SOFIA UNIVERSITY ST. KLIMENT OHRIDSKI
FACULTY OF BIOLOGY
YOUTH SCIENTIFIC CONFERENCE
“KLIMENT’S DAYS”
8TH NOVEMBER 2019
ABSTRACTS

FUNDED BY:
PROJECT № 80-10-76/2019 SOFIA UNIVERSITY;
PROJECT № 3360/2018 SCIENTIFIC RESEARCH DEPARTMENT, SOFIA UNIVERSITY
YOUTH SCIENTIFIC CONFERENCE

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8TH NOVEMBER 2019

ABSTRACTS

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|               | Laboratory of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 139 Plovdiv, Bulgaria |            |
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CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF POLYSACCHARIDES AND OTHER METABOLITES FROM CYANOBACTERIA

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Keywords: Anabaena laxa, Oscillatoria limosa, Phormidium molle, polysaccharides, biological activity.

Cyanobacteria are ancient prokaryotes, which are a promising source of physiologically active metabolites, potentially useful for the human nutrition, cosmetics and healthcare. The physiology, chemistry and bioactivity of their metabolites are still poorly explored. Therefore, the aim of the current study was to investigate the chemical composition and biological activity of polysaccharides and smaller molecules isolated from three different cyanobacterial species. Three water-extracted polysaccharide complexes were obtained from pretreated with 80% (v/v) ethanol and acetone biomasses of Anabaena laxa (AL-PSC), Oscillatoria limosa (OL-PSC) and Phormidium molle (PM-PSC). AL-PSC, OL-PSC and PM-PSC were analyzed for total sugars, monosaccharide composition, ester content, molecular weight distribution, protein and total phenol contents. FT-IR spectra of the complexes were also studied. Ex vivo immunomodulatory activity against human leukocytes and in vitro cytotoxic activity against different human-derived tumour cell lines of the three complexes were evaluated. The chemical profile and antioxidant activity of the total extracts, obtained from the biomass pretreatments, were investigated by GC-MS, HPLC-UV, ORAC and HORAC methods, respectively.

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 DCM # 577/17.08.2018.
DISTRIBUTION OF THE BALKAN-ANATOLIAN EARTHWORMS (CLITELLATA, MEGADRILI) DENDROBAENA COGNETTI (MICHAELSEN, 1903) AND EISENIA KATTOLASI ZICSI & MICHALIS, 1981

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Among the 226 earthworm species which were registered from Balkan Peninsula, Anatolia, the Levant and Cyprus, only two of them are Balkan-Anatolian species: Dendrobaena cognettii and Eisenia kattoulasi. D. cognettii is widely distributed in Europe. It is also recorded only in one locality in Thrace part of Turkey. E. kattoulasi is common in Balkans, Caucasus and also in Levant region. But it was recorded only one locality in Inner Anatolia so far. Keywords: Dendrobaena cognettii, Eisenia kattoulasi, earthworms, Balkan-Anatolia species.
COMPARATIVE STUDY OF THE BEHAVIOR OF THE BROWN BEAR, *URSUS ARCTOS* UNDER DIFFERENT CONDITIONS IN CAPTIVITY

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Keywords: brown bear, behavioral response, new object exploration, captivity

The brown bear, *Ursus arctos* is a species that has been reared in captivity for a long time in Bulgaria. The aim of this work is to study how the behavior of bears is affected by different rearing conditions in captivity by studying the stereotypic behavior, exploratory behavior and reactions of bears to visitors in the Dancing Bears Park, Belitsa and Sofia Zoo. A total of 13 bears were studied - 10 in the Dancing Bears Park, Belitsa and 3 in Sofia Zoo. The exploratory behavior was studied by providing a new object and a new odour stimulus. More than half of the animals tested reacted to the visual stimulus. However, mainly male bears responded to the odour stimulus. Stereotypic behavior was observed both in Dancing Bears Park and Sofia Zoo. The most severe case was observed in a male bear in Sofia Zoo. The results obtained demonstrated that the different conditions in captivity have significant effect on bear behavior. Studies of animal behavior in captivity are important for improving their welfare.
MORPHOLOGICAL AND KINETIC CHANGES IN SPERMATOZOA DURING IN VITRO INDUCED DECAPACITATION WITH SEMINAL PLASMA PROTEINS

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In order to be able to fertilize the egg a number of biochemical, physiological and morphological changes take place in the sperm cells that precedes the acrosomal reaction. This process is known as capacitation and it can be detected by observing a significant increase in the kinetic parameters of the male gametes, called hyperactivation, and is followed by changes in membrane integrity of the acrosome. Under different physiological conditions, the reverse process of decapitation is observed. It is represented as a partial or complete inhibition of the hyperactivation and affects the efflux of cholesterol from the apical part of the acrosome. The aim of our research is to determine the role of the proteins in the seminal plasma, with different molecular weight, regarding the processes of in vitro induced decapacitation, and its detection in terms of morphological and kinetic profile of the spermatozoa. Experimental model in our studies were clinically healthy individuals of the species Canis lupus familiaris (domestic dog). Fresh ejaculates were obtained and then examined by Computer assisted sperm analyzer (CASA) to determine the kinetic and morphological status of the sperm cells. Seminal plasma from the ejaculates was selected, purified and then subjected to High Performance Liquid Chromatography (HPLC). As a result of the chromatographic analysis, three fractions of seminal plasma were selected based on the molecular weights of the proteins. After the selection we incubated fresh ejaculates with a capacitation medium to induce capacitation in vitro, and upon its onset, re-incubated the already capacitated sperm cells with each protein fraction. The incubation was carried out and then evaluated by CASA based on the VCL and VAP- parameters every 30 min for 2 hours. The results strongly demonstrated that the HPLC- Fraction 3, which contains proteins with molecular weight 60-100 kDa, returned more than 25% of the spermatozoa to a decapacitated state, decreasing the level of hyperactivation. In terms of morphology, we detected a reduction in sperm count with impaired membrane integrity. As a result we can conclude that seminal plasma proteins from HPLC- Fraction 3, with certain molecular weight, can lead to changes in the kinetic and morphological status of spermatozoa during in vitro decapacitation. With gratitude to: Program for Support of Young Scientists and Postdoctoral Students-2018-2020.
EFFECT OF ULTRA-LOW CRYOPRESERVATION TEMPERATURES ON THE LIPID PEROXIDATION OF EJACULATES OBTAINED FROM RAMS OF TWO BREEDS - LACAUNE AND SYNTHETIC POPULATION BULGARIAN MILK (SPBM)

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Keywords: ejaculates, cryopreservation, lipid peroxidation, rams

The aim of the present study was to evaluate the effect of ultra-low cryopreservation temperatures of -196°C on the lipid peroxidation of ejaculates obtained from rams of two breeds - Lacaune and Synthetic Population Bulgarian Milk (SPBM). For the study, 12 ejaculates from 6 Lacaune rams and 9 ejacules from the 5 Synthetic Population Bulgarian Milk (SPBM) rams were used. The ejaculates were obtained by the method of artificial vagina and were diluted 1:24 with 6AG medium. The obtained ejaculates were examined by a computer sperm analyzer (CASA) to determine total motility, movement type and concentration before freezing and after thawing. Levels of lipid peroxidation were also determined before and after cryopreservation by measuring the amount of thiobarbituric acid reacting products (TBARs) in ejaculates by the method of Hunter et al. (1963), via Spekol 11 (Carl Zeiss, Jena) at a wavelength of 532nm. The results showed an increase in levels of lipid peroxidation and a decrease in sperm motility after cryopreservation in the ejaculates of both breeds compared to pre-freezing values.

Acknowledgments: 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).
TRANSCRANIAL MAGNETIC STIMULATION

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Transcranial magnetic stimulation (TMS) is a non-invasive, painless and safe method for assessing the physiological and functional status of the cerebral motor cortex and the neural pathways from cortex to spinal motoneurons. TMS may be used for investigation of the activity of motor cortex, and the amplitude of motor evoked responses shows the number of activated cortical motoneurons. A lot of studies have proven that TMS is a safe method and does not lead to long-term effects, and only causes a short-lasting changes in neuronal activity. No changes were found in heart rate, blood pressure, cerebral blood flow and brain activity in electroencephalograms. Relatively rarely, are observed negative effects, most commonly as painless discomfort and headache. The method was first applied in 1985 and since then it has been proven that studies of motor excitability and changes that occur in it, in addition to fundamental knowledge, have been applied in clinical practice. At some neurological diseases is observed different from the normal nervous excitability. Worldwide with this method are examined people with brain lesions, Parkinson’s disease, multiple systemic atrophy, multiple sclerosis, amyotrophic lateral sclerosis, dystonia, schizophrenia, epilepsy, dementia, migraine, chronic pain, etc. Through TMS could investigate the processes during different motor tasks, at different moods and attention, at visual perception and speech, to examine the sensory-motor integration, and even the action of neuroendocrine hormones. TMS is also used in the study of cognitive functions of the brain. It is suggested that the use of TMS could improve specific cognitive skills in people without neurological diseases. After TMS may be observed a positive change in skills, which include continuous visual and hearing attention, improvement of speech, and a positive change in the performance of much more complex tasks, such as threat recognition, improving associative and analytical thinking.
STUDENTS'S CLUB SKOREC - A DECADE OF VOLUNTEER SCIENCE AND EDUCATION IN FACULTY OF BIOLOGY

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The different types of non-obligatory students related initiatives have always been an important part of the education of Sofia University, motivating the students and improving their knowledge and experience, gained in lections and practical classes. At the same time, many of these activities are not constant in time, since they require a serious maintaining efforts from lecturers, which should provide sustainability and constant replacement of the graduating students with new ones and coordinate a vast range of activities. The Student's club for education and development with ecological center (SKOREC), was founded in Department of zoology and anthropology, but during the years it gained strong support from different lecturers an the Student's council from the Faculty, as well as scientists, NGO's, etc. The main activities of the club can be classified as follows: 1. Maintaining of educational collection of live animals - visited by University and high school students. 2. Organizing of field trips, dedicated to wildlife observations. 3. Involving students in working on project-based fundraising. 4. Providing volunteers for nature conservation and educational activities. 5. Improving the skills of the students for writing scientific papers and participating in conferences. As a result of the club's work, c.300 young people are visiting the animal collection yearly, tens of field trips were successfully organised, many of the most active volunteers started working in institutions and organizations, related to biological education. Skorec co-organized the Faculty’s annual scientific conference “Kliment’s Days” and participated in Biofest, European night of the scientists, University botanical garden anniversaries, etc. The green belt around the Faculty was also modified in biodiversity-friendly way, with new species of plants, bird boxes, wildlife information billboard. The club have a group of 1000+ members in the social network - an interactive area, where useful information, work proposals, etc. are regularly published.

After ten years of active work, we can confirm that organizing students in such activities proved as a powerful tool for additional education and motivation and the efforts should continue in order to improve the achieved results.
POSTER SESSION
ASSESSMENT OF BODY COMPOSITION CHARACTERISTICS IN BULGARIAN RHYTHMIC GYMNASTS BY BIOELECTRICAL IMPEDANCE ANALYSIS

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Keywords: body composition, rhythmic gymnasts, BIA, young athletes

The aim of the study is to determine the influence of sport activity on the body composition compartments in young rhythmic gymnasts (RG) at different age categories. A total of 27 RG, who had trained at least for two years and not less than 20 hours weekly, were tested. Athletes were divided into two experimental groups: pre junior (mean age 8.87 ± 0.72 years) and junior (mean age 11.55 ± 0.52 years). The method of bioelectrical impedance analysis (BIA) was used to evaluate the body composition. Statistical analysis was performed by SPSS16. T-test was used to identify statistically significant intergroup differences. A correlation analysis was applied to establish the relationship between body composition compartments. The gymnasts in pre junior group had significantly lower mean values (p <0.05) of height, weight, muscle mass (MM), total body water (TBW) and fat free mass (FFM) compared to junior group. Significant intergroup differences were not identified in regards to fat mass (FM), body fat percent (PBF), visceral fat (VF), waist- to- hip ratio (WHR) and body mass index (BMI). A significant positive relation with high intensity between muscle mass and height, weight, TBW and FFM (p <0.01) in both assessed groups was established. The results of present study indicate that the muscle mass of the gymnasts rises with increasing the sport experience but the PBF and the other obesity parameters keep relatively constant mean values.
POPULATION STATUS OF HIMANTOGLOSSUM CAPRINUM (M. BIEB) SPRENG (ORCHIDACEAE) ON ZEMENSKA MOUNTAIN, WEST BULGARIA

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Conservation species included in Bulgarian biodiversity act (Annex 2, 3), Red list of Bulgarian vascular plants (VU), CITES (Annex 2), Council directive 92/43 EEC (Annex 4); Bern Convention (Annex 2). Field surveys were carried out in the period 2006-2015. The transect method was used. As a result of the study it was found that the Zemenska Mountain is the largest habitat of its kind in Bulgaria, known to date. 1200 generative individuals were identified in several fragments of a branch population that spans the northern part of the mountain. Two major fragments were identified - in the area of Glamen peak (Fragment 1) and in the area of the St Peter's saddle (Fragment 2) Fragment 1: on the east slopes of Glamen peak /42.479706, 22.688084/. It covers an area of 1 decare. Number of about 700 generative individuals. Average density 10 individuals per m². Fragment 2: the saddle St. Peter /42.499202, 22.692694/. Area of 2 decares. Number of 500 generating individuals. Average density 10 individuals m². There are also quite a few individuals found in the area of St. Peter saddle, Mechkata Peak, Ivanov Peak, Vilna Mogila Peak and others.
APPLICATION OF A MICROINVASIVE METHOD FOR THE EXAMINATION OF THE GASTRIC CONTENT OF STURGEON SPECIES (PISCES: ACIPENSERIDAE) FROM THE DANUBE RIVER

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Keywords: tabanids, fauna, structure of horse flies assemblages, zoogeographical aspects, Bakadzhitsite hills, South- East Bulgaria

Microinvasive methods are widely used in studies of rare and endangered animals. All sturgeon species currently living in the Danube River are either critically endangered or at the edge of extinction. The reasons for this are complex, but one of the possible is changes in food resources. Although sturgeons have been studied on a systematic and zoogeographical point of view, the diet of sturgeons in the Lower Danube waters is quite unknown. The aim of the present study is testing application of non lethal methodology for investigation of the diet composition of the sturgeons. During a three months sampling (from June to August 2019), 56 specimens (N – 56; mean total length (TL) - 367 mm; range: 180 – 580 mm; mean weigh (W) - 274,5 g; range: 29 – 1145 g) from species Acipenser gueldenstaedtii, A. stellatus and A. ruthenus were collected from Danube River near the village Vetren. The study has tested a methodology for examination of gastric content of sturgeon species in field conditions using the gastric lavage method. Gastric lavage involves inserting a probe down the esophagus in to the stomach where water is flushed to induce regurgitation. The results show that extraction of gastric contents was 91 % successful and more research is needed to test the efficiency of the method for specimens up to 45 grams. No lethal or negative effects were detected during the recovery period after the procedure.
TERATOLOGICAL CHANGES IN CYCLOPS VICINUS (ULYANIN, 1875) (COPEPODA, CYCLOPOIDA) FROM VAYA LAKE

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The zooplankton of Lake Vaya was investigated and a specimen of C. vicinus was found in 2005 with teratological changes concerning shortening of the wax and changing the number of bristles on it. This type of change is first reported in zooplankton organisms in Lake Vaya, which is under intense anthropogenic pressure.
NEW RECORDS OF PTEROMALIDAE (HYMENOPTERA: CHALCIDOIDEA) FOR LOZENSKA MTS., WITH SOME CONTRIBUTIONS TO THE FAUNA OF BULGARIA

