International Conference of Polish Botanical Society
on the "Biological diversity -
from cell to ecosystem - interdisciplinary study of genetic,
pharmacological, and biological diversity"

ABSTRACT BOOK

Edited by Grażyna Łaska

Organized by Bialystok University of Technology
in collaboration with University of Bialystok
and Medical University of Bialystok

Bialystok, Poland, July 9-11, 2017

International Conference of Polish Botanical Society
on the "Biological diversity - from cell to ecosystem - interdisciplinary study of genetic, pharmacological, and biological diversity"

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A b s t r a c t B o o k

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Białystok, 2017

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Table of contents

Program

Conference place:
Center of Modern Education (Building I, Ground floor, Hall 33)
Bialystok University of Technology
Zwierzyniecka Str. 16, 15-333 Białystok

Sunday, 9 July 2017

15:00-18:00	**Registration opens, Welcome Reception**
18:30-21:00	**Excursion to special places in Bialystok City with a professional guide** (included in the basic fee) **Meeting at the conference and registration place** (Reserve Zwierzyniecki Forest, Planty Park, Branicki Palace, Town Hall, Kosciuszko square, Catholic Cathedral)

Monday, 10 July 2017

8:00-15:00	Registration and poster set up
9:00-9:30	**Opening Ceremony, Welcome speeches**
9:30-10:00	Coffee Break
10:00-11:30	**Plenary Session 1 -** Invited Speakers
11:30-12:00	Coffee Break
12:00-13:30	**Plenary Session 2 -** Invited Speakers
13:30-15:00	**Lunch** (Buildings H, Beta Student Hostel, Zwierzyniecka Street 12)
15:00-17:00	**Poster Sessions** (Number poster in Conference bag) **Selected posters will be presented orally 10-15 minutes**
16:00-16:30	Coffee Break
19:00-24:00	**Conference Party** (Ticket in Conference bag, information about transport and place)

Tuesday, 11 July 2017

10:00-11:30	**Plenary Session 3**
11:30-12:00	Coffee Break
12:00-13:30	**Plenary Session 4**
13:30-15:00	**Lunch**
15:00-16:00	**Sessions 5**
16:00-16:30	Coffee Break
16:30-17:00	**General Discussion and Closing Ceremony**

Detailed program

Monday, 10 July 2017

8:00-15:00	**Registration and poster set up**
9:00-9:30	**Opening Ceremony, Welcome speeches**
9:30-10:00	**Coffee Break**
10:00-11:30	**Plenary Session 1 - Biodiversity in interdisciplinary molecular research** **Chair: Prof. Marc-Andre Selosse**
10:00-10:45	Prof. Jordan Zjawiony Therapeutic potential of psychoactive plants. The case of *Salvia divinorum*
10:45-11:30	Prof. Sergey Dromashko Molecular methods in assessing genetic diversity
11:30-12:00	**Coffee Break**
12:00-13:30	**Plenary Session 2 - Biodiversity in interdisciplinary ecological research** **Chair: Prof. Vladimir Titok**
12:00-12:45	Prof. Marc-Andre Selosse, Julita Minasiewicz, Alicja Robionek, Alzbeta Novotna, Michal May, Marcin Jakalski Mycoheterotrophy and mixotrophy: plants eating mycorrhizal fungi
12:45-13:30	Dr Jason Hoeksema, Melanie Roy, Grażyna Łaska, Aneta Sienkiewicz, Amber Horning, Matthew Abbott, Joshua Mattox, Claire Tran Sharing of ectomycorrhizal fungi between a coniferous tree (*Pinus sylvestris*) and an herbaceous perennial (*Pulsatilla patens*)
13:30-15:00	**Lunch**
15:00-17:00	**Poster Sessions - Scientific Committee** **Selected posters will be presented orally 10-15 minutes**
16:00-16:30	**Coffee Break**
19:00-24:00	**Conference Party** **(Ticket in Conference bag, information about transport and place)**

