT OF SALT STRESS ON THE PHOTOSYNTHESIS OF DIFFERENT HYBRID LINES SORGHUM

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Soil salinity is an increasing problem in the world, but its impact on the plants depends on the plant species. The effects of different NaCl concentrations (0 - 250 mM) on the photosynthesis of three hybrid lines of sorghum (ES Albanus, ES Shamal and ES Foehn) with different drought tolerance were investigated. Chlorophyll a fluorescence (PAM and OJIP fluorescence transitions) was used for evaluating the impact of salt stress on the functions of photosynthetic apparatus. The influence of the salt stress on the oxido-reduction state of P700, pigment composition, electrolyte leakage and stress markers (MDA and H2O2) was also estimated. Data revealed effects of different NaCl concentrations on: the effective quantum yield of a photochemical energy conversion of PSII (ΦPSII), the ratio of regulated (ΦNPQ) and non-regulated (ΦNO) energy loss in PSII, the amount of the closed PSII centers (1-qp) and their efficiency (Φexc), the apparent antenna size of the active PSII centers (ABS/RC), the electron flux reducing end acceptors at the acceptor side of PSI (REo/RC), the electron transport flux from QA to QB per PSII, the oxido-reduction state of P700 and the cyclic electron flow around PSI. All these changes vary depending on the salt concentration, as well as the hybrid line. The relationship between the degree of inhibition of the functions of the photosynthetic apparatus and the level of stress markers and pigment composition are discussed.

Acknowledgements: *This studies for hybrid lines sorghum ES Albanus and ES Shamal was supported by the Bulgarian Science Research Fund, КП-06-К36/9, 2019 and for hybrid line sorghum ES Foehn by the Bulgarian Academy of Sciences, National Research Programme "Young scientists and postdoctoral students" (PMC 577/17.08.2018) 2018-2020. The authors thank EURALIS company for providing sorghum seeds.*

BIOACTIVE COMPOUNDS FROM MEDICINAL PLANT EXTRACTS WITH ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY

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Infectious diseases and antimicrobial resistance are problems that require continuous search for new sources of bioactive substances. Terpenoids, phenolics, steroids, alkaloids are secondary metabolites that exhibit a broad spectrum of pharmacological activity, including antimicrobial. Extracts of Amaryllidaceae, Asteraceae and Lamiaceae species were studied against plant pathogens: Alternaria solani, Alternaia alternate, Botrytis cinerea, Fusarium oxysporum, Fusarium solani and Neocosmospora keratoplastica. Methanolic extracts of Artemisia santhonicum L., Origanum vulgare subsp. hirtum (Link) Ietsw. and Leucojum aestivum L. as well as acetone exudates of Salvia sclarea L., and Thymus pulegioides L., showed antifungal potential. These extracts were analyzed by GC/MS for the detection of bioactive compounds. In the acetone exudates of T. pulegioides and S. sclarea, respectively triterpene acids and diterpene (sclareol) were found as the main components. Alkaloid galantamine was determined as active compound of L. aestivum extract. Chlorogenic, quinic, azelaic, protocatechuic and caffeic acids as well as polyols were detected in large amounts in the extract of A. santhonicum whereas carvacrol was determined as active compound in the extract of O. vulgare subsp. hirtum. Also the studied extracts were scanned for bactericidal and bacteriostatic activity against Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis. Significant activity were found to exhibited methanolic extracts of Artemisia lerchiana Weber, Salvia officinalis L., Micromeria dalmatica Benth, Achillea clypeolata L., Thymus jankae Celak., Tanacetum parthenium L. Phenolic acids, sterols, flavonoids, terpenes and triterpenes acids were identified in the selected extracts. The received data characterize triterpene acids, terpenes, alkaloids, flavonoids, polyols as potential compounds with antimicrobial activity.

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

E&SD-5 POSTER SESSION

FUNCTIONAL RESPONSE OF INDIGENOUS BACTERIA TO A FUNGICIDE QUADRISR ADDED TO A LOAMY SAND SOIL AT INCREASING APPLICATION RATES

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Soil microbes play crucial role in nutrient cycling, maintaining soil fertility and soil carbon sequestration. However, contemporary agriculture practices include extensive application of fungicides for control of pathogens. The broad-spectrum mode of action of fungicides causes impacts not only on target but also on non-target organisms. One of the most globally used strobilurin fungicides against mold, rot, early and late leaf spot, rusts and rice blast is azoxystrobin (Az). The aim of the study was to analyze the non-target effects of fungicide QuadrisR (active ingredient – Az) on bacterial metabolic activity using the technique of soil mesocosms laboratory experiment. Mesocosms were constructed with loamy sand soil (pH 5.6) and increasing concentrations of QuadrisR calculated according to Az as follows: Az1 (field dose (FD) - 2.90 mg/kg), Az2 (14.65 mg/kg) and Az3 (35.0 mg/kg), and unpolluted soil as a control (Az0). Soil mesocosms were cultivated 120 days and sampling was conducted one day after fungicide treatment (D1), and on days 30th (D30), 60th (D60), 90th (D90) and 120th (D120). Bacterial functional response was tested using Biolog EcoPlate™, and it was expressed as total catabolic activity (AWCD – average well color development) and community level physiological profiling (CLPP). DT50 and DT90 values showed that QuadrisR applied in field recommended dose (Az1) will affect directly or indirectly soil inhabitants for minimum of 37 – 122 days, whereas expected effects caused by Az2 and Az3 will be much more lasting - from two to ten months. The low fungicide concentration (Az1) caused changes in AWCD which lasted for at least four months. The difference between both Az1 and Az2, and Az3 in AWCD was more pronounced than that between Az1 and Az2 and increased over time. Biolog Ecoplate technique manifested the inability of soil bacteria to utilize D-Xylose. SIMPER analysis showed that only 12 of 30 Biolog carbon sources contributed to around 53 % of the dissimilarity between CLPPs. The most variable utilization rate was measured for the carbohydrates (41 %), carboxylic acids (25 %) and amino acids and amines (17 % each) carbon guilds. QuadrisR affected non-target soil bacteria by influencing on their metabolism and capacity to utilize carbon sources, hence leading to disturbance of the soil nutrient cycling and negative influence on soil health.

Acknowledgements: *The study was funded by the BMES-NSF - (Grand D11/6-December 2017)*

POSTER SESSION **E&SD-6**

EFFECT OF TWO ASTERACEAE SPECIES ON PLANT PATHOGENIC FUNGI

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Keywords: *plant extracts, Centaurea finazzeri, Artemisia absinthium, phytopahtogenic fungi*

Extracts, essential oils, and other compounds with plant origin are proved to exhibit biological activity against plant fungal pathogens in vitro and in vivo and recently are more extensively surveyed as potential fungicides. Members of Asteraceae family are among the most promising plant species in this respect. Here we report the effect of two Asteraceae species – one limited distributed (Centaurea finazzeri Adamović) and one widespread (Artemisia absinthium L.) on three economically significant fungal plant pathogens, possessing wide host range - Alternaria alternata, Fusarium oxysporum and Botrytis cinerea. Extracts of the studied species were analyzed for the content of bioactive compounds by GC/MS and TLC. Phenolic, organic and fatty acids, polyols, terpenes and flavonoids were detected. The effect of plant extracts of both studied plant species was evaluated in vitro based on their influence on the mycelium growth of the pointed three phytopathogenic fungal species.

Acknowledgements: *This work was supported/(partially supported) by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018*

E&SD-7 **POSTER SESSION**

SNAPSHOT OF THE BACTERIAL MICROBIOTA WITHIN THE GASTROINTESTINAL TRACT OF THE HONEYBEES FROM 4 DIFFERENT LOCATIONS IN BULGARIA

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Agricultural production strongly depends on pollinators, and among them one of the central roles is played by the honeybees Apis mellifera, which are negatively influenced by the anthropogenic pressure and industrialization of the agriculture. In order to partially assess the role of the anthropogenic factors on the health of the hives we performed an NGS-based metagenomics research of the eubacterial microbiota composition within the gastrointestinal tract of the honeybees from 4 different locations in Bulgaria which are differently influenced by human activities. Total DNA was isolated from the content of the stomach of the bees and intestines which were subjected to 16S rRNA metagenomics based on the V3-V4 region, and performed on the Illumina 2 x 150 paired end reads. Despite the fact that within the four locations the most predominant genera were Lactobacillus, Gilliamella, Bifidobacterium and Snodrassella, differences were observed not only in their relative abundances, but also in the total number of the bacterial species observed. A clear tendency of an inversely proportional dependence between the anthropogenic pressure and microbiota richness was observed.

Acknowledgments: *This study was funded by the Bulgarian National Research Fund under Grant No. KП-06-H26/8 from 17.12.2018.*

EFFICIENCY OF FOUR BULGARIAN ISOLATES OF ENTHOMOPATHOGENICNEMATODESOFGENUSSTEINERNEMA (RHABDITIDA: STEINERNEMATIDAE) AGAINST CERATITIS CAPITATA

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Entomopathogenic nematodes (EPNs) have their own symbiotic bacteria in their intestine. When they enter insect hosts the released bacteria depress insect immune capacity and protect nematodes and bacteria from cellular and humural host reaction. The entomopathogenic nematodes Steinernema feltiae (origin Kresna) and three strains of S. arenarium (origin Zemen, Rupite and Varna) were evaluated against last larval instar of the Mediterranean fruit fly Ceratitis capitata under laboratory conditions (13% humidity and To 21oC). 50 specimens of IV larval instar of medfly per strain were placed in 2 ml tubes filled with sand infested with 100 and 200 invasive juveniles of EPN and the same number for untreated control. Each specimen was checked on 24, 48 and 72 h after infestation.
The strain of S. feltie caused 96% mortality on the 24h and only 16% of them died as prepupae. S. arenarium strain "Varna" provided 72% mortality, respectively "Rupite" - 52 % and Zemen 49%. Mortality caused by S. arenarium strains took place after 48h and more medfly specimens were able to pupate. No one of the nematode isolates was able to infect the medfly pupae within the puparia.

Acknowledgments: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018". The authors thank Dr Panagiotis Milonas from Benaki Institute for initial laboratory population C. capitata and to Dr. Denis Gradinarov for EPN infestation material.*

E&SD-9 **POSTER SESSION**

LONG TERM EFFECTS OF SOIL FUMIGATION ON NEMATODE COMMUNITY IN A GREENHOUSE CONTINUOUS CYCLE OF PRODUCTION

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Effects of DMDS fumigation of soil on nematode communities was followed in 5 successive crops (cucumber-lettuce- lettuce- cucumber- cucumber) in greenhouse experiment. The trial was conducted in 3 variants: 1) DMDS – soil fumigant; 2) Nematicide used by farmer and 3) Untreated control. The replicates of each of the variants were randomly placed in 170 m2 of vegetable greenhouse established in 2007. Soil nematodes were isolated from four bulk samples per each replicate. After Identification to genus level specimens were separated in trophic groups and functional guilds. Impact of fumigation was estimated on the base of analysis of food web condition in 6 different samplings – one before and five after the treatment with DMDS.

Root knot nematodes and Bacterial feeders dominated in all variants before treatment and in 2th and 3rd variants after fumigation. There was no significant difference between dominant groups between variants with conventional nematicide and control. Total abundance rapidly fell down after fumigation with DMDS in variant 1st and only single bacterial feeders and root knot nematodes were isolated in some of the subsamples. Abundance of bacterial feeders recovered on the 4th month but diversity of other groups was recovered after the 6th month. On the other hand, abundance of gall nematode was significantly lower than other variants till a year after fumigation and four successive crops.

Food webs in whole trial area were characterized as N-enriched, with the prevalence of bacterial decomposition channels, low C:N ratio and disturbed. Stress in variant 1 after DMDS application corresponded with a depleted enrichment and degradation of food web conditions. In all following samplings food web conditions recovered and were similar to those in untreated plots. In plots treated with nematicide usual for farmer practice a winter degradation of food web conditions was observed at sampling 4. Generally a higher variability was recorded there and degradation of food webs were registered even the last sampling – a year and four months after beginning of the experiment. Final conclusion was that regardless pronounced negative effect of DMDS on the target group of root knot nematodes that fumigant had not long term effect on soil nematode communities in studied greenhouse area.

Acknowledgments: *This work was partly supported by the bilateral contract ISSAPP-CERTIS /01.06.2018 (80%) and by BNSF under contract № КП-06-Н-36/12 2019 (20%).*

BLOOD CELLS COMPOSITION OF DIFFERENT DEVELOPMENT STAGES OF CERATITIS CAPITATA

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Mediterranean fruit fly is is considered to be one of the world most destructive fruit pests because of its global distribution, wide host range and rapid dispersal. The insects have very effective defense system against different pathogens and parasites. The cellular immune response is realized by some types of circulating hemocytes. During study of immune answer of C. capitata to enthomopathogenic infestation difference in immune answer between prepupa and IVth instar larvae was observed. According to our knowledge, no investigation on immune cells structure of different development stages of the species was reported. The aim of present study is to follow changes of blood cell structure in all larval stages and imago of C. capitata.

Hemolymph of the hundred specimens of each instar larvae stage of laboratory population of C. capitata were taken by cutting the end of abdomen. Hemolymph of imago was obtained by centrifuging of punctured specimens. Hemolymph of each 5 specimens was were stained by Gimza's method on a slide. The hemocyte identification and counting was performed on micro photograph images taken with camera DP70 under microscope Olympus BX51. Stage values means and variances were analyzed by Kruskal-Wallis ANOVA and Tukey HSD test by pairs.

In early development stages I and II larval instar plasmocytes and granulocytes were less than 35% of all heamocytes. In IV larval instar plasmocytes decrease significantly as far as granulocytes increase their relative part more than 50%. No significant difference of hemocyte composition was observed between IVth larval instar and imago and female and male specimens.

Acknowledgments: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".*

E&SD-11

POSTER SESSION

ECOLOGICAL AND ECONOMICAL IMPORTANCE OF PHAEOPHYTA (BROWN ALGAE)

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Algae are the main primer producers in aquatic environments and they also constitute the first step of the food chain. Phaeophyta or the common name as "brown algae", is one of the important groups of eukaryotic marine algae which include chlorophyll a, chlorophyll c, abundant fucoxanthin, phycocolloid algin, and reserve food in the form of laminarin. Brown algae which are mostly filamentous or thalloid algae are multicellular marine species, ranging in length up to more than 100 meters and only a few species live in freshwaters. The importance of brown algae comes from both of their ecological and economic usages. The main areas of use of brown algae are food, agriculture, cosmetics, medicine, pharmacy, and industry. Alginic acid and alginates can be listed as the leading products obtained from brown algae. Since brown algae are very rich in nutrients, various forms of them are consumed all over the world. Especially species of Sargassum, Laminaria, and Focus are used as fodder for cattle and poultry. While Focus and Laminaria are rich in iodine, Macrocystis is a good source for increasing the fertility of the soil for agriculture. Brown algae are widely used in the pharmaceutical industry. For example, Ascophylum is used in the production of antibiotics and Laminaria for goiter medicines. Also, the composition of Laminaria is used to prepare medicines that prevent blood clotting. Alginates obtained from brown algae are beneficial for intestinal health. Besides, alginate polysacrites bind metal ions very well and reduce the absorption of heavy metals from the system. A specific acid called alginic acid, which is obtained from brown algae is used as a thickening agent in industries like baking, rubber, cosmetics, and the medical field. Brown algae, which are very rich in nutritional content and value, can create an important resource to meet the nutritional needs of people in the future due to their easy production and rapid development under suitable conditions. Nowadays, biotechnological studies on brown algae, which are of great ecological and economic importance, need to be developed and increased.

