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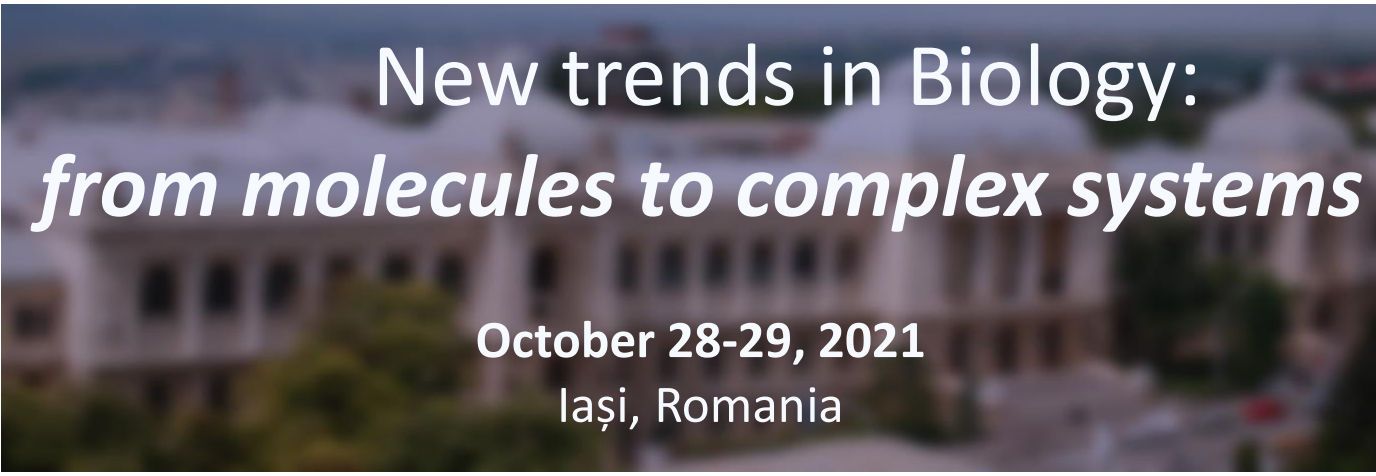
Universitatea „Alexandru Ioan Cuza" din Iași, Facultatea de BIOLOGIE
Laboratorul de Biochimie și Biologie Moleculară
Bulevardul Carol I, Nr. 20A, 700506, Iași, România
www.jemb.bio.uaic.ro



ABSTRACTS

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FOREWORD

This volume is a compilation of the scientific work abstracts that have been presented in the Scientific Session of the Faculty of Biology (SSFB), *New Trends in Biology: from Molecules to Complex Systems*, successfully held on October 28th-29th, 2021.

The Faculty of Biology of the "Alexandru Ioan Cuza" University of Iași, in partnership with the "Anastasie Fătu" Botanical Garden and the Iași Museum of Natural History, organised the SSFB as fully virtual conference, due to the COVID-19 pandemic conditions.

SSFB included 57 presentations with over 170 authors from Romania and abroad (i.e., Canada, Cameroon, China, Egypt, France, Georgia, Italy, Morocco, Russia, Spain, Tunisia, Ukraine, USA). The oral and poster presentations have been grouped in two sections named *Taxonomy and Ecology*, and *Experimental and Molecular Biology*. The diverse and consistent scientific topics discussed in the SSFB contributed to dissemination of new research results, reflecting the plurivalent dimensions of the current biology. We mention the plenary lecture, *Edible and medicinal plants from the Upper Palaeolithic to Bronze Age on the territory of Georgia according to palynological study*, given at the beginning of the session by Inga Martkoplishvili, PhD researcher at Georgian National Museum.

The SSFB also included associated events, such as round tables and exhibitions. *Round Table Educational Resources in Biology* had three topics: "The role of botanical gardens in preventing the disappearance of plant species", organised in collaboration with the Romanian Academy – Iași Branch, Subcommission for Nature Monuments; "The new exhibition of the Museum of Natural History in Iași: directions, strategies, perspectives"; "Using molecular models for better education in life sciences". *Round Table Pollution and its effects on Organisms* was focused on the subject of "Plastic – an emerging pollutant in the Black Sea". The 45th edition of *Autumn Flowers Exhibition* has been organised by the "Anastasie Fătu" Botanical Garden of Iași, being dedicated to its 165th anniversary – the first botanical garden founding in Romania. The *Virtual Exhibition 3D Printed Models of Macromolecules* was open during the session, on the SSFB website (http://cercetare.bio.uaic.ro/ssbio/program.html).

The collection of abstracts in this volume covers a wide variety of subjects, from fundamental to applied research. The Section of *Taxonomy and Ecology* brought together oral presentations that addressed topics on plant ecology, phytosociology, flora and fauna of NATURA 2000 habitats, entomology, ornithology, aquatic ecology, bioarchaeology. The Section of *Experimental and Molecular Biology* included both oral and poster presentations, and the subjects covered various areas of interest, such as antimicrobial activity and antibiotic resistance, biomarkers discovery, cyto-compatibility and -toxicity, neurobiology, proteomics, genomics, pharmacology, food and microbial biotechnology.

This scientific event occasioned the strengthening of the collegial and professional links between the participants. The success of the SSFB 2021 was due to the effort and enthusiasm of the organizing committees, the moderators, as well as the authors, whom we thank once again!

We are confident that this event will be continued, and we are looking forward to the next SSFB in 2022 to debate relevant issues, challenges, opportunities, and new findings in the research field of Biology.

On behalf of the organizers,
Luminița Bejenaru, Mihaela Danu, and Marius Mihășan.

SECTION
TAXONOMY AND ECOLOGY

EDIBLE AND MEDICINAL PLANTS FROM THE UPPER PALEOLITHIC TO BRONZE AGE ON THE TERRITORY OF GEORGIA ACCORDING TO PALYNOLOGICAL STUDY

INGA MARTKOPLISHVILI[1,*]

[1]Georgian National Museum, 3 Purtseladze st. Tbilisi 0105, Georgia
*Corresponding author: ingamartkoplishvili@gmail.com

Nutrition ration and medication of a man of Upper Paleolithic, Neolithic and Bronze Age of Georgia have been revealed for the first time relying on factual data and using palynological research. Material of 13 archaeological sites was studied, which comprises 153 samples obtained from cultural layers.

The study has shown that a man of the Upper Paleolithic mainly ate meat, as bone material is richly presented in all studied caves. As for vegetative food, he used fruits, sprouts, leaves, seeds and bulbs of many plants. Man of Stone Age already knew 32 species of medicinal plants.

Improvement of man's nutrition took place in the Neolithic because he started cultivating cereals and other plants at that time. Horticulture and viticulture were developed. Flax oil and wine appeared. Folk medicine made progress in the Neolithic period. In comparison with Upper Paleolithic, the Neolithic man studied and used far more medicinal plants. Pollen of 56 medicinal plants was defined from Neolithic layers in all, that is, approximately twice more than in Paleolithic ones. According to palynological analysis, cereals and other kinds of vegetative food were of fundamental importance in the nutrition ration of the Bronze Age population, which was also confirmed during the isotopic analysis of bones of man's skeleton.

Palynological spectra of grain-grinders of Kura-Araxes culture showed that, besides cereals, dry leaves, seeds and fruits of numerous medicinal plants (48 taxa) were rubbed and ground on them. Those medicinal plants, which were found on grain-grinders, were also discovered in the abdomens of deceased persons in burial grounds of settlements. That means that these remedies were prepared in the same place.

A new tradition of funeral appeared in the period of Bedeni culture, according to which some medicines, put in baskets or wooden boxes, were placed in burial alongside with deceased. These are first aid kits and they are found in three burial mounds of Bedeni plateau.

The first great leap in the development of folk medicine during the last 35 thousand years happened in the Neolithic. The second most important change in folk medicine occurred in the period of Bedeni culture. Man used 61 medicinal plants during that time.

According to palynological research, 97 medicinal plants were revealed in all, in the period from Upper Paleolithic to Late Bronze.

ASPECTS REGARDING THE ECOLOGY AND IMPORTANCE OF *ECHIUM VULGARE* L. AND *ECHIUM ITALICUM* L. SPECIES

ALEXANDRA ROXANA SAVA[1,*], ANIȘOARA STRATU[1], MARIA-MAGDALENA ZAMFIRACHE[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Carol I, No11, 700506, Romania
*Corresponding author: shealex95@gmail.com

This study brings to attention aspects of ecology, and the importance of the species *Echium vulgare* L. and *Echium italicum* L. (family Boraginaceae). The study is based on the analysis of data from the literature.

The genus *Echium* is represented worldwide by 68 species, and in Romania there are 3 species (*Echium italicum*, *Echium russicum*, *Echium vulgare*).

Echium vulgare is a biennial plant species. This species is an Eurasiatic geoelement. It is spread in Europe and Asia, but according to Plants of the World Online (2021) it is also present in other regions of the globe. From an ecological point of view, it is a heliophilic, eurythermic, xero-mesophilic, oligo-mesotrophic, euriionic species.

Echium italicum is a biennial plant species. This species is a southern Eurasiatic geolement. From an ecological point of view, it is a heliophilic, subthermophilic, xero-mesophilic, neutrophilic and eurinitrophic species.

In Romania, *Echium vulgare* is spread from the plain area to the mountain floor (through meadows, ruderal places, shrubs, gravelly places) and *Echium italicum* from the plain to the oak forests area (through meadows, vineyards, ruderal places, shrubs).

Echium vulgare is a species that has the following potential uses: melliferous (medium economic-beekeeping weight), tinctorial, edible, fodder (in the young stage), ornamental, medicinal. Some parts of the plant (flowers, leaves, stem, root) have uses in traditional medicine. Recent studies have highlighted its antioxidant, antimicrobial, analgesic and antidepressant potential.

Echium italicum has a potential use as edible (leaves) and medicinal, some parts of the plant (flowers, leaves, stem, root) have uses in traditional medicine. Recent studies have highlighted its anxiolytic, sedative, antimicrobial, antioxidant, analgesic, anti-inflammatory and wound healing potential.

Echium vulgare and *Echium italicum* also have the ability to extract heavy metals from polluted soils.

HABITATS OF COMMUNITY AND NATIONAL INTEREST FROM EASTERN PART OF THE NATURA 2000 SITE "SĂRĂTURILE JIJIA INFERIOARĂ – PRUT" (ROSCI0222)

OANA ZAMFIRESCU[1,*]

[1]Alexandru Ioan Cuza University of Iaşi, Faculty of Biology,
Bd. Carol I, No. 20A, 700505, Iaşi, Romania
*Corresponding author: oana.zamfirescu@uaic.ro

Humid areas of the lowland reaver courses have been altered through draining, damming and channel digging and in order to render them suitable for agriculture, grazing and fish farming. These profound modifications result in natural habitat loss with further negative consequences on local fauna. Conservation of biodiversity through habitat diversity preservation is one of the most modern and successful approaches on the matter. Humid areas include a great variety of habitats and favour faunistic concentration. The study site is such an area, encompassed between the valleys of Prut and Jijia. Field studies were conducted in the vegetation seasons of the years 2018 and 2019 and employed the method of Zürich-Montpellier School for vegetation study. In total, 21 associations have been identified. Habitat analysis revealed the presence of six habitats of community interest and another six, of national interest.

A little less than 30% of the studied area is transformed and 59% of the area is covered by habitats of European and national interest (23.5% and 35.5%, respectively). Thus, the conservative value of the study area is quite high given the absence of any kind of management, and therefore it could be easily improved with minimal effort.