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Keywords: avian behavior, birds, alpine swifts, camera trap, parental behavior

The family Pteromalidae is poorly studied in Lozenska Mountain and only three species were recorded from this area since now. We sampled fifteen localities in grasslands using classic sweep-netting techniques for collection of small insects. Also, a number of cynipid galls (Cynipidae) were gathered in six other localities from Quercus cerris L. and Q. pubescens Willd trees for subsequent rearing of their inhabitants. Twenty pteromalid species were established, all considered as new for the fauna of the mountain. Six species are recorded here for the first time from Bulgaria - Catolaccus ater (Ratzeburg), Norbanus meridionalis (Masi), Psilocera confusa Graham, Pteromalus albipennis Walker, Stenomalina laticeps (Walker) and Stinoplus etearchus (Walker). In addition, we discuss the distribution of the species and their zoogeographical classification.
POSTER SESSION

PHYSICAL DEVELOPMENT OF STUDENTS IN FACULTY OF BIOLOGY OF SOFIA UNIVERSITY "ST. KLIMENT OHRIDSKI"

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The purpose of this study is to characterize anthropometric physical development of Bulgarian students at the beginning of the XXI century, by comparison with the results of previous studies of students in Sofia. An anthropometric survey of 77 students, 18 to 25 years old, 31 males and 46 females in the Faculty of Biology at Sofia University, was conducted in 2016-2017. The results show stagnation of the secular changes in height, which should be attributed to the long-term negative impact on the growth and development of the students during the poor years of their early childhood. Secular weight changes are positive, thus BMI increases sharply as well. This indicates that weight and BMI respond in a shorter time to changes in living conditions than height. Most physical development traits show the similar dynamics as weight and BMI. Inter-age differences in height during the comparison period (1986-2017) increase and exceed those described as normal differences in human populations. The observed secular changes are probably due to the large socio-economic changes during the period under review (1986-2017).
CEPHALOSCOPIC INVESTIGATION OF SCHOOL CHILDREN FROM CENTRAL GREECE

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Keywords: cephaloscopic traits, Greek population

For the purposes of this study, 6 cephaloscopic traits of 2683 school children from the regions of Thessaly and Epirus of Central Greece were described. The assessment of the differences in the frequencies of occurrence of these features between the studied subgroups using the $\chi^2$ test, showed that in some cases there are statistically significant differences by gender, socio-demographic and territorial-geographical aspect. Considering the frequencies of all features together (in combination) enhanced the differentiation between the compared groups according to $\chi^2$, with single lack of differences between boys and girls from the villages of Thessaly ($p = 0.603$). It is emphasized that these differences characterize only the frequency of occurrence of a given trait, and do not reflect a different form of this trait.
WHAT DO WE KNOW ABOUT MARINE ANIMALS FROM SOUTH BAY, LIVINGSTON, ANTARCTICA? THE EXPERIENCE FROM THE XXVIITH BULGARIAN ANTARCTIC EXPEDITION

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Livingston Island is part of the South Shetlands, Maritime Antarctica. There, on the shore of the South Bay are located the Bulgarian Antarctic Base "St. Kliment Ohridski". Numerous marine mammals and birds are known from the South Bay. There is a feeding site of the humpback whale *Megaptera novaeangliae* (Borowski, 1781). At Hannah Point there are colonies of the southern elephant seal *Mirounga leonina* (Linnaeus, 1758) and the gentoo penguin *Pygoscelis papua* (Forster, 1781). The Weddell seal *Leptonychotes weddellii* (Lesson, 1826), the leopard seal *Hydrurga leptonyx* (Blainville, 1820) and the chinstrap penguin *Pygoscelis antarcticus* (Forster, 1781) are also common in the region. However, the Bulgarian researches on the marine biota of the Livingston Island are just beginning. With this study we aim to provide initial data on the most common groups of marine animals (invertebrates and fishes) from different habitats as a result of the XXVIIth Bulgarian Antarctic expedition:

1. **Lithotelms.** Water-filled depressions in seashore rocks present a favourable habitat for harpacticoids (order Harpacticoida, Crustacea): high water temperature and oxygen concentrations, refugee, food resources. There we found four harpacticoid species, two of which were new for the region. Also we found many oligochaete worms (family Enchytraeidae) and Antarctic mites.

2. **Interstitial habitats.** The waters from the sandy beaches were inhabited by many harpacticoids, as well as by a small number of mites and oligochaetes. Common species were invertebrates from the sea littoral zone (molluscs, amphipods) or planktonic ones (Copepoda).

3. **Tidal zones (0 to -2m depth).** In this area there is a very strong abrasive effect of the sea ice, therefore benthic fauna (flora also) are not presented well. Two exceptions were the Antarctic limpet *Nacella concinna* (Strebel, 1908) and the active swimmers of the order Amphipoda.

4. **Marine pelagial zone (0 to -50m depth).** This zone is characterised by a large number of “krill” crustaceans (*Euphausia superba* Dana, 1850). Pteropod gastropods were also recorded: the sea angel *Clione antarctica* E. A. Smith, 1902 and the sea butterfly *Limacina rangii* (d'Orbigny, 1835). The microzooplankton was extremely poor in terms of species and with very low abundance. Only single representatives of the orders Calanoida and Cyclopoida (Copepoda) were found there.

5. **Shallow littoral zone (-2 to -20 m depth).** On the stony bottoms (in front of the Bulgarian base) were found mainly: the order Amphipoda, molluscs (incl. *N. concinna*), the sea star *Odontaster validus* Koehler, 1906 and the sea spiders *Decolopoda australis* Eights, 1835. In the muddy bottom (Johnsons Dock Bay) were found: the polychaete scale worm *Barrukia cristata* (Willey, 1902) and catworm *Agaephampus trissophyllus* (Grube, 1877); the bivalves *Laternula elliptica* (P. P. King, 1832) and *Aequiyoldia eightii* (Jay, 1839); the isopods *Spinoserolis beddardi* (Calman, 1920) and the corrugated ribbon worm *Parborlasia corrugatus* (McIntosh, 1876). In the shallow littoral zone (Johnsons Dock Bay and Rezervnato Bay) were found five fish species: of family Nototheniidae - *Notothenia coriiceps* Richardson, 1844; *N. rossii* Richardson, 1844; *Trematomus tokarevi* Andriashev, 1978, *T. rematomus nicolai* (Boulenger, 1902) and of family Channichthyidae - *Chaenocephalus aceratus* (Lönnberg, 1906).
CAMERA TRAPPING MARTENS IN TWO DIFFERENT MOUNTAINS – EFFECT OF THE LURE

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Stone marten (Martes foina) and pine marten (Martes martes) are two sympatric European species which overlap their distribution and inhabit similar habitats in the Bulgarian mountains. The study of their interactions, in sense of interspecific competition, is difficult due to their similar external appearance and similar tracks and signs of their presence. Camertrapping provides valuable data on the species presence and behaviour, but to intensify the data collection lure is often used. During May-August 2019 equal number of camera traps were set in two mountains with different habitats, Vitosha (n=10) and Rhodopi (n=10) with three different methodological approaches: 1. Without lure; 2. Applying valeriana lure with attenuating effect for a month (two times for Rhodopi and once for Vitosha); 3. Intensive luring session for 7 days. The camera traps in Rhodopi Mtn recorded two times more martens (n=72) than those in Vitosha (n=38). Both locations yielded a significant number of non-identifiable martens (n=27 for Vitosha and n=40 for Rhodopi). The pine marten was not assuredly confirmed for Vitosha, although having been registered in previous studies. No martens were registered in Vitosha Mtn during the intensive luring session, and only few (n=5) were registered after the lure was applied. Five registrations of martens were made during the intensive luring session in Rhodopi mountain (one stone marten confirmed) and 44 registrations were made after the application of the lure, as all confirmed pine martens were registered with a lure present. We hypothesize that the higher number of martens’ registration in Rhodopi Mtn is due to the higher number of pine martens in this mountain, which are probably more attracted to the lure.
Testate amoebae are a group of free-living unicellular protozoans that possess enclosed, morphologically distinct shell. They are present in a wide range of wetland environments and are especially numerous and diverse in peatlands. Testate amoebae distribution is a result of their specific adaptation to a wide variety of environmental factors such as moisture content, nutrient status and pH. The aim of this study is to investigate the taxonomic diversity, frequency of occurrence and dominant structure of Sphagnum-dwelling testate amoebae in Pirin Mountains. Samples were taken from three spatially separate regions. A total of 112 testate amoebae species belonging to 35 genera were identified. There are considerable differences in community composition between sampling regions. Two new species for the Bulgarian fauna were registered – Arcella bathystoma and Sphenoderia ovoidea. A new locality of the rare species Arcella dentata was established in Popovi Livadi area.
B&BE-11

WOLF (CANIS LUPUS L.) DIET IN OSOGOVO MTN. (BULGARIA) OVER TWO YEARS

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Studying the diet of an apex predator such as the wolf (Canis lupus L.) is important for revealing the relationships between this species and its ungulate prey. Between April and September, a total of 50 wolf scats were collected for two consecutive years (2018 and 2019) in the Osogovo Mountains (n= 26 for 2018 and n=24 for 2019). The comparison between the two years shows that in 2018 the wolf consumed only larger species - wild boar (Sus scrofa L.) (53.8%) and domestic horse (Equus ferus caballus L.) (46.2%), the latter being eaten mainly as carrion due to the harsh winter. In 2019 the wild boar dominated in the food spectrum (80.8%), as the other species, such the domestic horse and the roe deer (Capreolus capreolus L.) (46.2%), the latter being eaten mainly as carrion due to the harsh winter. In 2019 the wild boar dominated in the food spectrum (80.8%), as the other species, such the domestic horse and the roe deer (Capreolus capreolus L.) are represented less (11.5% and 3.8% respectively). The lower content of domestic horse remains may be explained by the increased care which the free ranging horses have received over the considered period. The results from a previous survey (conducted in 2005) in the same area showed that the roe deer accounted for a much higher proportion – 71.9% in the wolf’s diet. The decline in the wolf’s preferences for roe deer, as well as the increase in the percentage of higher-mass species in their food over the years may be due to the increasing number of wolves in the region. They consequently form larger packs with higher energy demand. This assumption is confirmed by the official game census. Despite the fact that a recent camera trap study in the same region indicates a higher density of roe deer (3.08-3.22 ind./km²) compared to the wild boar (0.82-0.90 ind./km²), the roe deer with its smaller average mass provides less energy per hunting effort for the wolf.
MORPHOLOGICAL VARIATION OF METOPIC SUTURE IN ADULT HUMAN SKULLS FROM THE NECROPOLIS OF ANTIQUE SALONA (CROATIA)

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Keywords: skull, Metopic suture, Metopism, variation

The Metopic (frontal) suture passes between unfused frontal halves and rarely persist into adulthood. Have a different opinion for a full ossification of the frontal suture. After Romanes (1972) metopic suture disappear by the fifth or sixth year, after Hamilton (1976) by the seventh and according Warwick and Williams (1980) disappear in the eight year and after Vikram (2014) is between 1 to 8 years of birth. Generally, metopic suture is defined to disappear to the early childhood. The incidence of metopic suture varies by races and has been reported in many populations. The complete Metopic suture extending from nasion to bregma and when is presented in adulthood this condition is known a Metopism. The incomplete metopic suture varies by forms and in this study was classified as linear, double and ‘V’ shape.

The aim of this work is to describe different type of Metopic suture in adult skulls from the Early Christian necropolis in the Late Antique site Salona, Croatia (IV-VI AD), and variation of the incidence of metopic suture in the European population. For the present study from 468 human skulls 104 (60 male and 44 female) were differentiated by sex, age and macroscopically observed for Metopic suture and his variation by types.

From 104 skulls between 30 – 56 years presence of metopic suture was found in 46 skulls (44.23%). The different variation of the Metopic suture was found in 16 (36.36%) female and 30 (50.00%) male skulls. 7 skulls (6.73%) are with complete Metopic suture (Metopism 4 females and 3 males) and 39 (37.5%) with incomplete (27 males and 12 female). The skulls with complete Metopic suture were observed for meet the condition end-to-end with the median saggital suture, at Bregma and it is present in 4 (3.84% 2 male and 2 female) skulls. The other condition, not end-to-end is observed in 3 (2.88%, 2 females and 1 male) skulls.

The present study show the anatomical variation of metopic suture and determine the incidence in adult dry skulls from the early Christian necropolis in Salona site, Croatia. The knowledge of the variation of the metopic are important for neurosurgeons, forensic, and anthropological point of view.
EVALUATION OF BIOINSECTICIDES AGAINST THE GREY MAIZE WEEVIL, TANYMECUS DILATICOLLIS (COLEOPTERA: CURCULIONIDAE), UNDER LABORATORY AND FIELD CONDITIONS

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Tanymecus dilaticollis is an important insect pest of maize and sunflower in Eastern and Central Europe. Currently, as a consequence of the restriction of the European Commission through Regulation 485/2013 for the use of specific neonicotinoid insecticides, efforts are directed toward reduction in the use of synthetic insecticides in pest control. Two different experiments were performed to examine effectiveness of two commercially available bioinsecticides, Naturalis® (Bio Intrachem, Italy) based on the entomopathogenic fungus Beauveria bassiana and azadirachtin-based botanical product NeemAzal T/S® (Trifolio – M, GmbH, Germany), against field-collected T. dilaticollis adults under laboratory conditions. Our results suggested that the adults of the pests are more susceptible to B. bassiana strain than to azadirachtin. Naturalis®, tested in six concentrations (2.3 x 10² - 2.3 x 10⁷/ conidia/ ml), caused 16 - 100% mortality of T. dilaticollis at 2-9 days. The median lethal concentration (LC50) after ten days of fungal treatment was 2.0 x 10³ conidia/ ml. NeemAzal T/S®, tested in four concentrations (0.5%, 1%, 2 % and 4%) with two applications, caused generally low mortality during all treatments – the percent of mortality ranged between 6% and 31% within 16 days. The mortality slightly increased with concentration after the second application. Furthermore, a field experiment consisted of two spraying of maize experimental plots with Naturalis® at a concentration of 200 ml/ 0.1 ha and recording the number of T. dilaticollis adults (alive and dead) in treated and control plots was conducted in Knezha, north-western Bulgaria. First spraying was carried out immediately after the maize seeds were planted, with two-week-interval between sprayings. Twenty days after exposure the mycoinsecticide caused higher mortality of the adults compared to the control treatment.

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DETERMINATION AND COMPARISON BETWEEN BIOLOGICAL RELATIVES WITH RESPECT TO DERMATOGLYPHIC PATTERNS ON GENERAL FINGERPRINT TYPES

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Keywords: dermatoglyphic patterns, correlations, fingertip patterns, Bulgarian population, frequencies

Dermatoglyphic patterns are good material for genetic studies because they are stable throughout life. Given that the dermal ridges developing in the period of intrauterine life, the genetic information contained in the genome can be decoded during this period and is reflected by dermatoglyphics. This study was undertaken to compare the dermatoglyphic patterns in related subjects, 104 families from the West Bulgaria.

247 parent-child pairs are analyzed for occurrence of the major dermatoglyphic patterns on each of ten fingers. There are 294 healthy children; 104 males and 147 females. Fingertip prints were taken from each participant using the ink method, and the counts of arches, loops and whorls were determined.