PHYTOCOENOLOGICAL STUDIES OF THE RARE COMMUNITIES OF SPIRAEA SALICIFOLIA IN RHODOPI MTS AND THEIR SIGNIFICANCE FOR VERTEBRATE ANIMALS

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Key words: *plant communities, field study, vertebrates, indicators for climate change*

The communities of Spiraea salicifolia are rare for Bulgaria and little is known about their composition, structure and significance for vertebrates. In this regard phytocoenological investigations as well as field studies about the presence of vertebrates were conducted. Totally ten phytocoenological relevés were made in the communities during the vegetation seasons of 2019 and 2020. Terrain work and further phytocoenological analysis followed Braun-Blanquet methodology. Ecological indicator values which characterize the ecological optimum of plant species along major environmental gradients were extracted and used as an effective way of characterization of the abiotic conditions of the studied vegetation. Biological (on the basis of Raunkiaer's life form) and chorological spectra were also constructed in addition to the ecological analysis and in order to give any basic information on the origin and history of this vegetation. The presence of vertebrate species in this vegetation was registered both through direct observations and traces of their activity. The habitat was studied in terms of its potential as a resource - food base and shelters. Species diversity of vertebrates was studied by the transect method through Shermann live traps and camera traps. The communities of Spiraea salicifolia are poor of species, acidophilic on wet terrains and with high vegetation cover. The boreal and subBoreal floristic elements prevail in relation to their relic origin in glaciation times. In the communities of Spiraea salicifolia the bank vole (Myodes glareolus) which is characteristic for the studied region was captured. There are data that climate change could lead to an increase of the calamities in this species, causing damage to plant communities as well as an increased risk of hantavirus in humans to whom they are carriers. The Balkan wall lizard (Podarcis taurica) was also registered in the habitat by direct observation. The species is found in Bulgaria mainly up to 500 m above sea level, but in the present study it was registered at 1220 m above sea level, which gives a reason to consider the species as a potential indicator for climate change.

Acknowledgments: *This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № ДО-230/06-12-2018).*

E&SD-13 **POSTER SESSION**

PRELIMINARY DATA ON THE ECOLOGICAL FEATURES OF THE COMMUNITIES OF SATUREJA PILOSA FROM CENTRAL BALKAN MTS.

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Satureja pilosa is a Balkan endemic species which forms open petrophytic communities with fragmented distribution on the territory of Bulgaria. These communities develop on rocky slopes and screes or occasionally gravel deposits mainly in Eastern Rhodope and Central Balkan Mts. As an aromatic plant the species has been investigated on its pharmacological activities. Its taxonomic status is widely accepted and its range is well known. However, the information on the ecology, phytosociology and syntaxonomy of its communities is limited. During the summer of 2020 almost all potential habitats in the lower parts of Central Balkan Mts were visited. Totally 25 phytocoenological relevés were made in rocky outcrops with high abundance of Satureja pilosa. Field work and further analysis followed the principals and methodology of Braun-Blanquet. Biological and chorological spectra (based on Raunkiaer's life forms and geographical range of species, respectively) were constructed in support to the preliminary syntaxonomical decision. The communities of Satureja pilosa are xerophytic with open vegetation structure on steep and sunny slopes. They are distributed on acidic substrates that originate from granite, gneiss, schist or migmatite rocks with high silica content. Their vegetation density is relatively low and the distribution patterns of the plants are mosaic. About 200 vascular plant species and subspecies and 10 bryophyte taxa take part in their composition. Most of the constituents cover about 1-5% of the examined sites. Exceptions are Satureja pilosa and Logfia arvensis, Sedum annum, Scleranthus perrenis in certain plots. These communities are strongly influenced by the Mediterranean climate. More than 1/3 of the taxa in their composition are sub-Mediterranean (including Mediterranean) elements. Balkan endemics and subendemics are about 8%. Syntaxonomically this vegetation probably belongs to a new Balkan alliance Saturejon pilosae with arguable affiliation to the order Stipo pulcherrimae-Festucetalia pallentis of the class Festuco-Brometea. Satureja pilosa is intensively collected by local people and it is not clear whether and how this influence its communities. Their ecological importance in preventing slope erosion however is undoubted.

Acknowledgements: *The research is carried out under the project: "Ecological and floristic structure of the communities of Satureja pilosa sensu lato in Central Balkan Mts." – Contract №80.10-146/23.04.2020 г.*

STRUCTURAL CHANGES IN CHLOROPLASTS OF ELODEA NUTTALLII (PLANCH). H. ST. JOHN LEAVES IN RESPONSE TO CADMIUM

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The effect of cadmium (Cd) on the ultrastructure of plastid apparatus in leaves of Elodea nuttallii (Planch). H. St. John was investigated in experimental conditions using transmission electron microscopy (TEM). The experimental plants were cultivated in a green house environment after preliminary adaptation. The plants were exposured to Cd during 5 days period wih concentrations 1 mg/l and 3 mg/l, consistented with previous studies. Cultivated plants under condition of the experiment without Cd were used as the controls. The results showed that at concentration 1 mg/l Cd chloroplasts have a well organized internal membrane system relative to the control, but fragmentation and weak swollen thylakoids were observed. Increasing of cadmium concentration at 3 mg/l Cd show hardly affected plastid ultrastructure, as swollen thylakoid membrane and reduction of grana stacks. The established structural changes of photosynthetic apparatus of E. nuttallii are analyzed and compared to previous researches of cadmium toxicity on E. canadensis under the same conditions.

E&SD-15 **POSTER SESSION**

PACIFIC OYSTER INVASION ALONG BULGARIAN BLACK SEA COAST

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In the last ten years, finding non indigenous, feral oysters in natural habitats (outside shellfish farms) of the Bulgarian Black Sea coast has attracted the interest of zoologists, hydrobiologists, and ecologists. In this regard, the focus of our research is 1) to document and present in detail all data on live oysters found so far, identified by morphological features such as Pacific oyster Magallana gigas (Thunberg, 1793) (= Crassostrea gigas) and 2) to confirm the species identity of this invasive oyster by molecular methods. As a result of our study, 8 localities of M. gigas were identified along the Bulgarian sea coast. These sites include, from North to South, the seawaters of Bulgarevo Village, Kavarna Town, Sts. Constantine and Helena Resort, Varna Town (Karantinata locality), Burgas Town (Sarafovo district, Port of Bourgas Shipyard and ship-repair factory), Cape of Maslen Nos, and Port of Kiten Town. Oysters were found attached to a hard substrate like boulders and bedrock, breakwater tetrapods, mussel collectors, a hull of a boat, and props of a quay. Morphological data were collected for 82 specimens of M. gigas from all localities. Shell height varied between 1.84 and 12.46 cm. In addition to single isolated specimens, were found for the first time two relatively small wild colonies of M. gigas in the seawaters of Burgas Town and Kiten Town, included 19 and 10 specimens, respectively. The pathways for the introduction of Pacific oysters in Bulgarian Black Sea waters were also commented. The information given in this article can be used as a basis and reference point for future targeted studies on this species of mussel on Bulgarian seacoast. Also, this can be used in monitoring's projects and projects related to biosecurity, because the long-term effects of current oyster invasion in our Black Sea-habitats at this moment are unknown.

POSTER SESSION **E&SD-16**

MONITORING AND EVALUATION OF CAVE-DWELLING BAT COLONIES FOR EUROPEAN BAT LYSSAVIRUSES SCREENING. FIRST SURVEY IN BULGARIA

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Rabies is a global neurological zoonotic disease in mammals and humans and one of the oldest human diseases, causes acute encephalitis and cerebral damage. The rabies viruses belong to the Rhabdoviridae family, genus Lyssavirus. It was found that bats are reservoirs of all lyssaviruses except Mokola and Ikoma. Five Lyssavirus species have been identified in European bats, as most common are European bat 1 lyssavirus (EBVL-1) and European bat 2 lyssavirus (EBVL-2). 33 bat species out of a 35 known in Europe are found in Bulgaria. Among them are all 13 species in which EBVL-1 and EBVL-2 and rarely other viruses, such as Bokeloh bat lyssavirus (BBLV), Kotalahti bat lyssavirus (KBLV) and Lleida (LLEBV) have been detected in other European countries. The aim of this study is to select the appropriate cave-dwelling bat colonies for European bat lyssaviruses screening. 14 Bulgarian caves (6 in North and 8 in South Bulgaria) and 3 species of cave-dwelling bats (Rhinolophus ferrumequinum, Myotis myotis and Myotis blythii) were chosen. These species are form a large summer colonies that can be trigger the spread of lyssaviruses among their members. In the first year of the project a winter monitoring was carried out in 4 caves in North Bulgaria (Orlova chuka, Zorovitsa, Emenska, and Magurata) and 1 cave in South Bulgaria (Ruzhishka) and also a summer monitoring was carried out in 2 caves (Orlova chuka and Zorovitsa). Significant winter colonies of Rh. ferrumequinum were found in the caves Orlova chuka, Magurata and Ruzhishka. Significant summer colonies of M. myotis and M. blythii were found in Orlova chuka and Zorovitsa. For this reason, last season sampling was carried out in 3 of the studied caves (Orlova chuka, Zorovitsa and Ruzhishka). The forthcoming analyses will confirm or reject the presence of rabies viruses in cave-dwelling bat species in Bulgaria. This will contribute to evaluation of the risk and protection of the health of humans, as well as the health of domestic and wild animals.

Acknowledgements: *Supported by Research Grant КП-06-Н31/14 „The role of the cave-dwelling bats as a vector and reservoir of the European lyssaviruses in Bulgaria", Bulgarian National Science Fund*

E&SD-17

POSTER SESSION

DOES THE STEREOTYPIC BEHAVIOUR IN A MALE EURASIAN BROWN BEAR (URSUS ARCTOS ARCTOS) IN CAPTIVITY IS INFLUENCED BY VARIOUS STIMULI?

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Stereotypic behaviour in wild animals kept in zoos is one of the major problems for their captive housing and welfare. Stereotypies are repetitive behaviours, invariant in form, and have no apparent functional consequences. Members of the family Ursidae often exhibit stereotypic behaviour in captivity. In this study we present our observations on the occurrences of stereotypic behaviour in a male Eurasian brown bear (Ursus arctos arctos), kept in Sofia Zoo under the influence of various stimuli. Usually the male bear displays persistent stereotypic behaviour "pacing" combined with "head-tossing". To try to reduce the duration and frequency of this behaviour, olfactory and visual stimuli were provided, as well as nutritional environmental enrichment. The influence of the visitors on the demonstration of stereotypic behaviour was also tested. The results showed that the nutritional enrichment has the greatest importance for minimizing the stereotypic behaviour and that the influence of the visitors does not significantly affect the duration and frequency of stereotypic behaviour. Studies of stereotypies and ways of minimizing them in wild animals in captivity are important for their welfare and conservation value. Key words: stereotypic behaviour, brown bear, environmental enrichment, captivity

Acknowledgements: *This study was done thanks to the Scientific Research Fund of Sofia University "St. Kliment Ohridski", Project grant № 80-10-87/15.04.2020 and the staff of Sofia Zoo.*

POSTER SESSION **E&SD-18**

CHARACTERISTIC OF AIRBORNE MICROBIOTA IN HIGHLY URBANIZED LOCATIONS IN SOFIA CITY, BULGARIA

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Airborne microbiota (AM) associated with particulate matters (PM), are widespread in the lower atmosphere layers. Since some airborne microorganisms have pathogenicity, they can lead to a wide range of diseases in human and other organisms. The intend of this study highlights the problem of composition and characteristics of AM with size-distribution, diversity and quantity in central urban site in Sofia (Faculty of Biology) during the summer season. Six-stage Andersen Impactor was used for the quantitative analysis. This sampling device collects particles in the range from > 7 μm to 0.65 μm. This can be used as a visualization of particles penetration in respiratory tract. The quantitative analysis showed the concentration ranging from 101 CFU/m3 to 495 CFU/m3 for bacteria, and respectively 298 to 1482 CFU/m3 for fungi. The tendency mentioned was that the predominant airborne bacteria were identified on stage 1 (>7 μm). On the other hand, the prevalent airborne fungi usually had the highest concentration on stage 3 (3.3-4.7 μm) and stage 4 (2.1-3.3 μm). The increasing of the microbial concentrations is closely related with increasing of PM concentration into the air. The analysis of microbiota has a significant contribution in air quality analysis of Sofia city and the present study can be an important stage in the strategy for prevention of human health and better air quality.

E&SD-19

POSTER SESSION

BIOREMEDIATION OF ACID DRAINAGE WATERS (AMD) BY LABORATORY-CONSTRUCTED PASSIVE SYSTEM

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A major environmental concern to the mining industry, both during the operational period and after closure is the generation of acidity, resulting from the oxidation of sulphide mineral wastes, catalyzed by acidophilic microorganisms. Acid drainage waters (AMD) in sulfide deposits are a serious environmental problem and their formation is associated with the activity of the hemolitotrophic microflora, including bacteria oxidizing reduced sulfur compounds and ferro ions. Due to its damaging effects several strategies for control and remediation of AMD have been developed. One of the most efficient method for bioremediation of such waters is their treatment in natural or constructed passive systems. The main objective of the research was the treatment of acid drainage water samples by means of a permeable multibarrier in a laboratory-constructed passive system. The treated samples originated from Kurilo uranium deposit and Tsar Asen sulfide deposit, Bulgaria. The multibarrier had a volume of about 0.5 m^3 and was filled by a mixture of solid biodegradable organic substrates and crushed limestone and was inhabited by a microbial community consisting mainly of sulphate - reducing bacteria and other metabolically interdependent microorganisms. The taxonomical profile and quantitative share of the established microflora into the passive system was strictly monitored. An efficient removal of the heavy metals was achieved at residence times within 28-46 hours depending on metal concentrations. The removal of the pollutants was due to different mechanisms such as chemical neutralization, microbial dissimilatory sulphate reduction, sorption and accumulation by means of living and dead plant and microbial biomass, sorption by some inorganic sorbents such as clays, hydroxides, carbonates, etc., as well as by bacterial oxidation of Fe^{2+} and Mn^{2+}, followed by the precipitation of the of the oxidation forms Fe^{3+} and $M^{4+,}$ mainly as $Fe(OH)_3$ and MnO_2. Due to the described treatment the concentrations of the heavy metal ions in the AMD water samples were reduced to permissible levels for industrial application of the waters.

RANKING OF PRIORITY POLLUTANTS IN SURFACE WATERS OF ISKAR RIVER CATCHMENT – APPROACH FOR OPTIMIZATION OF POLLUTION CONTROL

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Keywords: *ranking of pollutants; priority substances; Iskar River; metals*

The determination of the ecological state and the assessment of the pollution in the surface water bodies of the Iskar river valley are of key social and economic importance due to the role of the hydroecosystem for the functioning of the technological water cycle of Sofia. The direct dependence of all elements in the cycle on water quality and the state of the ecosystem makes an adequate control of the degree of pollution and the risk of various pollutants extremely urgent. Prioritization/ranking of pollutants is an approach that facilitates the assessment process by providing information on the level of risk of the presence of each pollutant. In this approach, to the individual pollutants are given ranks/measures depending on the measured concentrations in water, taking into account the specific characteristics of the pollutant itself - ecotoxicological data, tendency for bioaccumulation and biomagnification. In the present study, 13 monitoring sites are selected in the Iskar river basin valley. The sites are directly related to the technological cycle of the city of Sofia through water abstraction of drinking water or discharge of waste streams, as well as some main tributaries of the main river flow. Data on the content of metals, metalloids and some organic pollutants in 2018 and 2019 are compared with the environmental quality standards (EQS). The highest levels of risk in the Iskar valley are found for trichloromethane, lead and cadmium. The highest value has been registered for the organic pollutant, but only once, while with regard to lead there is a constant risk for all sites - it is registered in both years at a significant number of sites. At the catchment level, the identified levels of risk from the presence of lead in surface waters are the most significant and the lead content is the risk factor with the highest potential for damage to the ecosystem and human health.