Tuesday, 11 July 2017

10:00-11:30	**Plenary Session 3 - Biodiversity in interdisciplinary ecological research** **Chair: Dr Jason Hoeksema**
10:00-10:45	Prof. Vladimir Titok, Alesya Kruchonok, Boris Anoshenko Methods for assessing environmental conditions for the creation of artificial coenopopulations of rare and endangered species of the flora of Belarus
10:45-11:30	Dr Emma Arapetyan Cryopreservation of Seed Herbaceous and Conifer Species in Ivam Franko Lviv National University
11:30-12:00	**Coffee Break**
12:00-13:30	**Plenary Session 4 - Biodiversity in interdisciplinary pharmaceutical research, Ethnobotany and ethnopharmacology** **Chair: Prof. Jordan Zjawiony**
12:00-12:45	Prof. Zinaida Klestova, Marina Marchenko, Alla Voronina Plants against animal virus diseases
12:45-13:30	Dr Milen Georgiev, Alina Ortan, Radu Claudiu Fierascu Plant biotechnology and metabolomics: towards accelerated lead finding
13:30-15:00	**Lunch**
15:00-16:00	**Session 5 - Biodiversity in interdisciplinary pharmaceutical and biological research** **Chair: Prof. Sergey Dromashko**
15:00-15:30	Dr Konrad Celiński, Hanna Kijak, Aneta Gmur, Aleksandra Wojnicka-Półtorak, Joanna Sokołowska, Ewa Chudzińska Karyological study of three closely related pines from the *Pinus mugo* complex
15:30-16:00	Mgr Aneta Sienkiewicz, Grażyna Łaska, Marcin Stocki, Andrzej Bajguz, Alicja Piotrowska-Niczyporuk, Jordan Zjawiony, Melissa Jacob, Shabana Khan Biologically active secondary metabolites from *Pulsatilla* species collected in Poland
16:00-16:30	**Coffee Break**
16:30-17:00	**General Discussion and Closing Ceremony**

Poster Sessions - Monday, 10 July 2017
Selected posters will be presented orally 10-15 minutes

No	Title and authors
Session 1 (S1) - Biodiversity in interdisciplinary molecular research	
S1-1	Endopolyploidy of *Dianthus arenarius* in Latvia and neighboring countries Nikole Krasņevska, Dace Grauda, Alesya Kruchonok, Isaak Rashal
S1-2	Phytotoxic activity of a fungus isolated from *Basella alba* (Malabar spinach) Elaine Smith, Brandon Clausen, Kumudini Meepagala, Stephen Duke
Session 2 (S2) - Biodiversity in interdisciplinary pharmaceutical research	
S2-1	L-Ascorbic acid and seleno-L-methionine modulate toxic effect of doxorubicin metal complexes as anticancer compounds Marzena Matejczyk, Grzegorz Świderski, Renata Świsłocka, Włodzimierz Lewandowski
S2-2	Phytochemical and pharmalogical studies on the leaves of a Nigerian Mistletoe, *Tapinanthus globiferus* V.K. Sharma, Amber Forsman, J.O. Fajemiroye, S.V.S. Radhakrishnan, Z. Ali, C. Thornton, C.A. Elusiyan, K.L. Willett, I.A. Khan, J.K. Zjawiony
Session 3 (S3) - Biodiversity in interdisciplinary biological research	
S3-1	Plant-microbe interactions in a heavy metals bioremediation context – associations of Fabaceae with rhizobia as an example Ewa Oleńska, Wanda Małek
S3-2	How might different research methods show metabolic diversity between C4 plants of the NADP-ME subtype? Paweł Rogowski, Wioleta Wasilewska, Elżbieta Romanowska
S3-3	The effects of diversified phosphorus nutrition on the growth of oat (*Avena sativa* L.) Ewa Żebrowska, Kamila Zujko, Anna Kuleszewicz, Iwona Ciereszko
Session 4 (S4) - Biodiversity in interdisciplinary ecological research	
S4-1	New data for reconstructing of the environment and climate in vicinity of the Wigry National Park at the end of the last interglacial Magdalena Fiłoc, Mirosława Kupryjanowicz, Magdalena Suchora
S4-2	Changes in natural renewal of trees and tree planting in secondary succession of the oak-hornbeam community on the basis of 27-year experimental study Grażyna Łaska

PS 1-1

Molecular methods in assessing genetic diversity

Sergey Dromashko, Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Belarus, S.Dromashko@igc.by

The lecture deals with molecular methods in assessing genetic diversity. Genomic registration of microorganisms, plants and animals is becoming increasingly important. The development of genotyping methods for living objects, valuable from the ecological, pharmaceutical and agricultural point of view, is actively underway.