The relationship of fingertip patterns of the four groups showed significant variations between father and daughter (p < 0.00001) and father and son (p<0.05), but not for groups of mother - daughter and mother - son. Correlations between father and daughter, father and son, mother and daughter, and mother and son regardless of sex were calculated for the occurrence of arches, loops and whorls. Parental influence varied widely in the specification of the different dermatoglyphic patterns. Father-son and father-daughter correlations were higher for the three major traits - whorls, loops and arches, mother-daughter correlations were higher only for arches and mother-son were weak for arches, but moderate strong for loops and whorls. These influences could be both genetic and non-genetic and must be further quantified and studied before pattern determination on fingertips can be correctly understood.
EPIDERMAL PALMAR RIDGES COUNT ON THE PALM IN BULGARIAN POPULATION

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Keywords: palmprint patterns, Total palmar ridge count (TPRC), Bulgarian population

The aim of this study is to investigate the variations in the epidermal ridges number on the palm into three cohorts/communities (Bulgarians Christians, Bulgarian Muslims and Bulgarian Turks) of Bulgarian population.

Present study includes 1395 healthy individuals, aged from 17 to 35 years - Bulgarian Christians (300 males and 300 females), Bulgarian Muslims (199 males and 199 females) and Bulgarian Turks (198 males and 199 females) from all over the country.

Comparison between each of the three communities was carried out using the following patterns: Ridge count of II (RC a-b), III (RC b-c), and IV (RC c-d), interdigital palm area, Ridge count left and right (a-b, b-c, c-d, a-d) and Total palmar ridge count (TPRC).

Based on the available data, no significant differences were found in the palmar ridge number between the two genders in the three communities of Bulgarian population. Sexual differences in palmprints for intra-groups comparison showed statistical significance between Bulgarian Muslims females and Bulgarian Christians and Bulgarian Turks for Total palmar ridge count (TPRC), (p <0.05).
ROLE OF BACTERIAL EXOPOLYSACCHARIDE IN PHAGE INFECTION

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Keywords: testate amoebae, biodiversity, dominant structure, Bulgaria

The members of the genus Xanthomonas are phytopathogenic bacteria that produce exopolysaccharide (EPS) - xanthan. The main purpose of the following study is to determine the role of this EPS in phage infection on xanthomonads. The amount of xanthan produced by the bacteria depends on the sugar content of the cultivation medium. For the purposes of this study two different media were used: NA (Nutrient Agar) where the bacteria produce less xanthan and PSA (Potato Sucrose Agar) where the quantity of the xanthan is more. The efficiency of plating (EOP) of 8 specific phages (105д/1, 105д/2, 105д/3 – specific to Xanthomonas euvesicatoria (Xe), 62т/1, 62т/2 – specific to Xanthomonas gardneri (Xg), 68т/1, 68т/2 and 68т/3 - Xanthomonas vesicatoria (Xv)) was tested via Double agar overlay plaque assay. As specific hosts 25 phytopathogenic xanthomonads strains (Xe – 17, Xv – 6 and Xg – 3) were used. The phytopathogenic bacteria used differ in their pathotype (Pepper – Tomato PT, Tomato T or Pepper P) and races. The results obtained showed that generally the cultivation of the bacteria on NA medium make them more susceptible to phage infection. The EOP of phage isolate 105д/1 showed that 63% of PT Xe strains and 89% of P Xe strains were more susceptible to phage infection when were cultivated on NA medium. Almost the same were the results for phage isolates 105д/2 and 105д/3, as the percent of more sensitive on NA medium PT and P strains were 88% and 89% for phage isolate 105д/2 and 63% and 67% for phage isolate 105д/3, respectively. A similar trend was observed for the efficiency of Xg specific phages 62т/1 and 62т/2. Phage isolate 62т/1 showed less activity than 62т/2 as it was active against only one phytopathogenic strain (62t) but the EOP on NA was 100% while on PSA – 60%. Phage isolate 62т/2 turned to be active against two phytopathogenic strains (62t and 76t) as for strains 62t there was no activity on PSA medium. For strains 76т phage activity was observed on the two media, but the EOP of the phage was greater on NA medium than on PSA. The EOP of phage isolates specific to Xv was not determined due to its inability to produce pure lysate. At this stage of the experiments we assume that these are lysogenic phages, not lytic. The main conclusion from this study is that the amount of the EPS produced from the bacteria plays a key role in phage efficiency.

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ASSESSMENT OF THE IMPACT OF ESSENTIAL FACTORS IN THE TRANSFORMATION OF THE BODY AND THE FORMATION OF SEXUAL DIFFERENCES DURING PUBERTY

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During the intensive morphological growth in the puberty, the age and sex (both as main effects and in their interactions) play an essential role in the phenotypic trait development. In the present work, we analyze data on the influence of age and sex on the dimensionality of 15 main height-length, width, and circumferential anthropometric features in 9-15-age-old schoolchildren from Sofia (Bulgaria). The influence of both factors on the morphological growth was estimated by MANOVA. The results indicate that almost all of the morphological features are statistically significantly influenced by age and sex. The interaction of these factors has been found to be statistically significant for 10 of the measured features. So the growing boys are on average higher, have longer body parts, as well as larger chest circumference and breadth, wider shoulder and pelvic girdles, and a larger forearm circumference compared to the growing girls. From the combination of the two factors (age & sex), apart from calf circumference are not significantly affected also dimensions of the waist, hip, thigh, and upper arm - relax. The size of the other circumferential dimensions that we have analyzed was influenced separately by age and by sex, while both these factors influenced relatively more equally both sexes only in the areas of hip and of the thigh.
SOIL MICROBIOTA AS AN INDICATOR OF SOIL QUALITY AND LAND USE IMPACTS IN AGROECOSYSTEMS OF RAZLOG MUNICIPALITY

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Keywords: Razlog Municipality, grasslands, soil quality, soil microorganisms, soil enzymes

Maintaining or improving soil quality is crucial if agricultural productivity and environmental safety are to be preserved for future generations. A study was conducted in the region of Razlog Municipality and the sampling points were from: 1) arable lands in the surroundings of Razlog town (p.R2), village of Dolno Draglishte (p.DD) and village of Bachevo (p.B); 2) grasslands in the surroundings of Razlog town (p.R1), village of Gorno Draglishte (p.GD) and village of Godlevo (p.G). At the present study, key indicators taken into account to evaluate the impact of different management practices on soil quality were soil chemical and microbiological properties, both of them subjected to slight (soil chemistry) and relatively strong (soil biology) seasonal fluctuations. The texture of the soils was dominated by the sand fraction (49% - 71%). Soil pH varied from acidic (p.DD) through slight acidic (p.R2, p.GD, p.G and p.B) to neutral (p.R1). Soil was with high (p.R1 and p.G - 20.89 g/kg), medium (p.GD, p.B and p.DD - 13.92 g/kg, on average) and low (p.R2 - 9.74 g/kg) content of organic carbon. Total nitrogen varied from 2.21 g/kg (p.R1) to 1.08 g/kg (p.DD), whereas the average concentration of total phosphorus was 0.72±0.27 g/kg. No heavy metals and pesticides pollution were recorded in the soils. Cluster analyses of soil chemical properties grouped soils according to their location, while microbial clustering clearly distinguished the soils by their use to grasslands and arable lands. In grasslands, soil enzyme activities (dehydrogenase (45.19 µg/g) and phosphatases (45.28 µg/g)), bacterial number (31.33 x 108 cells/g), and bacterial capacity to utilize Biolog™s carbons (AWCD - 1.38 OD) were two, four, and one and a half times higher than that of arable lands, respectively. Our study proved that the sustainment of grasslands on the territory of Razlog region is a good agricultural practice, maintaining in a good balance the man-nature relationships. The food and ecological incomes will increase if farmers from the region increase the share of organic production - forage, meat and milk.

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INVESTIGATION OF MICROBIOTA IN ATMOSPHERIC AEROSOLS DURING LIDAR MONITORING OF HIGHLY URBANIZED AREA IN SOFIA CITY

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Keywords: LIDAR monitoring, bioaerosols, particulate matter (PM)

The air quality in polluted areas in Sofia city with fine particle matters (PM) become a serious problem in the last few years. The microbial fractions associated with particulate matter (bioaerosols) is also very important for air quality.

The purpose of the present study was to conduct microbiological analysis of airborne microbiota in outlined by LIDAR characteristic zones of increased concentration of aerosol pollutants using classical taxonomy and 16S rDNA sequencing analysis.

The lidar mapping was performed by the two-wavelength lidar system developed at the Laser Radar Laboratory (LRL) of the IE-BAS that is capable of scanning the horizontal aerosol distributions and the vertical long-distance transport of air masses.

Lidar beam was directed to Tzarigradsko shose bvd and the samples were collected from three location points – Techpark, Lavov most and Dragan Tzankov bvd.

A Hygitest 106 (Maimex) for air sampling as well as Koch sedimentation method were used for collection of the samples. Four different filters (Gelatin, Cellulose-ester, Polymer-fibrous and Glass fibrous) and different elective media were used to enumerate and identify the isolates.

The obtained results show dominant presence of genera Bacillus, Erwinia, Acidovorax, Enterobacter, Arthrobacter, Streptomyces, Kocuria, Rathaybacter. Among the isolates are established opportunistic pathogens referred to Bacillus cereus, Bacillus pumilus, Enterobacter aerogenes, Erwinia herbicola. In all samples the fungi from genera Penicillium and Aspergillus were permanently found.

As a conclusion, it must be noted that the approach used is effective for analyzing the microbiota in air bioaerosols during the Lidar monitoring of particulate matter (PM) and is of great importance for air monitoring and qualitative evaluation of air in urban areas.

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MAPPING OF NATURAL HABITATS AND PLANT SPECIES WITH CONSERVATION IMPORTANCE ON THE TERRITORY OF ETROPOLSKA STARA PLANINA MTS.

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Keywords: Natura 2000, Braun-Blanquet, habitat diversity, mapping.

The main aim of the current research is to reveal habitat diversity in Etropolska Stara Planina Mts, especially these habitats included in Annex 1 of the Bulgarian Biodiversity Act with special attention to the plant species with conservation importance. For this purpose, the dominant plant communities were studied according to the floristic methodology of Braun-Blanquet (1964). On the basis of species composition and ecological characteristics, the relevant NATURA 2000 habitats were determined. As a result, a thematic map of the natural habitats and species with conservation importance for the territory of Etropolska Stara Planina Mts was made.

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THE EFFECTS OF MEDIA ON NATURAL HABITATS IN TURKEY

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Keywords: Honey bee, Apis Mellifera, atypical open-air nests

Both of human life and natural life are build on various balances in the world. The relationship between humankind and the environment, is the most important of these balances. As a result of the deterioration of the natural balance, the ecosystem is also disturbed and ecological problems arise. Undoubtedly, the deterioration of the ecosystem balance in the world is caused mainly by the increase in human population and activities. Nowadays, technological developments also have significant effects on this subject. While technological developments improve and facilitate the living conditions of people, they also play an active role in the disruption of natural balance. People who want to get away from the noise and chaos of the city are looking for new natural recreation areas. However, the natural areas which are discovered by human beings begin to deteriorate rapidly due to over population. Investors who start to see potential profit income in places that people visit frequently tend to the construction of food and beverage and accommodation facilities by reducing the existing green spaces. Media and especially widely used social media, due to it reached in a short time to a large audience, is unfortunately accelerating this process of deterioration also in Turkey. These regions rapidly lose their natural beauty due to increasing demand and start to face pollution problems. The aim of the study is to reveal how the places which are only well-known by local people in Turkey, are affected by the influx of visitors through the media. For this reason, situations of before and after sharing in the media of selected natural areas from various geographic regions of Turkey are given comparatively.
INFLUENCE OF THE RAPID TEMPERATURE INCREASE ON THE BINDING OF CO-60 AND CS-137 TO SOIL ORGANIC MATTER

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Studies of the geochemical behavior of technogenic radionuclides under the conditions of abrupt changes of the temperature are necessary for a more adequate assessment of the risk of their spreading through the food chains, especially during the first months after radioactive contamination, when radionuclides are less strongly bound to the soil. Co-60 and Cs-137 are technogenic radionuclides that can enter the environment as a result of accidents involving nuclear power plants, gamma irradiators, or radioactive waste. The purpose of this study is to determine the effect of the rapid temperature increase over a period of one month on the binding of Co-60 and Cs-137 with humic and fulvic acids in soils from ten regions in Bulgaria. This paper presents the results from a model study, which was carried out with soil samples from the surface 0-10 cm layer. The samples were contaminated with a radioactive solution in the laboratory and stored at conditions simulating hot summer and spring/autumn using a climatic chamber. The results obtained show that abrupt warming reduces the relative Co-60 content associated with humic and fulvic acids in soils with low cation exchange capacity (CEC) and higher sand content. In the other soils characterized by normal CEC and clay texture, the organic-bound Co-60 increases with increasing temperature. It has been found that rapid warming has little influence on the binding of Co-60 to humic and fulvic acids in acidic pH soil. Conditioning at elevated temperature affects differently the binding of Cs-137 with humic and fulvic acids in different soils. It is necessary to analyze the mineralogical composition of the studied soil samples to explain the observed changes.
Phytoremediation is defined as the cleaning process for contaminated areas from pollutants by using growing plants. Organic and inorganic materials can be eliminated with plants from the polluted area with this technology. It is preferred to use plants that absorb environmental pollutants, accumulate high levels in their tissues and detoxify them through physical, chemical and biological processes. In this way, it is aimed to regulate or stabilize contaminated waters and lands. Phytoremediation has become widespread in many countries in recent years, which is a passive technology for cleaning the heavy metals from contaminated soils and waters. The plants used in these studies should be able to grow in a healthy way in polluted areas without being affected by the existing pollutants and be able to form their roots and green parts at sufficient levels. For this reason, there are extensive scientific researches on the treatment processes which are economically inexpensive and practical in the treatment of such wastes which are important problems of many industrial organizations. As a result of these researches, it has been seen that microorganisms, algae, fungi and some plants can be used in metal biosorption and there is a need for more detailed research on this subject. Phytoremediation is defined as the use of hyperaccumulator plants to remove or neutralize the pollutants in the environment. Compared to different improvement methods, it has many advantages such as its low cost, aesthetically pleasing, ease of application and short application time. Also this method can be used in conjunction with other methods as a completion phase. Another condition to be successful in phytoremediation is the development of suitable plants and determination of the tolerance mechanisms of the plants to heavy metals. In phytoremediation technique, hyperaccumulator plants are used which absorb heavy metals and accumulate high levels in their tissues and neutralize them after various processes. The aim of this study is to draw attention to phytoremediation, one of the most effective and important environmental friendly technology, in water pollution problems. In this way, it is thought that the studies related to phytoremediation applications will be increased and improved. Additionally, detailed studies on genetics and physiology of hyperaccumulator plants need to be increased.
PLANT-DERIVED PROTEIN HYDROLYSATES STIMULATE FUNCTIONAL ACTIVITY AND GROWTH OF YOUNG CUCUMBER PLANTS

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Plant biostimulants are a promising tool and sustainable approach for promoting nutrient uptake, photosynthetic assimilation and plant productivity. Biostimulants can mitigate the adverse impact of abiotic factors by improving the biochemical, morphological and physiological processes in crop plants. Plant-derived protein hydrolysates are important plant biostimulants because they can increase seed germination and plant productivity. The present work was aiming at studying the effect of three commercial protein hydrolysates Kaishi, Naturamin and Terra-Sorb on functional activity and growth of young cucumber plants. Cucumber seeds were soaked for 4 h in distilled water (control) and in a range of different concentrations (0.01%, 0.05%, 0.5%, 5% and 10%) of Kaishi, Naturamin and Terra Sorb. The effects of biostimulant treatments were studied in 30-day-old cucumber plants. Changes in quantum yield of PS II (ϕPSII), quantum yield of non-regulated heat dissipation (ϕNO), quantum yield of nonphotochemical quenching (ϕNPQ) and plant growth parameters were monitored. It was found that two hydrolysates had a positive effect for the functional activity of the young cucumber plants. The optimal concentration for Terra Sorb was 0.05% and 0.01% for Kaishi. Naturamin treatment didn’t significantly influence the analyzed fluorescence parameters. Kaishi and Terra-Sorb stimulated the root growth more significantly at 10% for Kaishi and 0.5% for Terra-Sorb, while Naturamin did not have any effect on roots. All of studied substances positively affected leaves and stem biomass accumulation with optimum 5% for Kaishi and Terra-Sorb and 0.01% for Naturamin.