THAUMATORYMUS NOTANISOIDES FERR. & NOV. (HYMENOPTERA: TORYMIDAE) NEW TO ROMANIA, A FAUNISTIC ELEMENT WITH MEDITERRANEAN AFFINITIES, WITH ADDITIONS TO ITS BIOLOGY

IONEL ANDRIESCU[1,*], ION ȘCHIOPU[2]

[1]Alexandru Ioan Cuza University of Iaşi, Faculty of Biology,
Bd. Carol I, No. 20A, 700505, Iaşi, Romania
[2]Comarna, Str. Bisericii nr. 1, Jud. Constanța, Romania
*Corresponding author: anion@uaic.ro

On leaves of *Pilosella piloselloides* (Vill.) (Asteraceae) the cynipid wasp *Aulacidea pilosellae* (Kieff.) (Insecta: Hymenoptera: Cynipidae) is forming galls on the mid vein. *Aulacidea pilosellae* is parasitized by *Thaumatorymus notanisoides* Ferr. & Nov. (Insecta: Hymenoptera: Torymidae). The data presented here were gathered by studying the biological material collected in South-Eastern Romania, in Comorova forest near the Balck Sea coast. The material consisted of 57 unilocular galls of *P. piloselloides*, from which 5 ♀♀ and 6 ♂♂ specimens of *T. notanisoides* emerged, with a parasitism rate of 19.3%. Of the trophic relations found in this complex the folowing are new: the relation between *T. notanisoides* and *P. piloselloides*, as well as the whole complex mentioned above; the relation between *A. pilosella* and the host plant *P. piloselloides* as well as the presence of *T. notanisoides* (genus and species) are new to Romania.

REVIEW OF *SCOLELEPIS* (POLYCHAETA: SPIONIDAE) FROM THE BLACK SEA

VICTOR SURUGIU[1,*]

[1]Alexandru Ioan Cuza University of Iaşi, Faculty of Biology,
Bd. Carol I, No. 20A, 700505, Iaşi, Romania
*Corresponding author: vsurugiu@uaic.ro

Currently, four species of *Scolelepis* have been mentioned in the Black Sea: (1) *S. squamata* (as *Nerine cirratulus*), (2) *S.* (*Parascolelepis*) *tridentata* (as *Nerinides tridentata* or as *Pseudomalacoceros tridentata*), (3) *S. cantabra* (as *Nerinides cantabra* or as *Pseudomalacoceros cantabra*), and (4) *S. foliosa*. The comparison of specimens from the Black Sea identified as *S. squamata* with specimens of *S. cirratulus* from the Mediterranean, of *S. squamata* from the North Sea and the Atlantic coast of Spain, and with the syntypes of *S. mesnili* from the Atlantic coast of France did not revealed significant morphological differences to treat them as distinct species. Slight morphological differences that were evidenced ranged within a size-related variability of taxonomic characters. However, the analysis of sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) of small- and large-bodied specimens of *S. squamata* from the Black Sea revealed the presence of two genetically distinct species, but none of them was identical to *S. squamata* whose sequences exist in GenBank. The only way to ascertain to which species of *Scolelepis* do belong specimens from the Black Sea is to collect specimens of *Scolelepis* from their type localities and undertake a comprehensive study of their morphology and information on molecular markers. For the moment, only collection of *Scolelepis squamata* from its type locality (island of Helgoland, North Sea, Germany) was successful and as the result a neotype was designated and deposited in the collection of the Zoological Museum at Hamburg University. Examination of the available material in the collection of the "Grigore Antipa" National Museum of Natural History, Bucharest, identified as *S. cantabra*, revealed the presence of two species in the same vial, one of them corresponding to *S. cantabra*, and the second one, a previously unrecognized species, is under way to be described as a new species.

AMPHIBIANS FROM THE NATURA 2000 SITE "SĂRĂTURILE JIJIA INFERIOARĂ – PRUT" (ROSCI0222)

ȘTEFAN R. ZAMFIRESCU[1,*], ALEXANDRU STRUGARIU[2], RALUCA MELENCIUC[3], PAUL C. DINCĂ[3], PETRONEL SPASENI[3], TIBERIU SĂHLEAN[4]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology,
Bd. Carol I, No. 20A, 700505, Iași, Romania
[2] Alexandru Ioan Cuza University of Iași, Institute of Interdisciplinary Research, Iași, Romania
[3]*Moldavica Herpetological Group*, Iași, Romania
[4] National Museum of Natural History "Grigore Antipa", București, Romania
*Corresponding author: zamfi@uaic.ro

Amphibians are one of the most threatened groups of animals in Europe. Because most of them are protected, amphibians may act as umbrella and flagship species in certain areas. The Natura 2000 site "Sărăturile Jijia inferioară – Prut" (ROSCI0222) is such an area that, despite its conservation status, is largely exposed to human activities and alterations. Thus, the aim of this study is to update the knowledge on the local amphibian fauna. Therefore, we carried out field surveys (visual diurnal and aural nocturnal) in the site area from April to August, 2018 and from March to July, 2019. We identified seven amphibian taxa (*Triturus cristatus, Lissotriton vulgaris, Bombina bombina, Bufotes viridis, Hyla orientalis, Pelobates fuscus* and species of *Phelophylax esculentus* Complex) that are typical for the major relief forms of the area. Five of the identified species are strictly protected according to European and national regulations, whereas the standard data form of the area lists only two species. Thus, the management plan of the site should consider all the strictly protected species as the standard data form fail to reflect the actual richness of the amphibian assemblages.

MIGRATION OF BIRD FAUNA IN THE PERIMETER OF THE ROSPA0072 SIRET MIDDLE FLOODPLAIN (ROMANIA)

CARMEN GACHE[1,*]

[1]Alexandru Ioan Cuza University of Iaşi, Faculty of Biology,
Bd. Carol I, No. 20A, 700505, Iaşi, Romania
*Corresponding author: cgache@uaic.ro

Our ornithological study presents data on the diversity of bird species recorded during the migration time on the territory of ROSPA0072 Siret Middle Floodplain, starting from July 2016 until June 2017. We identified 150 bird species during the spring migration, and 153 bird species in the autumn migration. We notice the presence of some bird species only during the spring, respectively autumn migration in the area, 14 of them included in the Romanian Red Book of Vertebrates. We also present quantitative data for observed bird species, and we recorded bigger populations during the autumn migration than in the time of spring migration but smaller than appear in the official standard forms of the Natura 2000 site. The typical forest species are dominant by diversity but some bird species related to the wetland habitats present significant populations. We notice the relevance of this territory for the migration of the white stork (*Ciconia ciconia*), diurnal raptor bird species, and some waterfowl species in the Eastern Romania.

PATHOLOGICAL EVALUATION OF THE SKELETON M190 DISCOVERED IN THE NECROPOLIS OF THE "VULPE" CHURCH (17TH-19TH CENTURIES) FROM IAȘI (ROMANIA)

VASILICA-MONICA GROZA[1], LUMINIȚA BEJENARU[1,2,*], GEORGE BILAVSCHI[3], LUDMILA PÎRNĂU[3], OZANA-MARIA PETRARU[1,2], MARIANA POPOVICI[1]

[1]Romanian Academy – Iași Branch, "Olga Necrasov" Center of Anthropological Research, Th. Codrescu, No. 2, Iași, Romania
[2]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Carol I Blvd., no. 20A, 700505, Iași, Romania
[3]Romanian Academy – Iași Branch, Institute of Archaeology, Th. Codrescu, No. 6, Iași, Romania
*Corresponding author: lumib@uaic.ro

In this study, the authors present an evaluation of pathologies identified in skeleton discovered in the necropolis of "Vulpe" Church in Iași (Romania) during 2019-2020 archaeological campaign. According to information provided by the archaeologists, the limits of use of the necropolis over time are set between the 17th and 19th centuries.
The lesions of a bone neoplasm are described in this work, that were identified in a skeleton (M190) belonging to a male aged 30-35 years (young adult). There are several perforations/lesions in the cranial bones (frontal, parietal and maxillary bones) and postcranial ones (femur, tibia, clavicle, sternum, and sacrum). The macroscopic, stereomicroscopic, and histological analyses were followed by imaging analysis (i.e. radiology and computed tomography). For histological analysis, bone samples were cut using a Dremel 3000 variable speed multi-tool. Samples were cleaned in multiple sonic baths and embedded in Buehler Epothin 2 epoxy resin. The sections were ground and polished using carbide paper and visualized through a transmitted light microscope.
The preliminary macroscopic analysis suggested the diagnosis of multiple myeloma, characterized by an abnormal accumulation of malignant plasma cells in the bone marrow, which causes damage, fragility, and bone destruction, caused by the local imbalance between osteoblasts and osteoclasts. Cases reported in several regions of the world, dating from the Neolithic to the Middle Ages, have increased knowledge about this pathology. Lesions of multiple myeloma are described as uniform and circular in size, unless they join, without the formation of new bone or bone reaction, giving them a "hit" appearance.
However, the subsequent microscopic analysis revealed histologically new periosteal bone formations, which would indicate not a myeloma but a possible carcinoma.

ARCHAEOZOOLOGICAL DATA ON THE BRONZE AGE COMMUNITY OF WIETENBERG CULTURE FROM UROI-SIGHETI (HUNEDOARA COUNTY, ROMANIA)

DANIEL MALAXA[1,*], ANTONIU MARC[2], SIMINA STANC[1], LUMINIȚA BEJENARU[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology,
Bd. Carol I, No. 20A, 700505, Iași, Romania
[2]Museum of Dacian and Roman Civilisation Deva, Archaeology Department, Romania
*Corresponding author: danielmalaxa@yahoo.ro

The Uroi village, from where the studied site comes from, is located on the right bank of the Mureș river, near the Simeria city (Hunedoara County, Romania), to which it belongs from administrative point of view. The archaeological site is located between 20+280 km and 20+512 km on the highway route, right in the Mureș river meadow, in the flooding area. Archaeological research taken in Uroi-*Sigeti* led to the discovery of 230 complexes belonging to Bronze Age, Iron Age and Middle Ages.

The studied archaeozoological material is dated to the Middle Bronze Age (i.e., phases II and III of the Wietenberg culture).

For the phase II of the Wietenberg culture (W II) were identified 103 faunal remains, and for the phase III (W III) 647 fragments. Animal husbandry was the main activity to obtaining food, domestic mammals having frequencies of 93% in W II, and 87% in W III. The identified domestic mammals are: *Bos taurus* (cattle), *Ovis aries* (sheep), *Capra hircus* (goat), *Sus domesticus* (pig), *Equus caballus* (horse) and *Canis familiaris* (dog).

With a frequency of only 7% wild mammals, the hunting was a minor occupation for the inhabitants of the W II settlement, but relatively more important in W III, with 13% of remains. The identified wild species are: *Cervus elaphus* (red deer), *Sus scrofa* (wild boar), to which are added *Capreolus capreolus* (roe deer) and *Lepus europaeus* (hare) in the W III level.

The presence of mollusks in both cultural levels, as well as the identification of a fragment of *Silurus glanis* (catfish) in the W III assemblage both indicate that the prehistoric communities of Uroi-*Sightei* were also using aquatic resources for food procurement.