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

E&SD-21 **POSTER SESSION**

SPECIATION OF U AND TH IN NATURAL WATERS AFTER SPECIFIC EXTRACTION WITH IONIC LIQUID AND ICP-MS/ GAMMA SPECTROMETRY MEASUREMENT

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Uranium is a widespread natural radionuclide causing adverse physiological effects after entering the human body. In nature it can be found both in geological and aqueous environment. Because of its radiotoxicity the determination of its total concentration in natural water resources has been studied extencively. Although the knowledge of uranium speciation in natural waters is essential for predicting the migration and health effects, this question has not been studied in detail. Furthermore, the daughter radioisotopes of 238U - e.g. 234Th, have very different environmental migration properties which needs to be conside. The chemistry of U and Th in natural waters can be very complicated and is mainly controlled by pH, redox potential, and type of available complexing agents, such as carbonates, phosphates, and sulfates, etc. Therefore, the aim of this research is to study the formation of different U and Th chemical species in water samples in dependence on pH, the presence of complexing agents and/ or buffer solution. Applying HYDRA calculation software possible chemical species of the two radionuclides in accordance to experimental conditions were generated. The obtained theoretical models were used as a basis for optimization and development of an analytical procedure for extraction of specific chemical species of U and Th with the ionic liquids 1-methyl-3-octylimmidazolium saccharinate from water solutions. After quantitative analysis with ICP-MS/ Gamma spectrometry measurements, the extraction efficiency was determined and assumptions about the mechanisms of exchange of specific species under varying experimental conditions have been made.

Acknowledgements: *This work is part of project BG05M2OP001-1.002-0019:„Clean technologies for sustainable environment – water, waste, energy for circular economy" (Clean&Circle) 2018 – 2023, for development of a Centre of Competence, financed by the Operational programme "Science and Education for Smart Growth" 2014-2020, co-funded by the European union through the European structural and investment funds. The financial support of INFRAMAT (Modern research infrastructure in support of science, culture and technological development) for upgrade of the scientific equipment is acknowledged. INFRAMAT is part of the National Roadmap for Scientific Infrastructure and is financially supported by the Bulgarian Ministry of Education and Science (Contract D01-155/28.08.2018 and D01-284/18.12.2019).*

BIODRYING OF MUNICIPAL SOLID WASTE - SUSTAINABLE TECHNOLOGY FOR WASTE TREATMENT AND MANAGEMENT

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Keywords: *biodrying; waste management; aerobic transformation; RDF*

Biodrying is a new method of biomass waste treatment, which is developed in recent years as an effort to solve problems of waste management. Biodrying is a pre-treatment technology that uses natural and forced aeration along with the heat generated by natural aerobic biotransformation of organic matter to reduce the water content of the waste. The technology has attracted extensive attention and undergone rapid development, especially for its potential to reduce the amount of waste for landfilling and to provide a substitute for the conventional fossil fuels – refuse derived fuel (RDF). It is considered, that because of the presence of biogenic fraction in RDF, greenhouse emissions of the burned fuel are partially carbon neutral, which can reduce the global CO_2 emissions. A disadvantage of RDF fuels is the need of temporary storage and transportation of the fuels, which can lead to undesired problems like spontaneous ignition and leachate production, with the consequences of health effects of the population and environmental contamination. The biodrying is part of mechanical biological treatment in Municipal Enterprise for Waste Treatment – Sofia. The reduction of moisture content in the biodrying process includes two main steps. The first one is the evaporation of liquid water and the second one is the removal of water vapor by the airflow through the material. Aerobic biodegradation of organic matter is the main process, in which heat for evaporation is released. The energy needed for the heat production is obtained from the metabolic processes of aerobic microorganisms. Biodrying technology that removes water by microbial activities has a good potential for pre-treatment of organic wastes with high water concentration.

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

E&SD-23 **POSTER SESSION**

SWOT ANALYSIS OF TECHNOLOGIES APPLIED FOR THERMAL TREATMENT OF MUNICIPAL SOLID WASTE

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Keywords: *Municipal solid waste, Waste-to-Energy, thermal treatment, SWOT analysis*

Sustainable management of rapidly growing quantities of Municipal solid waste (MSW) is one of the crucial challenges facing the modern world. MSW management system cannot be sustainable only with one technical solution, but an integrated approach is needed. Thermal treatment of waste being a main part of Waste-to-Energy (WtE) field is essential for integrated waste management systems not only because the potential to reduce the quantity and volume of MSW, but also because the prospective for utilizing the energy potential of waste. The aim of this study is to perform a basic SWOT analysis of thermal waste treatment technologies, focused on future opportunities for selection, realization, environmental efficiency, perception by social communities in regional, national and global scale. An important focus of this analysis is the possibility of creating and implementing softening intelligent solutions in the harmony of thermal waste treatment technologies and clean environment. There are variety of thermal treatment technologies that can be used to convert waste into energy. The conventional approach for energy production from waste is direct combustion or incineration. Typical combustion plant is equipped with grate on which the waste is burnt with the heat generated being captured in a boiler. Advanced methods for deriving energy from waste by thermal treatment are pyrolysis, gasification and plasma-based technologies. Pyrolysis involves heating the waste material in the absence of oxygen. Depending on the temperatures, a range of solid, liquid, and gaseous products are formed. Gasification involves partial combustion of waste in a limited supply of oxygen to produce a combustible gas as a product. Plasma-based technologies enables high destruction efficiency. There are number of issues that should be considered, including strengths and weaknesses of each of these technologies for the right selection. Therefore, a SWOT analysis is required, as a part of strategic planning process for implementation of facility for thermal treatment of waste.

Acknowledgment: *This document was supported by the Grant № BG05M2OP001-1.002-0019: "Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy", financed by the Science and Education for Smart Growth Operational Program (2014-2020) and co-financed by the EU through the ESIF.*

POSTER SESSION **E&SD-24**

URBAN GREEN SPACE AND ITS IMPACT ON HUMAN HEALTH

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Open urban spaces play an important role in the lives of modern city residents. Their aesthetic importance helps determine the characteristic of suburbs, and ameliorates the built-up character. Living in a city provides many opportunities for residents to connect with each other in many different ways. In order to take advantage of all the benefits that the big city can provide, it is especially important to design the urban environment, using urban planning solutions in the interest of the citizens. A well-thought-out design and healthy environment contribute to increasing the positive indicators of life. This relates both to a person's physical health and to the development of a sustainable social environment, affecting people's behavior and usage of outdoor spaces. Through the tools of urban planning, it is necessary to create an environment that includes all the requirements for a modern city, and pay attention of the thermal, visual and sound comfort, as well as the needs of activities and entertainment of the residents. Open urban spaces should be planned in terms of the daily life that takes place in them. Quality urban design is necessary to promote social cohesion, create living and safe spaces, and maintain a sustainable living environment. It is extremely important in their design to preserve the identity of the settlement. Both the architectural ensembles and the design of urban spaces have a leading role in determining the appearance and character of the city, respectively, to define the opportunities that the place can offer to its users.

E&SD-25

POSTER SESSION

VALUE OF URBAN GREEN SYSTEMS IN PARIS AND SEVLIEVO CITIES

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Cities themselves represent both problems and solutions to the challenges of sustainability for an increasingly urbanized world. Society is constantly changing the ecosystems that underlie the understanding of natural capital, embodying the civilizational nature of our planet. Sevlievo is a small sustainable town, European capital of pumpkin that in 2012 turned its old barracks into a beautiful urban park system. On the other hand, Paris has a rich biodiversity with diverse natural flora and vegetation. Since the 10th century, Paris has been one of the main cities of France: it is located in the heart of a rich agricultural region (like Sevlievo), with royal castles, abbeys and a cathedral; in the 12th century, with the opening of the Sorbonne, the city became one of the first university centers as well as an arts center. In such an urbanized world, the role of green systems in cities is indispensable in providing important ecosystem services for human well-being. Still, the value of urban green systems is overlooked in urban environment and green infrastructure management decisions. The urban green systems of Paris and Sevlievo (on the order of every other city in the world) have the following types of value – Ecological, Economic and Social. Increasing the sustainability of cities, require reducing the degradation of their ecosystems. Here the concept of sustainable development intervenes, meeting the needs of the present without compromising the capabilities of future generations.

A SURVEY OF A BIOINSECTICIDE FOR CONTROLLING INSECT PESTS UNDER LABORATORY CONDITIONS

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Several Lactobacillus species are accepted as microorganisms with Qualified The purpose of this study was to evaluate the efficiency of the mycoinsecticide Naturalis® (BioIntrachem, Italy) against field-collected adults of the grey maize weevil, Tanymecus dilaticollis, the cereal leaf beetle, Oulema melanopus, and the multicolored Asian lady beetle, Harmonia axyridis. Naturalis® was tested in six concentrations (2.3 × 102 - 2.3 × 107 conidia/ml) in glass Petri dishes. The median lethal concentration (LC50) after ten days of fungal treatment ranged from 2.0 × 103 conidia/ml to 2.1 × 106 conidia/ml for the different species. Naturalis® was most efficient against T. dilaticollis adults causing significantly higher mortality at all concentrations tested compared to the control treatment (water). The possibility of using mycoinsecticide to control coleopteran pests in Bulgaria is discussed.

Acknowledgements: *This study was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".*

EVALUATION OF IN VITRO CYTOTOXIC AND PROAPOPTOTIC PROPERTIES OF MEDICINAL PLANT COTINUS COGGYGRIA SCOP. TOWARDS A375 MELANOMA SKIN CANCER CELLS

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Malignant melanoma is a highly metastatic type of skin cancer that is characterized with a low response to available chemotherapeutics and plenty of efforts are directed to the finding of novel, more effective and less toxic drugs. Medicinal plants have promising antineoplastic potential due to their large range of chemical and biological variety. The present study was undertaken to explore the cytotoxic capacity of crude aqueous ethanolic extract from leaves of Cotinus coggygria and its chloroformic and aqueous fractions on human melanoma cell line A375. Normal dermal cell line BJ was used in the analysis to assess the selectivity in the action of plant substances. The cytotoxic properties of C. coggygria were examined through MTT cell viability assay performed in parallel with cell morphology observation under light microscope, and Trypan blue test after 24 h treatment period in a wide range of extract and fractions concentrations. The obtained results revealed statistically significant selective cytotoxic activity of plant substances against A375 cancer cells with the highest inhibitory effect on cell viability detected for the chloroformic fraction of the extract. Further investigations of the chloroformic fraction proapoptotic potential against A375 cells by fluorescent microscopy observation after staining with Annexin V and propidium iodide detected considerable increase in the number of cells in early and late apoptosis after treatment. Future studies will be focused on more detailed assessment of anti-melanoma therapeutic potential of medicinal plant C. coggygria.

Acknowledgements: *This work was supported by the Bulgarian National Science Fund, Ministry of Education and Science, contract KP-06-M21/5. The authors are grateful to Vemo 99 Ltd. for providing the extract of Cotinus coggygria.*

ELECTRO-ASSISTED REDOX SENSITIZATION OF COLON CANCER CELLS TO SN38 VIA INDUCTION OF APOPTOSIS/ FERROPTOSIS AND UPREGULATION/DOWNREGULATION OF ABC TRANSPORTERS

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Over the past few years, more experimental data suggest that the regulation and control of cellular redox-status during the chemotherapy and/or radiotherapy of malignancies could be a critical to the development of new and effective therapeutic strategies. The explanation is a long-known fact – one of the basic mechanisms of the anti-tumor activity of conventional chemotherapeutics is related to redox-signaling influence and induction of oxidative stress into the cancer cells, leading to apoptosis and/or necrosis. Nowadays for a number of generally used anticancer drugs has been reported oxidative stress-related mechanism of action. On other hand, the development of multidrug resistance to chemotherapeutics remains one of the major challenges in the treatment of cancers. Anticancer drug resistance is advanced by triggering a wide range of cellular mechanisms, including: drug inactivation of the active drug substances; modification of drug target; quantitative reduction drug uptake; increased drug efflux; activation of different detoxifying/neutralizing systems; activation of DNA repair mechanisms; overcoming drug-induced apoptosis/cell death and other. The aim of this study was to elucidate the possibilities for sensitization of colon cancer cells to the chemotherapeutic drug SN38 and investigation of mechanism of action after combination with electroporation (EP). In conclusion we found that the combine treatment of colon cancer cells with EP and SN38

affects cell viability and cytoskeleton integrity. This effect was accompanied by: (1) high production of intracellular superoxide and hydroperoxides and depletion of glutathione; (2) changes in the expression of membrane ABC transporters – upregulation of SLCO1B1 and retention of SN38 in the cells; (3) increased DNA damages and apoptotic/ferroptotic cell death. In conclusion, the anticancer effect of combined treatment is related to changes in the redox-homeostasis of cancer cells, leading to activation of signal pathways for induction of cell death via apoptosis and/or ferroptosis and influence on multidrug resistance proteins. Thus, the electroporation has a potential to increase the sensitivity of cancer cells to conventional anticancer therapy with SN38.

Acknowledgement: *This work is partially supported by the Bulgarian Ministry of Education and Science under the National Research Program "Young scientists and postdoctoral students" (granted to SS for performing of ELISA assays) (PMS№ 271/29.10.2019).*

DETERMINATION OF INTERLEUKIN 6 (IL-6) SECRETED FROM PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

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Keywords*: PBMC, IL-6, culture media, ELISA*

In the recent years the intrauterine administration of autologous peripheral blood mononuclear cells (PBMC) was proposed as an effective approach to improve embryo implantation in patients with repeated IVF failures. The isolated PBMC mainly consist of T lymphocytes, B lymphocytes, and monocytes. These cells are sources of chemokines such as IL-6, IL-1β, and TNF-α that promote the invasion of the trophoblast into the maternal tissue. The Th-2 cells may facilitate a more permissive immune-inlammatory profile for implantation. Actually the Th-2 integral modulator IL-6 is known to favour the embryo implantation. However there is no exact information on the secreted levels of IL-6 from PBMC in the literature.

The aim of this study was to investigate whether PBMCs secrete IL-6 when cultured at physiological conditions (37ºC) and to measure its concentrations after 2 hours of incubation.

Blood samples were obtained from 10 women patients of Nadezhda Women's Health Hospital after signing written informed consent. For PBMC isolation, 9 ml of heparinised whole blood from each patient were processed by density gradient centrifugation using Pancoll (P04-60100, Pan-Biotech) for 25 minutes at 400g. The buffy coat of PBMCs were collected and washed with phosphate buffer saline and counted in Makler counting chamber. A total of 10x106cells were cultured in 500µl StemLife™ basal medium (LS-1060, Lifeline Cell Technology) for 2 hours at 37ºC. Quantification of IL-6 in the PBMC medium was measured by sandwich enzyme-linked immunosorbent assay (ELISA) (E-EL-H0102, Elabscience) according to the manufacturers' instructions. Descriptive statistics was conducted using SPSS v.21.

No IL-6 was detected in the fresh LL media control. The presence of IL-6 was confirmed in all PBMC samples. The IL-6 concentrations in the PBMC culture media after 2 hours incubation varied between 4.71 pg/ml and 8.73 pg/ml with range of 4.02 pg/ml. The mean IL-6 secreted from the PBMCs was 7.14 pg/ml ± 1.86 pg/ml SD.

In conclusion, this study shows that PBMCs secrete IL-6 in detectable amounts in the culture media. Furthermore, the measured IL-6 concentrations could be used as an indicator for the functionality of the PBMC used for intrauterine applications.