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MINING NEAR AN URBANIZED AREA - PROJECT BASED TRAINING

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Keywords: Project-based learning, school, Human activity, economic interest, Russian federation, ecology

Project-based learning at school is necessary because students work much more successfully outside of the classroom hours. The freedom of the student and the actuality of the problem give a sense of importance and the benefit of the effort. Human activity has a very significant impact of nature and this fact is the reason why are required measures for the rational use of natural resources. Unfortunately, in many cases economic interest plays a leading role. This is the case that we have chosen to explore and realise at school through a debate on topic “For and Against mining in the near urban area- project-based learning. In block hours (2 hours) students will participate in a debate. They are pre-divided into three groups: Group 1- protect the economic interest of the Russian federation from mining (diamonds). Group 2- protect the economic interest of the population (providing jobs), but also the interest of the population in terms of how this activity affects the quality of life in this area. Group 3- environmentalists-protect the interest of nature. Students have two weeks to research all the details, to form an opinion, to prepare their arguments, to suggest the arguments of their opponents and to rebut them, to develop strategies.
POSSIBILITIES FOR BIOLOGICAL CONTROL OF FUSARIUM ROOT ROT ON CUCUMBER WITH ANTAGONISTIC FUNGI FROM TRICHODERMA AND GLIOCLADUM GENUS

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Key words: cucumber, Fusarium root rot, immune response, antagonistic fungi, combine application

Potential disease control method was evaluated against root and stem rot of cucumber (Cucumis sativus) caused by Fusarium oxysporum f. sp. radicis-cucumerinum. The efficacy of strains of antagonistic fungi of the genus Trichoderma and Gliocladium, applied separately and combined, on the growth and immune response of plants to Fusarium root rot (Fusarium oxysporum f. sp. radicis-cucumericum D.J. Vakalounakis) were investigated. The symbiotic properties of the strains were investigated in co-cultivation on an artificial culture medium (potato-dextrose agar) by the co unter colonies method (Waterhouse). The biometric indices of the seedlings and plants during the vegetation were made, the percentage of Fusarium root rot of the variants was calculated. For this purpose, the biological control agents Trichoderma viride (Gliocladium virens) were added at seeding time followed by inoculation with the pathogen. It has been found that combined introduction of several strains of Trichoderma viride and Gliocladium virens in substrate improves biometric parameters of seedlings and plants, accelerates flowering and fruit formation in cucumbers and has a prophylactic effect on the development of Fusarium root rot in this crop. The results from this study indicate that several different strains of Trichoderma viride and Gliocladium virens can be used at seeding to control Fusarium root and stem rot on greenhouse cucumber.
ON THE USE OF GAMMA-IRRADIATION FOR DISINFESTATION OF LEATHER, PAPER AND WOOD MATERIALS IN BULGARIA

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Artifacts made from organic materials (natural polymers) are at risk of biological attack under improper storage conditions. Their destruction by insects and microorganisms such as fungi and bacteria represents a major threat to their long-term preservation, causing degradation, pigmentation and physical damage to the materials. Chemical treatment with ethylene oxide, methyl bromide, have disadvantages, such as incomplete material penetration as well as risks related to long-term document stability, environmental pollution and health of the staff.

In the recent decades it was shown that ionizing radiation is extremely suitable for disinfestation and preservation of organic-based artifacts. This study summarizes the main results of studies on the influence of gamma-irradiation on the structure and properties of leather, paper and wood materials. Analytical data, obtained with SEM, EPR, FTIR, DSC and TG are presented. It was found that the application of doses 5-10 kGy does not cause significant changes in the structure of the studied materials. The leather samples were found to be the most stable, incl. after administration of doses up to 25 kGy. Effects on wood and paper patterns depend on the age, composition and extent of biological damage. On the basis of the results obtained, it can be concluded that gamma irradiation with appropriately selected doses can be successfully applied for the purpose of preserving materials of leather, wood and paper without disturbing their structure.

Acknowledgments: This study was performed with the financial support of the IAEA, Coordinated Research Project F23032, Research Contract N– 20567.
THERMOACIDOPHILIC CHEMOLITOTROPHIC ARCHAEA ISOLATED FROM DIFFERENT SULFIDE ORE DEPOSITS IN BULGARIA

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Keywords: Thermoacidophilic archaea, Sulfolobus, Bioleaching, Chemolithotrophs

Acidophilic chemolithotrophs oxidizing sulfide minerals, ferrous iron, elemental sulfur and reduced sulfur compounds are the typical microbiota of the sulfide ore deposits. On the same time the acidophilic chemolithotrophic archaebacteria also can be found in such habitats because of their specific environmental factors. These archaebacteria belong to genera Sulfolobus, Metallosphaera and Ferroplasma.

In this study the diversity of acidophilic archaebacteria and the possibility for practical application are investigated.

Samples from five sulfide deposits were collected for analyzing the presence of these archaebacteria. Elective media and specific conditions of cultivation were used for obtaining the enrich cultures and identification of the pure cultures. The taxonomic status of the isolates was achieved by the PCR methodology, using specific primers (F1_SuM (Sense) и R1_SuM (Antisense) and sequencing. Optimal parameters for growth and favorable energy substrates were determined by classical microbiological methods.

It was found that the archaebacteria did not present in all deposits tested but they are permanently present in acid mine drainage. The dominant species based on molecular taxonomy done were Sulfolobus metallicus, Metallosphaera sedula and Ferroplasma acidiphilum.

Being acidophiles, they can not only withstand the acidic conditions necessary for bioleaching, but also produces sulfuric acid that can used for the bioleaching process and maintains the necessary levels of oxidized iron through its metabolism.

Bioleaching of pyrite and chalcopyrite as well as desulfurization of the coal at temperatures conducive of Sulfolobus metallicus and Metallosphaera sedula were more effective than treatment with mesophilic bacteria.

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NEGATIVE EFFECTS OF WASTE SHOES ON ECOSYSTEM

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In order to protect the foot from external influences in the early ages, weed-ivy and leather and footwear that entered human life were exposed to change with the effect of the Industrial Revolution. The materials used such as plastic and the increase in the number of shoes used brought about the problem of shoe waste. Materials used in shoemaking and shoes that cannot be used have harm to nature and human health. The materials used in shoes such as leather, glue, plastic, metal damage the ecosystem when they are disposed of in the trash, and large manufacturers continue to work on the subject. Shoes that are old and can’t be used are burned and buried in the ground around the World. Reuse of the shoe by repairment and reuse of upper and sole materials as a shoe or producing a different production through recycled is important. Used shoe sole materials are used as a ground surface material in sports halls, overpasses, playgrounds etc. in the world as well as in our country. Shoe parts containing recyclable plastic materials can be used as additives in the production of different products. Incineration, if unused shoes are destroyed, the effect on air pollution must be eliminated. As another method, it takes a long time for the materials used to be destroyed according to the type in burying. In this way, the shoes are mixed with soil and become pollutants that will harm the living life. As a result, giving the shoes to people in need of wearable shoes, collecting waste shoes, separation of shoe materials and transport of these materials to other areas for reusing will contribute to the ecosystem and economy. In the production of footwear, it will be appropriate to direct the works from the raw material to the product and the disposal of old shoes without harming the ecosystem. For this: • Considering environmental and health of living in the production of materials required for shoe production • Shoe designers prioritize environmental impacts, use of environmentally friendly materials • Informing manufacturers to determine how to reuse of old shoes • Supporting investments in the sector for encouraging recycling It should be provided that storing the wastes separately in the shoe factories. Collecting separately of wast shoes will ensure that products remain clean and not lose their economic costs. Ecological sustainability is important for the economy of our developing country.
FARMERS’ EDUCATION – AN INSTRUMENT FOR IMPROVING AGRICULTURE PRODUCTIVITY AND ENVIRONMENTAL SUSTAINABILITY: A CASE STUDY FROM BLAGOEVGRAD REGION

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Keywords: agricultural management, farmers’ education, agricultural sustainability

To develop a successful agricultural production scheme requires specialized knowledge and skills. Such abilities provide opportunities for farmers to identify their priorities, establish contacts with colleagues, researchers, companies and national authorities. The aim of the present study was to identify the needs of specialized training for the farmers in Blagoevgrad region. A total of 248 farmers were interviewed by “Risk Analysis” Foundation using a questionnaire and the obtained results were statistically processed. The largest relative shares of the surveyed farmers were in the municipalities of Sandanski (14.9%), Razlog (12.1%), Blagoevgrad (11.7%), Simitli (10.1%) and Yakoruda (8.5%). Women accounted for 47%, while men were 53% of the respondents. The largest part of the farmers was from the age group of 35-44 years (29.6%), followed by persons aged 45-54 (23.5%) and those aged between 55 and 67 (22.3%). Most of the respondents (42.1%) pointed out the maintenance of local traditions as a main reason for their occupation as farmers. 71% of the interviewed have not received any specialized training. The main part of them (50.8%) used their colleagues as a source of information or private consultants (37.4%). 75% of the farmers expressed willingness to be trained, and the most preferable forms for training were seminars and lectures. The survey showed that farmers had no specific knowledge, connections or experience to support their agricultural activities. More information campaigns and training courses should be organized, which will lead to expanding and improving the quality of their production, hence increasing their competitiveness on the market.

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E&SD-14

WATER MICROFLORA FROM THE SOUTH-WEST PART OF BULGARIA

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The aim was to study the microflora of drinking water from springs, fountains, and wells from the Southwestern region of Bulgaria around Sofia and Dupnitsa. The following tasks were planned to fulfill this goal: To study the microflora of spring waters from the villages of Kopilovtsi, Bistrica, Verinsko and Dupnitsa. The quality of water was assessed using selective media for enumeration of E.coli, Enterococcus faecalis, Pseudomonas aeruginosa, Clostridium, and total microbial number according to European water standards. Water from Dupnitsa and Bistrica have shown an increase in total microbial number, families Enterobacteriaceae, Pseudomonadaceae, and Enterococcaceae during the investigated spring and summer period. Chromogenic coliform agar has greater selectivity than the MacConkey medium for bacteria of the Enterobacteriaceae family and is recommended for use in future studies. Cetrimide agar and King B medium showed similar results in a parallel water study. The waters from the tested fountains and wells are not suitable for drinking or agricultural use. The seasons have a strong impact on the microflora of the water in the well from Dupnitsa and in the water of the river Bistritsa, where the water catchment is located for water supply to the village.
MOLECULAR MECHANISMS OF THE PROTECTIVE EFFECT OF BIOSTIMULANT KAISHI AGAINST OSMOTIC STRESS

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Keywords: scrub communities, new alliance, Genista lydia, Bulgaria, Greece

Plant biostimulants are natural substances that can promote plant growth and development, and enhance tolerance to abiotic and biotic stress. These substances operate through not well characterised mechanisms that differ from the effects of conventional fertilisers. To improve the efficacy of biostimulants, there is urgent needs to advance our understanding of molecular mechanisms of their action. We investigated some of the molecular responses of the important food crop Zea mays and the model plant species Arabidopsis thaliana to the biostimulant KAISHI, focusing on the expression profiles of genes encoding protective proteins (dehydrins and heat shock proteins). Exposure to low concentrations (10-12 and 10-6) of the biostimulant resulted in enhanced postembryonic root growth and development in both plant species. Pre-treatment or simultaneous KAISHI treatment partially mitigated the negative effects of the applied osmotic stress, which is consistent with the observed changes in gene expression patterns.

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E&SD-16

EFFECT OF WASTEWATER TREATMENT PLANT DISCHARGE ON MICROBIAL COMMUNITY STRUCTURE AND UPTAKE OF NUTRIENTS IN ISKAR RIVER

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The upper part of Iskar River and its reservoir are the water sources in Bulgaria with the most important economic and social meaning because of their role for a drinking water supply of Sofia. The most critical factor with potential high-risk levels for water quality in this hydro system is the discharge of Samokov Wastewater Treatment Plant (WWTP) and disposal of untreated sewage from the small villages in different ecotone zones. On the ecosystem level, the adequate response to this organic pollution is based on the self-purification capacity and the joint full functioning of biocoenoses. In this study, we focus on the structure of the microbial community as functional diversity related to the main transformation processes. Microbial communities in surface waters and sediments with their associated metabolic activity have a fundamental role in the metabolism of streams and biodegradation of all pollutants. The obtained results show that the microbial groups participating in organic carbon transformation and specific indicators (bacteria from g. Pseudomonas and coliforms) have higher quantitative parameters in sediments near to discharge of WWTP Samokov. In sediments of the Palakaria River, the bacteria from the nitrogen cycle are detected in a permanently high number. These data are indicators for significant nitrogen loading and represent an adequate response of microbial community structure to this type of disturbance. The modeling of ecosystem response to risk situation of overloading with nutrients and organics from WWTP discharge confirms the high self-purification capacity of Iskar River. The importance of studied sub-catchment for the functioning of urban water cycle and quality of drinking water of Sofia enforces the extension of an existing monitoring program with assessment of functional microbial diversity and kinetics of self-purification processes in critical ecotone zones.

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INDUCTION OF PHOTOSYNTHETIC ACTIVITY IN THE NON-PHOTOSYNTHETIC CUSCUTA CAMPESTRIS BY GALL-FORMING INSECTS SMICRONYX

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Species of the genus Cuscuta parasitize on a variety of plants of agricultural importance thus reducing their yield. Being holoparasitic, their photosynthetic activity is greatly diminished. Cuscuta spp. on their own are subject to parasitism by the gall-forming insects from the genus Smicronyx. While the main function of the gall is to support larval feeding, it was noticed that it is surprisingly rich in chlorophyll. We have conducted a research on the photochemical reactions and chlorophyll content in galls of Smicronyx sp. formed on Cuscuta campestris, and have characterized the thylakoid electron-carrier complexes and distribution of RuBisCO. It was shown that the main phenotypic distinction between the galls’ tissue and the uninfected stems of C. campestris is the accumulation of chlorophyll, especially in the greenish inner cortex of the galls. The results from chlorophyll fluorescence induction curves (JIP test), as well as separation of the thylakoid complexes by 2D BN PAGE electrophoresis proved the functionality of the galls’ photosynthetic apparatus and the predominant functioning of cyclic electron flow. Western Blot analysis showed the 55 kDa RuBisCO subunit (RbcL) was equally distributed in galls and in non-gall tissue. The combination of biophysical and biochemical analysis led to the conclusion that the parasitic plant C. campestris has a conserved photosynthetic capacity, which is not manifested during the regular lifecycle. When parasitized by Smicronyx spp., however, the photosynthetic activity is induced in the inner cortex of the galls, which is possibly related to better nutrition of the larva.
MB&BT-2

STUDY OF THE BACTERIOCIN GENETIC DETERMINANTS OF ENTEROCOCCUS DURANS EDD2

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Enterococcus durans EDD2, isolated from a beehive and previously determined by polyphase approach including genus- and species-specific PCR and Sanger sequencing of the 16S rRNA gene, along with biochemical and physiological tests, showed by the agar well-diffusion test an antimicrobial inhibitory activity against the bee pathogen Paenibacillus larvae. Due to the interest on the inhibition of the beehive pathogen, potentially due to bacteriocin activities, in order to characterize the bacteriocin genetic determinants, a next-generation sequencing (NGS) of the whole genome was performed. The analysis of the NGS data showed that genetic determinants for enterocin P and enterocin L50A were present while no genetic determinants for virulence factors were found. The stain was also subjected to several antibiotics and no antibiotic resistance was observed. All these data suggest that Enterococcus durans EDD2 probably is not a result from a faecal contamination by humans or livestock, and possibly is maintained within the hive because of its protective properties against pathogenic bacteria.