The process of determining the genotype of an individual is carried out using a variety of molecular methods/markers. Some commonly used types of genetic markers are: RFLP (restriction fragment length polymorphism); SSLP (simple sequence length polymorphism); AFLP (amplified fragment length polymorphism); RAPD (random amplification of polymorphic DNA); VNTR (variable number tandem repeat); SSR microsatellite polymorphism (simple sequence repeat); SNP (single nucleotide polymorphism); STR (short tandem repeat); SFP (single feature polymorphism); DArT (diversity arrays technology); RAD markers (restriction site associated DNA markers).

There are examples of genomic identification and certification of agricultural and wild plants, animals, birds, and fish at the Institute of Genetics and Cytology (Minsk, Belarus).

DNA barcoding is a system for fast and accurate species identification that makes ecological system more accessible with the use of short DNA sequence instead of whole genome and it is used for eukaryotes. The short DNA sequence is generated from the standard region of a genome known as a marker. This marker is different for various species like CO1 cytochrome c oxidase 1 for animals, matK+rbcL (chloroplast maturase K and ribulose-1,5-bisphosphate carboxylate) for plants and internal transcribed spacer (ITS) for fungi.

This method underlies a number of international research projects. The Consortium for the Barcode of Life (CBOL) is an international initiative devoted to developing DNA barcoding as a global standard for the identification of biological species. CBOL has 200 member organizations from 50 countries. The International Barcode of Life project (iBOL) is the largest biodiversity genomics initiative ever undertaken. Hundreds of biodiversity scientists, specialists in genomics, technologists and ethicists from 25 nations are working together to construct a richly parameterized DNA barcode reference library that will be the foundation for a DNA-based identification system for all multi-cellular life. ECBOL – European Consortium for the Barcode of Life was established as part of the research infrastructure efforts of EDIT, European Distributed Institute of Taxonomy.

DNA barcoding has many applications in various fields like preserving natural resources, protecting endangered species, controlling agricultural pests, identifying disease vectors, monitoring water quality, the authentication of natural health products, and the identification of medicinal plants. The advantage of DNA barcoding also consists of identification of all life stages, fragments/products of organisms, stomach contents, etc.

PS 1-2

Therapeutic potential of psychoactive plants. The case of *Salvia divinorum*

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Psychoactive plants and fungi attracted humans' attention for millennia. They were used for centuries by shamans and priests in folkloric medicines and religious practices. In modern times they are used mostly as recreational drugs. However, many of them became addictive drugs of abuse. Psychoactive natural products from plants play an important role in the discovery and development of new drugs for the treatment of central nervous system (CNS) disorders. They have led to the discovery of a number of new receptor systems and their endogenous ligands. Development of useful biological probes of CNS receptors helped in understanding the causes of many CNS disorders. For many of them the mechanism of action in CNS and their toxicity to vital organs and systems of human body is still unknown.

Our research focuses on identification of plant metabolites responsible for CNS activity and the design of new ligands with high affinity to CNS receptors. A major part of our research is dedicated to the chemical modification of salvinorin A (SA), the active metabolite of *Salvia divinorum*. SA is an agonist with high affinity to the k-opioid receptor (KOR). Molecular modeling of the SA binding site led to the design and synthesis of 22-thiocyanatosalvinorin A. It represents the most potent KOR agonist to date, which covalently binds to the receptor. This finding facilitated a better understanding of the binding site and initiated further development of potential CNS drugs. SA is also known for its antinociceptive and antidiarrheal properties in animal models of abdominal pain and functional GI disorders. Because of its short duration of action and strong hallucinogenic effects, SA never advanced to human clinical trials. We have found that simple chemical modification of the structure of SA significantly alters the pharmacological profile from CNS to gastrointestinal (GI) activity. This led to the discovery of PR-38, an analog with dual affinity to KOR (9.6 nM) and MOR (52 nM).