DENTAL MICROWEAR PATTERNS – EVIDENCE IN A HUMAN POPULATION OF 17TH CENTURY FROM IASI CITY (ROMANIA)

OZANA-MARIA PETRARU[1,2], MARIANA POPOVICI[2], VASILICA-MONICA GROZA[2], LUMINIȚA BEJENARU[1,2,*]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology,
Bd. Carol I, No. 20A, 700505, Iași, Romania
[2]Romanian Academy – Iasi Branch, "Olga Necrasov" Center of Anthropological Research,
Th. Codrescu, no. 2, Iași, Romania
*Corresponding author: lumib@uaic.ro

Dental microwear is used in attempts to characterize the diet of past human populations and it refers to the microscopic marks left on the teeth hard tissues (i.e., enamel, dentine), usually on buccal and occlusal surfaces. The microwear patterns provides insight into the physical properties of the food and abrasives consumed in the weeks or even months before death. Several studies address dental microwear analysis of past human populations in diachronic and synchronic approach, but the knowledge of within-group microwear variation is still precarious. The aim of this research is to identify, and describe patterns of the within-group dental microwear variation, on M2 molars, depending on sex and anatomic position (i.e., left/right, superior/inferior) in a 17th century archaeological population discovered in Iasi city (Romania). The dental microwear analysis was performed on the "facet 9" of the distobuccal cusp (mandibular molars) and mesiolingual cusp (maxillary molars) through three nonoverlapping consecutive micrographs/molar, using a scanning electron microscope (SEM) Tescan Vega II SBH. The microwear features were identified as linear marks (fine and coarse scratches) and nonlinear marks (small and large pits) using an early version of MicroWeaR software. The microwear features along with their dimensions (i.e., length and width) were subjected to multivariate analysis. The microwear profile of the analysed archaeological population can be described as "abrasive" due to the high proportion of pronounced traces such as coarse scratches and large pits. Our findings show a within-group microwear variation: two microwear profiles based on molar laterality in females (left and right for both maxillary and mandibular M2 molars), and one less heterogeneous microwear profile in males. The microwear pattern in males is similar to that of the right molars in females.

ASSESSING THE SEXUAL DIMORPHISM IN AN ARCHAEOLOGICAL HUMAN POPULATION OF 17TH CENTURY FROM IAȘI (ROMANIA): GEOMETRIC MORPHOMETRICS OF THE SECOND MOLAR TEETH

MARIANA POPOVICI[1], VASILICA-MONICA GROZA[1], OZANA-MARIA PETRARU[1,2], LUMINIȚA BEJENARU[1,2,*]

[1]Romanian Academy – Iași Branch, "O. Necrasov" Center of Anthropological Research, Th. Codrescu no. 2, 700479, Iași, Romania
[2]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Bd. Carol I, No. 20A, 700505, Iași, Romania
*Corresponding author: lumib@uaic.ro

The dental features are highly heritable and evolutionarily conservative, representing an excellent anthropological material in the study of phylogeny, migrations and morphometrical variability. The dental variability is a very debated topics, especially in correlation with the sexual dimorphism. Although the sexual dimorphism has been intensively studied on the molar size (i.e. mesiodistal and buccolingual diameters), the molar shape by sex is not yet a topic addressed at the same scale. This study aims to evaluate the size and shape in correlation, in order to obtain predictable dental models in terms of sexual dimorphism. For this purpose, the Geometric Morphometrics method was applied on a sample of permanent second molars M2 (upper/maxillary – M2, and lower/mandibular – M2). The dental material belongs to human skeletons of 17th century from the "Curtea Domnească" necropolis of Iaşi city (Romania). The bidimensional data were collected using a set of landmarks placed on the occlusal surface, at groove junctions, and a set of semilandmarks on the periphery of the same surface. Our results highlight how different regions of the tooth are associated, and how patterns of the M2 shape variation are defined in the two sexes. The size is found to be a poor indicator for sexual dimorphism, contrary to the shape which additionally provides a high degree of discriminant role. According to our results, the upper M2 tooth is stronger sexual dimorphic than the lower M2. Discriminatory features for the upper second molar (M2) are described by the variability of buccolingual area, shape variability of hypocone and paracone cusps, while in the lower second molar (M2), the distal cusps have a greater dimorphic role.
The dental phenotypic patterns described in our study could be applied in a complementary method to identify sex in skeletal samples, when classical methods cannot be applied.

ECOPHYSIOLOGICAL AND LIFE-HISTORY ADAPTATIONS OF *GAMMARUS BALCANICUS* (Schäferna, 1922) IN A SINKING-CAVE STREAM FROM WESTERN CARPATHIANS (ROMANIA)

OCTAVIAN PACIOGLU[1,*], ȘTEFAN-ADRIAN STRUNGARU[2], GABRIEL PLĂVAN[2]

[1]National Institute of Research and Development for Biological Sciences,
Splaiul Independenței 296, 060031, Bucharest, Romania
[2]Alexandru Ioan Cuza University of Iași, Department of Biology, Faculty of Biology,
Bd. Carol I, 20A, 700505, Iași, Romania
*Corresponding author: octavian.pacioglu@incdsb.ro

Freshwater gammarids are known to colonise occasionally sinking-cave streams, providing contrasting morphological, life-history and ecophysiological adaptations compared to their surface conspecifics. In this study, a subterranean and a surface population of the species *Gammarus balcanicus* was surveyed for one year in a sinking-cave stream from the Western Carpathians (Romania). The results showed that the cave-dwelling population comprised individuals that were significantly larger compared to their surface conspecifics, had larger body-size at sexual maturity and that the females produced fewer, but larger eggs, compared to the population situated outside the cave. The trophic position and the omnivory were significantly higher for the cave-dwelling compared to surface population and the elemental imbalance for C:P molar ratios lower, but similar for C:N. However, the subterranean population did not present troglomorphic characters or longer lifespan as known for other cave-surface paired crustaceans. This, together with the rather extensive hydrological connection of the habitats, suggests active gene-flow between populations and similar response to seasonality for body-size distributions, indicating that the observed ecophysiological and life-history differences are rather the consequence of phenotypic plasticity than the result of genetic adaptation.

RESPONSE OF AQUATIC MACROINVERTEBRATES COMMUNITIES TO MULTIPLE ANTHROPOGENIC STRESSORS IN A LOWLAND TRIBUTARY RIVER

ȘTEFAN-ADRIAN STRUNGARU[1], CORNELIU MIHĂIȚĂ POHONȚU[2], MIRCEA NICOARĂ[3,5], CARMEN TEODOSIU[4], EMANUEL ȘTEFAN BALTAG[6], ROXANA JIJIE[1], GABRIEL PLĂVAN[3,*], OCTAVIAN PACIOGLU[7], CATERINA FAGGIO[8]

[1]Alexandru Ioan Cuza University of Iași, Institute for Interdisciplinary Research, Science Research Department, Lascăr Catargi Str. 54, 700107, Iași, Romania
[2]University Ștefan cel Mare of Suceava, Department of Forestry and Environmental Protection, University Street, No. 13, 720229, Suceava, Romania
[3]Alexandru Ioan Cuza University of Iași, Department of Biology, Faculty of Biology, B-dul Carol I, 700505, Iași, Romania
[4]Gheorghe Asachi Technical University of Iași, Department of Environmental Engineering and Management, 73, "Prof. Dr. D. Mangeron" Street, 700050, Iași, Romania
[5]Alexandru Ioan Cuza University of Iași, Doctoral School of Geosciences, Faculty of Geography-Geology, B-dul Carol I, 700505, Iași, Romania
[6]Alexandru Ioan Cuza University of Iasi, Marine Biological Station "Prof. dr. I. Borcea", Nicolae Titulescu Street, No. 163 907018, Agigea, Constanța, Romania
[7]National Institute of Research and Development for Biological Sciences, Splaiul Independenței 296, Sector 6, 060031, Bucharest, Romania
[8]University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Italy
*Corresponding author: gabriel.plavan@uaic.ro

In the current study the response of aquatic macroinvertebrate communities to multiple anthropogenic stressors in a typical lowland river that crosses pristine sectors situated toward headwaters, as well as densely populated urban areas was assessed. We wished to develop an effective bioassay procedure for assessing water and sediment quality in lotic ecosystems from Romania with the aid of macroinvertebrate organisms correlated with physicochemical parameters and pollutants in both dissolved fractions and material bonded to sediment. A fast scanning approach of the river, from springs to the mouth, was employed. We found significant changes in physicochemical parameters along a longitudinal gradient, the highest values being registered within the urban area and heavily agriculturally developed areas. The macroinvertebrates showed affinities for certain abiotic factors, emphasising their potential use for future studies as reliable ecological indicators, shaped by a synergic combination of urban effects and magnitude of type of land use.

SECTION
EXPERIMENTAL AND MOLECULAR BIOLOGY

DIETARY INTERVENTION EFFECT OF DIHYDROMYRICETIN ENCAPSULATED WITH D-A-TOCOPHERYL POLYETHYLENE GLYCOL 1000 SUCCINATE-SAPONIN SELF-MICROEMULSION DRUG DELIVERY SYSTEM

LEI CHEN[1,*], JIANBO XIAO[2]

[1]Guangdong Ocean University, College of Food Science and Technology, Zhanjiang 524088, China
[2]University of Vigo, Faculty of Food Science and Technology, Department of Analytical Chemistry and Food Science, 36310 Vigo, Spain
*Corresponding author: chenlei841114@hotmail.com

Dihydromyricetin (DMY) has a good effect on extenuating hyperlipidemia, which is highly related with high fat diet (HFD), but its poor absorptivity in blood and intestines restrained its availability and application.
The present study aims to design a self-microemulsion delivery system (d-α-tocopheryl polyethylene glycol 1000 succinate - quillaja saponin) to enhance the absorptivity of DMY (DMY-S), and to investigate its dietary intervention effect on HFD fed mice.
High-throughput sequencing analysis of intestinal microbiota and UPLC-QTOF/MS-based liver metabonomics were applied to disclose the preventive effect of DMY-S on hyperlipidemia in HFD mice and to figure out the possible mechanism.
DMY-S can inhibit the increase of bodyweight and fat mass, preventing non-alcoholic fatty liver disease. Compared to the model group, the abundance of mice intestinal flora is mainly changed in certain bacterial genera of *Firmicutes* and *Bacteroides*, including *norank_f_Muribaculaceae* and *Blautia*. The result of metabolism analysis indicated that the expression levels of cincassiol B, creatine, pantothenic acid and aminobutyric acid in the liver tissues of mice treated with DMY-S showed a down-regulation. The DMY-S prevented hyperlipidemia in HFD mice mainly by affecting the metabolic pathways including glycerophospholipid metabolism, sphingolipid metabolism and pantothenate and CoA biosynthesis.
Taken together, the results suggest that TPGS-QS-SMEDDS can improve the biological activity of DMY in the body, and DMY-S can prevent related diseases caused by HFD by regulating lipid metabolism and excretion. These findings may contribute to improving the bioavailability of DMY and understanding the metabolic mechanism of DMY-S.

THE EFFECT OF SECONDARY METABOLITES ON ANTIMICROBIAL ACTIVITY IN WATER TREATMENT

SARA ER-RAHMANI[1,2,*], HASSAN LATRACHE[2], SAAD IBNSOUDA[1]

[1]Sidi Mohamed Ben Abdellah University, Faculty of Science and Technology, Microbial Biotechnology and Bioactive Molecules Laboratory, B.P. 2202 - Route d'Imouzzer FES - Morocco
[2]Sultan Moulay Slimane University, Faculty of Science and Technology, Laboratory of Bioprocesses and Biointerfaces, PO Box 523 Beni Mellal, Morocco
*Corresponding author: sara.errahmani@usmba.ac.ma

Microorganisms can be found naturally in nature (soil, air, water). Through our food and air, we are exposed to these species from birth. The greatest health risk from the presence of these microorganisms in water is from drinking contaminated water. Outbreaks of waterborne disease have been associated with inadequate treatment of water supplies and unsatisfactory management of water distribution. The main objective of this work is to study the antimicrobial activity of secondary metabolites on *Escherichia coli* and *Bacillus subtilis*.

The antimicrobial activity of various compounds against *Escherichia coli* and *Bacillus subtilis* was determined by the broth micro dilution method. This method is one of the most fundamental methods for analyzing the susceptibility of microorganisms to antimicrobial agents. To apply this method, it was based on: the preparation of solutions of the compounds to be tested, preparation of the inoculum, micro dilution in broth of the compounds, inoculation and incubation.

The results of the antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* of 12 compounds of natural origin (secondary metabolites and major compounds), showed a good antimicrobial activity of thymol and carvacrol as curative means against the formation of biofilms of these two bacterial species on the channels of water treatment.