SEROEPIDEMIOLOGICAL AND MOLECULAR IDENTIFICATION OF THE AGENT OF Q FEVER IN CLINICAL SAMPLES FROM HUMANS AND CATTLE IN BULGARIA DURING 2018 – 2019

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Introduction: Q-fever, caused by Coxiella burnetii, a type of bacterium found worldwide, included Bulgaria. The interest in it is growing steadily due to unclear yet facts about the nature of the epidemic process manifested in humans, often with no apparent connection to probable sources of infection, self-limiting sporadic outbreaks with variable intensity; similar clinical symptoms with other diseases, options for chronification, etc. The aim of our study is to summarize the last two-year (2018-2019) experience of NRL "Cell cultures, rickettsia and oncogenic viruses" NCIPD, Sofia and BAS - NDRVMI- Sofia the diagnosis of C. burnetii from clinical samples of Bulgarian patients and cattle. Materials and methods: Between January, 2018 until December, 2019 we tested a total of 345 clinical samples (sera and blood) of ambulatory and hospitalized in different hospitals of the country patients with symptoms of atypical pneumoniae, fever of unknown origin, variety of cardiac complications, hepatitis and others as well as 200 cattles from different regions of the country. Diagnostics of C. burnetii in clinical samples was performed by indirect ELISA and by the conventional PCR for the detection of the sodB C. burnetii gene using the specific primers CB1 and CB2. Results: The indirect enzyme immunoassay analysis (ELISA) of the tested clinical samples obtained from patients with various symptoms and cattle have been proven anti-C. burnetii positive IgM antibodies. A relatively high percentage affected people were in active age 40 – 50 years old (61.54%). When examined serum samples positive IgG result was proven at 33.54%. A positive PCR signal of C. burnetii-DNA was obtained. The male/female ratio was 1.4. No seasonality or trend in reported cases was observed. Conclusion: Because of the experience in the NRL and BAS, was developed diagnostic scheme including complex methods to improve early laboratory diagnosis of C. burnetii, allowing taking proper treatment of suspected patients.

Acknowledgements: *This work was supported by the Bulgarian National Science Fund under Grant KP-06-H33/3 from13/12/19.*

MORPHOLOGY OF COMPOSITE HBEST1/POPC AND HBEST1/ SPHINGOMYELIN MONOLAYERS

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Human bestrophin-1 (hBest1) is a transmembrane Ca^{2+} - dependent anion channel, associated with the transport of Cl−, HCO_3- ions, γ-aminobutiric acid (GABA), glutamate (Glu), regulation of cell volume and retinal homeostasis. Mutations in the corresponding gene lead to retinal degenerative diseases, defined as Bestrophinopathies, but recent research suggests that hBest1 is also connected to neurodegenerative pathologies, such as Alzheimer's and Parkinson's diseases. The lipid environment is essential for the proper function and regulation of hBest1. Using Brewster Angle Microscopy (BAM) we are able to observe differences in the morphology of composite hBest1/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) or hBest1/sphingomyelin (SM) monolayers, under specific surface pressure values and constant temperature. In order to throw a light on hBest1 channel function, we also studied the effects of calcium ions, glutamate (Glu) and γ-aminobutirate (GABA) on mixed hBest1/ lipid films.

Acknowledgments: *This work was financially supported by grants from Bulgarian Science Fund (№ KP-06-N23/7, 18.12.2018) and by the Bulgarian Ministry of Education and Science under the National Program for Research "Young Scientists and Postdoctoral Students" (PMC № 577 / 17.08.2018).*

MB&BT-6 **POSTER SESSION**

THERMODYNAMIC FEATURES OF BLOOD PLASMA DERIVED FROM PATIENTS WITH NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases (NDDs) are an important medical and social problem as their relative share has been steadily increasing in recent years. Despite advances in diagnostic and therapeutic methods, there are still no reliable biomarkers identifying the complex pathways contributing to the pathology and early diagnosis of NDDs. Therefore, the development of new approaches for early diagnosis of these diseases is of great importance. Differential scanning calorimetry is a technique highly sensitive to the protein concentration and conformation, as well as to the interactions of proteins with other molecules. In the recent years, this was exploited in order to identify disease specific changes in the plasma proteome that might serve as biomarkers. The main objective of this work is to identify specific features in the thermodynamic behavior of blood plasma derived from NDDs patients compared to healthy ones. The obtained results reveal that for most of the studied NDD cases the plasma thermograms exhibit shift of fibrinogen and albumin assigned transitions to higher temperatures (by 1–2 °C) and increased values of the temperature weighted average center of the thermogram, as compared to those of healthy individuals. Two groups of NDD thermograms are defined, based on the derived thermodynamic parameters. For the first group the application of Wilcoxon non-parametric test shows statistically significant difference in globulin assigned region compared to the healthy controls, whereas for the second group difference is also detected for the albumin assigned transition and in addition, a new peak at ca. 60 °C is detected. The deconvolution analysis reveals alteration in the enthalpy and the midpoint temperature of the constituent components contributing to the albumin and globulin assigned transitions of all NDD thermograms as compared to that of controls . In summary, our data demonstrate that the calorimetric profiles of blood plasma derived from patients with NDDs exhibit specific calorimetric features. Their clinical potential as biomarkers needs further in-depth studies.

Acknowledgement: *This work is supported by the Bulgarian Ministry of Education and Science under the National Research Programme „Young scientists and postdoctoral students" (DCM 167/19.03.20, M.G.)*

ASSOCIATION BETWEEN CD4+ AND CD8+ CELLS IN THE ENDOMETRIUM OF RIF PATIENTS

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OBJECTIVE: It is known that human endometrial receptivity is affected by T cell subpopulations and CD4+ (T-helpers) / CD8+ (T-killers) ratio. To ensure successful embryo implantation, an active state of maternal immune tolerance mediated by regulatory T cells in the endometrium is essential.
The purpose of this research was to quantify and compare endometrial CD4+ (T-helpers) and CD8+ (T-killers) cells during the mid-luteal phase in women, with repeated implantation failures (RIF) using immunohistochemistry.
MATERIALS AND METHODS:The endometrial biopsies were obtained from 36 women with RIF (at least 3 unsuccessful attempts) during the mid-luteal phase of the cycle. The presence of CD4+ and CD8+ positive cells was evaluated by immunohistochemistry using rabbit polyclonal CD4 antibody (E-AB-65682, Elabscience) and rabbit polyclonal CD8 antibody (E-AB-60717, Elabscience) and visualized by Novolink Max Polymer Detection System (RE7280-K, Leica). Results are presented as percentage of CD4+ or CD8+ positive cells of the total number of stromal cells.
Statistical analysis was performed by Spearman's correlation test using SPSS v.21 (IBM Corp., Armonk, NY, USA). $P<0.05$ was considered to be statistically significant.
RESULTS: CD4+ and CD8+ cells were found in all endometrial samples. The percentage CD4+ cells and CD8+ cells in the endometrial stroma varied between 0.01% and 4.05%, and between 0.16 and 1.52%, respectively. The mean CD4+ percentage and CD8+ percentage were 0.58% ± 0.84% and 0.45% ± 0.30%, respectively.
Spearman correlation analysis showed significant positive correlation between the percentage of CD4+ and CD8+ cells in the endometrial samples of the studied RIF patients ($r=0.52$; $p=0.01$).
CONCLUSIONS: Our study showed that CD4+ cells (T-helpers) are more abundantly distributed than CD8+ (T-killers) in the endometrial stroma of RIF patients during the mid-luteal phase. The observed association between CD4+ and CD8+ could provide a valuable data on the endometrial receptivity.

MB&BT-8 **POSTER SESSION**

CALORIMETRIC MARKERS FOR IGD MULTIPLE MYELOMA DETECTION

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Key words: *IgD Multiple Myeloma, Differential Scanning Calorimetry, blood serum Multiple Myeloma (MM) is malignant lymphoproliferative disorder, which exists in secretory and nonsecretory form.*

Specific characteristic of secretory MM is the production of monoclonal immunoglobulins – IgM, IgG, IgA, IgE or IgD. In the focus of this work is the rare isotype IgD MM associated with aggressive clinical course, presence of Bence Jones protein, osteolytic lesions, hypercalcaemia, amyloidosis. IgD MM affects younger population and has lower survival compared to other isotypes. In order to identify calorimetric markers we applied differential scanning calorimetry on blood serum samples from IgD MM patients and determined the thermodynamic parameters (temperature of denaturation and excess heat capacity of the major serum proteins). Pearson correlation coefficient, standardized Euclidean distance and metric similarity were used to quantify the differences in the shape and space between the control and IgD MM thermograms. Based on these parameters, two groups of calorimetric profiles are established: (i) the thermograms in group 1 resemble those of healthy controls, and the main calorimetric peak (assigned to albumin denaturation) is centered at 61-63 °C; (ii) for group 2 the main peak is shifted to higher temperatures (64-65 °C) and the albumin transition overlaps with that assigned to immunoglobulins. All cases in group 2 exhibit a sharp transition below 62 °C, which was previously found for only a few MM cases with secretion of monoclonal free light chains. In addition, the results show that for 50 % of the IgD MM cases (irrespective of the calorimetric group) the high temperature transition at 85 °C (due to transferrin and IgG denaturation) is more pronounced than the controls - an observation that appears to be specific for IgD MM. Our previous large scale investigations of the other MM isotypes revealed a similar transition, however located at 82 °C. In conclusion, the high enthalpy 85 °C transition is identified as a IgD MM specific calorimetric feature that might be used as complementary marker for IgD MM detection, however its validation requires further studies.

Acknowledgement: *This work is supported by the Bulgarian Ministry of Education and Science under the National Research Programme „Young scientists and postdoctoral students" (DCM 168/19.03.2020, A.D.)*

DEVELOPMENT OF A NOVEL GENE EXPRESSION-BASED PROGNOSTIC SCORE IN MALIGNANT PLEURAL MESOTHELIOMA

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Introduction: Malignant pleural mesothelioma (MPM) is a rare cancer type with an increasing incidence worldwide. There are no curative therapies for advanced MPM. Most of the MPM cases are associated with previous occupational exposure to asbestos. From a genomic standpoint, MPM is characterized by a high level of tumor suppressor genes alterations. Different genomic alterations however can converge on a limited number of dysregulated gene expression profiles. That makes a prognostic gene expression based scores an appealing approach to develop novel biomarkers in rare cancers such as MPM.
Aim: We aimed to develop a rational gene expression based prognostic score in MPM using publicly available datasets.
Materials and methods: We developed the prognostic score using processed RNASeq data for 87 newly diagnosed MPM patients analyzed through the Cancer Genomes Atlas (TCGA-MESO dataset) project. The prognostic model was built using the Robust Likelihood-Based Survival Modeling with Microarray Data method, which was implemented through the rbsurv package for R.
Results: We narrowed down the model building to analysis to 179 genes, which have been shown previously to be of importance to MPM development. Our statistical approach showed that a model including two genes was the best predictor for overall survival (OS) ($p<0.0001$). Receiver operating characteristics (ROC) curve analysis also showed that the score was a very good predictor of 2-year survival (Area under the curve (AUC) = 0.815). The discrete score based on the median of the continuous score stratified the patients into low and high score groups. The discrete score also correlated with OS ($p<0.0001$). Furthermore, we were able to demonstrate that low and high score groups of patients had differential gene expression profiles as well as differential mircoRNA expression profiles.
Conclusion: This is the first study to demonstrate a very good performance of only 2-genes expression model in MPM. We are currently extending our work to validate the score on a number of gene expression datasets from different analytical platforms.

NATURALLY-OCCURRING MODULATORS OF CORONAVIRUS INFECTIONS: VIRTUAL LIBRARY DEVELOPMENT

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Introduction and aims: Plant biotechnologies can support the production of promising molecular scaffolds for the design of anti-coronavirus agents. The therapeutic strategies that could be addressed involve different approaches, including virus-targeted, immunotherapy, etc. The aims of this study were to: (i) perform screening of research literature for potential modulators of coronavirus infections naturally-occurring in plants and (ii) prepare a virtual library of selected modulators. Data and methods: Data on naturally-occurring compounds of plant origin were retrieved from the NIH PubMed and PubChem systems (https://www.ncbi.nlm.nih.gov/). Analysis of the collected data was done based on the chemical scaffolds and the proposed mechanisms of action of the compounds. Results: So far, chemical and biological data about 30 compounds (a core collection), investigated for their potential activity against various coronaviruses (SARS-CoV, MERS-CoV, HCoV 229E and others), have been harvested and organized in a virtual library. The latter contains data about experimentally tested plant-derived and commercial compounds which are naturally-occurring in plants. The library is characterised by chemically diverse scaffolds presented by the following chemical classes: triterpenoids, phenols and alkaloids. The biological activities were analysed in the context of distinct virus-directed therapeutic targets. The library is constantly updated. Conclusions: The developed virtual library could serve as a mechanistically justified, highly curated resource of naturally-occurring compounds which could be subjects for further pathology-oriented in silico studies, relevant to modulation of coronavirus infections.

Acknowledgements: *The work is supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" [grant number DCM # 577/ 17.08.2018].*

PPARγ SIGNALLING IN VIRUS-INDUCED RESPIRATORY DISEASES: MODE OF ACTION NETWORKING

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Introduction and aim: The peroxisome proliferator-activated receptor γ (PPARγ) is a key regulator of inflammatory responses and is ubiquitously expressed in many tissues and organs including lung. Several studies were reported in the last two decades pointing out the role of PPARγ signalling in respiratory viral infections. The aim of this study was development of mode of action networks, reflecting the relationships between the progression or alleviation of virus-induced respiratory diseases and PPARγ activity or PPARγ expression levels. Data and methods: Data mining was performed using the resources of the NIH PubMed and PubChem systems (https://www.ncbi.nlm.nih.gov/) with emphasis on available experimental evidence supporting PPARγ targeting in virus-induced pulmonary disorders. The mode of action networking approach was then applied to link the observed biological effects associated with signalling pathways and biomarkers of relevance. Results: Several mode of action networks were proposed, linking PPARγ modulation to pulmonary diseases induced by influenza virus, respiratory syncytial virus, and HIV infection. Multiple levels of biological organisation were discriminated in the developed networks to reflect known molecular, cellular, tissue, organ and system effects. The networks were organized in the context of: ligand-induced PPARγ activation, PPARγ up- or downregulation and PPARγ deficiency. Distinct host- and virus-related therapeutic modes of action were outlined. Conclusions: The reported results allow for a better understanding of the potential role of PPARγ in virus-induced pulmonary pathologies. The proposed mode of action networks present a mechanistically justified basis for subsequent in silico drug design studies focused on PPARγ-targeting in virus-induced respiratory diseases.