Our studies showed that PR-38 significantly inhibits GI motility in physiological and pathophysiological conditions and produces a potent, analgesic effect in mice models of abdominal pain. Moreover, PR-38 showed a remarkable bioavailabilty being orally available and active after oral administration without producing any adverse effects in CNS. PR-38 has a great potential to become a powerful drug candidate for the treatment of abdominal pain in irritable bowel syndrome (IBS) and other GI disorders.

Support: This work was financially supported by National Institutes of Health grants R01DA017204 and the Inventus Plus program of the Polish Ministry of Science and Higher Education (0089/P01/2010/70).

PS 2-1

Plants against animal virus diseases

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Alla Voronina, The Institute of Pharmacology and Toxicology, Ukraine

Viral infections still occupy a leading position in infectious diseases. Despite significant advances in vaccine prophylactic can not completely eradicate of infectious diseases.

In recent years, a growing number of new infectious viral disease characterized by unusual symptoms or the appearance of new infectious diseases. In most of these infections are common to humans and animals, there are first in animals, birds, and humans. This so-called “emergent diseases”, including about 75% – a zoonosis.

The problem today is not only the lack of rapid diagnostic methods for identifying of new agents, but no practical means of dealing with them. Unfortunately, in the world developed a small number of commercial antiviral drugs.

Viruses which caused diseases worldwide spread and have possibility for mutations. More dangerous viruses attack animal and human organisms, causing economic losses. The aims of each countries are creating biosafety systems for preventing of viral infections. One of the ways in these measures developed are new prophylaxis and antiviral means obtained.

We investigated the possibility of using some plants and dosage forms in anti-viral therapy in sensitive model systems.

We used *in vitro* system:1. continuous cell culture of versenised swine embryonic kidney, 2. cell culture BHK-21. Test-model virus was the member of Coronaviridae family. Propilenglicol extracts of plant – *Camellia sinensis* var. *assamica.*

The chemical composition of *Camellia sinesis* is complex.

In the study we tested the system in vitro commercial propylene glycol extract of leaves of the plant *Camellia sinesis* from a dose of 40 ml/cm^3 for its antiviral activity.

In the chosen cell cultures transmissible gastroenteritis virus infectious activity was 7.5 ± 0.03 lg $TCID_{50}/cm^3$, which is consistent of test conditions.

Results of experimental researches of cytotoxic and antiviral action of preparations are presented. MTD is 10^{-5} μl/cm^3. Index of CC_{50} – 10^{-4} μl/cm^3.

Propylene glycol Camellia sinesis extract had a significant ability to reduce infectious properties of the virus in the system in vitro. After 96 hours it was found that the titer of infectious virus activity was 7.5 ± 0.06 $TCID_{50}/cm^3$, while a D holes with propylene glycol extract of *Camellia sinesis* – 3.2 ± 0.04 $TTSD_{50}/cm^3$. Thus, we first proved effective antiviral effect in respect of coronavirus propylene glycol extract of *Camellia sinesis.* In vitro experiments we obtained the data that was shown plant antiviral activity to 4.3 ± 0.04 lg $TCID_{50}/cm^3$.

The results confirm the perspective of tested means for use by clinical exams by viral infection caused by coronovirus.

PS 3-1

Plant biotechnology and metabolomics: towards accelerated lead finding

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Per definition metabolomics represents a comprehensive holistic approach, comprising of systematic identification and quantification of all metabolites in an organism, at given conditions. The comprehensive analysis of the chemical fingerprints left by metabolic processes started to play a crucial role in the personalized medicine [1].

Since the rise of the omics age several techniques for high throughput analyses of targeted metabolites have been developed. Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, because it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of ^{1}H NMR-spectrometry over the other analytical platforms is the possibility for quantification and thus the direct comparison of concentrations of all compounds present in the sample, as the signal intensity is only dependent on the molar concentration of the solutes [2–5].

Some case studies, from author's laboratory, on the application of NMR-based metabolomics concept in natural products research, plant biotechnology and lead finding [3–8] will be presented and discussed.