WHERE SHOULD THE LINE BE DRAWN WHEN CONSIDERING THE BENEFITS AND DISBENEFITS OF USING PESTICIDES FROM A FUNGAL PERSPECTIVE?

TIBERIUS BALAEȘ[1,*], CĂTĂLIN TĂNASE[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, Iași, Romania
*Corresponding author: tiberius.balaes@uaic.ro

The introduction of pesticides, and particularly the insecticides, have changed the modern life in many ways. Such commercially available products have a wide range of applications, from crop protection and husbandry up to the domestic use. Modern intensive agriculture has benefited the most from their use.

The industry of pesticides has a long history of big mistakes, with widely marketed compounds that were highly toxic to both humans and wildlife. Negative effects such as carcinogenic, teratogenic, endocrine-disrupting effects, among many others, combined with a strong resilience and persistence in natural ecosystems are subjects of important public concerns. There is a long list of products that have been used in the past but are currently being banned from commercialization due to their toxicity. Today, in most countries, there are governmental bodies and authorities involved in regulation and approval of pesticides before entering the market.

When assessing the negative side-effects of such products, many scholars are focusing on the toxicity over human health or aquatic animals, for practical reasons, while other types of organisms are often being ignored, although very important in environment.

In the present study we assessed the toxicity of currently market-available insecticides over saprotrophic beneficial fungi. Several saprotrophic yeasts, as well as filamentous fungi (Ascomycota and Basidiomycota) were cultured under the influence of insecticides. Insecticides were added in different concentrations as commercial formulation and not as pure compounds, through filter sterilization. Standard media were used according to the species requirements. The toxicity was evaluated through biomass accumulation rate and minimum inhibitory concentration of insecticides, which were verified using both disc-diffusion and serial micro-dilution methods.

The used insecticides containing synthetic pyrethroids presented varied effects over fungal growth and development in our study, suggesting a significant negative impact over such non-target organisms.

COMPARATIVE ANALYSIS OF SOME MORPHOLOGICAL AND BIOCHEMICAL TRAITS IN 37 TOMATO CULTIVARS GROWN IN ECOLOGICAL GARDEN OF THE BOTANICAL GARDEN „VASILE FATI", JIBOU

CRISTINA-MIRELA COPACI[1], TÜNDE-ÉVA JAKÓ[1,*], GABRIEL VERDE[1], PAUL-MARIAN SZATMARI[1], IULIA CHIȘ[1], LIA MLADIN[1], OANA SICORA[1], COSMIN SICORA[1]

[1]Babeș-Bolyai University, Biological Research Center - "Vasile Fati" Botanical Garden Jibou, 16 Wesselényi Miklós Street, Jibou-455200, Romania
*Corresponding author: denes.tundeeva@gmail.com

The aims of this study were a brief description of the tomato cultivars collection grown in ecological conditions in Jibou Botanical Garden and a comparative study of the morphological and biochemical features of the fruits. The collection consists of cultivars and varieties of tomatoes from Romania and Europe, obtained by seed exchange with other botanical gardens.

One of the purposes of our botanical garden is to preserve in its collection the seeds of these tomato cultivars and varieties. In order to analyze the impact of the ecological growth on the cultivars and varieties, a comparative analysis of the morphological characteristics and the content of some bioactive compounds (ascorbic acid, lycopene and beta carotene) of the fruits was performed.

Results from this preliminary study will be used for a comprehensive description of our collection during the next years.

FIBROUS GELATIN-BASED SCAFFOLDS ENRICHED WITH MNPs DISPLAY CYTOCOMPATIBILITY WITH HUMAN ADIPOSE-DERIVED STEM CELLS

ALEXANDRA-ELENA DOBRANICI[1], AIDA ȘELARU[1], ELENA OLĂREȚ[2], IZABELA STANCU[2], MARIETA COSTACHE[1], SORINA DINESCU[1,*]

[1]University of Bucharest, Faculty of Biology, Department of Biochemistry and Molecular Biology, Spl. Independenței 91-95, 050095 Bucharest, Romania
[2]University Politehnica of Bucharest, Advanced Polymer Materials Group (APMG), Str. Gh. Polizu 1-7, Bucharest, Romania
*Corresponding author: sorina.dinescu@bio.unibuc.ro

Human adipose-derived stem cells (hASC) are promising candidates for nervous tissue engineering grace to their ability to transdifferentiate into cells of ectodermal origin, such as neurons or glial cells, considering their mesodermal origin. Moreover, magnetic biomaterials are currently investigated as potential scaffolds to promote nerve regeneration. Therefore, we aim to develop and investigate the cytocompatibility and potential of natural-based materials enriched with magnetic nanoparticles (MNPs) to support hASCs growth, viability and proliferation. Gelatin composites, generated through electrospinning technique, were enriched with different concentrations of MNPs (0%, 0.5%, 1%, 2%), seeded with hASC and maintained in standard culture conditions for one week. In order to assess their biocompatibility, cell viability and proliferation were investigated using MTT test, while cytotoxic profile was evaluated based on lactic dehydrogenase (LDH) release in culture medium. The extent of both live and dead cells was revealed through Live/Dead assay and changes in cytoskeleton arrangement and focal adhesion formation were investigated by immunolabeling and confocal microscopy. All tested composites manifested good biocompatibility when tested in contact with hASCs, without inducing significant cytotoxic effects for up to seven days of *in vitro* culture. However, results indicated increased cell viability and proliferation in the presence of MNPs, with a proportional increase dependent on MNPs concentration. Cytoskeleton and focal adhesion immunolabeling revealed an elongated shape of actin microfilaments and highlighted paxillin distribution, indicating beneficial effects of MNPs-enriched scaffolds. Therefore, materials with MNPs content manifest good interaction with hASC and could be used in further studies of hASC transdifferentiation towards neuronal lineage.

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RESVERATROL-LOADED VESICULAR ELASTIC NANOCARRIERS GEL IN IMIQUIMOD-INDUCED PSORIASIS TREATMENT: *IN VITRO* AND *IN VIVO* EVALUATION

MAHMOUD A. ELGEWELLY[1], SOHA M. ELMASRY [1], NESRINE S. EL SAYED[2,*], HAIDY ABBAS[1]

[1]Damanhour University, Faculty of Pharmacy, Pharmaceutics Department, Egypt
[2]Cairo University, Faculty of Pharmacy, Pharmacology and Toxicology Department, Egypt
*Corresponding author: nesrine.salah@pharma.cu.edu.eg

This work aimed to develop a new efficient approach for safe treatment of psoriasis. To achieve that, resveratrol-loaded spanlastics(F1-F12) were prepared and evaluated by complete in vitro characterization. The two optimal formulations (F10 and F11) had their particle size in the nano range with high entrapment efficiency and sustainable drug release. These two formulae were incorporated in carbopol 934 gel formulations (G1-G8) with different concentrations of drug and carbopol 934 polymer. G1 and G5 (1% w/w Carbopol 934 gel and 0.1% resveratrol) showed 40.13% ± 2.017% and 73.76% ± 2.46%,8 hours drug release, respectively. Their pH was accepted and non-irritant. At a shear stress of 500 s−1, G1 and G5 showed a reasonable viscosity of 1048.5 ± 2.12 cps and 954 ± 2.15 cps, respectively. In the in vivo psoriasis study, mice treated by G5 gel showed significant improvement of erythema and scaling compared to positive control group and they maintained healthy skin as shown in histopathological observations. Moreover, this group showed the least changes in mRNA expression of inflammatory cytokines. Concisely, our results suggest that selected carbopol gel of resveratrol-loaded spanlastics could maximize resveratrol topical anti-psoriatic effect.

MTT AND CLONOGENIC ASSAYS TO EVALUATE THE IN VITRO EFFECT OF BLEOMYCIN IN DIFFERENT TUMOR CELL LINES

GABRIELA VOCHIȚA[1,*], COSMIN-TEODOR MIHAI[1,2], ALLA BOREYKO[3], EUGEN KRASAVIN[3], DANIELA GHERGHEL[1]

[1]Institute of Biological Research Iasi, branch of NIRDBS, 47 Lascăr Catargi, 700107 Iași, Romania
[2]"Grigore T. Popa" University of Medicine and Pharmacy Iași, Advanced Centre for Research and Development in Experimental Medicine (CEMEX), 9-13 Mihail Kogălniceanu, 700259 Iași, Romania
[3]Joint Institute for Nuclear Research, Laboratory of Radiation Biology, Dubna, Russia
*Corresponding author: gabriela.vochita@icbiasi.ro

Bleomycin (BLM) is an anti-tumor antibiotic glycopeptide synthesized by *Streptomyces verticillus*, inducing DNA double strand breaks, similar to those obtained by radiotherapy. It is considered a radiomimetic drug indicated, in association with other cytotoxic agents, for slow or stop the growth of cancer cells. The aim of this study is to assess the bleomycin effect using the MTT and the clonogenic assays, both tests being applied in chemoradiation and radiosensitivity analyses. Clonogenic assay is commonly used to investigate survival of irradiated cancer cells, whereas MTT method is performed for the study of chemosensitivity or toxicity of the drugs on different cell lines. Despite its long duration, the colony formation assay is reliable method used in cancer research, allowing the *in vitro* screening to determine therapeutic radiation doses and drug concentrations.

In present study the HeLa cervical cancer cell line and MCF-7 breast carcinoma cells were treated with logarithmic bleomycin doses (0.1, 1.0 and 10 μg/mL), for 24 and 48 h. For the colonies formation experiment, 100 cells/well were seeded in 12-well plates and allowed to grow for two days before treatment. After the expiration of the treatment period (48 hours), the fresh growth medium was added, the procedure being repeated at each 3 days. Experiment was stopped when the number of the colonies at the Control reached over 50 cells/colony (two weeks). At this moment, the colonies were washed with PBS, fixed in ethanol, and stained with Trypan blue. The number and size of the colonies were analyzed using ImageJ plugin.

The cell viability generally had a dose-dependent diminution, the effect being more intense after 48 h of treatment. Regarding the colonies formation, the number of the colonies decreased at high doses (10 μg/mL), and the MCF-7 cell line was more sensitive to the radiomimetic treatment than HeLa cells.

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IN VITRO ASSESSMENT OF CYTOTOXICITY IN HEALTHY CELLS FOLLOWING EXPOSURE TO HEAVY METALS

DANIELA GHERGHEL[1,*], CRĂIȚA MARIA ROȘU[1], COSMIN-TEODOR MIHAI[1,2], GABRIELA VOCHIȚA[1]

[1]Institute of Biological Research Iasi, branch of NIRDBS, 47 Lascăr Catargi Street, 700107 Iași, Romania
[2]Advanced Centre for Research and Development in Experimental Medicine (CEMEX), "Grigore T. Popa" University of Medicine and Pharmacy Iasi, 9–13 Mihail Kogălniceanu Street, 700259 Iași, Romania
*Corresponding author: daniela_gherghel@yahoo.com

Heavy metal pollution is one of the main problems of contemporaneity, difficult to manage, due to their chemical stability and high degree of toxicity. The present study aims to investigate cytotoxic events occurring as a result of direct exposure of mammalian cells to the action of some heavy metals (cadmium and lead), emphasizing the importance of investigation of intracellular damages, for which data from the literature are limited. Thus, the effect of heavy metals, administered in form of salts, respectively cadmium chloride (CdCl2) and lead nitrate (Pb(NO3)2) was *in vitro* tested, on certain cytophysiological processes – cell viability, protein synthesis, enzymes involved in protection against oxidative stress, cell cycle, the complex process of apoptosis, integrity of genetic material – in normal cell lines, kidney cells from the African monkey *Cercopithecus aethiops*, Vero (ATCC® CCL-81) and Chinese hamster lung fibroblasts, V-79 (ATCC® CCL-93 ™), at different doses and time intervals.
All our *in vitro* research has revealed that both cadmium and lead are cytotoxic and genotoxic, by decreasing cell viability and protein synthesis, as well as by damaging DNA in Vero renal cells and V-79 fibroblasts, the toxicity of these heavy metals can be the result of complex mechanisms of action involving the induction of programmed cell death (apoptosis) and oxidative stress.
These results represent the scientific support of future research directions, which on the one hand, will highlight the effect of heavy metals on other cell lines, healthy or tumor, in order to use *in vitro* cell systems as methods for rapid testing of heavy metal toxicity, and on the other hand, they will contribute to the development of effective protection and treatment methodologies for diseases related to exposure to heavy metals.