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

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Keywords: Obestatin, Cardiac, Hormone, Autonomic nervous system, In vitro, Sympathectomy, Vesicular monoamine transporter

The aim of this study is to investigate the inotropic effect of obestatin in the presence of reserpine 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 10 μmol/l reserpine.

It is concluded that, the treatment with reserpine inhibits vesicular monoamine transporters (VMATs) and so it depletes adrenergic stores and abolishes positive inotropic effect of obestatin

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MB&BT-4

ENHANCEMENT OF HIPPEASTRUM PAPILIO GROWTH USING HYDROPONIC SYSTEMS

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Hippeastrum papilio (Ravenna) Van Scheephen (Amaryllidaceae) is an endangered endemic species from the rainforests of Brazil. It was considered extinct, but in 1990 was rediscovered with about 50 individuals. The species is not only an attractive ornamental plant, but the one with the highest content of the valuable alkaloid galanthamin. Beautiful hybrid cultivars have been created whose galanthamin content is very low; on the other hand, multiplication of native high productive individuals by in vitro clonal propagation is very slow. The aim of the present study was to enhance the growth of H. papilio bulblets using two different hydroponic systems and to compare their effectiveness. Sixty 2-month old seed-deriving bulblets (weight 0.2 to 1.3 g and diameter 3 to 10 mm) were evenly distributed in 3 groups, 20 bulblets each, and putted in perlite on Flood & Drain hydroponic system, clay pebbles on Cutting board hydroponic system, and terrine with soil as a control, in a room phytotron under 23±2ºC, air humidity of 56±18% and mixed daily and artificial light. After 16 weeks all bulbs were lifted, measured, and transferred to pots with soil mixture. Best results were obtained on the Cutting board hydroponic system where all plants survived and the average weight and bulb diameter were 27.2 ± 16.1 g and 20.8 ± 4.2 mm, respectively. Plant growth was medium on the Flood & Drain hydroponic system, with 17 survival plants, an average weight of 15.4 ± 9.2 g and an average bulb diameter of 15.8 ± 3.4 mm. Growth was lowest in the control variant, with an average weight of 7.9 ± 3.3 mm and an average bulb diameter of 13.1 ± 2.3 mm, while 19 plants survived. Differences in plants’ growth in the 3 variants were significant (P < 0.001, Anova single factor); however, new leaves appeared in all variants during the experiment. One month after the plants’ transfer to pots with soil mixture all of them continued to successfully develop new leaves. Plants will be further analyzed for selection of high galanthamin producing individuals. In addition, slow-growing in vitro bulblets obtained by clonal multiplication of the best H. papilio individuals will be transferred to the Cutting board hydroponic system to shorten their growth time.

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MECHANISMS OF IMMUNE DEFENSE IN INSECTS, AN OVERVIEW

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The topic of immune mechanisms in insects has not been presented so far in the Bulgarian scientific literature. As the largest group of animals on Earth and in view of their economic importance as pests of forest and agricultural plant species, as well as carriers of many infectious diseases in humans and animals, these issues are becoming increasingly important. In view of the development of biological control, as well as the relationships between infectious agents and the insects subject to infection, they are becoming increasingly significant and related to the degree of resistance of the insect to natural conditions. Which tissues and organs are involved in immune processes and how they are carried out in insects? These are important questions considering their role in nature. Basic data are presented related to their innate immunity, cellular and humoral responses, immune pathways and cascades, antiviral responses and processes of phagocytosis, encapsulation, nodulation and melanization. Interesting questions have been considered, the answers to which would give opportunities and perspectives related to the future development of entomological science in Bulgaria.
The cytotoxic activity of eight newly synthesized complexes of Cu(II) (four) and Co(II) (four) with Schiff bases derived by a condensation reaction of o-Vanillin (val) with S-Tyrosine (Tyr), L-Threonine (Thr), DL-Tryptophan (Trp) or L-Serine (Ser) was evaluated in our study. The following cell lines were used as model systems: MCF-7 (human luminal A type breast cancer); MDA-MB-231 (human triple negative breast cancer); HeLa (human cervical carcinoma), LSR-SF-SR (rat sarcoma) and Lep-3 (non-tumor human embryonic fibroblastoid cells).

The investigations were performed by methods with different molecular/cellular targets and mechanisms such as MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, double staining with propidium iodide and acridine orange, Comet assay, Annexin V – FITC test, immunocytochemical test for the detection of Ki-67 marker of proliferation (in short-term experiments, 3-72h, with monolayer cultures) and colony-forming method (in long-term experiments, 30-40 days, with 3D cancer cell colonies).

Our results indicate that the complexes investigated decrease viability and 2D/3D growth of the treated tumor cells in a time- and concentration-dependent manner. Cu(II) complexes (effective at concentrations of 10-200 µg/ml) have been found to be more pronounced cytotoxic agents as compared to Co(II) complexes with the same Schiff bases (active at a concentration range of 50-400 µg/ml). MDA-MB-231 human triple negative breast cancer cells are more sensitive to the cytotoxic activity of CuVanThr as compared to Lep-3 non-tumor human cells.

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EX SITU CONSERVATION AND COMPARATIVE DETERMINATION OF ESSENTIAL OIL COMPOSITION IN THE BALKAN ENDEMIC PLANT STACHYS THRACICA DAVIDOV

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Stachys thracica Davidov. (Thracian woundwort) is a Balkan endemic species included in the Red Data Book of Bulgaria with the national conservation status: “rare” (R). The plants from genus Stachys have long history of use in ethnomedicine under the form of extracts, decoctions, ointments for treatment of genital tumors, sclerosis of the spleen, inflammatory diseases, cough, ulcers and infected wounds. Essential oils have been of great interest in recent years due to their diverse pharmacological effects. Still there is no available information about ex situ conservation of S. thracica and little is known about its chemical composition and biological activity. The aim of the present work is to access effective protocol for ex situ conservation of the Thracian woundwort and subsequent comparative determination of the essential oil composition in in situ, in vitro cultivated and ex vitro adapted plants. Successful micropropagation was achieved on basal MS medium with 30 g/L sucrose and 7 g/L agar. Ex vitro adaptation was accomplished in growth camera with 83% survival. GC-MS analysis of the essential oils resulted in the identification of 41, 37 and 38 compounds in in situ grown, in vitro cultivated and ex vitro adapted plants respectively, constituting 80.1%, 85.2% and 86% of the total oils. Germacrene D, β-elemene, α-cadinol, α-limonene and (Z)-β-ocimene were the principal components representing 65.3%, 71.1% and 70.3% of the oil of in situ, in vitro cultivated and ex vitro adapted plants respectively. All three groups of plants seem to store predominantly sesquiterpenes at the expense of monoterpenes. Different growth conditions did not affect significantly the composition of essential oils. The comparative determination of essential oil composition in in situ, in vitro and ex vitro adapted plants is a prerequisite for further research in order to reveal the medicinal potential of the Thracian woundwort. A collection of in vitro tissue and ex vitro cultures has been established which is an alternative approach for preservation of S. thracica.

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Plants are able to modify their growth, development, and physiology according to a variable environment. This ability of plants plays a key role in maintaining efficient growth rate. Responses to variable environmental conditions, involve modifications in the photosynthetic apparatus, to maintain high photosynthetic efficiency of plants under particular light regimes. Apart from the well studied effect of light intensity, quality of light also affects photosynthesis and other developmental and biochemical processes. Light-emitting diodes (LEDs) are solid-state semiconductor devices that produce narrow spectrum light. LED technology has developed rapidly over the past decades. The increasing availability of LEDs provides the researchers the opportunity to optimize wavelength selection for photomorphogenic as well as photosynthetic responses. There are many studies on the effect of different wavelengths on plant growth and the results are very promising thus the interest in using LEDs in horticulture is rising rapidly. It is established that manipulating light correctly can optimize plant growth and chemical composition and can increase the economic efficiency. The aim of our study is to investigate the effect of light quality on photosynthesis and plant growth of rocket salad (Eruca sativa L.). Therefore three light conditions were used: white light (WL) provided by fluorescent lamps, red-blue (RB) and red-green-blue (RGB) provided by LEDs. Photosynthetic performance was analyzed by pigment content, chlorophyll fluorescence and thermoluminescence. To asses the economical productivity under the three light treatments leaf area and fresh biomass accumulation were measured. The most intensive thermoluminescence emission after excitation by flashes, indicative for electron transfer activity was obtained in RB leaves, followed by WL. The results from the thermoluminescence study are in correspondence with the chlorophyll fluorescence data. Our results also show higher leaf and biomass accumulation in RB and WL treatments. Based on the conducted experiments it can be concluded that the selection of appropriate wavelengths of the spectrum can optimize growth quality and morphogenic responses of rocket salad plants.

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THE ASSOCIATION BETWEEN THE PRESENCE OF P16-POSITIVE CELLs IN HUMAN ENDOMETRIUM DURING THE MID-LUTEAL PHASE AND WOMAN’S AGE

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P16INK4a is considered as a robust biomarker of cellular aging in different human tissues. This biomarker also is commonly measured in order to explore the level of senescence in the human endometrial tissue. Previous studies have shown that p16-positive senescent cells are involved in endometrial receptivity and in the acute cellular remodelling during the implantation event. The objective of the present study was to assess the association between the presence of p16-positive senescent cells in the endometrial compartments during the mid-luteal phase and woman’s age.

This is an observational study of 350 fertile women aged between 24 and 56 years who had an endometrial biopsy during the mid-luteal phase (7 days after LH surge) of the natural cycle. Patients with BMI<18 kg/m2 or BMI≥30 kg/m2, endometriosis, polycystic ovary syndrome, endometrial polyps, myoma, abnormal uterine development and hydrosalpinx were excluded from the study.

Endometrial biopsies were obtained by pipelle suction and they were immediately fixed in 10% formalin, then embedded in paraffin for histological determination and subsequent analysis. Immunohistochemical analysis was performed on the paraffin-embedded sections by Novolink Polymer Detection System (Leica Biosystems, Wetzlar, Hesse, Germany). Tissue sections were stained with an antibody against p16 ink4a (Master Diagnostica, Granada, Spain). 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.

No correlation was found between the frequencies of occurrence of p16+ stromal cells and the woman’s age (R=0.056; p=0.61). In contrast, the frequencies of occurrence of p16+ glandular cells and p16+ luminal epithelial cells showed significant correlation with woman’s age (R=0.38; p<0.01 and R=0.47; p<0.01, respectively).

There is a significant age-dependent increase of the presence of p16-positive senescent cells in the glands and luminal epithelium of the human endometrium. This rise is more evident in the luminal epithelium in comparison with the endometrial glands.
Multidrug resistant (MDR) bacteria are among the worst threats to public health. It bears high economical and sociological burdens expected to significantly rise in the near future. Estimations point that drug resistant infections will kill 10 million people worldwide annually by 2050 unless active actions are taken. One of the most important questions to solve is the identification of novel compounds with antimicrobial properties that can substitute for the classical antibiotics in various areas of our environment. One of the most promising alternatives of this type that have a proven potential for fighting MDR strains are antimicrobial peptides (AMPs). They are naturally expressed in all domains of life, but their purification and characterization from natural sources is usually a challenging task. To overcome this, we suggest a novel approach that combines genome mining and heterologous expression in order to obtain novel putative AMP sequences and produce the needed peptide quantities for functional tests.

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EVALUATION OF SOME WEIGHT INDICES IN RAT MODEL OF DIABETES

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Diabetes is a metabolic disease that induces serious complications as microangiopathy, nephropathy, neuropathy or retinopathy. Changes in macroparameters as organ/body weight indices are the first sign that the organ was affected. The aim of our study was to evaluate the effect of hyperglycemia on some weight indices in rat. Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin at a dose of 100 mg/kg b.w. on day 1 (neonatally, NDM) or day 10 (prepubertally, PDM) in rats. Treated animals were sacrificed on day 25 or day 50. Spleens, livers, kidneys, brains, testes and epididymides of control and diabetic rats were sampled, weighed and organ/body weight ratios were calculated. We observed significant elevation of liver, kidneys and brain indices on day 25 after induction of both NDM and PDM. On day 50 kidney index remained considerably higher in NDM but not in PDM. Spleen/body weight ratio was reduced in PDM. Testicular index in NDM and epididymal index in both NDM and PDM were significantly decreased in adulthood unlike mid puberty when these organs were not affected. Future studies would elucidate the histopathological and functional changes in the investigated organs in conditions of experimentally induced diabetes in rat.

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PREPARATION AND PROTEIN ADSORPTION OF DIFFERENT INORGANIC HYDROXYPHOSPHATE VACCINE ADJUVANTS: A COMPARATIVE STUDY

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Vaccine adjuvants are components of vaccines that, when combined with an antigen, accelerate, prolong, or enhance the antigen-specific immune responses. Although inorganic adjuvants do not constitute specific immune targets themselves, they are fundamental in the formulation and the vaccine success could be attributed to them. The only inorganic adjuvants currently used in human and veterinary vaccines are aluminium compounds (hydroxides and phosphates), however there is literature data and practical experience with other successful inorganic adjuvants, such as calcium phosphate. Aluminium-based adjuvants have certain limitations, such as ineffectiveness for some antigens, induction of eosinophilia, augmentation of specific and total IgE antibodies, induction of primarily humoral immune response and inability to augment cell-mediated, especially cytotoxic T-cell responses. Adverse effects have been reported, such as induction of local hypersensitivity reactions, various local and systemic immunotoxicity reactions, association with macrophagic myofasciitis syndrome (MMF) and autoimmune syndromes induced by adjuvants (ASIA). These limitations prompt extensive search and development of non-aluminium (2nd generation) adjuvants over the last few decades. Here, we report on the preparation, characterization and comparative analysis of the protein adsorption capacity of different inorganic hydroxyphosphates, such as those of calcium(II), magnesium(II), iron(III) and aluminium(III). The different adjuvant dispersions were prepared by controlled precipitation and sterilized by autoclaving. Structural analyses were performed by transmission electron microscopy, EDX spectroscopy, X-ray powder diffraction and infra-red spectroscopy. The obtained results suggest that different non-aluminium hydroxyphosphates can be successfully used as protein carrier systems. Their capability for protein antigen delivery to antigen-presenting cells needs to be evaluated in future studies.