Acknowledgements

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PS 4-1

Cryopreservation of seed herbaceous and conifer species in Ivam Franko Lviv National University

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Seeds of species of Ukraine flora were stored in liquid nitrogen from one to six months. The eppendorfs and paper tubes used for seed storage. The biologically (physiological, biochemical, genetic, microbiological), physical, mathematical methods were held for seed viability characteristic after cryoconservation. The studied seeds included into the Red Book of Ukraine, endemic, under disappearance, protected wild or introduced flora can be storage at –196°C without the loss of their viability and germination viability.

PS 4-2

Sharing of ectomycorrhizal fungi between a coniferous tree (*Pinus sylvestris*) and an herbaceous perennial (*Pulsatilla patens*)

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The sharing of species of ectomycorrhizal fungi (EMF) between different co-occurring host plant species could allow the formation of common mycorrhizal networks, which can alter the mechanisms and outcomes of plant–plant interactions, potentially increasing or decreasing the intensity of plant–plant competition. On the other hand, host-specific EMF could allow their host plants to explore unique niches in the soil, facilitating coexistence between co-occurring plant species. Sharing of EMF species among woody species is thought to be common in many forests, but very few herbaceous plants form EMF, so they are assumed to be excluded from EMF networks on forests. Thus, discovery of sharing of EMF between woody and herbaceous plants in forests can change our overall view of species interactions in those ecosystems. We studied the EMF on roots of a common coniferous tree, *Pinus sylvestris*, and a co-occurring rare herbaceous perennial, *Pulsatilla patens*, in northeastern Poland. We examined roots from paired samples of the two plant species, visually classified EMF into morphotypes, studied tissue sections of mycorrhizal structures using compound microscopy, and used Sanger sequencing of fungal DNA and matching with public databases to classify EMF taxonomically. On both host plant species, we observed abundant EMF colonization, with root tips of *P. patens* exhibiting swollen appearance on colonized tips of short roots, as well as a variety of colors and textures of fungal mycelium covering and emanating from those swollen tips. Several EMF morphotypes appeared to commonly colonize both host plant species, including the globally common *Cenococcum geophilum*, which was confirmed on roots of both host plant species using Sanger sequencing. In addition, sectioning and microscopic examination of another EMF morphotype common on *P. patens* confirmed the presence of a mantle and Hartig net, indicating the likely presence of functional ectomycorrhizal structures. This observation of sharing of EMF between a coniferous tree and an understory herb indicates the potential for common mycorrhizal networks to alter interactions between these two species, and has implications for our general understanding of tree–herb interactions. In this particular case, it may also indicate a unique way in which the distribution and abundance of a rare herbaceous plant may be influenced by shared mutualisms with a common co-occurring woody plant.

PS 4-3

Mycoheterotrophy and mixotrophy: plants eating mycorrhizal fungi

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The evolution of land plants provided repeated emergences of mycoheterotrophy, where achlorophyllous plants exploit carbon from their mycorrhizal fungi. This condition, suggested to be an adaptation to forest environments where little light is available, recently made strong achievements due to two tools: fungal molecular barcoding allowed identification of the (often uncultivable) mycorrhizal fungi; natural isotopic abundances supported which fungal guild was giving carbon to the mycoheterotrophic plants. Temperate and Mediterranean species, mainly orchids and Montropoideae (Ericaceae), have specific basidiomycetous fungal partners that usually form mycorrhizae with surrounding trees (ectomycorrhizal fungi). By contrast, subtropical and tropical species often connect to arbuscular-mycorrhizal (AM) fungi or even to saprotrophic, wood- or litter-decaying basidiomycetes. Their specificity is often lower.

More recently, intermediate evolutionarily steps were found to exist, where the plant is still green and photosynthetic, but partly uses carbon from its fungal associates. This strategy, called mixotrophy, is now well described for green temperate orchids and Montropoideae species associated to ectomycorrhizal basidiomycetes or sometimes ascomycetes. Phylogenetic frameworks suggest that mixotrophy pre-dispose to evolution of mycoheterotrophy. In some mixotrophic *Cephalanthera* and *Epipactis* spp. (orchids), the rare survival of achlorophyllous plants (albinos) further supports their use of fungal carbon. More recently, our investigations of albinos‘ nutrition and fitness revealed why emergence of mycoheterotrophy is rare in evolution of mixotrophs: photosynthesis is not used for survival, which is supported by fungal carbon, but mainly for seed production. Thus, photosynthesis loss drastically reduces fitness and, as a result, mixotrophy is evolutionarily metastable.