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COGNITIVE FACILITATION AND ANTIOXIDANT EFFECTS OF AN ESSENTIAL OIL MIX ON SCOPOLAMINE-INDUCED AMNESIA IN RATS: MOLECULAR MODELING OF *IN VITRO* AND *IN VIVO* APPROACHES

LUCIAN HRIȚCU[1,*]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, 700505 Iași, Romania,
*Corresponding author: hritcu@uaic.ro

The present study investigated the capability of an essential oil mix (MO: 1% and 3%) in ameliorating amnesia and brain oxidative stress in a rat model of scopolamine (Sco) and tried to explore the underlying mechanism. The MO was administered by inhalation to rats once daily for 21 days, while Sco (0.7 mg/kg) treatment was delivered 30 min before behavioral tests. Donepezil (DP: 5 mg/kg) was used as a positive reference drug. The cognitive-enhancing effects of the MO in the Sco rat model were assessed in the Y-maze, radial arm maze (RAM), and novel object recognition (NOR) tests. As identified by gas chromatography-mass spectrometry (GC-MS), the chemical composition of the MO is comprised by limonene (91.11%), followed by γ-terpinene (2.02%), β-myrcene (1.92%), β-pinene (1.76%), α-pinene (1.01%), sabinene (0.67%), linalool (0.55%), cymene (0.53%), and valencene (0.43%). Molecular interactions of limonene as the major compound in MO with the active site of butyrylcholinesterase (BChE) was explored via molecular docking experiments, and Van der Waals (vdW) contacts were observed between limonene and the active site residues SER198, HIS438, LEU286, VAL288, and PHE329. The brain oxidative status and acetylcholinesterase (AChE) and BChE inhibitory activities were also determined. MO reversed Sco-induced memory deficits and brain oxidative stress, along with cholinesterase inhibitory effects, which is an important mechanism in the anti-amnesia effect. Our present findings suggest that MO ameliorated memory impairment induced by Sco via restoration of the cholinergic system activity and brain antioxidant status.

VALORIZATION OF ESSENTIAL OILS FROM *TETRACLINIS ARTICULATA* AND *PINUS HALEPENSIS*: APPLICATION IN AN ANIMAL MODEL WITH ALZHEIMER'S DISEASE INDUCED BY B-AMYLOID (1-42)

FATIMA ZAHRA SADIKI[1,*], MOSTAFA EL IDRISSI[1], LUCIAN HRIȚCU[2], PAULA ALEXANDRA POSTU[2], OANA CIOANCĂ[3], ADRIANA TRIFAN[3], MOHAMMED SBITI[4]

[1]Moulay Ismail University, Faculty of Sciences of Meknes, Department of Chemistry, Bp. 11201, Zitoune, Meknès, Morocco
[2]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, Bv. Carol I, no. 11, 700505 Iași, Romania
[3] University of Medicine and Pharmacy "Gr. T. Popa", Faculty of Pharmacy, Department of Pharmacognosy, Iași, Str. Universității no. 16, 700115 Iași, Romania
[4]Faculty of Medicine and Pharmacy of Fez, Fes, Morocco
*Corresponding author: fatizahrasad@gmail.com

Many new drugs derived from secondary plant metabolites have been applied for the treatment and prevention of various diseases. Aromatic plants are often used in traditional medicine. Essential oils are widely used as antibiotics to treat infectious diseases due to bacterial contamination; they are also used as antioxidants to treat neurodegenerative disorders including Alzheimer's disease.

The objectives of this work were therefore to analyze the chemical compositions, to evaluate, *in vitro*, the antimicrobial and antioxidant activities and to examine, *in vivo*, the neuroprotective effects in rats with Alzheimer's disease induced by β-amyloid (1-42) of essential oils from *Pinus halepensis* Mill. and *Tetraclinis articulata* (Vahl) Masters.

The essential oils were extracted by the hydrodistillation technique using a Clevenger type apparatus. The ability of essential oils to inhibit microorganisms was tested using three different methods: the disk diffusion method, the liquid macrodilution method and the solid state dilution method. The antimicrobial activity was variable depending on the nature of the strain and the essential oil. Subsequently, in vitro antioxidant activity was evaluated by two methods: the DPPH free radical scavenging test and the ABTS test. Based on the results, both oils tested have an important antioxidant potential. Also, we demonstrated that our essential oils reversed the Aβ1-42-induced decreasing of the spontaneous alternation in the Y-maze test and the Aβ1-42-induced increasing of the working and reference memory errors in the radial arm maze test. Biochemical estimates of brain homogenates for acetylcholinesterase and biomarkers of oxidative stress were also performed. Furthermore, the oxidant-antioxidant status in the rat hippocampus was recovered after treatment with essential oils. These results suggest that the essential oils of *P. halepensis* and *T. articulata* have neuroprotective activity and can be considered as a therapeutic tool for the mitigation of amyloid Aβ toxicity and neuronal dysfunction.

AMELIORATIVE EFFECTS OF COTININE AND 6-HYDROXY-L-NICOTINE IN A SCOPOLAMINE-INDUCED ZEBRAFISH MODEL OF ALZHEIMER'S DISEASE

RĂZVAN ȘTEFAN BOIANGIU[1,*], MARIUS MIHĂȘAN[1], DRAGOȘ LUCIAN GORGAN[1], BOGDAN ALEXANDRU STACHE[1,2], LUCIAN HRIȚCU[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, Carol I bd., no. 20A, Iași, Romania
[2]Regional Institute of Oncology, Department of Molecular Genetics, Center for Fundamental Research and Experimental Development in Translation Medicine—TRANSCEND, General Henri Mathias Berthelot Street, no. 2-4, Iași, Romania
*Corresponding author: boiangiu.razvan@yahoo.com

Alzheimer's disease (AD), a progressive neurodegenerative disorder, affects over 50 million people worldwide and represents the most common form of dementia and the fifth leading cause of death. AD is associated with memory impairments and cognitive decline, although several neuropsychiatric symptoms, such as anxiety, depression or apathy were also observed in the early stages of the disease. Zebrafish (*Danio rerio*) was successfully used to simulate AD pathology. Cotinine (COT) and 6-hydroxy-L-nicotine (6HLN) are two nicotinic derivatives that possess pro-cognitive and antioxidant properties in different rat models of AD, eluding the side-effects of nicotine (NIC), their precursor. The current study aimed to evaluate the effects of COT and 6HLN on memory deficits, anxiety-like behavior and oxidative stress in the scopolamine (SCOP)-induced zebrafish model of AD. For this, 1 and 2 mg/L of COT and 6HLN were administered to zebrafish that were previously treated with SCOP (100 µM). The memory performances were assessed in Y-maze and novel object recognition (NOR) tasks while the anxiety-like behavior was assessed in novel tank diving test (NTT). The biochemical parameters of oxidative stress were monitored from brain samples and RT-qPCR analysis was used to measure the *egr1* and *nrf2a* gene expression. Data were statistically analyzed using one-way ANOVA and Tukey's multiple comparison tests. Our results indicated that COT and 6HLN mitigated the SCOP-induced memory deficits and anxiety in the specific behavioral tasks. The biochemical analysis revealed that both compounds reduce brain oxidative stress. Also, COT and 6HLN increased the *egr1* and *nrf2a* genes expression in the brain of the zebrafish treated with SCOP suggesting a potential pro-cognitive and antioxidant effect of the treatment. Taken together, our results suggest that COT and 6HLN might represent new therapeutic agents for ameliorating AD-like condition.

IN SEARCH OF BIOMARKERS OF EMOTIONAL BURNOUT (EMOTIONAL EXHAUSTION STAGE)

SERGII TUKAIEV[1,*], DMYTRO HARMATIUK[2], ANTON POPOV[2], MYKOLA MAKARCHUK[1]

[1]Taras Shevchenko National University of Kyiv, Educational and Scientific Centre "Institute of Biology and Medicine", 64/13, Volodymyrs'ka St., Kyiv, 01601, Ukraine
[2]Igor Sikorsky Kyiv Polytechnic Institute Department of Electronic Engineering, 37, Peremogy Ave., 03056, Kyiv, Ukraine
*Corresponding author: tukaev@univ.kiev.ua

The neurophysiological mechanisms of emotional burnout remain insufficiently studied. It was found that EEG changes during burnout differ from biomarkers of depression and chronic fatigue syndrome. To establish the EEG markers of burnout was our aim. 182 volunteers, first-third year students from the Taras Shevchenko National University of Kyiv aged 18 to 24 years participated in this study. The EEGs were recorded during the resting state (3 min, closed eyes condition) monopolarly using EEG 23-channel system Neurocom. To establish EEG correlates of emotional burnout during rest state we used a special software written in Python 3.6 to implement Power Spectral Density calculation, the interhemispheric and intrahemispheric average coherence and Detrended Fluctuation Analysis (DFA). We analyzed separate artifact-free EEG segments in all frequency bands from 0.2 to 45 Hz. Psychological testing was performed before the registration of EEG. To determine the formation of emotional burnout Boyko's "Syndrome of emotional burnout" Inventory was used. The Exhaustion phase of emotional burnout was formed in 13 participants, and it was under development in 47 participants. In background EEG activity during the development of the exhaustion variations in EEG spatial synchronization were observed in low- and high-frequency EEG components. Development of exhaustion includes breaking of parietal-occipital links (alpha2,3-subbands) and formation of interhemispheric prefrontal, anterior frontal, and frontal links (alpha1,2-subbands). There were interruptions of coherence in the beta1 (interhemispheric frontal-frontal and parietal-parietal links) and beta2 subbands (right interhemispheric frontal link). DFA describes the long-term temporal correlations in the cortex, which are involved in different aspects of brain functioning. We detected a high resting state DFA scaling exponent values (up to 0.90-0,95) under exhaustion development in the theta1 (left prefrontal area), theta2 (right frontal area), alpha3 (posterior regions), beta2 (left temporal region). Obtained values of DFA exponent and average coherence suggest the exhaustion formation is accompanied by the changes in visual and verbal processing, emotional processes (discretion and analysis).

A LINK BETWEEN BRAIN CANCER AND NEURODEGENERATION

PAULA ALEXANDRA POSTU[1,*], ADRIAN TIRON[1], CRINA ELENA TIRON[1], LUCIAN HRIȚCU[2]

[1]Regional Institute of Oncology, Center for Fundamental Research and Experimental Development in Translation Medicine-TRANSCEND, Iași, Romania
[2]Alexandru Ioan Cuza University of Iași, Department of Biology, Faculty of Biology, Romania
*Corresponding author: paulaalexandra.postu@gmail.com

Aging may be defined as a time-dependent accumulation of cellular damage, requiring an equilibrium between the promotion of tissue renewal and the suppression of cell cycle. Advanced age is recognized as the dominant risk factor for most human diseases, including malignant neoplasms and neurodegenerative disorders. A growing body of evidence indicates a negative association between carcinogenesis and neurodegeneration, suggesting that the predisposition for one family of diseases may decrease the risk for the other. Therefore, the focus of this study has been to assess the impact of β-amyloid peptide, a key molecule in Alzheimer's disease pathogenesis, on glioblastoma within an in vitro system.