Acknowledgements: *The work is supported by the Bulgarian Ministry of Education and Science under the National Research Programme 'Young scientists and postdoctoral students' [grant number DCM # 577/ 17.08.2018].*

POSTER SESSION **MB&BT-13**

STUDY OF THE KYNETIC OF THE BIMOLRCULAR INTERACTION "HOST-CELL-HERPES SIMPLEX VIRUS" IN THE CONDITION OF ONE STEP VIRUS GROWTH INFECTIOUS PROCESS

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Introduction: Since viruses are obligate intracellular parasites, they may exist and reproduce only in living host cell. In this bio-complex, the cell, in the role of host, provides its own structures and biosynthetic activity, but in accordance with the viral genetic program and with the direct participation of viral regulatory structures. Herpes simplex virus infections (HSV) are ubiquitous and widespread in the human population and represent a suitable model for study of virus-cell interactions. Aim: The aim of the present study is to detect and to evaluate the kinetics of a bimolecular "cell-host – herpes simplex virus" interaction in a condition of one-step virus growth infectious process using the surface plasmon resonance (SPR) method at different multiplicity of infection (MOI) and time of exposure. The obtained results are compare with other widely applicable methods such as microscopic observation of structural changes of infected cells and assay for detection of cell proliferation and survival. Materials and methods: Human embryonal lung cells (Lep cell line) cultured at a density 3 x 103 cells/ml and infected with HSV type 1 (HSV-1) with different MOI (0.01, 0.03, 0.05 and 0.1) were used. The relative cell survival and the structural-morphological changes associated with the different phases of the progression of the infectious process obtained by the SPR method are compare with microscopic observation of changes in the morphology of the cell monolayer and with assessment of survival rate by MTT-assay. Cell survival and cytopathic changes are recorded at different intervals, which coincide with the different stages of the replication cycle of HSV-1 (3h, 6h, 12h, 24h, 48h and 72h). Results: The obtained results from the SPR-method indicate accurate determination of the latent period at different MOI. The SPR method allows differentiation of the eclipse period from the latent phase, as well as determination of its duration at different MOI. The beginning of the latent and exponential phase (released virus) determined by the SPR method coincide with the structural-morphology changes registered by microscopic observation of the cell monolayer and with the reported survival rate by the MTT-assay. Conclusions: From the obtained results, can be concluded that the SPR method is promising and could be used to assess the bimolecular interaction "host-herpes virus" in a single-cycle infectious process.

MB&BT-14 **POSTER SESSION**

EFFECT OF RUTA GRAVEOLENS AND ASTRAGALUS GLYCYPHYLLOS EXTRACTS AGAINST HUMAN HERPES VIRUS – 1

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Objective: Human Herpes Virus (HHV) type 1 and 2 are cause of hidden pandemics in global scale, as well as sever clinical symptoms associated with active replication in the human host. As until now there are 11 license anti-herpes drugs. Most of them are based on acyclovir and his derivative. Their frequent usage leads to the selection of drug resistance strains and patients offen experience unwanted side effects. Natural products (for ins. plant extracts) are tolerated better by living organisms and their complex composition prevent appearance of resistant virions. The aim of our work is to study the effect of Ruta graveolens and Astragalus glycyphyllos extracts against Human Herpes Virus – 1. Materials and methods: The extracts are obtained from in vivo cultivated plants, using methanol extraction. All tests are done in in vitro experimental settings. We use MDBK cell line, and also laboratory strain F of HHV – 1. The following methods were applied: MTT assay to determine cell survival and modified MTT assay to determine effect against virus replication in cell culture. Results: Obtained data shows that two of the extracts from both plants behave very differently. The extracts from R. graveolens shows much higher cytotoxicity compared to A. glycyphyllos. The two plant extracts also behave differently when testes for inhibition of the viral replication, A. glycyphyllos showing higher efficiency. Conclusions: Tested extracts differ from each other in high degree, and the one with antiviral potency is the methanol in vivo extract from Astragalus glycyphyllos.

Acknowledgements: *This work was financially supported by the grand № 80-10-198/2020 of Scientific fund, University of Sofia "St. Kl. Ohridski", Bulgaria. Gained results are due to long term working by the team of laboratory of Virology, department of Biology, Sofia university.*

ASSESSMENT OF THE RADIOPROTECTIVE EFFECT OF CURCUMIN

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Curcumin is a polyphenol component in the natural spice Curcuma longa. It is well known that curcumin possesses antioxidant, anti-inflammatory, immunostimulating, and anti-carcinogenic activities.

Aim

Our aim was to evaluate the clastogenic or radioprotective effect of curcumin in non-cancer cells against radiation-induced chromosome aberrations. On the other hand, we assessed the secretory levels of radiation-induced inflammatory cytokines from lymphocytes pre-treated with curcumin.

Methods

Human peripheral blood samples were pre-treated with different concentrations of curcumin (0.5μg/ml; 10μg/ml; 20 μg/ml and 100 μg/ml) and were exposed to 60Co γ-rays using various irradiation doses (0.05 Gy; 0.5 Gy; 1 Gy and 2 Gy). Both γ H2AX/53BP1 foci assay and FISH analysis were used to evaluate the frequency of DNA double-strand breaks (DSBs). The extracellular protein levels of inflammatory cytokines Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Tumor Necrosis Factor α (TNFα), and Monocyte Chemoattractant Protein-1 (MCP-1) were measured using specific ELISA kits.

Results

Our results showed that all curcumin pre-treated samples exhibited significantly lower γ H2AX/53BP1 foci appearance and reduced translocations frequency in irradiated compared to untreated samples. At 1 Gy irradiation and 10 μg/ml curcumin, the reduction of total translocations frequency was 42%. At samples treated with 2 Gy irradiation and 0.5 μg/ml curcumin, translocations frequency declined almost twofold compared to non-treated cells. In samples treated with 10, 20, and 100 μg/ml curcumin we observed a dose-independent decrease in the secretory levels of proinflammatory cytokine IL-8.

Conclusion

This in vitro study showed that curcumin reduces both γ H2AX/53BP1 foci and DNA translocations occurrence in peripheral blood lymphocytes, after γ irradiation. There was no dose-dependent change in the secretory levels of cytokines IL-6, IL-10, MCP-1, and TNF-α 24 hours after irradiation. Interestingly the secretory levels of cytokine IL-8 in curcumin-treated cells decreased independent from dose. Based on this preliminary scientific data, curcumin exhibits a protective effect on normal human lymphocytes from γ irradiation, but more research is needed to better understand this dependence.

Acknowledgements: *This research is being funded by the Bulgarian National Science Fund (Contract № KП-06-H23/3/2018).*

MB&BT-16 **POSTER SESSION**

POLYFUNCTIONAL CLICKABLE COMPOUNDS AS A USEFUL TOOL FOR BIOACTIVE MOLECULES LABELING

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The coming age of personalized medicine needs reactions and reagents with fast kinetics, excellent orthogonality and biocompatibility. Click-chemistry processes such as inverse-electron demand Diels–Alder (IEDDA) reaction – have become extremely popular with promises to revolutionize chemical biology, radiochemistry and materials science. The exceptional fast kinetics of this catalyst-free reaction, using low concentrations of highly strained unsaturated dienophiles and 1,2,4,5-tetrazines coupling agents, make it appropriate for in vivo radiolabelling using pretargeting methodologies. The IEDDA reaction can be tuned to reach rate constants from 1 up to 106 M-1 s-1 by changing the electron deficiency of the 1,2,4,5-tetrazine precursors (Tz), or by manipulating the ring strain and electronic effects on the dienophiles (TCO). Significant efforts have focused on accelerating the reaction rate by synthesizing new derivatives of TCO and Tz. The present study is aimed to the synthesis of new TCO and Tz moieties appropriate for further bioconjugation via oxime or hydrazone formation.

POSTER SESSION **MB&BT-17**

ASSOCIATION BETWEEN HUMAN SPERM DEFECTS AND AGE

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OBJECTIVE:Sperm morphology and the frequency of sperm abnormalities have been strongly linked with human fertilisation. Furthermore, age-relating infertility continues to be an increasing problem associated with semen quality. However, the influence of male age on sperm morphology is still barely studied. The aim of this study was to assess the association between male age and sperm abnormalities.
MATERIALS AND METHODS:This study includes 263 fertile men aged between 18 and 73 years. Tygerberg strict criteria were used to evaluate sperm morphology. Totally 23 types of morphological abnormalities of the head, midpiece and tail were recorded. The analysed sperm defects were large, small, tapered, pyriform (pear-shaped), round, and amorphous heads (formless), heads with a small cap area, bent neck, asymmetrical insertion of the midpiece into the head, irregular midsection, and abnormally thin and thick midsection, cytoplasmic droplet, short, multiple, hairpin, broken or bent tails, coiled tails and terminal droplet. The total number of head, midpiece and tail defects found in a sample was expressed as cumulative head, cumulative midpiece and cumulative tail defects, respectively.
Statistical analysis was performed using SPSS v.21 (IBM Corp., Armonk, NY, USA). $P<0.05$ was considered to be statistically significant.
RESULTS:The most common sperm defects were small head (19.25%±12.42%), amorphous head (21.21%±11.32%), cytoplasmic droplets (33.88%±17.44%), while the rarest sperm defects were double head (0.14%±0.57%), double tail (0.83%±1.38%), thin midpiece (1.52%±2,36%), and acephalic spermatozoa (1.55%±2.69%).
The performed Spearman correlation analysis revealed weak but significant positive correlation between men age and the appearance of coiled tail ($R=0,138$; $p=0.027$) and the frequency of occurrence of acephalic spermatozoa ($R=0.134$; $p=0.031$). Moreover, the age negatively correlates with the cumulative head defects ($R= -0.146$; $p=0.019$).
CONCLUSIONS:Relatively small number of sperm abnormalities has relation to men age. Our study has shown that, the aging is associated with an increased percentage of spermatozoa with coiled tail or acephalic cells. Further studies are required to clarify the influence of the aging on the impairment of the spermatogenesis leading to morphological defects.

MB&BT-18 **POSTER SESSION**

CHARACTERIZATION OF A TRIMETHOPRIM/ SULFAMETHOXAZOLE-RESISTANT STENOTROPHOMONAS MALTOPHILIA CLINICAL ISOLATE FROM BULGARIA

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Stenotrophomonas maltophilia is globally recognized as an emerging multidrug-resistant (MDR) pathogen in healthcare facilities, especially in the intensive care units (ICUs) where it predominantly causes respiratory tract infections, and less commonly urinary tract infections, wound and soft tissue infections, bacteraemia, sepsis, endocarditis, and meningitis. Here, we report the isolation of a trimethoprim/sulfamethoxazole (SXT)-resistant isolate, which was later identified to be class 1 integron-carrying strain. SXT has been recognized as the first-line agent for symptomatic S. maltophilia infections, therefore SXT resistance is a serious therapeutic challenge for clinicians.

INFLUENCE OF CRYOPRESERVATION ON THE ACTIVITY OF ANTIOXIDANT ENZYMES SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) IN EJACULATES OF RAMS OF INDIGENOUS BULGARIAN SHEEP BREED

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Keywords: *ram, sperm, Sofia breed, CAT, SOD*

The aim of the experiment is to examine the influence of cryopreservation on the activity of enzymes of antioxidant protection - SOD and CAT in ejaculates of rams of breed - Sofia (Elin-Pelin). The breed is insufficiently studied in terms of its reproductive qualities. It is endangered of extinction and it is important to explore the possibilities for its protection. For the experiment, 10 ejaculates from 5 rams were examined. Ejaculates were obtained by the method of artificial vagina, during the insemination campaign of the breed and diluted with medium 6A-G. Sperm motility was examined before freezing and after thawing of ejaculates by sperm analyzer (CASA). The activity of enzymes SOD and CAT was examined spectrophotometrically also before freezing and after thawing of ejaculates Sperm motility after cryopreservation decreased by 26.66% ($P \leq 0.001$). The SOD activity after cryopreservation was reduced by about 30% ($P \leq 0.01$) and CAT activity was reduced by 50% ($P \leq 0.01$). In conclusion, we can say that the ejaculates of rams breed Sofia (Elin-Pelin) have good cryotolerance, which is expressed in the ability to reduce the induction of oxidative stress in the ejaculate and preserve their fertility after the freezing-thawing process.

Acknowledgement: *The financial support of National Scientific Program for young scientists and postdoctoral fellows, funded by the Bulgarian Ministry of Education and Science (MES) with PMC № 577 (17.08.2018).*

MB&BT-20 **POSTER SESSION**

STUDY OF THE RELATIONSHIP BETWEEN THE AGE OF THE BREEDERS AND THE QUALITY OF THEIR EJACULATES OBTAINED OUTSIDE THE INSEMINATION CAMPAIGN

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The age of the breeder is one of the determining factors for the quality of his ejaculate, given the development of his reproductive system. Therefore, the present study aimed to investigate the effect of age on the quality of ejaculate from rams of the breed Synthetic population of Bulgarian dairy. Raids divided into two age groups were used for the experiment: G1 (2.5 years) vs G2 (6 years). The ejaculates were prepared by the artificial vagina method and diluted with medium 6A-G. The studied ejaculates were obtained outside the insemination campaign of the breed. A Sperm Class Analyzer (Microptic Systems) was used to study sperm motility. The biochemical activity of the enzymes lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) was determined using an automatic Mindrai BA88 spectrophotometer. The activity of the enzymes was studied in sperm plasma, in aqueous and newt extract. Significant differences between the groups were found in the total sperm motility ($P \leq 0.01$) and the activity of the LDH enzyme in the sperm plasma ($P \leq 0.01$). Regarding the activity of the enzyme LDH in aqueous and newt extract, as well as the activity of the enzyme GGT in sperm plasma, aqueous and newt extract, no significant differences between the groups were found. In conclusion, the age of rams outside the insemination campaign affected sperm motility, but did not significantly affect the activity of the enzymes LDH and GGT.

EFFECT OF OBESTATIN ON CONTRACTILITY OF EXCISED FROG HEART PREPARATIONS AFTER TREATMENT WITH DESIPRAMINE

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Keywords: *Obestatin, Autonomic nervous system, In vitro, Desipramine; Heart; Adrenaline transport*

The aim of this study is to investigate the inotropic effect of obestatin in the presence of desipramine in heart preparations of Pelophylax ridibundus frog. It is known that, the myocardial β-adrenoreceptors and cAMP-dependent protein kinase targets downstream are responsible for the observed positive inotropic effect of obestatin. The application of obestatin in concentrations of 1 nmol/l and 100 nmol/l significantly enhances the force of contraction of excised and cannulated frog hearts. This effect was completely blocked by in vitro application of 1 μmol/l desipramine. Desipramine, a tricyclic secondary amine, inhibits the neuronal uptake of adrenaline and noradrenaline by binding to the noradrenaline transporter. In amphibians, adrenaline is the main sympathetic transmitter. In the frog heart, the endogenous adrenaline levels greatly exceed the noradrenaline levels and sympathetic stimulation results in the release of adrenaline but not noradrenaline. It is concluded that e treatment with desipramine abolishes the positive inotropic effect of obestatin due to the inhibition of adrenaline re-uptake in frog heart autonomic neurons.

Acknowledgment: *This work was supported by grant № 80-10-129/16.04.2020 from Sofia University "St. Kliment Ohridski".*

MB&BT-22 **POSTER SESSION**

METAGENOMICS STUDY OF THE MICROBIOTA OF THE TRADITIONAL BULGARIAN GREEN CHEESE FROM CHERNI VIT

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The Bulgarian green cheese is a traditional product from the village Cherni Vit near the town of Teteven in the Northern part of the Balkan mountain. It is produced from raw fats rich sheep or goat milk only within the village's area because of the specific local microclimate in combination with the lack of use of starter cultures. The metagenomics studies were accomplished on the Illumina HiSeq 2 x 250 bp paired end reads platform. In order to assess the eubacterial and the fungal microbiota of the traditional Bulgarian Green cheese the V3-V4 region of the 16S rRNA genes and the ITS2 region were targeted respectively. Between 97 and 137 eubacterial species and between 12 and 19 fungal species were observed in the four samples from 4 different cheese' batches. We found that the beneficial eubacterial and fungal species were predominant while the counts of the potentially pathogenic species were negligible. Based on these results we found that the Bulgarian green cheese is a safe for the customer dairy product, despite being prepared from raw milk.