PS 4-4

Methods for assessing environmental conditions for the creation of artificial coenopopulations of rare and endangered species of the flora of Belarus.

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Botanical gardens play a significant role in the implementation of programs for the conservation and reproduction of biodiversity of natural flora. The most effective ways to restore the wealth of the plant world is a set of methods *in situ* and *ex situ* conservation. To develop adequate models of translocation activities (reintroduction and repatriation), studies of the ecological space of the territory, promising for translocation activities, are necessary.

Objects and methods: artificial coenopopulations (CP) of rare plants *Lunaria rediviva* L.; *Hedera helix* L.; *Hepatica nobilis* Mill.; *Allium ursinum* L.; *Lilium martagon* L.; *Tulipa sylvestris* L.; *Matteuccia struthiopteris* (L.) Tod.; *Iris sibirica* L.; *Campanula latifolia* L.; *Aruncus dioicus* (Walter) Fernald; *Vicia pisiformis* L.; *Drymocallis rupestris* (L.) Sojk; *Digitalis grandiflora* Mill.; *Geranium phaeum* L.; *Astrantia major* L.; *Pulmonaria mollis* Wulfen ex Hornem.; *Clematis recta* L.; *Iris aphylla* L.; *Epipactis helleborine* (L.) Crantz. in *ex situ* condition in the Central Botanical Garden NAS of Belarus (CBG)

Using phytoindication methods (Optimum – point scale by Ellenberg, amplitude scale by Tsyganov and sum of the amplitudes of the plant community) it is possible to determine the specificity of the plant community of the selected habitat and the set of conditions that determine this specificity. Also, these methods can determine the ecological tolerance and the biocompatibility of the repatriated species, which will help to more accurately fit it into the ecological space of the future habitat.

Results: Ecological analysis was performed for localities of rare and endangered plant species in the CBG. Artificial cenopopulations of *Astrantia major* L., *Allium ursinum* L., *Lunaria rediviva* L., *Hedera helix* L. were analyzed in ecological space of the CBG by estimating ecological valence, tolerance and biocompatibility. Comparison between ecological amplitudes and optimums of species studied allows environmental stress points to be identified towards their ecomorphs.

The studied CP during their existence on the territory of the CBG showed a different degree of development of habitat conditions. The ecological field of the CBG has the same climatic conditions, but all habitats are insignificant, but differ in soil characteristics and degree of illumination. In a number of cases, these factors are crucial for the success of the development of artificial CP.

The most sensitive CP to the complex of factors is *A. major*. The use of Tsyganov's amplitude scales and Ellenberg's point scales allow objectively to assess and justify the eco-morphology of the plant for rare and protected species in *ex situ* conditions, and a more accurate selection of a new habitat for stenovalent species.

SL-1

Karyological study of three closely related pines from the *Pinus mugo* complex

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Pinus mugo complex comprises 16 species, 91 varieties, and 19 other forms, commonly occuring in the main European mountains. This large and polymorphic complex of closely related pines constitutes a big challenge for botanists and taxonomists due to highly variable phenotype characteristics within a particular taxon, ongoing hybridization in sympatric populations, lack of distinct morphological taxa-specific determinants as well as absence of genetic differentiation among them. Moreover, cytogenetic data on pines from this complex are very scarce.

The aim of this study was to describe karyotypes of three closely related pines from the *Pinus mugo* complex, i.e., *Pinus mugo* (Turra), *Pinus uliginosa* (Neumann) and *Pinus* ×*rheatica* (Brügger) using C-banding methods in order to characterize phylogenetic relationships between these taxa. This is the first report on morphometric data for *Pinus uliginosa* and *Pinus* ×*rheatica* karyotypes.

Our results confirmed that basic chromosome number is the same amongst the taxa under study, $2n = 2x = 24$ and karyotypes have a similar morphology. On the other hand, the number of secondary constrictions (SCs), NORs and nucleoli differed between *Pinus mugo*, *Pinus uliginosa* and *Pinus* ×*rheatica*. For each of these pines some taxa-specific determinant was identified. Based on here obtained results, *Pinus uliginosa* and *Pinus* ×*rheatica* represent two different taxa, and therefore should be not used as synonymuous names.