U87 cells were grown in RPMI-1640 media supplemented with 10% foetal bovine serum. 24 h from seeding a β-amyloid peptide suspension in different concentrations has been administrated to the cells and incubated for different periods of time, as follows: 6h, 12h, 24h, 48h and 72h. After treatment removal, cell viability and the level of reactive species of oxygen (ROS) were evaluated using standardized kits. Moreover, on fixed cells, immunofluorescence protocols were applied in order to assess different molecular markers.

Cellular viability has been found impaired by β-amyloid administration in a time-dependent manner. Also, a time-dependently increase in ROS level was detected as response to β-amyloid peptide suspensions. Additionally, the exposure of U87 cells for 72h to β-amyloid resulted in downregulation of AKT3 and BDNF expressions.

β-amyloid peptide presents the potential to impair U87 cells survival capacities and to ameliorate their characteristic aggressive behavior.

SWEROSIDE CAN PREVENTS SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT AND BRAIN OXIDATIVE STRESS IN ZEBRAFISH (*DANIO RERIO*)

ION BRÎNZA[1,*], LUCIAN HRIȚCU[1], ELDAHSHAN OMAYAMA[2]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, Romania,
[2]Ain Shams University, Department of Pharmacognosy, Egypt
*Corresponding author: ionbrinza995@gmail.com

Alzheimer's disease (AD) is the most common neurodegenerative disorder, being associated with memory disorders and cognitive decline that ultimately affect thought, reason, video-spatial orientation, and behavior. AD is associated with mitochondrial dysfunction and a significant difficulty in cholinergic transmission, therefore, there is a special interest in the selection of different compounds capable of regulating acetylcholinesterase (AChE) activity. Recently, it has been discovered that several plant iridoids have significant neuroprotective effects, being able to slow down the process of neurodegeneration. The mechanisms of action of iridoids have been shown to be mediated by the inhibition of cholinesterases including AchE. We focused on one natural iridoid, Sweroside (Swe) which is described in studies as an iridoid with beneficial effects on the SNC, without adverse effects. The Swe was administered to zebrafish, chronically by immersion, immersion in concentrations of 1 μg/L, 3 μg/L and 5 μg/L. The zebrafish dementia model was established by treating the zebrafish with Sco (100 μM), 30 min before behaviorally tested. As a positive control we used Galantamine (Gal, 10 mg/L). Anxiety was measured using the Novel Tank Test (NTT), spatial memory was assessed using by Y-maze test, and recognition memory was assessed using by the Novel Object Recognition Test (NOR). We also evaluated the impact of the Swe on the cholinergic and oxidative status of this animal model. Our results show that Swe can effectively restore the antioxidant defense mechanism by increasing the level of antioxidant activity in the brain and can improve cognitive dysfunction of amnesic fish by inhibiting AChE, which is also correlated with improved memory parameters, as shown in behavioral approaches (NTT, Y maze and NOR).

The authors declare no conflict of interest.

INVESTIGATION OF THE ACTION OF ROIFOLINE ON COGNITIVE PROCESSES IN ZEBRAFISH

DANIELA-ADRIANA TANASĂ[1,*], LUCIAN HRIȚCU[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, Animal Physiology Laboratory, Iași, Romania
*Corresponding author: adrianatanasa7@gmail.com

Alzheimer's disease (AD) is a global priority according to the World Health Organization. As the average age of the population increases, the rate of AD also increases [1]. AD affects people from the age of 65, being a neurodegenerative disorder involving language, memory, understanding, judgment, and reasoning [2]. According to reports of AD, the histopathological characteristics associated with Alzheimer's dementia are represented by extracellular accumulations of beta-amyloid leading to the formation of neuritic plaques or senile plaques, but also by internal accumulations of neurofibrillary tangles formed by hyperphosphorylated microtubules associated with tau proteins. Rhoifolin (Rho), a flavone glycoside, exerts many biological activities such as anticancer, antidiabetic, hepatoprotective, antirheumatic, antibacterial, and antiviral properties. The goal of this study was to investigate the improvement impact of Rho (1, 3, and 5 μg/L) on scopolamine (Sco, 100 μM)-induced zebrafish amnesia. Zebrafish have been used as an animal model because it has a high degree of similarity to the human brain model. The behavioral tests used were the Y-maze test that examines the spatial memory of the animal model and their response to novelty, but also the new object recognition test (NOR) - through which we evaluate the memory of zebrafish (*Danio rerio*) recognition of a new object.

Our study data highlighted the beneficial effects of Rho treatment on the spatial memory in the Y maze test and recognition memory in the NOR test, thus suggesting the beneficial effects of Rho on cognitive processes, but also on the amelioration of neuropsychic symptoms of AD in the animal model of dementia represented by zebrafish (*Danio rerio*).

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THE NATURAL NEUROPROTECTIVE COMPOUNDS USED IN THE 6-HYDROXYDOPAMINE INDUCED PARKINSON'S DISEASE IN ZEBRAFISH: THE CURRENT APPLICATIONS AND PERSPECTIVES

SARA ABIDAR[1,*], LUCIAN HRIȚCU[2], AMINA AMAKRAN[1], GABRIELA DUMITRU[2], ELENA TODIRAȘCU-CIORNEA[2], MOHAMED NHIRI[1]

[1]Université Abdelmalek Essaâdi, Faculté des Sciences et Techniques, Laboratoire de Biochimie et Génétique Moléculaire, Tanger Principal BP 416, Morocco
[2]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, 700506 Iasi, Romania
*Corresponding author: sara.abidar91@gmail.com

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a degeneration of the dopaminergic neurons in the *substantia nigra pars compacta*, resulting in the loss of dopamine in the striatum, leading thus to the PD classic movement symptoms: resting tremor, rigidity, and bradykinesia/akinesia. Furthermore, Levodopa's efficacy declines with long term use, generating therefore serious motor complications. Neuroprotection implies the use of different agents exhibiting various neuroprotective strategies: iron chelators, antioxidant, anti-inflammatory, anti-lipid peroxidation, monoamine oxidase inhibitors, dopamine receptor agonists, neurotrophic factors…

The aim of the present study is to summarize and analyze the neuroprotective activities of natural compounds, including extracts, formulations, fractions and bioactive drugs investigated using the 6-OHDA induced lesions in zebrafish model, providing thus future applications in the clinical trials. All the natural drugs tested were retrieved from PubMed Database, via pre-set searching strings. The relevant compounds were included and analyzed according to their biological activities.

Several natural compounds were analyzed, they were reported to modulate different signaling pathways involved in the pathogenesis of PD; the main compounds found were bioactive drugs, as acteoside, berberine, chrysin, the derivatives from Danshensu, Hesperidin, Naringenin, Panaxatriol saponins, Protocatechuic, quercetin, Schisantherin A, Tetramethylpyrazine, Theacrine and Xanthotoxin…; the plant extracts investigated were the aqueous and methanolic extracts of leaves from *Ceratonia siliqua*, the ethanolic extract of Fructus *Alpinia oxyphylla*, and its bioactive compound: oxyphylla A, *Ginkgo biloba* L., preparations, the Hexane fraction from *Spondias mombin* L.

The herbal medicines studied deserve further consideration as examining the optimum concentrations, bioavailability and the ability of these agents to cross the blood brain barrier to exert their effects against PD. A complete understanding of the molecular mechanisms involved in PD, and larger epidemiologic and randomized clinical trials in humans are also required.

APPLICATIONS OF PROTEOMICS IN LIFE SCIENCES

COSTEL C. DARIE[1,*], MADHURI JAYATHIRTHA[1], DANIELLE WHITHAM[1], BRÎNDUȘA ALINA PETRE[1], EMMA BAXTER[1], BAILEE UNDERWOOD[1], BAILEY BRENNEN[1], SHELBY ALWINE[1], GEORGE CULVER[1], YASHVEEN RAI[1], PANASHE MUTSENGI[1], MARY DONNELLY[1], HANNAH YORKEY[1], ISABELLE SULLIVAN[1]

[1]Clarkson University, Department of Chemistry & Biomolecular Science, Biochemistry & Proteomics Group, Potsdam, NY, 13699-5810, USA
*Corresponding author: cdarie@clarkson.edu

The central dogma in biology is that DNA directs transcription of the coding information to RNA, which is then translated into proteins. So, one gene can lead to one protein. However, combination of various exons from the same gene can lead to formation of protein isoforms, which sometimes are tissue or organ specific. Individual proteins are also post-translationally modified by methylation, acetylation, phosphorylation, myristoylation, etc., which leads to formation of modified proteins, sometimes with various functions. Lastly, unmodified or modified proteins also interact with other proteins, to form stable or transient protein-protein interactions. Here, we show how the use of mass spectrometry-based proteomics can investigate the structure and function of proteins and entire proteomes.

INVESTIGATION AND CHARACTERIZATION OF THE JUMPING TRANSLOCATION BREAKPOINT (JTB) PROTEIN USING MASS SPECTROMETRY BASED PROTEOMICS

MADHURI JAYATHIRTHA[1,*], DANIELLE WHITHAM[1,*], DEVIKA CHANNAVEERAPPA[1,*], COSTEL C. DARIE[1,*]

[1]Clarkson University, Department of Chemistry and Biomolecular Science, Potsdam, NY, USA
*Corresponding author: whithad@clarkson.edu; channad@clarkson.edu; cdarie@clarkson.edu

Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in various types of cancer including prostate and breast cancer. Hence this protein could be a biomarker for tumor malignancies and a potential target for their treatment. However, the biological function and the pathway through which this protein causes increased cell growth and proliferation is not entirely clear. Investigation and comparison of the proteomes of cells with upregulated and downregulated JTB can be a good approach to understand the function of the protein and also its contribution to tumorigenesis. In this study, MCF7 breast cancer cell lines were transfected with the sense orientation of the JTB cDNA in HA, His and FLAG tagged CMV expression vector as well as with shRNA plasmids. Proteins extracted from transient and stable transfected cells were separated using SDS-PAGE. The expression of JTB was confirmed by western blotting technique. In gel digested peptides were analyzed by a Nano Acquity UPLC coupled with QTOF Xevo G2 Mass Spectrometer. Data processing was done using Mascot 2.4 server and Scaffold 4.1 software. We found several proteins that were dysregulated. Furthermore, we will do Immunoprecipitation to look at JTB protein interacting partners. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a potential drug target for malignancies.

PROTEOMIC ANALYSIS OF HUMAN BREAST MILK TO REVEAL POTENTIAL PROTEIN BIOMARKERS FOR BREAST CANCER

DANIELLE WHITHAM[1], ROSHANAK ASLEBAGH[1], DEVIKA CHANNAVEERAPPA[1], BRIAN PENTECOST[2], KATHLEEN F. ARCARO[2], COSTEL C. DARIE[1,*]

[1]Clarkson University, Department of Chemistry & Biomolecular Science, Biochemistry & Proteomics Group, Potsdam, NY, 13699-5810, USA
[2]University of Massachusetts Amherst, Amherst, MA, USA
*Corresponding author: cdarie@clarkson.edu

Breast cancer (BC), one of the most common cancers, is a leading cause of death for women in the United States. An estimated 1 in 8 women in the United States will develop BC in their lifetime. Early diagnosis and treatment of BC is crucial, and protein biomarkers for this disease could make this possible. Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of protein biomarkers. This study employs MS-based proteomics to study the protein differences in human breast milk from women with BC and matched controls. If significant protein dysregulations are revealed, they could be considered potential future protein biomarkers of BC for diagnosis and treatment. In this study, six human breast milk samples were analyzed by performing a 2D-SDS-PAGE and further analyzed via nanoLC-MS/MS. The human breast milk samples consisted of three comparison pairs of BC vs. control. There were one Coomassie blue-stained gel (6 total gels) and two replicate silver-stained gels (12 total gels) for each sample of breast milk. The silver-stained gels were scanned using a laser densitometer and the images were analyzed using Progenesis Same Spots and Progenesis PG240 software. The spot percentages were measured for each dysregulated spot., An in-gel trypsin digestion was performed for each spot with statistically significant dysregulation. This was followed by nanoLC-MS/MS, data processing, database search and statistical analysis. The dysregulated proteins found in this study can be investigated as BC biomarkers for future clinical methods for early diagnosis and treatment of BC.