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Shortwave UV radiation is a main abiotic environmental factor that affects plant development. UV light is a signal for the photomorphogenesis but also induces DNA damage (cyclobutane pyrimidine dimers, CPDs) in the plant genome, which if not effectively eliminated inhibit plant growth and productivity. In plants, chromatin modifications play a key role in the regulation of gene expression and adaptability to stress, which determines the need to understand the link between chromatin dynamics and plant DNA repair mechanisms. The role of histone acetylation is essential as the modulation of genes associated with adaptation to stress factors is observed. Our previous studies have shown that the phase of photomorphogenic development influences the capacity of barley seedlings to repair CPDs and therefore determines their ability to grow under conditions of UV stress. It can be suggested that factors related to chromatin structure contribute to the differences in the repair kinetics between etiolated and green barley seedlings. To our knowledge, no studies have been conducted on the expression of genes engaged in the modulation of chromatin structure in relation to the UV stress response in barley. The aim of this work was to elucidate the molecular basis of barley UV tolerance by examining the role of genes, coding histone acetyltransferases (HATs) in the processes of photomorphogenesis and DNA repair after UV stress. Transcriptional response of three HAT genes belonging to the GNAT/MYST family, namely HvGCN5, HvELP3 and HvMYST was investigated. Etiolated and green seedlings were UV-C irradiated and incubated under different light conditions. RNA was isolated at different time-points after treatment and the mRNA levels were assessed by real-time RT-PCR. Increased expression of HvELP3 gene was observed in the UV-C treated seedlings during their post-radiation recovery. In conclusion, our results provide insight into the factors related to the chromatin structure and their reaction to UV stress in barley.

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ADAPTATION OF ACTIVATED SLUDGE TOWARDS STEPWISE TOXICITY INCREASE IN LANDFILL LEACHATE TREATMENT

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Keywords: activated sludge, bacteria, micro- and metafauna, leachate, adaptation

Very important and insufficiently studied subject is the activated sludges (AS) in WWTP of the landfills storing household wastes and of the plants treating the same wastes. The leachate generated in these WWTPs contains various xenobiotics in high concentrations. This results as lower efficiency and difficult management of the leachate treatment. Whereas the bacterial community in the AS has better adaptation abilities in such conditions, the communities of the micro- and metafauna consists of higher organisms. This reflects to their lower survival when landfill leachate is treated. The goal of this study was to evaluate the influence of the landfill leachate on the bacterial, micro- and metafauna communities in activated sludge from Sofia WWTP and to assess their adaptation potential towards increasing concentrations of the leachate pollutants. The approach of stepwise adaptation of the AS was used in a model leachate treatment process. In 168 hour of the experiment the obtained results for micro- and metafauna showed higher count of the attached and free-swimming ciliates and also – of the small flagellates. At this time the metabolic activity of the communities (total dehydrogenase activity – TDA) reached its highest value for the experiment (19.97 µgH+/ml*min*mgP). In 336 hour the rotifers were added amongst the dominant groups of the micro- and metafauna. In the same time TDA decreased 3 times which was an early indication for intoxication changes in the AS. At the end of the process (504 hour) when highest concentration of the landfill leachate was applied, a decrease of the values of the AS biotic index was found. This was caused by the decrease of the number of the small flagellates and the free-swimming ciliates. The metabolic activity of the communities was hardly registered by the conventional TTC method (TDA was only 0,03 µgH+/ml*min*mgP). All this corresponded well with the lowered biodegradation ability of the AS. The adaptation of the communities of the micro- and metafauna was impeded. Considering the results for the aerobic heterotrophic bacteria, the macroorganisms in the AS had an important buffering and protective role as xenobiotic accumulating organisms. After their death, the protozoa and rotifers representatives gradually released the xenobiotics. This helped in the processes of adaptation of the bacteria and establishment of stable biodegradation.

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RELATIONSHIP BETWEEN SUPEROXIDE DISMUTASE ACTIVITY AND GLUTATHIONE PEROXIDASE ACTIVITY IN HUMAN SEMINAL PLASMA AND SPERMATOZOA

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Oxidative stress-related sperm damage caused by reactive oxygen species (ROS) is one of the main causes for male infertility. Superoxide dismutase (SOD) and glutathione dismutase (GPx) are essential components of the antioxidative defense system that present in sperm cells and seminal plasma. The objective of our study was to examine the relationship among SOD activity and GPx activity in human seminal plasma and human spermatozoa.

Sixty one men aged between 19 and 59 years were selected. Samples were collected by masturbation after sexual abstinence for 3-5 days. Sperm SOD and GPx activities were determined using Ransod and Ransel diagnostic kits (Randox Laboratories Ltd., Antrim, UK). An aliquot of the corresponding sperm suspension (20x106 sperm/mL) was centrifuged at 600xg for 5 minutes and the supernatant (seminal plasma) was separated and stored frozen until use. The remaining pellet was treated with 0.5 mL of 0.1% Triton X-100 in PBS and vortex-mixed three times for 20 seconds followed by centrifugation at 1,000xg for 5 minutes. Aliquots of the supernatant (obtained intracellular extract) and seminal plasma were added to the wells of the microplate and the assay was performed according to the manufacturer’s instructions. Statistical analysis was performed by Spearman’s correlation test using SPSS v.21 (IBM Corp., Armonk, NY, USA). Descriptive parameters and patient characteristics were reported as mean ± SD and median. P<0.05 was considered statistically significant.

The determined SOD activity was 134.23 ± 215.89 U/109 spermatozoa and 14.76 ± 8.69 U/ml seminal plasma. The observed GPx activity was 5.02 ± 14.91 U/109 spermatozoa and 106.76 ± 34.26 U/ml seminal plasma.

There was a significant but relatively low positive correlation between sperm GPx and seminal plasma GPx activities (R=0.44; p=0.01). In contrast, sperm SOD activity showed stronger positive correlation with seminal plasma SOD values (R=0.77; p<0.01). The degree of positive correlation between seminal plasma SOD and GPx activity (R=0.23; p=0.02) was similar to that between sperm SOD and sperm GPx activity (R=0.27; p=0.05).

Sperm SOD activity appeared to be more strongly linked to seminal plasma SOD activity compared to the association between sperm and plasma GPx activity.
IN VITRO ASSESSMENT OF LACTOBACILLUS PLANTARUM STRAINS, AS SAFETY CANDIDATE-PROBIOTICS

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Several Lactobacillus species are accepted as microorganisms with Qualified Presumption of Safety (QPS) in the EFSA’s list. Lactobacillus plantarum is one of them and it is widely distributed species with proven probiotic (pro –for and bios- life) potential and technological relevance. In addition, every strain must complete safety requirements, before implementation. Antibiotic susceptibility or resistance is one of EFSA’s important criteria for the safety of probiotics. The reason is to avoid any possibility for antibiotic resistance genes transfer to opportunistic pathogens in the gut. As a part of its QPS, EFSA has introduced a mandatory assessment of the sensitivity of probiotics to different antibiotics with corresponding cut-off values. In the present study we determine antibiotic susceptibility patterns of lactobacilli isolated from traditional fermented dairy products and with human origin. With this aim 18 Lactobacillus plantarum strains (6 from breast milk, 6 from home-made “katak”, 6 from other fermented dairy products) are assessed for antibiotic susceptibility to Nalidixic acid, Gentamycin, Vancomycin, Streptomycin, Ciprofloxacin, Ampicillin. All tested strains are resistant to Nalidixic acid and ~70% of them to Vancomycin and Ciprofloxacin. Concerning other antibiotics, strain-specific antibiotic-sensitivity patterns are observed. Only 3 L. plantarum, isolated from dairy products possess resistance to Streptomycin. The EFSA’s cut-off values have to be also determined for them. A high variety in effects of Gentamycin and Ampicillin, from full inhibition to absence of effect, has to be pointed. Antibiotic resistance may also be considered an advantage in the selection of probiotic strains, however only when it is not transferable. In this respect, the established resistance of the tested strains of breast milk or dairy lactobacilli to the antibiotics discussed (except for those involved in horizontal gene transfer) would not create safety problems with their possible inclusion in the food chain. Estimated susceptibility patterns of tested candidate-probiotic strains are also important, considering the use of the latter as agents accompanying antibiotic therapy.

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PROTEIN ADSORPTION OF NEWLY FORMULATED POTENTIAL VACCINE ADJUVANTS OF MIXED CALCIUM-MAGNESIUM HYDROXYPHOSPHATES

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Immunological adjuvants are key components of various inactivated vaccines, which in combination with suitable protein antigens potentiate their immunogenicity and therefore are of key importance to vaccine efficacy. Aluminium hydroxide and phosphate have been used for decades as immunological adjuvants in various vaccines for human and veterinary practice. Such vaccines include those against tetanus, diphtheria, pertusis, hepatitis A/B, HPV, conjugated pneumococcal vaccines, inactivated rabies vaccines, etc. Despite their successful use, there are still unsolved problems with adjuvanted vaccines, such as falling antibody titers with time, inefficiency with some antigens, selective induction of polarized Tn2-type of immune response and inefficient cytotoxic T-cell response, as well as some local and systemic immunotoxicity. These limitations determine the need for new immunological adjuvants with improved efficacy and safety. Here, we present the preparation and characterization of novel nanostructured mixed calcium-magnesium hydroxyphosphates as potential alternatives to the aluminium hydroxyphosphate adjuvants. The suspensions were prepared by precipitation from mixed Ca(II)/Mg(II) solutions and were sterilized by autoclaving. Structural characterization was performed by means of transmission electron microscopy with elemental and electron diffraction analysis, X-ray powder diffraction and infra-red spectroscopy. The protein adsorption properties were studied by using a model protein (albumin) in order to evaluate the suitability of the prepared materials for application as protein antigen carrier systems. The development of new calcium-magnesium hydroxyphosphate adjuvants was motivated by data from the scientific literature and patents describing effective immunological adjuvants based on calcium and magnesium phosphates that result in combined humoral and cellular immune response. This is especially important for inactivated virus vaccines. In addition, calcium phosphate is a natural body constituent with already proven efficacy and safety as an immunological adjuvant in some human vaccinations.

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ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACTS OF ACHILLEA THRACICA VELEN. PLANTS

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Achillea thracica Velen. (Asteraceae) is perennial Bulgarian endemic species with valuable medicinal properties. The plants from genus Achillea have long history of use in ethnomedicine under the form of extracts, decoctions, ointments for treatment of genital problems, diseases of the digestive system, inflammatory diseases, to stop bleeding, ulcers and infected wounds. The aim of the present study was to investigate the antimicrobial activity of methanolic extracts of in vivo and in vitro grown, and ex vitro adapted Thracian yarrow plants. A. thracica samples were prepared by Soxlet extraction using methanol as solvent. The antimicrobial activity of the methanolic extracts was determined by disk-diffusion method against seven Gram-positive bacteria, five Gram-negative bacteria, two yeast strains and one phytopathogen bacterial strain. This study revealed different antimicrobial activity of tested samples. All three tested extracts showed antibacterial activity only against Gram-positive bacteria: B. cereus, B. pumilus, S. aureus, M. luteus and to Gram-negative bacterium E. coli strain 3397. Our data revealed that Gram-positive bacteria were more sensitive than Gram-negative ones toward the plant methanol extracts.

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ANALYSIS OF POSSIBILITIES FOR DATA EXTRACTION FROM FREELY ACCESSIBLE DATABASES WITH RELEVANCE TO FOOD SAFETY

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Introduction and aim: Assuring safety along the food supply chain is crucial for maintaining high quality of life. The increasing numbers of chemical constituents that are being introduced in food and feed make safety evaluation by means of classical toxicological approaches a challenging task. In silico hazard identification has proved as a promising first-line approach in this process and computational analysis of large data pools has become a key step. In this study we aim to perform an analytical overview of current freely accessible databases with relevance to food safety.

Databases provide systematised collections of information concerning chemicals and their adverse effects, which significantly support modern hazard identification. Various manual or automated data extraction and analysis techniques are applied depending on the diverse databases’ architecture and on how the search, data processing and reporting are conducted.

Currently, a considerable number of food/feed-relevant databases have been developed to organize data on chemicals either deliberately (food ingredients, additives, flavourings, supplements) or unintentionally (pesticides, food contact materials, contaminants) introduced in the final products. Analysis of freely accessible resources, hosted by WHO, EFSA, US FDA and US EPA [1-4], allowed comparison of main features of the databases. An overview of the toxicological endpoints, chemical domains, search and data retrieval options was performed to provide solid basis for future development of in silico hazard prediction tools.

There is a constantly increasing interest in developing freely accessible databases, as an organized source of chemical and toxicological information, relevant to food safety. Application of automated data extraction and analysis strategies increases the efficiency of big data exploitation thus providing ready to use, curated data for the development of computational tools to support food safety evaluation.

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3. US Food and Drug Administration, https://www.fda.gov/

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INFLUENCE OF HEAVY METALS ON THE STRUCTURE OF BACTERIAL COMMUNITIES IN LONG-TERM CONTAMINATED SOILS IN THE REGION OF CHELOPECH, BULGARIA

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Keywords: heavy metals; soil contamination; bacterial communities; 16S rRNA gene.

Heavy metals (HMs) are the main contaminants of Bulgarian soils, being widely distributed as a result of agricultural and industrial activities. Heavy metal pollution alters the composition and activity of soil microbial communities, and in most of the cases it correlates with their concentration and bioavailability. Studying the bacterial diversity and its dynamics in relation to soil properties is essential for clarifying the links between soil environment and its microbiome.

The aim of the work was to study the influence of heavy metal (Cu, Pb, Zn) pollution on bacterial community structure of two soil samples, collected in the vicinity of Chelopech village. The studied samples were chosen from a collection of soil samples along the cooper contamination gradient in this region.

DNA was extracted from two soil samples - Karl_5.1. (very highly contaminated) and Chel_1.1. (control sample). 16S rDNA clone libraries were constructed for both samples and RFLP analysis was performed. Clones from representative RFLP groups were sequenced. Based on the obtained sequences, two phylogenetic trees were constructed with MEGA 7.0 software.

The dominant phyla in the control sample were Proteobacteria, Bacteroidetes and Acidobacteria, whereas in Karl_5.1. Acidobacteria was not detected, but Actinobacteria was present. The dominant classes were Chitinophagia, γ-Proteobacteria, α-Proteobacteria and Rubrobacteria in Karl_5.1. compared to Chel_1.1., where α-Proteobacteria, Acidobacteria subdivision 6, β-Proteobacteria and Chitinophagia were demonstrated. The high level of heavy metal pollution (Cu, Zn, Pb) in Karl_5.1 dramatically changed the structure of the bacterial communities at phylum and class levels towards reduction of their abundance compared to the control sample.

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EFFECT OF THE FUNGICIDE AZOXYSTROBIN ON THE Fungal Community Structure in Loamy Sand Soil

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Keywords: azoxystrobin, ITS clone libraries, mesocosm

Azoxystrobin (Az) is a new fungicide of the strobilurins, which is one of the most popular chemicals used for control of fungal plant pathogens. Az is a broad – spectrum fungicide effective against Ascomycota, Deuteromycota, Basidiomycota and Oomycota, inhibiting their mitochondrial respiration and spore germination. The aim of the study is to evaluate the effects of Az on the fungal community structure.

A mesocosm experiment was conducted using agricultural soil (loamy sand) amended with 28.93 mg kg\(^{-1}\) Az (Quadris\(^{R}\)), and untreated soil as a control. The fungicide effect was studied on the 1st (D1) and 30th (D30) day of soil mesocosms’ incubation. ITS clone libraries were constructed to evaluate the effects of Az on fungal community structure.

The dominant divisions, presented in fungal communities on D1 were Ascomycota, Basidiomycota and Mucoromycota, which remained relatively unaffected in both studied mesocosms - with azoxystrobin (Az3) and without azoxystrobin (Az0). On D30, the dominant divisions in both mesocosms were Ascomycota and Basidiomycota, whereas Mucoromycota was represented only by 4%. The main change in the structure of Az impacted fungal community on D30 was an increase in the abundance of Ascomycota representatives. At the subdivision level Pezizomycotina, Saccharomycotina, Agaromycotina and Mortierellomycotina were demonstrated in both mesocosms at D1 and D30. At subdivision level changes were obtained in their percentage redistribution. Pezizomycotina and Mortierellomycotina on D1 in Az treated soil decreased compared to the untreated soil. In contrast, the representatives of Agaromycotina increased. The response of the fungal communities on D30 after Az treatment was the increase of representatives of Pezizomycotina and Agaromycotina, and a decrease in Mortierellomycotina. The presence of representatives of subdivision Saccharomycotina in both mesocosms was not affected by Az on D1 and D30.