Acknowledgment: *This research was performed under Sofia University Research Fund Grant № 80-10-27/18.03.2020.*

EFFECT OF TEMPERATURE AND LIGHT ON THE PHYSIOLOGY AND BIOCHEMISTRY OF THE NEW BULGARIAN CYANOPROKARIOTA CHROOCOCCUS SP. R-10

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Keywords: *Obestatin, Autonomic nervous system, In vitro, Desipramine; Heart; Adrenaline transport*

Cyanoprokaryota, also known as "blue-green algae" or cyanobacteria, are chlorophototrophic prokaryotes that are able to perform oxygenic photosynthesis. A significant number of the representatives exhibit high growth rate when cultivated under optimal conditions, and are also able to synthesize products of great economic importance which explains why the interest towards cyanoprokaryota is constantly increasing. In this study, our team has investigated the adaptive capabilities and the properties of the biomass of the Bulgarian strain Chroococcus sp. R-10. We have estimated how changes in the cultivation parameters (temperature, light) affect the PSII operation and the biomass composition. The physiological advantages of the optimal temperature (26 ºC) in combination with low light intensity were again confirmed by the functional integrity of PSII oxygen evolving complex. Also, it was observed that the joint effect of light and temperature did not affect the protein synthesis significantly ($P>0.05$), while the pigments and lipids levels dropped most noticeably at the optimal growth temperature and low light intensity (down to 0.67 and 10%, respectively, P

EVALUATION OF DIFFERENT CULTURE MEDIA FOR ENUMERATION AND DIFFERENTIATION OF LACTOBACILLUS SPECIES

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The growth and maintenance of microorganisms on solid agar media is a common practice in microbiology and is routinely used for isolation and enumeration of lactic acid bacteria from fermented foods as well as probiotic products. This is still the main method described in the ISO standards for quantification and control of microorganisms used in the food industry. The experiments include species of lactic acid bacteria, most often used in the food and pharmaceutical industries - Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus and Lactobacillus plantarum. Four commonly used culture media MRS, LBD, RCA, mRCA were tested. The analyses clearly demonstrate that the mRCA medium has the best reproducibility in total cfu/ml for all of the strains studied. This is followed by MRS medium, which shows relatively good reproducibility, but lower than mRCA. The next medium is RCA and the lowest efficacy is observed in LBD medium, with one exception in L. plantarum strain, where the lowest number is reported in RCA medium. Species L. rhamnosus has the best growth indicators, followed by L. plantarum, L. bulgaricus and the weakest growth was reported for L. acidophilus. We observed up to two log reduction of bacterial number especially for L. bulgaricus and L. acidophilus. The test media were also used to morphologically describe bacterial colonies and the micromorphology of bacterial cells. No significant morphological differences were found that could give discrimination between the species examined. There are minimal differences in the size of the colonies and the cells, but this could not be used as a clearly distinguishable indicator.

Acknowledgment: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".*

WHOLE-GENOME SEQUENCING-BASED CHARACTERIZATION OF A METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA ISOLATE FROM BULGARIA

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Pseudomonas aeruginosa is one of the leading nosocomial pathogens worldwide. It causes infections that are hard to treat due to the intrinsic resistance of the species and its remarkable ability to acquire additional resistance mechanisms to multiple groups of antimicrobial agents. Therefore, treatment options are narrowed down to only few antibiotics and carbapenems are arguably the most preferred among them. However, carbapenem resistance in Pseudomonas aeruginosa is increasing globally and the major reason for this is the metallo-beta-lactamases (MBLs) production. Here, we report the isolation of a MBL producing P. aeruginosa isolate from Bulgaria, which was later subject to a whole-genome sequiencing to explore its resistome.

MB&BT-26 **POSTER SESSION**

ELECTROINDUCED RELEASE OF LOW MOLECULAR COMPONENTS, TOTAL PROTEIN AND B-GALACTOSIDASE FROM KLUYVEROMYCES LACTIS 1470

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Kluyveromyces yeast genus is of considerable interest in biotechnology industry. This yeast genus is an important source of the enzyme β-galactosidase (EC.3.2.1.23), which is responsible for hydrolyzing lactose to its comprising monomers – namely glucose and galactose, thus finding application of the enzyme in food industry to process products intended for people with lactose intolerance. Development of methods for fast and efficient extraction of intracellular biologically active compounds from microorganisms is a field of great interest. The conventional extraction techniques can be divided in mechanical and non-mechanical methods with mechanical methods being widely used in the industry because of the ability to process large volumes of cell mass. However, mechanical methods lead to significant fragmentation of the cells and highly non-selective release of intracellular contents. An alternative method that achieves cellular permeabilization is treatment of cell suspensions with pulsed electric field with high intensity, which leads to the formation of pores in the cell membrane - electropermeabilization/electroporation. It has been shown that treatment of cell suspensions with pulsed electric field leads to efficient release of low molecular compounds such as aminoacids, antioxidants and nucleotides, as well as to the release of proteins, including cytoplasmic enzymes with preserved enzymatic activity. In the current study, cell suspension of the yeast Kluyveromyces lactis 1470 was treated in batch or in flow mode and the electrical parameters were optimized. We investigated the potential for producing water extracts of low molecular weight compounds after short-term (15-30min) incubation in water. Additionally, we show that initial incubation of electropermeabilized cells in water does not inactivate β-galactosidase. We furthermore studied the release of total protein and of β-galactosidase in potassium-phosphate buffer with varying incubation conditions to maximize protein extraction: cellular concentration during incubation, buffer concentration and pH, addition of thiol compound (dithiothreitol), which increases permeability of the cell wall.

ANTIBIOTIC RESISTANCE AND AGGREGATION ABBILITY OF LACTOBACILLUS STRAINS ISOLATED FROM TRADITIONAL FERMENTED FOODS

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Key words: *Lactobacillus, Antibiotic resistance profile, Autoaggregation ability and hydrophobicity*

Ten Lactobacillus strains newly isolated from traditional fermented foods from Gora region were evaluated in vitro for safety and functional properties that could enable their use as technologically significant strains or probiotics.
The assessment of antibiotic resistance in LAB is one of important criteria in their characterization. The antibiotic resistance profile of 10 strains was determined by using of test antibiotics from three main groups: Inhibitors of cell wall synthesis; Protein synthesis inhibitors; Inhibitors of DNA synthesis. Considering the safety aspects, the resistance to antibiotics varied among the examined strains. Resistance to three of test antibiotics has been established for more of the studied strains, which is a prerequisite for further study for establishment of the genetic determinants.
Microbial aggregation is a desirable property of LAB, especially with probiotic potential and therefore studies of the aggregation potential and coaggregation ability of probiotic strains are conducted. Physicochemical characteristics of the cell surface, such as hydrophobicity, can also affect the autoaggregation and adhesion of bacteria to different surfaces. Autoaggregation ability and hydrophobicity of the new isolated strains ware determinate. Four of the studied strains demonstrate a well expressed autoaggregation ability (over 50%) and in the other six studied strains it was in the range of 20 to 35%. Hydrophobicity over 15% was detected in three of studied strains.
The obtained data prove the presence of important functional properties and safety of the studied strains that determinate their technological applicability.

Acknowledgment: *This work was supported by Project contract 80-10-143/23.04.2020 г., Scientific Found of Sofia University "St. Kliment Ohridski" and the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 /17.08.2018".*

MB&BT-28 **POSTER SESSION**

EFFECTS OF PLANT SESQUITERPENE LACTONES ON QUORUM SENSING-REGULATED BACTERIAL PHENOTYPES

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While the development of antibiotics resulted in remarkable advances in the fight against infectious microorganisms, it was unfortunately paralleled with the highly increasing risks for the development of antibiotic resistance. Resistance development poses the urgent question for the discovery of novel prospective approaches to the fight against multidrug resistant bugs. Together with the search of novel antibacterials, a growing interest occurs in non-traditional approaches, among which are the attempts, instead of killing the bacteria, to suppress the bacterial virulence and the development of virulence-related phenotypes. A significant number of these phenotypes are regulated by the quorum sensing (QS) system. QS represents the cell to cell communication within the bacterial world that allows bacteria to share information about cell density and adjust gene expression accordingly. Therefore the QS system has lately been recognised as an important target for novel antibacterials. Among the substances that are expected to interfere with QS, important group are some low mwt metabolites of different medicinal plants. High are the expectations to sesquiterpene lactones that have structural similarities with the homoserine lactone signals of bacterial QS. The objectives of the study are to test the capacity of 10 purified sesquiterpene lactones isolated from Bulgarian medicinal plants to influence the development of QS-regulated phenotypes - production of the pigments violacein (C. violaceum) and pyocyanin (P. aeruginosa) and biofilm formation in two Gram-positive and two Gram-negative model strains. All of the tested sesquiterpene lactones were shown to influence the phenotypes in focus, however the effects differed between the bacterial species. Strong suppression of the production of the pigment violacein by C. violaceum was observed. However pyocyanin release increased in the presence of the plant molecules. The tested lactones tended to suppress the biofilm growth of the Gram-positive model strains, and had variable effects on biofilms of the examined Gram-negative bacteria. The observed species-specific responses to plant substances should be taken into account and studied in further detail in order to develop successful approaches to the suppression of bacterial virulence.

ANTIBIOTIC SUSCEPTIBILITY AND SURVIVABILITY IN ORAL ENVIRONMENT OF NEWLY ISOLATED LACTIC ACID BACTERIA STRAINS

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LAB express beneficial properties to the consumer and are widely used as probiotic substances to promote human gastrointestinal and oral healthcare.
This study's aim is to evaluate the susceptibility of newly isolated LAB strains to several commonly tested antibiotic agents and the survivability of these strains under simulated conditions of the oral cavity.
Seven LAB strains were used, previously identified as one strain of Leuconostoc mesenteroides and six strains of Lactobacillus plantarum. The main objective is to determine the potential of use of these LAB strains as probiotics for oral health.
The evaluation of the antibiotic susceptibility is important for LAB with probiotic potential for selecting strains to be used as regulators of the gastrointestinal microbiota. Ten test antibiotics from three main groups Inhibitors of cell wall synthesis, Protein synthesis inhibitors, and Inhibitors of DNA synthesis were used. Resistance to a wider range of antibiotics has been established in some of the studied strains identified as Lactobacillus planatarum, which is a prerequisite for further study for establishment of the genetic determinants.
With the oral cavity being the entrance point of the gastrointestinal tract, the taken probiotic substances are initially presented to the saliva. During this first contact the survivability and stability of the bacterial strains is of primary importance. The oral stress test was held under 37ºC in an electrolyte saline solution with added lysozyme as the model for the oral saliva. It was found that 6 strains manifest very good survivability under simulated conditions of oral stress. These results determine the potential of tested strains to show their useful properties in this environment.

Acknowledgement: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 /17.08.2018".*

MB&BT-30 **POSTER SESSION**

IN VITRO INVESTIGATION OF THE PRODUCTION OF INDOLE ACETIC ACID BY FIVE BACILLUS STRAINS

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Key words: *Bacillus, PGP activity, indole acetic acid (IAA)*

The ability of five Bacillus strains to produce indole acetic acid (IAA) was investigated in this study. Plant growth promoting activity (PGP) of these strains was determined in our previous work. IAA is refers to plant hormones auxins. Auxins are responsible for cell elongation, cell division, root and shoot growth, flowers and fruit development in plants.
During the batch fermentation process of studied strains were determinate the cell density (CFU/ml), pH, assimilation of carbon source and IAA production. Glucose assimilation as carbon source started slowly at the beginning of fermentation process and was exhausted up to 24 hours of the process. The pH of the cultural liquid varies between 4.5 - 5.5 during the culturing. The highest IAA production were measured by B. thuringiensis 13VR with 8,38 µg/ml followed by B. cereus 7VR (7.35 µg/ml) and B. subtilis VR (3.47 µg/ml). Detected IAA quality as product of Bacillus strains was prerequisite for further study.

Acknowledgement: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 /17.08.2018".*

IN VITRO AND IN VIVO EVALUATION OF PGP-ACTIVITIES OF NEWLY ISOLATED RHIZOSPHERE STRAINS

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Key words: *Bacillus subtilis, cell-free supernatants, seed germination, Lactuca sativa, Lycopersicon lycopersicum*

A germination test was performed to establish the biological effect of four newly isolated rhizosphere strains Bacillus subtilis (M1, SZ1, SZ2 and AZ5) in different concentrations on the growth and development of seeds of Lactuca sativa (lettuce) and Lycopersicon lycopersicum (tomato). The effect of cell-free supernatants (CFS) from newly isolated strains of the genus Bacillus has been studied.
Cell-free supernatants of strains B. subtilis AZ5, B subtilis SZ1, B. subtilis SZ2 and B. subtilis M1 has possitive effect on treated tomato and lettuce seeds.
A 144-hour cell-free supernatant of Bacillus subtilis (M1, SZ1, SZ2 and AZ5) strains was found to show a positive effect with about a 30% increase the length of the root in lettuce seed germination test compared to control variants. In all four used CFS of the bacterial strains, more than 35% positive effect was observed on the length of the root in germination seeds test of tomato compared to the control variant treated with water.
The obtained results shows that the studied strains have the potential to be used as an agents for promoting the growth and development of studied plants.

Acknowledgement: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 /17.08.2018".*

MB&BT-32 **POSTER SESSION**

CHANGES OF METABOLIC ACTIVITY OF BREVIBACILLUS LATEROSPORUS BT271 IN PRESENCE OF PHENOL AND ZINC

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Key words: *Bacillus, PGP activity, indole acetic acid (IAA)*

The biotechnological approach in which the addition of highly active biodegrading bacteria are added in bioremediation activities is a well-known one. What makes it even more attractive is the possibility to use the biodetoxification activity of some bacteria while it is combined with their abilities to promote the plants growth. An example of this is the bacteria from genus Brevibacillus. The presented study demonstrates an investigation of the metabolic activity of Brevibacillus laterosporus BT271 in presence of five phenol (5-1000 mg/L) and zinc (50-1000 mg/L) concentrations. The metabolic activity was determined by tetrazolium salts - 2,3,5-triphenyl tetrazolium chloride (TTC) and 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC). The bacteria count was monitored spectrophotometrically. The results showed that after 6 hours of incubation the increase in the number of bacteria is highest with 5 mg/L phenol (4.44 x107 cells/mL). But the concentrations of 100 mg/L and 250 mg/L also showed significant bacteria count (up to 4.35 x107 cells/L). The total dehydrogenase activity (determined by TTC concentration) was higher when concentrations up to 500 mg/l phenol were applied (up to 1.42 x10-10 μM H+/min/cell). The fluorescent analysis based on CTC also showed high respiratory activity in the bacterial cells when the applied phenol concentrations were up to 500 mg/L. The data obtained in the experiments with zinc showed almost complete inactivation of B. laterosporus BT271 in all the concentrations of the heavy metal. No total dehydrogenase activity was found and the CTC/DAPI based fluorescence analysis showed almost complete sporulation of the bacteria. The results from the study showed that B. laterosporus BT271 possesses high biodegradation potential when phenol is present even in relatively high concentrations. However, when zinc is in the environment it exerts strong inhibiting effect on the bacteria from the BT271 strain. This indicates that the bacterial strain has potential to be used as bioaugmenting microbial preparation when contamination with phenolic compounds happens in the environment. The described experiments were a first step towards creation of product for acceleration of the bioremediation in the context of the circular economy.