MASS SPECTROMETRIC CHARACTERIZATION OF THE ZEIN PROTEIN COMPOSITION IN MAIZE FLOUR „ECO- FRIENDLY" EXTRACTS

LAURA DARIE-ION[1], GABI DROCHIOIU[1], COSTEL C. DARIE[2], BRÎNDUȘA ALINA PETRE[1,2,*]

[1]Alexandru Ioan Cuza University, Faculty of Chemistry, Department of Chemistry, Iași, Romania
[2]Clarkson University, Department of Chemistry & Biomolecular Science, Potsdam, NY, USA
*Corresponding author: bpetre@clarkson.edu

Zein proteins, first identified in 1821, belong to the prolamine protein class that in cereals represents more than 60% of the total endosperm protein. Zein proteins are a family of related polypeptides encoded by a multigene family whose structural genes are located in maize in three main clusters on chromosomes 4, 7, and 10 [1]. Zein proteins have found wide application in refining food products, such as candies, nuts, and apples. For agricultural applications, microspheres are produced out of zein proteins which are filled with pheromones and/or pesticides for controlled release of the biologically active substances to diminish odor and to provide a safer working environment [2]. Zein proteins have been applied for coating of tablets and in zein-coated chitosan/tripolyphosphate nanoparticles for oral delivery of selenite [2]. Zein-coating has also been shown to protect peptides with antioxidant and antihypertensive activities.

In this study, a procedure for the extraction of corn grain zein proteins, which is considered environmentally friendly and which aimed at producing a highly homogeneous zein protein product, was applied to investigate the izoforms composition. To verify homogeneity of the extracted and defatted protein material from maize flour we applied direct ESI- QTOF mass spectrometric measurements.

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MICROPROPAGATION FROM CALLUS CULTURES OF *CAPSICUM ANNUUM* L.

SMARANDA VÂNTU[1,*]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Carol I Bd., 20A, 700505, Iași, Romania
*Corresponding author: s_vantu@yahoo.com

Capsicum annuum L. (Solanaceae) is a well known source of natural compounds with high antioxidant, antimicrobial, anticancer activities. One of the most important secondary metabolites, with medicinal properties is capsaicin synthesized in fruits. Plant tissue cultures are a better alternative for producing secondary metabolites. Indirect micropropagation through callus cultures has become a biotechnological approach for the improvement of economically important plants.

The initiation of "in vitro" cultures of *Capsicum annuum* L aimed to assess the dedifferentiation capacity, depending on explant origin and growth regulators and to develop a multiplication protocol, based on indirect regeneration through shoots, followed by roots development induction. The proliferative capacity was tested on plantlets explants, cultivated on Murashige-Skoog basal medium, testing two auxins: 2,4 dichlorphenoxyacetic acid (2,4 D) and indolylacetic acid (IAA) and a cytokinine: benzylaminopurine (BAP).

The MS medium with 1.0 mg/l IAA and 1.0 mg/l BAP proved to be the best for callus induction from hypokotyl explants. Shoot regeneration was achieved after subculturing the calli on MS medium, supplemented with 1 mg/l BAP and 0,1 mg/l IAA. It was found to be the best for multiple shoot regeneration from callus through organogenesis. Root system development was achieved on MS medium without growth regulators. The rooted shoots were transferred for acclimatization to pots, containing sterile mixture of perlite-soil.

ANALYSIS OF THE LAKE TROUT HEART AND BLOOD PROTEOME USING EVOLUTIONARY PROTEOMICS

SHELBY ALWINE[1,*], EMMALYN J. DUPREE[1], BERNARD CRIMMINS[2], THOMAS HOLSEN[3], COSTEL C. DARIE[1]

[1]Clarkson University, Department of Chemistry & Biomolecular Science, Biochemistry and Proteomics Group, Potsdam, NY, 13699-5810, USA
[2]AEACS, New Kensington, PA, USA
[3]Clarkson University, Department of Civil and Environmental Engineering, Potsdam, NY, 13699-5810, USA
*Corresponding author: alwinese@clarkson.edu

Salvelinus namaycush (lake trout) is a top-predator fish in the Great Lakes region. The Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) uses lake trout as bioindicators of the presence of persistent, bioaccumulative and toxic (PBT) chemicals. Elevated levels of PBTs can cause changes in transcribed genes, translated mRNAs, proteins produced, and post-translational modifications of these proteins in aquatic species. Though lake trout is used as a bioindicator of PBTs, there is currently no well-developed proteome database for the species that has been monitored for five decades. In this study, heart and blood samples from lake trout were analyzed by SDS-PAGE, followed by in-gel trypsin digestion and analysis by nanoLC-MS/MS. The data was searched against different NCBI and UniProtKB databases in Mascot Daemon and the output was analyzed by Scaffold 4.3 software. Databases used include *Actinopterygii*, *Salmonidae*, *Salvelinus*, as well as the highly studied species *Oncorhynchus mykiss* and *Danio rerio*. Using these better developed protein databases, we were able to identify many novel proteins for the lake trout, as well as explore evolutionary relationships for the lake trout species. This ongoing project will potentially lead to a more developed, comprehensive proteome database for lake trout that can be used for further proteomic studies on the effects of legacy PBT chemicals in the Great Lakes ecosystem.

IDENTIFICATION OF INVERTEBRATES USING DNA BARCODING. STUDY CASE: DANUBE RIVER-DANUBE DELTA-BLACK SEA SYSTEM

SELMA MENABIT[1,2,*], TATIANA BEGUN[1], ADRIAN TEACĂ[1], MIHAELA MUREȘAN[1], ANA BIANCA PAVEL[1], LAVINIA IANCU[2], CRISTINA PURCAREA[2]

[1]National Institute of Marine Geology and Geo-Ecology (GeoEcoMar), Bucharest, Romania
[2]Institute of Biology Bucharest, Department of Microbiology, Bucharest, Romania
*Corresponding author: selma.menabit@geoecomar.ro

In the last decades, the use of molecular techniques for the identification and classification of life forms has become quite popular. The most widely applied method is represented by DNA barcoding, a modern technique complementing traditional morphology-based taxonomic assignments, that uses a short, standardized fragment of genomic DNA in order to identify an organism up to the species level. Therefore, in the present study, we applied both morphological and molecular approach based on DNA barcoding (cytochrome c oxidase subunit 1 gene sequencing) in order to identify 5 marine and 16 freshwater invertebrate species, respectively, belonging to 9 major taxonomic groups of Porifera (1), Hirudinea (2), Gastropoda (5), Bivalvia (3), Amphipoda (3), Mysida (2), Isopoda (1), Insecta (3), Tunicata (1) inhabiting the Danube River-Danube Delta-Black Sea System. The study was of major interest due to the lack of molecular data on the investigated groups from the study area, which is shelter to many endemic and Ponto-Caspian species. The molecular method proved to be successful for all the analysed individuals, being very helpful to assign the exact taxon to those misidentified through morphotaxonomical criteria. Our results suggest that DNA barcoding represent a valuable and efficient tool for species discrimination and we strongly recommend that further specimens morphologically identified should be sequenced, to test the feasability of COI gene fragment amplification for species identification. Considering these aspects, we can assume that DNA barcoding is an effective approach for species diagnosis. However, combining phenotypic and molecular criteria increase the accuracy and reliability of results.

NEUROPROTECTIVE AND ANTIOXIDANT EFFECTS OF THE AQUEOUS EXTRACT OF *XIMENIA AMERICANA* STEM BARK ON MEMORY IMPAIRMENT INDUCED BY DIAZEPAM IN MICE

JEAN GALBA BEPPE[1,*]

[1]University of Maroua, Faculty of Science, Department of Biological Sciences,
P.O. Box 814, Maroua, Cameroon
*Corresponding author: beppe840@gmail.com

Ximenia Americana (XA) is a plant traditionally used for the treatment of headaches, rheumatoid arthritis, intestinal diseases and mental illness. The current study was undertaken to investigate the neuroprotective and antioxidant effects of the aqueous extract of X. americana on diazepam-induced amnesia. Thirty-six mice were randomly divided into six groups: normal control received distilled water (10 mL/kg), negative control received diazepam on day 14 (DZP 3 mg/kg, p.o.), the positive control received piracetam (PIR 150 mg/kg, p.o.), three tested groups received XA aqueous extract (25, 50 and 75 mg/kg BW, p.o.) for 14 consecutive days. Radial Arm Maze (RAM) and T-Maze were carried out to assess the behavioral response 30 min after treatment. At the end of behavioral tests, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) levels were assessed in mice brain homogenates. Brain was also isolated for histological studies. The administration of XA aqueous extract significantly ($p < 0,001$) decreased the reference memory errors and the working memory errors in the RAM, compared with normal control group. Additionally, XA aqueous extract-treated groups significantly ($p < 0,001$) increased the time spent in the preferred arm in the T-maze compared to DZP non-treated group. *X. americana* DZP-treated groups significantly ($p < 0,001$) increased CAT and GSH levels, and a decrease in MDA level was observed in brain homogenates compared to DZP non-treated group. The aqueous extract improved memory in behavioral tests and decreased the oxidative stress in the rat hippocampus. These results suggest that XA has protective and antioxidant effects.

THE STIMULATORY EFFECT OF THE ROMANIAN MONOFLORAL HONEY ON KERATINOCYTE PROLIFERATION

ANDREEA IOSĂGEANU[1,2,*], ELENA MIHAI[2], OANA CRĂCIUNESCU[2], ANIȘOARA CÎMPEAN[1]

[1]University of Bucharest, Faculty of Biology,
Splaiul Independenței 91-95, Bucharest, R-050095, Romania
[2]National Institute of R&D for Biological Sciences,
296, Splaiul Independenței, 060031 Bucharest, Romania
*Corresponding author: andreea.iosageanu@gmail.com

The main constituents of honey are sugars, which represent 95-99% of the dry matter, but it also contains phenolic compounds, proteins, organic acids, lipids, vitamins, water and minerals. Clinical studies have reported the successful use of honey in skin wound treatment [1]. However, more complex studies are required to identify and understand how certain compounds act on the wound healing process. The aim of this study was to evaluate the effect of different honey varieties on keratinocyte proliferation *in vitro* and to correlate their biological activity to the physicochemical characteristics.

Three honey samples of different floral origin were analysed in this study: linden, sunflower and rapeseed honey. For the melissopalynological analysis, we have used the nonacetolytic method [2]. For the physicochemical characterization, the moisture, pH and free acidity were analysed. The total phenolic content (TPC) was determined by Folin-Ciocâlteu method and the ascorbic acid content by the DCPIP method [3]. The effect of honey samples on HaCaT keratinocyte proliferation was evaluated by the Neutral Red assay after 24 and 48 h of incubation. Cellular morphology observation was performed in a similar experiment using phase-contrast microscopy.

The results confirmed the botanical origin of monofloral honey samples. The physicochemical characterization proved the quality of honey. TPC values ranged from 450 mg GAE/kg honey for linden honey to 560 mg GAE/kg honey for sunflower honey. The ascorbic acid content was 2.5 mg/kg honey for sunflower honey and undetectable for the other two samples. Cell viability tests indicated a wide range of biocompatibility (0.1-5 mg/mL) of all honey samples. A stimulation of keratinocyte proliferation was observed in case of all honey samples by up to 5%. No alterations in cell morphology were observed.

Based on these promising results we envision further studies to test of the honey potential for wound healing applications.