SL-2

Biologically active secondary metabolites from *Pulsatilla* species collected in Poland

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Natural products obtained from *Pulsatilla* species have been used for centuries in traditional folk medicine for the treatment of many diseases and ailments. Phytochemical studies have confirmed the high content of a variety of secondary metabolites from *Pulsatilla* species (*Pulsatilla patens* subsp. *multifida* (Pritz.) Zämelis, *Pulsatilla chinensis* (Bunge) Regel, *Pulsatilla koreana* Nakai, *Pulsatilla cernua* (Thunb.) Bercht. et Opiz., *Pulsatilla montana* subsp. *balcana* (Velen.) Zamels & Paegle, *Pulsatilla halleri* subsp. *rhodopaea* (Stoj. et Stef.) K. Krause, *Pulsatilla slaviankae* (Zimmer.) Jordanov & Kožuharov, *Pulsatilla nigricans* Storck). These compounds have shown a variety of biological properties including antitumor, antimicrobial, antifungal, anti-allergic, neuroactive, neuroprotective, cognition-enhancing, immunomodulatory, anti-oxidant and cytotoxic activities.

The present study was aimed on the identification of biologically active secondary metabolites from the rare plant species *Pulsatilla patens* subsp. *patens* and the cultivated *Pulsatilla vulgaris* subsp. *vulgaris*. Chromatographic analysis using GC-MS of the silylated methanolic extracts from the leaves and roots of this species revealed the presence of carboxylic acids, such as benzoic, caffeic, malic and succinic acids. HPLC analysis of the methanolic extract of the root of *P. patens* indicated the presence of saponins, including saponin D. The extracts of *Pulsatilla* species were evaluated for their antifungal, antimicrobial, antileishmanial and antimalarial activities, and cytotoxicity to mammalian cell lines. Both *P. patens* and *P. vulgaris* were active against the fungus *Candida glabrata* with IC50 values of 9.37 μg/mL and 11 μg/mL, respectively. Inhibitory concentration values (IC50) for cytotoxicity were in the range of 32–38 μg/mL for *P. patens* and 35–57 μg/mL for *P. vulgaris* for each cell line indicating a general cytotoxic activity throughout the panel of cancer and non-cancer cells.

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S 1-1

Endopolyploidy of *Dianthus arenarius* in Latvia and neighbouring countries

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Dianthus arenarius is endangered perennial plant species, included in Annex II of the European Council Habitats Directive 92/43/EEC as well as in the Latvian endangered plant list. In Latvia, Lithuania and Belarus *D. arenarius* create a complex of several perennial subspecies. Genetic diversity of populations is in many cases crucial for sustainable existence and also served as criteria to choose appropriate protection measures for rare and endangered species. Genetic diversity of *D. arenarius* has not been studied in Europe until now. Changes on chromosome level, including endopolyploidy, can reflect adaptation under pressure of different stress conditions and ongoing processes in population of *D. arenarius* through territory of Latvia and neighbouring countries. The goal of this study was to compare endopolyploidy level of populations of *D. arenarius* in target territory. Determination of DNA content (C-value) level of individual cells in leaves of mature plants was performed by the BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function. Samples for flow cytometry were prepared with the DNA staining kit (Sysmex Partec, PI Absolute, GmbH, Germany), cells nuclei were stained with 10 µL propidium iodide. Cell counting events were triggered by forward-scattered signal. The excitation of the cell fluorescence was made by 488 nm Coherent Sapphire Solid State (blue) laser. Flow cytometry analysis of DNA content in *D. arenarius* leafs from different localities revealed presence of several relative fluorescence peaks from 2C up to 18C. Percentage of high C-value DNA nucleus among all samples was very low, represented by only some specimens.