ASSESSMENT OF ACTIVATED SLUDGE FROM MUNICIPAL ENTERPRISE FOR WASTE TREATMENT OF SOFIA CITY DURING MODEL ADAPTATION PROCESS WITH LANDFILL LEACHATE

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Landfill leachate is generated from the waste degradation in landfill sites and rainwater infiltrates. Its treatment includes more often biological methods combined with physical and chemical methods. The availability of polycyclic aromatic carbohydrates, phenols, polychlorinated phenols, pesticides, heavy metals, and refractory organics in landfill leachate remains a critical technological problem during biological treatment. The effect of these toxic pollutants on activated sludge (AS) processes is related to deformations of AS structure /bulking or pin-point flocs/ and inhibition of biodegradation activity. One of the most economic and effective possibilities for problem solving is application of adaptation as a biological method. The aim of the study is to assess the activated sludge from Wastewater Treatment Plant to Municipal Enterprise for Waste Treatment of Sofia City during model adaptation process with landfill leachate. The duration of adaptation process was 21 days. It was accomplished in aerobic reactors and it started with 50 times diluted leachate with addition of 0.79 g/l glycerol as co-substrate. On the 7th day was added 25 times diluted leachate with 0.79 g/l glycerol and on the 14th day was added undiluted leachate with 0.79 g/l glycerol. The influent was changed on the 2nd, 4th, 9th, 11th, 16th and 18th day without addition of glycerol. Data showed that the most favorable conditions for adaptation of the AS to leachate are in the first 6 days, at the highest dilution of leachate. The availability of pin-point flocs showed a deformation of AS structure during the whole adaptation process. This deformation is an indicator for "starving" AS which is related to a decrease of biodegradable organics measured as BOD5 from 1200 mgO2/l to 10 mgO2/l.

The quantity of Pseudomonas sp. and Acinetobacter sp. was increased sharply after 14th day, with addition of more concentrated leachate, which was an answer of AS to higher concentrations of toxic pollutants. The results showed that AS would have a greater ability to adapt when the adaptation algorithm includes more intermediate steps with an addition of more diluted amounts of leachate.

Acknowledgements: *This research was supported by the Grant №BG05M2OP001-1.002-0019: "Clean Technologies for Sustainable Environment - Water, Waste, Energy for a Circular Economy", financed by the Science and Education for Smart Growth Operational Program (2014-2020) and co-financed by the EU through the ESIF.*

POSTER SESSION **MB&BT-34**

APPLICATION OF BIG DATA FOR IMPROVEMENT OF THE ASSESSMENT OF THE SELF-PURIFICATION POTENTIAL IN WATER AND SEDIMENTS OF CASCADE FROM SHPP SREDEN ISKAR

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Keywords: *big data, assessment, water, self-purification, sediments*

One of the professional environmental challenges of today is the assessment, control and biomanagement of river ecosystems used for renewable energy production. A similar ecosystem is the Iskar River in the area of the Cascade of 5 functioning HPPs "Sreden Iskar". Various problems are intertwined in this technological ecosystem, which require an interdisciplinary solution for control and management of the risk associated with the entry of pollutants into water and sediments in this area. Big data development offers realistic opportunities for more efficient operation, assessment and management of such complex technological ecosystems. Big data refers to all the data we have and we transform it into knowledge that we can use directly for management. A very important element is the right framework for the use of data, analysis and solutions, which is the basis of optimized efficiency.
The aim of this study is to use the accumulation of large databases to improve the assessment of the self-purification potential in sediments and waters of the Sreden Iskar cascade, with microbiological and hydrochemical parameters over a long period of time. From this point of view, the assessment of this potential and the forecasts for its impact from the operation of small hydropower plants is essential for preserving the ecological integrity of the region.
The use of big data in water management and control is a relatively new concept. Improvements in computerized analysis allow the large amount of data that is generated to be transformed so that problems can be identified earlier. Ultimately, big data applied in an ecological direction could increase the productivity and sustainability of technological aquatic ecosystems.

Acknowledgment: *This document was supported by the Grant № BG05M2OP001-1.002-0019: "Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy", financed by the Science and Education for Smart Growth Operational Program (2014-2020) and co-financed by the EU through the ESIF by means financing of labor. This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № Д01-322/18.12.2019).*

RELATIONSHIPS BETWEEN BACTERIAL SEGMENT AND FAUNA COMPLEXES IN ACTIVATED SLUDGE AS AN INDICATOR APPARATUS FOR TREATMENT OF LEACHATE FROM LANDFILL

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Key words: *activated sludge, bacteria, micro- and metafauna, leachate, control*

Landfills can be sources of various types of water pollutants. As the amount of waste in their mixed collection increases, so do the toxic compounds in it. One of the main problems for biotechnologists in wastewater treatment plants remains the control of the effective biodegradation of incoming pollutants from the leachate.
The aim of this study is to develop an indicator apparatus to control the treatment of infiltrate from landfills through the relationship of bacterial segment and fauna complexes in activated sludge. To achieve this goal, a model adaptation process of activated sludge to leachate from a landfill will be carried out.
The biodegradation potential of the bacterial segment is strongly inhibited due to the contained "bouquet" of xenobiotics (toxic organic and inorganic contaminants) in the leachate. Fauna complexes, which have an extremely important supporting role in activated sludge, are severely suppressed and in most cases die. This may be related to their function as a "protector" and xenobiotic distributor for bacteria. This is evidenced by studies in which the biotic index of micro- and metafauna decreases at the end of the experiment. From a maximum value of 10 in the middle of the process, to a value of 1 at the end of the process. In addition, there is a sharp decrease in the diversity of macroorganisms. All this is combined with relatively high amounts of microbiological indicators for aerobic heterotrophs, bacteria of the genus Pseudomonas and the genus Acinetobacter, in the middle and end of the experiment.
The main hypothesis of the study is that the control of wastewater treatment processes can be achieved by indicating and regulating the relationship between bacteria and micro- and metafauna, on a quantitative and qualitative level. Studying these micro relationships between pro- and eukaryotic organisms would help to overcome the possible inhibition of wastewater treatment processes due to the toxic substances contained in the leachate from landfills.

Acknowledgement: *This work has been carried out in the framework of the National Science Program " Young scientists and postdoctoral students", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № Д01-322/18.12.2019).*

RESPONSE OF TWO WINTER COMMON WHEAT VARIETIES TO DROUGHT STRESS

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The aim of the present work was to investigate the response of two winter common wheat varieties, created in Institute of Plant Genetic Resources in Sadovo, Bulgaria, to dehydration stress (Nadita and Ailzla). The sensitivity of investigated varieties to desiccation and ability to recover after re-watering was evaluated by following the alterations in the pigment content, desiccation-induced lipid peroxidation, generation of reactive oxygen species (H_2O_2) and the level of non-enzymatic and enzymatic antioxidants. Plants were grown in growth chamber at normal conditions (16/8h day/night photoperiod, illumination 300 μmol photons m-2 s-1 and 20º/18ºC day/night temperature). Watering of plants was stopped at fully developed third leave for 7 days followed by a period of 4 days re-watering to determine the ability of plants to recover after dehydration stress. The most prominent alterations in evaluated parameters were observed after 7 days of dehydration. The results in respect to drought sensitivity and ability to recover after stress of both wheat varieties could represent an interest for breathers for creating new and more drought resistant crops for agricultural application.

Acknowledgment: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018"*

SURFACE PROPERTIES OF AQUEOUS SOLUTIONS OF BIOCOMPATIBLE SAPONINS-BASED BIOSURFACTANTS MIXTURES

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Saponins are frequently encountered natural biosurfactants with specific chemical structure and various biological activity (they exhibit antiallergic, anti-inflammatory, antiviral, antibacterial activity, reduce the cholesterol and fatty acids absorption and are used as adjuvants in vaccines preparation etc.). Biocompatible foams, stabilized by saponins and their mixtures with other bioactive substances are widely applied in different processes in food, cosmetics, pharmaceutical industry and medicine. Thin liquid foam films – one of the basic elements of foams, are an appropriate model for studying the stability of disperse systems and an instrument for studying the properties of surface-active compounds at two approaching interfaces. The present work explores surface activity and interfacial dilational rheology of aqueous solutions of Quillaja saponins and their mixtures with apple pectin. The surface tension of the investigated systems, as well as the kinetics of adsorption of the biosurfactants at the air/water interface, are obtained using a tensiometer Kruss K20. For studying the dynamic surface tension and surface rheology of the adsorption layers a profile analysis tensiometer (PAT-1) in a bubble mode is applied. The properties of foam films (drainage kinetics, film thickness, disjoining pressure isotherm, critical pressure of rupture, etc.) are characterized using the microinterferometric method of Scheludko-Exerowa and the TLF Pressure balance technique. The results demonstrate the behavior of the investigated systems at water/air interface and in thin liquid films. The obtained information is essential for optimizing the composition and fine-tuning the properties of biocompatible foams based on Quillaja saponins and their mixtures with apple pectin in view of future industrial, pharmaceutical and biomedical applications.

Acknowledgements: *The investigation is part of Project "Biocompatible particle-stabilized foams and emulsions for biomedical applications", (2018-2021) between Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences and Institute of Physical Chemistry, Bulgarian Academy Sciences.*

PHYTOHORMONAL PLAYERS IN LAMIUM ALBUM PHYTOIMMUNITY

ZH. GEORGIEVA, Z. KARCHEVA, T. ANGELOVA, R. TODOROVA, P. SAVOURAT, E. MINOT, V. MITKOVA, K. ALEKSANDROV, D. PETROVA, G. CHANEVA, M. ZHIPONOVA

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During their lifetime plants experience different types of stress factors. The environmental signals are mediated inside the plant organism via phytohormones that cause cellular changes and subsequent adequate response. Although there are some general tendencies, each plant species has specific adaptation strategy towards the environmental changes. The aim of the present study is to investigate the white dead nettle Lamium album which is a medicinal plant enriched in secondary metabolites that take part into the phytoimmunity system. The effects of phytohormones (abscisic acid, ABA; cytokinin, CK; gibberellic acid, GA; jasmonic acid, JA; salicylic acid, SA) participating as abiotic and biotic stress mediators are studied in controlled in vitro conditions by estimation of their growth and antioxidant state. The application of selected concentrations of phytohormones showed that the growth of L. album in vitro plants is mostly reduced upon ABA treatment, while CK and GA had positive effect on growth. The growth changes caused by ABA and CK correlated with opposite changes in water content and biomass, and ABA and GA acted as antagonists of shoot growth. Oxidative stress, corresponding to higher content of reactive oxygen species, was present upon application of ABA, JA and GA. Polyphenols acting as non-enzymatic antioxidants were accumulated upon ABA and GA, and their level was reduced upon CK and JA revealing respective synergistic and antagonistic effects of the investigated phytohormones on the plant immunity. The hormones ABA, CK and JA decreased the total antioxidant activity associated with reducing molecules. This effect was proportional to the lower polyphenols content in the case of CK and JA. In conclusion, among the used phytohormonal concentrations, the plant immunity of L. album was mostly affected by ABA, CK and JA, as well as GA, which altogether could serve as a base for further detailed studies.

MB&BT-39 **POSTER SESSION**

TWO-DIMENSIONAL PROTEIN PROFILING OF VARIOUS SOYBEAN (GLYCINE MAX) PRODUCTS IN REFERENCE TO DIFFERENT HYPERSENSITIVITIES

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Soybean (Glycine max) products have increasingly made their way in the market and in people's diet. Such products are soybeans, dry minced meat, tofu, milk, sprouts, etc. The reason behind this wider access is the high-quality protein, vitamins, minerals, fiber etc., which makes the soybean a main part of various diversity of diets such as vegan and vegetarian. Even though soy products have their unmistakable advantages they are also strongly associated with respiratory diseases and food allergy together with other major foods – wheat, dairy and eggs. In addition, soybean is heavily included significantly in all foods, especially prepared. Deeply discussed as allergens amongst the dozen suggested ones (proteases, storage proteins, trypsin-inhibitors) are glycinin and β-conglycinin, which are the major globulins that account for about 80% of the total seed globulin fraction. The present research aims to provide information on characteristics of soybean allergenic proteins of various soybean products, commercially available on the market. This was obtained with two-dimensional electrophoresis for better resolution in regards to quality. Differences in these profiles were determined and characteristic protein fractions were detected as a result. Among them are representatives of various storage proteins (glycinin, conglycinin) and enzymes (lipoxygenase, amylase and trypsin inhibitors), which could cause a number of allergic and other pathological conditions. The weakest representation of the different protein classes was visible in soybean sprouts. The two main clinically important allergenic proteins in soybean are beta-conglycinin and glycinin. Soybean sprouts show a lower presence of these two types of protein, followed by tofu and minced meat, and are most common in seeds and milk. Similar data was obtained for lipoxygenase and agglutinin. The observed differences also suggest similar future results in regards to the allergenic and immunogenic potential, which is extremely important for a possible, properly conducted and consistent with the health status diet.

Acknowledgements: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".*

PHAGE DISPLAY APPLICATION IN IMMUNOLOGICAL ANALYSIS OF WHEAT-DEPENDENT PATHOLOGICAL CONDITIONS

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For the past decade the problems, associated with wheat (Triticum aestivum) consumption have been in the spotlight, because of their effect on people's health and everyday life. Among these problems are the following conditions - wheat allergy, baker's asthma, celiac disease, atopic dermatitis, WDEIA, etc. Despite this, the exclusion of said crop is very difficult due to the fact that wheat is main component of the human's diet, because of its bread-making properties and nutritional value. Wheat's proteins, capable of inducing an immune response, are representatives of different protein families – storage proteins (gliadins and glutenins), enzymes (proteases) and enzymes-inhibitors (trypsin-inhibitors), etc. In addition, the processing of said proteins in high temperature and low humidity conditions (baking) affects their digestibility and therefore the response of the immune system to the protein fractions. All of this is enough reason for researching the alternatives to wheat, which could be suitable for affected people. Such are other species of the Triticum family – spelt (Tr. spelt), kamut (Tr. turanicum) and einkorn (Tr. monococcum), as well as maize (Zea mays), which is a completely non-gluten crop. Differences in the protein profiles makes them potential substitutes for wheat, despite the existence of conflicting data – for example, for some einkorn is thought to be equally strong in inducing immune response in celiac disease, while for others, the non-existence of the D-genome makes einkorn the perfect wheat substitute. The stated so far is the reason for the present aim to decipher the connection between the protein content, digestibility and cross-reactivity of the said cultures. A phage display technology was used for selection of specific antibodies against whole wheat protein. Protein extraction, followed by hydrolysis with digestive enzymes was made. The obtained scFv antibodies from the phage display, were further used in Western blot technique for detecting cross-reactive protein fractions in hydrolysates of said alternative crops. Observed was almost complete proteins' hydrolysis and an absence of immunological cross-reactivity, which suggest minimization/elimination of the allergenic potential, although under physiological conditions this could be strongly compromised.

Acknowledgements: *This work was supported by grand 80-10-114/16.04.2020 Science Fund – SU – Research project in support of PhD students.*

MB&BT-41 **POSTER SESSION**

SUSCEPTIBILITY OF MEMBERS OF SOLANACEAE TO CUSCUTA CAMPESTRIS INFECTION

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Keywords: *host plant resistance, parasitic plants, salinity stress*

Cuscuta campestris is an aggressive aerial parasitic plant with North American origin, but widely spread as an introduced and invasive species on all continents except Antarctica. It is known as a common parasite on economically important crop plants, although some and especially tomato (Solanum lycopersicum) are naturally resistant. The molecular mechanisms of this resistance are still not fully understood. In the present study, a range of members of Solanaceae – eggplant (Solanum melongena), five pepper (Capsicum annuum) and five tomato Bulgarian cultivars were tested for their susceptibility to Cuscuta campestris infection. Moreover, the effect of different saline regiments – 0, 100 and 200 mM NaCl on the development of the parasite were analyzed. While eggplant and pepper were found to be susceptible hosts, only two individual C. campestris plants successfully developed on tomato. While in eggplant the growth of the parasite was remarkably higher on NaCl challenged hosts, the predominant effect in pepper was the opposite. Furthermore, water-soluble and cell wall bound proteins from the infection site in tomato were isolated and separated by two-dimensional gel electrophoresis. In both fractions numerous differentially presented proteins in response to infection were observed. They are promising putative factors, defining the remarkable resistance of this species to Cuscuta infection and will be subject to further analyses.