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DRAFT GENOME OF A USEFUL BACTERIA: PAENARTHROBACTER NICOTINOVORANS

IASMINA HONCERIU[1,*], ȘTEFAN RĂZVAN BOIANGIU[1], MARIUS MIHĂȘAN[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, BioActive research group, 700505 Iași, Romania
*Corresponding author: honceriu.iasmina@yahoo.com

Arthrobacter genus comprises Gram-positive soil bacteria in the form of cocci or rods. Several *Arthrobacter* species have a fully sequenced genome, interest that came from the meaningful role these bacteria have in the bioremediation of soil. *Paenarthrobacter nicotinovorans* is a member of this genus that exhibits the unique ability to degrade nicotine and used it as a C source. This ability is due to the presence of a *nic*-genes cluster on a megaplasmid: pAO1. Thus, *P. nicotinovorans* acquires a pivotal ecological role in detoxification of nicotine-contaminated soils. Also, it has important applications in the pharmaceutical industry: 6-hydroxy-nicotine, the first intermediate from the nicotine catabolic pathway, has been shown to possess a neuroprotective effect in laboratory rats.

Using *P. nicotinovorans* for biotechnology applications such as producing 6-hydroxy-nicotine is difficult due to the lack tools to perform metabolic engineering. Hence, we aim to overcome this issue by providing the complete genome of *P. nicotinovorans* pAO1+. Genomic DNA was isolated using the Qiagen DNeasy UltraClean Microbial Kit. The sequencing library was prepared by random fragmentation and 5' and 3' adapter ligation. Paired-end sequencing was performed on an Illumina NovaSeq 6000, read length 150 bp. QC was done with fastQ, while trimming, clipping and filtering were performed with fastp. SPAdes 3.14.1 was used for assembly, and both checkM and Quast was used to evaluate the results. The draft genome of pAO1+ is 4.4 Mbp in size and consists of 31 contigs. The largest contig is 778 kbp, the mean contig length is 143 kbp and N50 is 270 kbp. CheckM indicated completeness of 99.7% and no contamination. Currently, we are working on sequencing high molecular weight gDNA using nano-pore technology in order to provide a scaffold and perform the final assembly.

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CRISPR-CPF1 SYSTEM AND ITS UTILITY IN EDITING THE *PAENARTHROBACTER NICOTINOVORANS* GENOME

IUSTIN-TIBERIUS MUNTEANU[1], FAKHRI KALLABI[1], MARIUS MIHĂȘAN[1,*]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, BioActive Research Group, Carol I B-vd, 20A, 700505, Iași, Romania
*Corresponding author: marius.mihasan@uaic.ro

Paenarthrobacter nicotinovorans is a Gram-positive bacterium that is best known due to its ability to metabolize nicotine. The stain has a proven potential for converting nicotine containing waste into useful chemicals. Its applications in biotechnology are hampered by the lack of reliable gene editing systems that would permit rational engineering of the nicotine degradation pathway.

CRISPR systems have been extensively used for genomic editing of eukaryotic cells and proved to be reliable and accurate. The applicability of CRISPR system for genomic editing of *Paenarthrobacter* strains remains elusive. The main goal of this work is to evaluate the applicability and functionality of the CRISPR-Cpf1 system in *P. nicotinovorans*. CRISPR-Cpf1 is a class 2 type V CRISPR system known to work in the closely related *Corynebacterium glutamicum* strains. The draft genome of *P. nicotinovorans* (BioProject accession PRJNA693273) was used to screen for the presence of incompatible CRISPR systems using CRISPRs web server (https://crispr.i2bc.paris-saclay.fr/). A number of 4 CRISPRs candidates were found on different contigs, but none were related to CRISPR-Cpf1. Hence, we concluded that the system might work in this strain and the pJYS3_ΔcrtYf plasmid (Addgene 85542) containing a functional a CRISPR-Cpf1 system was electroporated into P. nicotinovorans. No transformants were obtained upon selection with kanamycin, indicating that the pJYS3 replicating origin might not be functional in *P. nicotinovorans*. Next, the CRISPR-Cpf1 genes from pJYS3_ΔcrtYf were amplified by PCR and ongoing work aims to clone these genes into a plasmid known to work in *P. nicotinovorans* – pART2.

ANTIBIOTIC RESISTANCE OF *PAENARTHROBACTER NICOTINOVORANS* – AN IN-SILICO AND IN-VITRO STUDY

MIHAELA ANDREEA MLEȘNIȚĂ[1,*], VERONICA CRISTINA MOLDOVAN[1], FAKHRI KALLABI[2], MARIUS ȘTEFAN[1], MARIUS MIHĂȘAN[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, BioActive research group, 700505 Iași, Romania
[2]University of Sfax, Faculty of Medicine of SFAX, Sfax, Tunisia
*Corresponding author: mlesnitaanda@gmail.com

The ability to metabolize nicotine brought the Gram-positive bacterium *Paenarthrobacter nicotinovorans* into the researchers' limelight since the 60's. Many advancements have been made since then and the potential applications of *P. nicotinovorans* nicotine catabolic pathway for the conversion of nicotine containing waste into useful chemicals have been proved. Yet, the applications of this strain in biotechnology are hampered by the lack of a complete genome and data on the physiology of the strain. Knowledge on the antibiotic resistance is especially important when attempting any metabolic engineering approaches that relies on plasmids as expression vectors.

Hence, our aim is to provide insights into the antibiotic resistance of *P. nicotinovorans* using the mixed approach: the draft genome of the strain was submitted to CARD (The Comprehensive Antibiotic Resistance Database) and the hits were tested in the lab using the Kirby-Bauer disk diffusion test. The draft genome was sequenced by NGS using the Illumina NovaSeq 6000 and the reads assembled with SPAdes 3.14.1. CARD gave a total of 255 loose hits (individual antibiotics and major antibiotic classes). All hits were filtered to 14 major hits based on an aminoacid identity level higher then 50%. A total of 15 antibiotics comprising 7 of the CARD major hits and 2 non-hits were tested in the lab. *P. nicotinovorans* was found to be resistant to 7 antibiotics (Kanamycine, Ceftriaxone, Erytromycin, Gentamicin, Neomycin, Spectinomycin, Vancomycin) corresponding to 5 CARD major hits. Although CARD also indicated a hit for tetracycline, the strain is sensible to this antibiotic at a concentration of 30 µg/ml. *P. nicotinovorans* was also found to be resistant to nalidixic acid, although CARD did not identify any hits for this antibiotic. One interesting find is that sensibility of *P. nicotinovorans* to certain antibiotics is modulated by the presence of nicotine in the growth medium.

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STUDY OF THE MECHANISMS OF ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM THE INFLUENT OF AN WASTEWATER TREATMENT PLANT

CRISTINA-VERONICA MOLDOVAN[1,*], MARIUS ȘTEFAN[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, Iași, România,
*Corresponding author: mcristina.veronica@gmail.com

Wastewater treatment plants (WWTP) are important hotspots for the selection of antibiotic resistant bacteria and antibiotic resistance genes. These plants collect wastewater from several industrial production process or from households, which are often polluted with various antimicrobial substances that represent a major factor involved in the selection of resistant microorganisms. Moreover, the reuse of wastewater after the treatment process leads to the dissemination of clinically important bacteria and resistance genes along surface waters (rivers, lakes, seas, oceans) and soil.
In this regard, the main goal of our study was to investigate the spread of resistant bacteria in the influent of an urban WWTP and to analyze the resistance mechanism of the tested bacteria. For this purpose, microorganisms were isolated on different culture media supplemented with some highly used antibiotics in Romania. The drug concentrations used were established according to the Clinical and Laboratory Standards Institute guideline for resistant bacteria. The antibiotic resistance was evaluated using minimum inhibitory concentration (MIC) assay. The presence of bacterial enzymes that hydrolyze β-lactam antibiotics was determined using chromogenic screening plates and phenotypic tests. The biofilm-forming capacity was assessed using crystal violet method.
Following the experiments performed, we obtained 96 resistant strains. The highest values of MIC were noted for ampicillin, tetracycline, chloramphenicol, erythromycin, and vancomycin. Only 55,96% of Gram-negative bacterial strains were carbapenemase-producing organisms. Among 11 strains that were tested for biofilm production, 3 formed a moderate adherent biofilm, while 5 formed a weakly adherent biofilm.
Our results indicate an increased presence of antibiotic resistant bacteria in analysed water samples, and the isolated strains presented several mechanisms of resistance with strong implication in resistance phenomenon.

ORGANIC ACIDS PRODUCTION BY PHOSPHATE SOLUBILIZING BACTERIA

LOREDANA-ELENA MANTEA[1,*], MARIUS MIHĂȘAN[1], MARIUS ȘTEFAN[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Departament of Biology, Carol I Avenue, no. 20 A, 700505, Iași, Romania
*Corresponding author: mantealoredana9@gmail.com

Microorganisms are involved in a range of processes that affect the transformation of soil phosphorus. Phosphate solubilizing bacteria (PSB) have the ability to convert insoluble phosphorus compounds in soluble forms using different mechanisms. It is generally accepted that the main mechanism of mineral phosphate solubilization by PSB strains is the decrease of soil pH due to the release of low molecular weight organic acids.Therefore, the main goal of this study was to identify the organic acids involved in tricalcium phosphate solubilization. The bacterial strains used were isolated from soils cultivated with maize (*Zea mays* L.) (P1.5S and P3.4S) and municipal wastewater (D12). The ability of the bacterial strains to solubilize tricalcium phosphate was determined at 28°C using phosphomolybdate ammonium method. Also, the pH of the bacterial cultures was measured and the organic acids were identified using HPLC (High Performance Liquid Chromatography). The amounts of solubilized phosphorus by the tested bacterial strains ranged between 8,36 and 14,59 µg/mL, the highest amount being recorded in the case of D12 strain. HPLC analysis of bacterial culture supernatants showed that all bacterial strains produce malic (or formic) acid. The other identified organic acids were tartaric, lactic, acetic, citric, succinic and oxalic acids. In conclusion, one of the mechanisms involved in tricalcium phosphate solubilization by the tested bacterial strains is the acidification of the culture medium due to the production of organic acids.

ANTI-*CANDIDA* ACTIVITY OF SYNTHETIC FLAVONOID Br-Cl

MIHAELA SAVU[1,*], LUCIAN BÎRSA[2], MARIUS ȘTEFAN[1]

[1]Alexandru Ioan Cuza University of Iași, Faculty of Biology, Department of Biology, 20A Carol I Bd., 700505 Iasi, Romania
[2]Alexandru Ioan Cuza University of Iași, Faculty of Chemistry, Department of Chemistry, 11 Carol I Bd., 700506 Iasi, Romania
*Corresponding author: mihaelasavu2@gmail.com

Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Therefore, the discovery of new drugs capable of overcoming the resistance mechanisms of microorganisms is crucial for overall health and safety. To discover new ”magic bullets”, researchers should turn their attention to the development of drugs belonging to a new class of compounds to which microorganisms have not developed resistance. A potential solution would be the use of flavonoids, compounds well known for their antibacterial, antifungal, antioxidant, antitumor and anti-inflammatory properties. In addition to natural flavonoids, synthetic flavonoids are very interesting due to their higher antimicrobial activity. In this context, we investigated anti-Candida activity of a new synthetic flavonoid Br-Cl. The antifungal activity was assessed by minimum inhibitory concentration (MIC) and minimum fungicidal concentrations. The mechanism of action was investigated using cell membrane permeability test, hyphal growth test and scanning electron microscopy (SEM). Our flavonoid showed a very good antimicrobial activity with a MIC of 15.62 μg/mL. After only one hour of incubation, the flavonoid Br-Cl caused significant and irreversible damage to the cell membrane. In the presence of the tested flavonoid, *Candida albicans* yeast to hyphal transition was prevented. Our results showed that flavonoid Br-Cl has a high potential to be used as an efficient antifungal agent.