Changes in the structure of fungal communities after treatment with azoxystrobin were reported at the level of division and subdivision on days 1st and 30th after the treatment with the fungicide azoxystrobin.

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CRISPR Cas9 is arguably the most used technology in modern genetics with numerous different applications like genome editing, base editing, transcriptional control and many others. A key property of this tool is its ability to be easily re-targeted to genomic regions of interest simply via cloning a novel 20-base pair gRNA compound. To fully use the CRISPR/Cas9 potential in different experimental scenarios, investigators need to have a simple, fast and cost-efficient way to modify the protein compound of the complex. Unfortunately, Cas9 even alone has quite large CDS that is beyond the current limits for the one piece gene synthesis services. In this work we use an alternative approach to create an optimized Cas9 BioBrick part that is compatible with the RFC12 standard. This element can serve as a backbone in many different constructs with various purposes.

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COMPARATIVE EFFECTS OF GLYPHOSATE AND ITS BASED HERBICIDE COSMIC® ON THE FUNCTIONAL ACTIVITY OF THE ATP-SYNTHASE COMPLEX OF LIVER MITOCHONDRIA

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Glyphosate (N-(phosphonomethyl) glycine) is the active substance in the composition of number of herbicides. It is also used in the creation of genetically modified crops, which are resistant to its action. Many studies have focused on the toxicity of glyphosate and its containing products related to changes in cellular morphology, viability and metabolic activity. Mitochondria are one of the most sensitive targets for glyphosate, which causes many functional changes and disturbance of cell energy balance. Objective data on the direct action of glyphosate on the ATP-synthase complex of mitochondria are still lacking. Our previous study showed that the herbicide Cosmic® containing glyphosate as the active substance stimulated ATPase activity of both intact and freeze/thawed mitochondria. The present work was undertaken to compare the effects of glyphosate on the ATP-synthase (ATPase) complex of rat liver mitochondria applied alone as a pure substance and in the form of Cosmic®. Our purpose was to investigate to what extent the observed effects are due to the glyphosate itself or determined by supplements in the composition of Cosmic®. Glyphosate and Cosmic® in concentrations 10, 25, 50 and 100 μM were applied on intact mitochondria, mitochondria decoupled by freezing/thawing, submitochondrial particles (SMPs) and unpurified soluble mitochondrial ATPase (F1). ATPase activity was determined by measurement of the inorganic phosphate increase in the reaction medium. We found that the pure glyphosate did not influence ATPase activity of intact liver mitochondria suggesting the lack of decoupling effect. In contrast of Cosmic®, glyphosate inhibited ATPase activity of freeze/thawed mitochondria. These discrepant effects may be due to some supplements in the composition of Cosmic®. Both glyphosate and Cosmic® inhibited ATPase activity of both SMPs and F1, which implies direct interactions of glyphosate with the enzyme part of ATP-synthase complex. This study provides a better insight into the mechanisms of action of glyphosate related to mitochondrial dysfunction.

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CATNIP (NEPETA NUDA) ANTIOXIDANT RESPONSE TO MICROPROPAGATION AND CRYOPRESERVATION

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Biotechnological approaches are useful tools for long-term conservation of medicinal plants with pharmaceutically valuable metabolites. The catnip (Nepeta nuda) is a plant widely used for its broad spectrum of therapeutic activities and here we compare the phytochemical response of in vivo grown N. nuda plants to plants undergone micropropagation (in vitro) and cryopreservation (cryo) followed by adaptation back to natural field conditions, i.e. ex vitro and ex cryo, respectively. We applied methanol as a solvent that is known to extract efficiently polar compounds with antioxidant potential. The quantity of phenolics was measured in N. nuda extracts from dry leaf material subjected to ultrasound. In comparison with the in vivo control, the highest level of total phenolic compounds was established in cryo and in vitro variants. This tendency was observed also in respect to the total flavonoid quantity. In the process of ex vitro and ex cryo adaptation, the total amount of these antioxidants was recovered back to the level of the in vivo control or higher. The respective total antioxidant activity (TAA) and antiradical (ABTS●+) activities in the N. nuda extracts was estimated, as well. Similarly to the total phenolics content, in cryo and in vitro variants was measured the highest TAA. The ABTS method confirmed maximal radical-scavenging activity in the cryo variant, while in the in vitro and ex cryo variants a significant decrease compared to the control was observed. Strong correlation was established between the total phenolics and TAA (R=0.912), while the correlation between TAA and the flavonoid content was lower (R=0.66). The ABTS approach showed significant correlation with the total phenolics content (R=0.602) and flavonoids (R=0.581). As expected, a significant correlation was observed between the total amount of phenolic compounds and flavonoids (R=0.744). Since the phenolic acids are a major class of phenolic compounds, we investigated their presence in methanol extracts from the different growth variants via Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The efficiency of the extraction was enhanced by using Soxhlet apparatus and in the obtained extracts were found to prevail acids such as caffeic, ferulic, cinnamic, vanillic, that are known to act as antioxidants. The overall data suggested that N. nuda is a plant enriched in antioxidants, which seem to assure its successful micropropagation and cryopreservation.
IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF NEWLY ISOLATED STRAINS OF LACTIC ACID BACTERIA FROM SPONTANEOUSLY FERMENTED FRUITS

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In traditionally prepared fermented products there are strains of the non-starter microbiota from different species of the Lactic acid bacteria, which are related to the organoleptic qualities of the products and their beneficial properties. LAB are commonly used as probiotic substances for promoting human gastrointestinal and oral health.

The present study aims for the isolation of new strains from LAB microbiota of spontaneously fermented fruits and assess their potential as antagonists against pathogens and spoilage microorganisms.

Seven strains were isolated and identified by morphological, physiological characteristics (cell morphology, catalase and oxidase activity, growth conditions) and biochemical characteristics by API® system and sequencing of 16S rDNA gene.

Screening of antibacterial and antifungal activity against pathogens and spoilage microorganisms was held. The main objective is to determine of potential of these LAB strains as probiotics for oral health. The assessment of antagonistic activity against oral associated pathogens is still in progress.

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The alkaliphilic strain Bacillus pseudalcaliphilus 20RF, producer of cyclodextrin glucanotransferase enzyme (CGTase, EC 2.4.1.19), converting starch and related substrates to cyclodextrins, was cultivated in nutrient media containing starch of potatoes, maize, wheat and tapioca (commercial products) as the main carbon source. Controls contained soluble potato starch. In order to optimize the composition of the culture medium, experiments were conducted with potatoes, maize, wheat, rice, spelt and tapioca flours. Cyclodextrin glucotransferase synthesis has been optimized by using secondary (waste) materials as an alternative to pure substrates. The cultivation of the strain was carried out with wheat and spelt bran, ground corn cobs and potato peels (0.2% and 1%). Bacillus pseudalcaliphilus 20RF was treated with an electric field (3-10 V) at different hours of the microorganism cultivation (from 0 to 24 hours) for 30 min. The effect of the variable components on the biosynthetic capacity of the producer was studied by measuring cyclodextrin glucotransferase activity (by determining β-cyclodextrins which are the product of the enzyme action) and total protein. The specific CGTase activity was calculated. In determining the effect of the electric field on the strain, microbial growth (OD650) was also taken into account. The comparative characterization of the substrates that showed the highest CGTase activity, applied at concentrations identical to the control (0.2%), showed that rice flour and potato peels significantly increased the synthesis of CGTase. On rice flour, the CGTase and the specific CGTase activities were higher by 90.6% and 86.4%, respectively, compared to control values. The maximum increase in both activities was observed when B. pseudalcaliphilus 20RF was cultivated on potato peels (0.2%). An increase of 99.6% in the CGTase activity and of 90.2% in the specific CGTase activity was observed, compared to control. Experiments conducted with an electric field imposing showed that at the specific operating parameters of the field, the growth and biosynthetic capacity of Bacillus pseudalcaliphilus 20RF did not change appreciably.

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SPERM-ZONA ADHESION PROPERTIES ARE RELATED TO THE CONVENTIONAL SEMEN PARAMETERS

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Keywords: spermatozoa, zona pellucida, semen analysis

The sperm adhesion to zona pellucida of the egg is a cellular interaction which represents the first direct physical contact between the male and female germ cells. The zona binding abilities of the spermatozoa are of crucial importance and can predict the assisted reproduction outcome. The conventional semen analysis which includes sperm volume, pH, sperm count, concentration, motility and morphology measurements does not provide enough data on the physiological properties of the semen. There are different sperm functional tests (e.g. sperm-zona pellucida binding test) trying to evaluate different stages of fertilization process developed for usage in the clinical practice to predict the pregnancy outcomes.

The aim of this study was to estimate how the sperm-zona adhesion score (SZAS) was related to the conventional semen parameters.

A total of 141 semen samples were collected from patients of Nadezhda Women’s Health Hospital. Regular sperm analysis was performed according to WHO 5th edition (2010). After 30 min of liquefaction at 37°C the semen volume and pH were evaluated. Sperm count, sperm concentration and sperm motility were assessed by CASA. Sperm morphology was evaluated by WHO (2010) criteria.

The sperm zona-adhesion ability was assessed using zona pellucidae from healthy donors’ germinal vesicles (GVs). Whole zonae were acid solubilised and immobilised in petri dishes. 250 000 motile spermatozoa from each patient were placed in the dishes and the adhered spermatozoa were counted. Sperm zona adhesion score (SZAS) presents the sperm zona adhesion rate evaluated by the number of adhered spermatozoa in 1mm² of zona pellucida coated area.

Spearman analysis revealed no relation between the SZAS and the physico-chemical properties of the semen (volume and pH), and the sperm count and concentration (p>0.05). However, significant correlation was observed between the SZAS and the sperm motility and morphology. The percentage of progressively motile spermatozoa and the zona adhesion rate showed slight positive correlation while the percentage of immotile spermatozoa was negatively related to the SZAS (r=0.25; P=0.03 and r=−0.16; p=0.05, respectively). The morphological evaluation also related positively to the zona adhesion ability of the spermatozoa (r=0.18; p=0.03).

In conclusion, reduced sperm mobility and morphological abnormalities may indicate dysfunctional zona adhesion properties of the spermatozoa.
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DISTURBANCES OF IRON HOMEOSTASIS IN HIV INFECTION

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Keywords: HIV, labile iron pool, lymphocytes, cART

Iron is an essential micronutrient as well as is a key factor for various stages of HIV life cycle. The hormone hepcidin plays a major role in the regulation of iron uptake and its distribution in cells.

To analyze the distribution of serum iron and intracellular labile iron pool (LIP) in T-cells in people with HIV infection.

41 men, aged 22 to 62 (mean, 39±12.1 years), were studied divided into two groups: HIV+ without treatment (A:n = 24) and healthy HIV(-) controls (B:n = 17). Hemoglobin (Hb) and serum iron (sFe) values in peripheral blood were determined by routine laboratory methods. The standard direct flowcytometriy was used to determine the percentage and absolute count (AC) of CD4+ and CD8+T cells. LIP was determined by flow cytometry in peripheral blood mononuclear cells using acetoxymethyl ester and subsequent incubation with Deferiprone. The difference between the mean fluorescence intensity (ΔMFI) of chelator-treated and untreated cells was used to evaluate the amount of LIP in CD4+ and CD8+ cells. The concentration of hepcidin was determined by ELISA.

The values of Hb in group A (mean 122 ± 17.8 g/l) were significantly lower compared to group B (mean 151 g/l ± 7.18) as well as sFe (12 vs. 21µmol/l, p<0.01). At the same time, the hepcidin was higher in HIV+subjects – 19.3 ng/ml compared group B – 3.1 ng/ml (p < 0.0001, T-test). Moreover HIV(+) patients had significantly higher CD4 and CD8 LIP values as compared to controls (ΔMFI 1007 vs. 128; 787 vs. 74, p<0.01 for both). In addition, we found a significant inverse correlation between hepcidin and Hb concentration (R=-0.5, p <0.01), and the CD4/CD8 ratio (R=-0.5, p <0.01).

Acute HIV infection causes an increase in the concentration of hepcidin, which leads to a decrease in serum iron concentration and anemia of inflammation.

Serum hepcidin level is a potential biomarker for the activity of HIV infection.

The elevated levels of LIP in CD4 and CD8 cells in HIV+ persons suppose potentially novel regulatory pathways in cellular iron metabolism in the regulation of HIV life cycle. These findings suggest a relationship between iron homeostasis and the HIV infection which needs further investigations.

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LABORATORY COMPARATIVE ANALYSIS OF SEROLOGICAL AND MOLECULAR METHODS FOR DETECTION OF MEASLES VIRUS IN BULGARIA

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Keywords: measles, RT-PCR, ELISA IgM

Measles is an acute human diseases caused by Measles virus (genus Morbillivirus, family Paramyxoviridae). Measles infection is highly contagious starts in the respiratory system. It remains a significant cause of death worldwide, despite the availability of a safe, effective vaccine. The present study aimed to compare serological and molecular methods for the detection of measles virus in Bulgarian patients.

During a measles outbreak in Bulgaria in 2019, the total 201 patients with collected two types of clinical materials were tested. The age of the patients studied was from 5 months to 65 years. Sera samples (n=201) and nasal swabs (201) from each patient were analyzed by serological (indirect EIA test for detection of the specific IgM antibodies) and molecular (extraction and detection of viral RNA) methods.

Measles virus was detected in 85% of tested nasal swabs by RT-PCR, compared to 70% in serum samples by serological method (ELISA IgM). Measles virus can be demonstrated in nasal swabs with greater frequency at the onset of clinical symptoms, and the collection method is less invasive and recommended in young children, infants and pregnant women.

Despite the WHO measles elimination strategy, the incidence rate has increased by more than 50% in the last year. For that reason, timely combined laboratory approach by serological and molecular assay to detect of measles virus is very important.
SCREENING FOR PEPTIDOLYTIC SYSTEM OF NEW ISOLATED LACTIC ACID BACTERIA FROM TRADITIONAL FERMENTED FOOD

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Traditional fermented foods containing lactic acid bacteria are associated with beneficial effects on human health, and some of those benefits are related to protein-derived products. The proteolytic system of LAB converts proteins to peptides and amino acid, which is essential for bacterial growth, play a major role in the development of flavor and texture of fermented products, and also contributes as immune modulators, antioxidants for human health.

Seven types of samples were collected from traditional fermented foods (yoghurt, white cheese and dried sausage) and 12 strains of LAB were isolated.

The newly isolated strains were evaluated for the presence of genetic determinants for six peptidases (pepN, pepR, pepO, pepT, pepX and pepQ) by PCR. The different combinations of these genes was detected at two strains identified as Lactobacillus sakei or Lactobacillus plantarum.

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NOVEL pUC19-BASED VECTOR THAT ALLOWS INTRODUCTION OF SYNTHETIC gRNA EXPRESSION CASSETTES VIA TA CLONING

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The rapid decrease in prices for gene synthesis services makes them a promising alternative for the conventional cloning approaches. The freedom guaranteed by gene synthesis allows the user to easily manipulate not only the sequence specific region of the gRNA molecule but also the remaining elements of the expression cassette, like promotors, terminators, etc. In this work we describe a TA cloning-based procedure for cloning such gRNA cassettes generated by gene synthesis in a simple and price efficient manner. To facilitate this process we developed a novel pUC-19 based cloning vector that eliminates the typical drawbacks of the TA cloning approach. In addition, this new tool allows classical blue-white screening for successful transformants that further simplifies the introduction of novel synthetic gRNA expression cassettes.