Acknowledgement: *The present study was funded by grant 80-10-96/16.04.2020 of the Science Fund of Sofia University.*

SALINITY STRESS RESPONSE OF CUSCUTA CAMPESTRIS IS AFFECTED BY THE HOST

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Parasitic plants represent numerous species of angiosperms with unique partial or full heterotrophic lifestyle. The negative impact on the host plants is devastating due to significant inhibition of host plant's growth and development. Little is known about the impact of abiotic factors, in particular salinity stress, on parasitic plants with limited or absent soil contact. As salinization of soil is one of the most important problem in agriculture, this topic represents an essential part of fundamental knowledge and understanding of parasitic plants strategy for sensing and choice of host plants. In the present study, the effect of different soil salinity regimens on Cuscuta campestris, an obligate stem parasite, in the presence of two different hosts was tested. The model plant Arabidopsis thaliana and the common host for Cuscuta spp., Medicago sativa were tested as hosts. In addition, the effect of salinity on the parasite in host absence was also analyzed. Growth characteristics were evaluated and comparative analysis with regards to the salt concentration was performed. Key biochemical markers for abiotic stress including L-proline, malone dialdehyde (MDA) and hydrogen peroxide were also studied. Further analysis of antioxidant activity was performed in terms of superoxide dismutase (SOD) and peroxidase activities (POX). It was estimated that the parasitic plant grows and develop with availability of both host plants, in contrast to the host absence. The growth is inhibited with increasing concentration of soil salinity (0, 100 and 200 mM NaCl), with the exception of M. sativa in which the parasite has the most intensive growth on 100 mM NaCl. This observation is in correlation with the hydrogen peroxide concentration and POX activity. With host A. thaliana the concentration of MDA decrease with higher concentration of soil salinity contrariwise with the concentration of L-Pro, whereas with host M. sativa this results are not in correlation with the observed growth characteristics. SOD activity is not affected with host availability. In conclusion, it was illustrated that the parasitic plant is affected by the different soil salinity regimens and this affection is dependent on the host availability and the type of the host with concomitant impact on the host quality.

Acknowledgement: *The present study was funded by grant КП-06-Н31/10 of the National Science Fund of Bulgaria.*

MB&BT-43 **POSTER SESSION**

ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACTS OF THE BULGARIAN ENDEMIC PLANT ACHILLEA THRACICA VELLEN

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Achillea thracica Velen. (Asteraceae) is a perennial Bulgarian endemic plant with medicinal properties. The species was found only near Manole village, Plovdiv, Bulgaria, and the number and size of populations appeared to be reduced mainly by human activities. Ex situ conservation of the Thracian yarrow was done by applying the micropropagation method. The aim of the present study was to investigate the antimicrobial activities of the extracts obtained from in situ grown, in vitro propagated and ex vitro adapted plants. A. thracica samples were prepared by thermostat extraction method using water as solvent. The antimicrobial activities of the aqueous extracts were determined by disk-diffusion method against seven Gram-positive bacteria, five Gram-negative bacteria and two yeast strains. This study revealed the presence of different antimicrobial activity of the samples. Our data reviewed that bacterial species were more insensitive than tested yeast strains towards the aqueous extracts of A. thracica Velen.

Acknowledgements: *This work has been done with the financial support of "Young scientists and post doctorants-2019" program and with the special permission №806/21.08.2019.*

POSTER SESSION **MB&BT-44**

COULD EVOLUTIONARY FACTORS AFFECT ENDOGENOUS CYTOKININ POOLS IN HYPERICUM SPECIES IN VITRO?

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The Hypericum genus comprises over 450 species distributed worldwide and classified into 36 sections. The most widely studied representative of the genus is H. perforatum, which has been applied in medicinal practice since the 1st century A.D. up to modern times. The species has been utilized for a wide array of pharmacological properties such as antimicrobial, antiviral, anti-inflammatory, wound-healing and anticancer, amongst many others. Research has shown that the production levels of condensed naphthodianthrones hypericin and pseudohypericin are related to the evolutionary development of the species in accordance with their sections' distribution. The flora of Bulgaria comprises 22 species of the genus, distributed within 11 sections. Of them, one species is Bulgarian (reported now as extinct), and five are Balkan endemics. In the present work, shoot cultures of hypericin non-producing H. calycinum L. (of the primitive Ascyreia section), hypericin producing H. perforatum L. and H. tetrapterum Fries (both section Hypericum) and H. richeri Vill. (section Drosocarpium - the most advanced in our study) were developed. For the purpose of multiplication stimulation, cytokinin (CK) N6-benzyladenine and auxin indole-3-butyric acid were exogenously applied in different combinations. The content of total endogenous CKs were studied and their pools and ratios between cis- and trans-zeatin types were compared. Results were indicative of the relations between evolutionary level and endogenous CK production. Thus, while the most primitive H. calycinum showed the lowest levels of total CKs, as well as of trans- and cis-zeatins, the highest total CK pools were recorded for the most evolutionary developed H. richeri. The analysis of data regarding the trans- and cis-zeatin types, however, showed an interesting interplay when comparing amongst the three hypericin producing species. Thus, while the highest levels of trans-zeatin types were detected for the most evolutionary developed hypericin producing H. richeri, on the contrary cis-zeatins dominated in H. tetrapterum. Our observations indicate that both complexity of evolutionary development, as well as hypericin production capacity, might be in close interplay with parameters of physiological adaptation in the plant organism such as endogenous CKs production.

Acknowledgements: *We are thankful to grants КП-06-H39/6, National Scientific Fund, Bulgaria and 19-12262S, the Czech Science Foundation, Czech Republic*

MB&BT-45 **POSTER SESSION**

AQUAPONICALLY CULTIVATION OF TOMATO PLANTS (SOLANUM LYCOPERSICUM L.)

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Aquaponics, being a synergistic integrity of hydroponics and aquaculture, has gained an increasing attention as a promising system for urban applications. The addition of algal component to aquaponic systems could lead to a number of positive effects – maintaining the pH balance, extra oxygen adding and water purification. Aquaponics, itself, had a beneficial effect on the tomato (Solanum lycopersicum L.) cultivation – an improved growth and slight increase of the amount of chlorophyll a and carotenoids was registered. Further, the exponentially growing green alga Coelastrella sp. BGV, a newly isolated strain, was used as a third component of the aquaponic system, to study the effect of microalgae on the growth and pigment composition of tomato plants. Cultivation in the aquaponic system equipped by the microalgal suspension resulted in a greater increase in the plant growth – about 20%, as well as a stronger effect on the pigment composition. Chlorophyll a and carotenoid content was almost equally enhanced - 20% and 17%, respectively. Interestingly, the most affected was the level of chlorophyll b, which was 40% higher than the control plants. Future research should focus on assessing the effect of microalgae on the quality of cultivated plants and the possible practical application of algal biomass.

Acknowledgements: *That study was supported by grant № 80-10-50/13.042020, Sofia University*

IMPACT OF UV-B LIGHT ON CANNABIS SATIVA LEAF METABOLITE CONTENT

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Cannabis sativa is an annual herbaceous species, which has been used for centuries and has recently seen a resurgence of interest due to its multi-purpose applications, including valuable medicinal opportunities. Light is a major factor controlling plant metabolism including growth and phytochemical potential. The present study aimed at investigating the effect of UV-B treatment, a high-energetic component of the solar radiation, on the plant performance. Cannabis sativa L. subsp. indica seedlings were grown in a phytochamber under white light as a control and white light with three variants of additional UV-B irradiation: low intensity (LI; 0,7 W/m2 UV-B); medium intensity (MI; 1,4 W/m2 UV-B) and high intensity (HI; 2,1 W/m2 UV-B). Plants treated with LI and MI had shown improved stem elongation, enhanced pigments and phenolics content in leaves compared to the control group. Chlorophyll a and chlorophyll b levels were highly increased, 43% and 46% respectively, in the LI variant. Maximum carotenoids content - 83% above the control, was measured under MI irradiation. Further, all plastid pigments were significantly decreased under the highest UV-B treatment. The level of reducing sugars in leaves did not follow that tendency and their concentration remained significantly lower than the control, regardless of the light intensity. Total leaf phenolic content and corresponding antioxidant activity were slightly increased under LI, and decreased in the MI and HI variants. The UV-B treatment did not lead to significant changes in the overall flavonoid content. Investigating the effect of high-energetic UV-B light on C. sativa demonstrated that LI additional UV-B treatment might be beneficial for its growth and metabolic performance, while the plant seems sensitive to higher doses of this radiation. The defence mechanism of C. sativa against UV-B light seems to involve sugar transport from the leaves towards the stem, as well as accumulation of non-enzymatic antioxidants such as the phenolic compounds.

Acknowledgements: *The plant material was kindly provided by ROMB Ltd.*

IMPACT OF SILVER NANOPARTICLES ON THE PHOTOSYNTHETIC ACTIVITY OF PEA (PISUM SATIVUM L.) UNDER SALT STRESS

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The aim of this study was to investigate the effects of silver nanoparticles (AgNPs) on the photosynthesis of pea (Pisum sativum L.) in the presence or absence of 100 mM NaCl in the nutrient solution. Different concentrations of AgNPs (10 mg/L, 25 mg/L and 50 mg/L) were applied by foliar spray on pea seedling. The treatments with NaCl and Ag NPs were carried out for 8 days. Pulse amplitude modulated chlorophyll fluorescence, P700 photo-oxidation, pigment composition, electrolyte leakage and some oxidative stress markers (amounts of MDA and H2O2) were used to assess the impact of AgNPs on pea seedling at physiological and salt stress conditions. Data revealed that the effects of AgNPs on the studied parameters under physiological conditions depend on the applied concentration, as concentration of 50 mg/L has a negative effect on the plants. Experimental results have also shown the protective effect of low concentrations of the studied AgNPs on the functions of photosynthetic apparatus under salt stress, as the protective effects were better expressed at 10 mg/L NPs. This study clarifies the reasons for the different effects of AgNPs under physiological conditions and salt stress on pea seedling as well as establishes the optimal protective concentration under this stress factor.

Acknowledgements: *This study was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" approved by PMC № 271/29.10.2019. The synthesis and characterization of the silver nanoparticles were carried out and provided by the joint project between the Bulgarian Academy of Sciences (BAS) and the Egyptian Academy for Science (ASRT).*

COMPARATIVE DETERMINATION OF ANTIMICROBIAL ACTIVITY OF ENDEMIC SPECIES FROM GENUS STACHYS DURING THE PROCESS OF EX SITU CONSERVATION

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Stachys bulgarica Degen & Neic and Stachys scardica Griseb. are Balkan endemic species included in The Red Data Book of Bulgaria with conservation status "endangered". Plants from Stachys genus have been widely used in the ethnomedicine for treatment of different inflammatory conditions including infected wounds. There is no available data on ex situ conservation of the species and little is known about their chemical composition and biological activity. The aim of the present work is ex situ conservation of the endemic species S. bulgarica, S. scardica and comparative study of antimicrobial activity of extracts isolated from in situ, in vitro cultivated and ex vitro adapted plants. In vitro cultures from the endangered species S. bulgarica and S. scardica were successfully induced by sterilized ripe dried seeds. The regenerated plants had vigorous growth and well-developed root system, but still poor growth index. All tested concentrations of the cytokinin BA (0.1 mg/L, 0.5 mg/L, 1 mg/L, 1.5 mg/L) stimulated shoot development in both S. bulgarica and S. scardica but most effective were 1 mg/L BA and 1.5 mg/L BA respectively. Ex vitro adaptation was successfully accomplished in phytotron chamber, greenhouse and experimental field with 96% for S. bulgarica and 92% for S. scardica. The antimicrobial activity of the methanolic extracts obtained from in situ, in vitro cultivated and ex vitro adapted plants from the three species was tested against three gram-positive bacteria Staphylococcus aureus, Staphylococcus epidermidis, Cutibacterium acnes, six gram-negative bacteria Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Acinetobacter calcoaceticus, Enterobacter cloacae, Escherichia coli and the yeast Candida albicans by agar disk diffusion method. A collection of in vitro cultivated and ex vitro adapted plants was established which is an alternative approach for the preservation of Stachys bulgarica and Stachys scardica.

Acknowledgements: *This work was financially supported by Grant № 80-10-4/18.03.2020 of Sofia University "St. Kliment Ohridski"*

MB&BT-49 **POSTER SESSION**

MEASLES AND RUBELLA VIRUSES - CLASSICAL AND MODERN METHODS OF LABORATORY DIAGNOSIS

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Measles and rubella are viral infections important to public health. Since the middle of the 20th century, they are subject to vaccine prophylaxis, but the number of patients remains high, especially among children under one year. The WHO has established a clear definition of their clinical and laboratory diagnosis. They are viruses that cause fever rash infection that can be detected by immunological, molecular and cell culture methods. The aim of the study is to present the methods for laboratory detection of measles and rubella virus. Materials and Methods: In the present study, 100 serum and oral fluid samples from patients with measles and rubella were tested. The samples were examined using a set of modern (ELISA and RT-PCR) and classical (virus isolation in Vero cells) methods. Results: The test samples were subjected to EIA analysis, RT-PCR test and viral isolation. Viral antibodies (IgM and IgG), rubella E gene and measles N gene were detected. In some of the samples a cytopathic effect was observed, it consists of either a broad syncytium or a stellate form with inclusion bodies visible. In most circumstances, routine testing for IgM will provide sufficient laboratory evidence to confirm a suspected measles and rubella cases, but PCR analysis and cell culture are the basis for proving infection at an early stage and determining of the virus circulation. Conclusion: The successful isolation of live virus from clinical specimens and detection of viral antibodies and RNA, depends on the timing and type of specimens and correct sample collection. Keywords: measles, rubella, RT-PCR, ELISA, viral isolation.

THE FLUORESCENCE IN SITU HYBRIDIZATION - AN INNOVATIVE METHOD IN THE CIRCULAR SOLUTIONS FOR RESOURCES RECOVERY IN MUNICIPAL ENTERPRISE FOR WASTE TREATMENT - SOFIA

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The circular economy and the sustainable development impose a waste minimisation and a continual use of resources. New technologies and innovative control systems have been developed and implemented in waste treatment plants. As one of the elements of each biotechnology and as one of the main parts of the management, the control system is really important. The choice of the right methods and indicators is a key moment to be assured exact and fast results reflecting the functioning of the biological systems.
The fluorescence in situ hybridization gives the opportunity to get deeper in the relationships between the microorganisms and to extract more information about their functioning and the biological processes at a whole. It is an innovative method that not just allow to quantify the selected microorganisms but also to localize them in the biological systems.
The fluorescence in situ hybridization have been used as a control tool in the management of different resource recovery technologies by the team of this study. One of its case studies is the Municipal enterprise for waste treatment – Sofia. This enterprise includes several technologies for recovery of different resources – waste, waters and energy. The fluorescence in situ hybridization for different groups of microorganisms (Pseudomonas sp., Acinetobacter sp., domain Archaea, Methanosaeta sp., Methanosarcina sp., family Methanobacteriaceae, Alcaligenes sp., Paracoccus sp., cluster Azoarcus-Thauera, ANAMMOX bacteria, etc.) have been proved its large potential as a method used in the management of such kind of technologies.

Acknowledgements: *This research was supported by the Grant №BG05M2OP001-1.002-0019: "Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy", financed by the Science and Education for Smart Growth Operational Program (2014-2020) and co-financed by the EU through the ESIF and by the Grant PT019-DG-56-22/22.05.19 "Gradual adaptation of activated sludge towards biodegradation of leachate from Sofia Waste Treatment Plant" funded by the Municipal enterprise for waste treatment.*