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INVESTIGATION OF THE ANTI-CANCER EFFECT OF EXTRACTS FROM PETASITES HYBRIDUS L. (BUTTERBUR) ROOT ON BREAST CANCER CELL LINES

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Keywords: ovarian cells, granulosa cells, co-culture, spermatozoa

Petasites hybridus L. (Butterbur) is a herbaceous perennial flowering plant of genus Petasites Mill (family Asteraceae). The main active constituents responsible for pharmacologic activity of Butterbur’s extracts are petasine and S-petasine and their iso- and neo-isomers. Unlike their relatively well-studied effects on urinary disorders, menstrual cramps, migraine headache, kidney stones, obstruction of bile flow and other gastrointestinal disorders associated with smooth muscle spasm, the anti-cancer effect of these extracts is very poorly studied. In this respect, the aim of the present study was to investigate and compare the anti-cancer activity of two types of extracts from Petasites hybridus L. (Butterbur) root differing mainly by their extraction method. Butterbur root extract by MedPlanta Ltd. Canada (B. root 1), was obtained from Bulgarian plant by an alternative extraction method (using ethyl alcohol) after the completely removing of the pyrrolizidine alkaloids. Butterbur root PE – Petadolex® extracts (Weber & Weber International GmbH & Co. KG, Germany) (B. root 2) was derivated by low pressure CO2 extraction. The anti-cancer activity of the two types of extracts was established on the triple negative breast cancer cell line MDA-MB 231 and human breast adenocarcinoma cell line MCF-7 in terms of inhibition of cell proliferation and induction of apoptosis. As a control a non-cancerous cell line L929 mouse fibroblasts was used. The cytotoxicity of the extracts was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and the effect on the cell nuclei was evaluated by DAPI staining. According to our results B. root 2 induced the stronger anti-cancer effect on the all three cell lines, MDA-MB 231, MCF-7 and L929, but without pronounced cell specificity. In contrast, B. root extracts 1 had a specific inhibitory effect on the viability of cancer cells, while the viability of normal cells was unaffected at the same drug concentration. The sensitivity to B. root 1 of cancer cells was more than twice as high as that of non-cancer cells. The high cell specificity of this extract has also been demonstrated by apoptosis analysis. In conclusion, the present data proved B. root 1 as a suitable anti-cancer agent with a high specific action to tumor cells and low effect on non-cancerous cells.

Acknowledgments: This work was supported by grant K11-06-H21/12/2018 of National Science Fund, Bulgaria.
The widespread application of antibiotics as antimicrobial agents is speculated to contribute to a rising incidence of infections with antibiotic resistance. The contemporary approaches for combating antimicrobial resistance crisis encompass synthesis of new compounds – natural derivatives of well-known drugs that exhibit antimicrobial activity and that can be applied as an alternative to antibiotics. The aim of the present study was to synthesize a series of derivatives of memantine and to evaluate their antibacterial potential. Memantine (MEM) is a FDA and EU approved drug for treatment of patients with moderate to severe dementia of the Alzheimer’s type. Its efficiency to block E. coli-caused bacteremia and meningitis in a mouse model has been recently reported. A series of novel MEM derivatives incorporating amino acids and peptidomimetics (Glycine, β-Alanine, Valine, 4-F-Phenylalanine, Gly-Thiazole, and Gly-Thiazolyl-Thiazole) have been synthesized. Their antibacterial effects in vitro have been evaluated in order to check a synergistic action able to elucidate bacteriostatic/bactericidal effect. The MEM hybrid molecules were tested against clinically significant conditionally pathogenic Gram (-) bacteria: Escherichia coli (NBIMCC 3397), Salmonella enterica (NBIMCC 8691) and Gram (+) bacteria: Staphylococcus aureus (NBIMCC 6538), Bacillus megaterium (BF 145), by agar diffusion method for antimicrobial susceptibility testing. The hybrid compounds 4-F-Phenylalanine-MEM, Valine-MEM, and Gly-Thiazole-MEM, when applied at 10 mM, were the most active against all test bacteria. The highest activity demonstrated 4-F-Phenylalanine-MEM with Minimal Inhibitory Concentration of 0.156 mM and Minimal Bactericidal Concentration of 0.3 mM against Salmonella enterica (NBIMCC 8691). Additionally, the effect of the cell density on bacterial growth inhibition by the MEM derivatives was determined. It was found that the resistance to inhibition and cell survival of the tested bacterial strains depends on cell density and incubation time. Full inhibitory effect was observed for 4-F-Phenylalanine-MEM for all tested bacterial strains after incubation with 1 mM for 7 h. Among the six novel MEM amino acids/peptidomimetics derivatives, 4-F-Phenylalanine-MEM is the most promising for possible application as a new anti-infective host-directed therapeutic agent against clinically significant conditionally pathogenic bacteria.
WHY PLANTS

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Herpes viruses are connected with human population as long as our first ancestors were walking the earth. Now over 95% of global population is carrier and suffering from herpes diseases. Of course there is a usable treatment in the face of acyclovir and its derivatives, but in the shadows is lurking the drug resistance. For now resistant strains are found mainly in immunodeficient patients, but for how long. Nevertheless laboratories all around the world are hoping to find the new miracle drug. And then there are all the plans: wells of different biological active compound waiting to be found and put to use. This is exactly our work. Until today we have found several champions in the field of antiviral activity. Nepeta nuda spp nuda has activity on adsorption and first stages of viral replication. Lamium album influence the IE ana E replication of herpes viruses and extracellular virions. And Vaccinium vitis-ideae inhibits viral replication.
BIOMIMETIC LIPOSOMES AS A TOOL TO REVEAL THE MEMBRANE STRUCTURAL CHANGES INDUCED BY OXIDIZED LIPIDS

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Keywords: membrane fluidity, oxidized lipids, liposomes, raft domains

Cell membranes are heterogeneous structures in lateral and transversal direction. They consist of various raft-like and non-raft domains with distinct compositions and properties. Lipid rafts are usually defined as small and highly dynamic plasma membrane entities that are enriched in saturated phospholipids, sphingolipids and cholesterol within a sea of unsaturated lipids. Physicochemical properties of the plasma membrane have been shown to play an essential role in cellular functionality. Among those properties, the lipid packing is of high importance because it spatially regulates raft formation. The membrane fluidity changes play a key role in regulation of membrane properties under physiological conditions and in the pathogenesis of disease. Membranes contain abundant phospholipids such as phosphatidylcholine (PC) in mammalian cells. Phospholipids, in particular those containing polyunsaturated fatty acids, are major targets subjected to the damage caused by free radicals. Cellular damage due to lipid oxidation is strongly associated with a number of pathological processes. Lipid oxidation elicits profound changes in the chemical structure of phospholipid fatty acids (FAs) thus opening the possibility that typical properties such as fluidity, ordering, transition temperature, lateral organisation, polarity and permeability might be modified.

In the present research we prepared liposomes, which are used extensively as model systems that mimic the lipid composition of biological membranes, to study the effect of oxidized lipid palmitoyl-oxo-valeroyl-phosphatidylcholine (POVPC) on membrane fluidity as a function of the degree of unsaturation of FAs at the sn-2 position in PC molecule. For this, we compared single component liposomes, binary and ternary lipid mixtures only differing in docosahexaenoic and oleic acid content by using Laurdan spectroscopy. The spectral shift in emission between different states of membrane order is used to calculate the generalized polarization (GP), which is a relative quantitative measure for lipid packing. The results showed that POVPC have a stronger effect on lipid ordering in cholesterol-containing monounsaturated matrix than polyunsaturated one. Flicker spectroscopy measurements of membrane bending rigidity revealed strong softening of PC bilayers upon addition of only 5 mol% of POVPC.

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COMBINED TREATMENT WITH MILTEFOSINE AND DIMETHYL SPHINGOSINE CAUSES CHANGES IN SPHINGOLIPID RHEOSTAT ON LUNG CANCER CELLS

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Modulation in the balance of the so called “sphingolipid rheostat” by increase of Cytohrome C reductase (Cyto. C red.), respectively ceramide levels at the expense of Shingosine-1-phosphate (S1P) levels, could alter the fate of tumor cells by inducing apoptosis. Therefore, the purpose of our study was to monitor the effect of the combined treatment with miltefosine and dimethylspingosine on Cyto. C red. and S1P levels in lung A549 cells. N,N-Dimethyl-D-erythro-sphingosine (DMS) could inhibit the activity of sphingosine kinase 1 (SK1), a key molecule responsible for the balance of ceramide and sphingosine-1-phosphate levels involved in cell differentiation and apoptosis. Miltefosine (HePC) belongs to antitumor lipids that bind to cell membranes and unlock a number of signaling pathways including apoptosis-related. Cytochrome c reductase (NADPH) activity was determined using Cytochrome c Reductase (NADPH) Assay Kit based on a colorimetric assay that measures the reduction of cytochrome c by NADPH-Cytochrome c reductase in the presence of NADPH. Shingosine-1-phosphate assay is based on the principle of double-antibody sandwich ELISA technique for detecting the amount of human S1P in biological samples. Western blot analysis of phosphorylation of protein kinase c (PKCα) was also performed in treated and untreated control like A549 cells. The results showed that the most marked release of cytochrome C was observed when the combination of HePC IC50 and DMS IC25 was used. The important new fact from medico-biological point of view is that both antitumor agents synergistically decrease the level of S1P, when applied in combination (specifically HePC IC50 and DMS IC25) to A549 cells. In addition, Western blot results showed that treatment with HePC induced a modest, but statistically significant reduction in the level of PKCα phosphorylation. The data also showed that HePC possibly acts as an indirect inhibitor of SK1, reducing levels of the pro-survival factor S1P. However, the molecular mechanism, underlying the interactions between miltefosine, protein kinase C and SK1 in A540 cells needs further studies and clarification.

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PLANT GROWTH PROMOTING EFFECT OF FIVE BACILLUS STRAINS ON ORIGANUM VULGARE SUBSP. HIRTUM

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Plant growth promoting rhizobacteria (PGPR) are a group of free-living bacteria that colonize the rhizosphere and contribute to increase growth and yield of crop plants. PGPR possess many traits that make them well suited as biofertilizers and biocontrol agents, such as: rapid growth and utilization of seed and root exudates, colonization as well as multiplication in the rhizosphere. Various bacterial species from Bacillus, Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter and Serratia genera have been reported to enhance plant growth and they were defined as plant growth promoting rhizobacteria (PGPR). Bacillus is the most abundant genus in the rhizosphere with known plant growth promoting activities. The aim of this study was to evaluate the effect on five bacterial strains of the genus Bacillus and the effect of phytohormones substances - indoleacetic acid and gibberellic acid on the development of the test plant Origanum vulgare subsp. hirtum. In greenhouse experiments with Origanum vulgare subsp. hirtum were evaluated the effect of strains and the substances on various parameters as root length, and stem length and the number of glandular trichomes on the leaves. The results were compared with the control variant and with plants treated with phytohormones (GA3 and IAA). Bacillus strains were increased the length on the roots and the number of trichomes on leaves of Origanum vulgare subsp. hirtum. The obtain results demonstrated that five Bacillus strains were appropriated as potential biofertilizers.

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ASSESSMENT OF THE HEALTH STATUS OF GRAPEVINE GENETIC RESOURCES IN TERMS OF VIRAL DISEASES

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Following requirements of the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the European Genebank Integrated System (AEGIS), a total of 172 accessions from the Agrobioinstitute grapevine genebank including varieties, rootstocks, wild grapes, local autochthonous varieties and new selected forms were periodically checked for their virus health status. The results obtained showed that total of 16.27% of the DAS-ELISA tested accessions were infected by one (89.29%) or more viruses (10.71%). The most widespread virus was Grapevine leafroll associated virus 3 (GLRaV 3) (14.53%), followed by Grapevine leafroll associated virus 1 (GLRaV 1) (2.91%) while the Grapevine fanleaf virus (GFLV) (0.58%) was scarcely represented. Mixed infection (GLRaV 1 + GLRaV 3) were detected in three samples (1.74%). All tested genebank accessions for Grapevine fleck virus (GFkV) were virus free.
THE INVASIVE PLANT \textit{Xanthium strumarium} L. – A POTENTIAL SOURCE FOR ESSENTIAL OIL

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Keywords: \textit{Xanthium strumarium} L., essential oil, invasive plant, antibacterial, antifungal, scolicidal activity

\textit{Xanthium strumarium} L. (Asteraceae, common cocklebur) is an annual plant, which reproduces only by seed. Its origin is thought to be Central or South America. Recent archaeological studies show that the fruits of \textit{X. strumarium} were used in Northwest China (IV–II century BC). This adventive for Europe plant suppresses the germination of different crops and demonstrates invasive behavior. It is the most often registered plant in the crop fields and in the agricultural adjacent territories of Bulgaria.

The aim of this study is to summarize the available information for \textit{Xanthium strumarium} as a potential inexpensive source for essential oil and its pharmaceutical effects. The essential oil is rich in limonene, borneol and cis-β-guaiene, and its composition differs depending on the plant substance. It is proved that the essential oil significantly inhibits the grow of Gram positive and Gram negative bacteria (most efficient against \textit{Staphylococcus aureus}), as well as some fungi (\textit{Candida albicans} and \textit{Aspergillus niger}). Moreover, the common cocklebur essential oil shows scolicidal activity against \textit{Echinococcus granulosus}. Only a small number of scientific publications are devoted to the research of \textit{Xanthium strumarium} L. essential oil, but they a shows good potential for future studies and practical use.

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STUDY ON CYTOTOXIC ACTIVITY AND AUTOPHagy INDUCTION ABILITY OF PIROXICAM AND ITS METAL [ZN(II), CU(II), CO(II), NI(II)] COMPLEXES IN HUMAN BREAST CANCER CELLS

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The aim of our study was to evaluate the influence of non-steroidal anti-inflammatory agent piroxicam and its metal [Zn(II), Cu(II), Co(II), Ni(II)] complexes on viability and proliferation of cultured human breast cancer cells as well as the ability of these compounds to induce autophagy. The cell line MDA-MB-231, established from human triple negative breast cancer, was used as a model system in our investigations. Short term experiments (24-72h, with monolayer cell cultures) were carried out by thiazolyl blue tetrazolium bromide (MTT) test and double staining with acridine orange and propidium iodide. Immunocytochemical analysis with polyclonal antibody against LC3B protein was used for the detection of autophagy. Long-term experiments (40 days, with 3D cell colonies) was performed by 3D colony-forming method. The results obtained reveal that the compounds investigated decrease in a time-and concentration-dependent manner viability and proliferation of the treated cells, metal complexes are more active as compared to piroxicam. Co(II) complex of piroxicam (Co-Pir) has been found to be the most pronounced cytotoxic agent followed by Zn-Pir. CC50 (48h) of Co-Pir and Zn-Pir are 130.2 µg/ml and 254.8 µg/ml, respectively. Applied at concentrations 250 µg/ml (Co-Pir) and 500 µg/ml (Zn-Pir) both compounds completely inhibit 3D growth of cancer cells in a semi-solid medium. Administered at concentrations of 50 µg/ml (24h and 48h, Co-Pir) and 100 µg/ml (48h, Zn-Pir) these complexes induce autophagy in the treated cells.

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