S 1-2

Phytotoxic activity of a fungus isolated from *Basella alba* (Malabar spinach)

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Novel pesticides are being increasingly developed from secondary metabolites isolated from natural sources. Natural products also offer diverse classes of molecules with various biological activities. This rationale has been adopted in the search for phytotoxic metabolites from plant pathogenic fungi. Plant pathogenic fungi have demonstrated herbicidal, antifungal, antibacterial, and insecticide activities. From an infected leaf of *Basella alba* (Malabar spincach), a colony of three fungi was cultured in potato dextrose agar (PDA) plates. Single colonies from this initial colony of fungi were isolated and cultured in PDA plates and identified via molecular techniques. One of these fungi was identified as *Phoma pomorum* and was grown on Czapek Dox (CDB) broth and Potato Dextrose broth (PDB) culture media for 7 days under continuous light at 24°C. The culture filtrates were extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and evaporated to obtain brown viscous liquids (850 mg from PDB and 920 mg from CDB). The ethyl acetate extracts were phytotoxic in a seed germination bioassay. The Potato Dextrose broth extract was found to be more active than that of the Czapek Dox. The major phytotoxic compound was isolated and identified as 8-hydroxy-3-(2-hydroxy-4-oxopentyl)-6-methoxy-isochromen-1-one **(1)** by spectroscopic techniques. **(1)** was found to be phytotoxic against both monocots and dicots. Isolation, structure elucidation, and biological activities will be discussed.

S 2-1

L-Ascorbic acid and seleno-L-methionine modulate toxic effects of doxorubicin metal complexes as anticancer compounds

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The most important problems of anti-cancer therapy include the toxicity of the drugs applied to healthy cells and the multi-drug resistance to chemotherapeutics [1]. One of the most commonly used anticancer drugs is doxorubicin, used to treat some leukemia and non-Hodgkin's lymphoma, as well as bladder, breast, stomach, lung, ovarian, thyroid, multiple myeloma and other cancers [2]. Doxorubicin rapidly penetrates into cells and accumulates mainly in chromatin of the cell nucleus, arresting mitosis and causing chromosomal aberrations [3]. Current research conducted in collaboration with the Medical University of Bialystok and MD Anderson Cancer Center (Houston, USA) is part of a broader theme, entitled “Study on improving the selectivity and activity of selected drugs and natural compounds with anticancer properties due to metal complexation”. Preliminary studies show that complexing with ligand metal with proven cytostatic activity results in changes in their molecular structure and distribution of electron charge, resulting in a change in their biological activity (including antitumor activity) [4]. Thus, there is a chance of receiving derivatives with decreased activity for the body and increased therapeutic selectivity.

In this study we examined the effect of simultaneous administration of seleno-L-methionine and L-ascorbic acid with known anticancer drug – doxorubicin and its new complexes with Mn, Mg, Fe, Co and Ni in prokaryotic model – *Escherichia coli* RFM443 with plasmid transcriptional fusion of *recA*promoter and *luxCDABE* as a reporter gene. Ascorbic acid and seleno-L-methionine as anticancer compounds modulate the activity of doxorubicin and its metal complexes. Cytotoxic potency of tested chemicals were calculated on the basis on the dose that confers inhibition percentage such as 20% for each concentrations of analysed chemicals. Genotoxic properties were calculated on the basis of the fold increase (FI) of SFI values normalized with control. Obtained results showed that doxorubicin metal complexes influenced biological activity of chemicals in comparison to DOX. L-selenomethionine and L-ascorbic acid modulated the cyto- and genotoxic activity of DOX and its metal complexes. Moreover, we noticed different sensitivity of *recA::luxCDABE* for 3 h and 24 h culture of bacteria strain. It suggests, that the potency of reactivity of genetic construct – *recA::luxCDABE* in *E. coli* depends on the age of bacterial culture.

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S 2-2

Phytochemical and pharmalogical studies on the leaves of a Nigerian mistletoe, *Tapinanthus globiferus*

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Nigerian mistletoe, *Tapinanthus globiferus* (Loranthaceae) known in Yoruba language as Afomo is a parasitic shrub growing on deciduous trees such as .guava, kolanut, rubber, cocoa and some citrus trees. Mistletoes find myriad of ethnobotanical uses and are practiced in different cultures to treat various ailments. Traditionally *T. globiferus* is used to alleviate several diseased conditions such as, hypertension, ulcers, **epilepsy**, vision enhancer and as a labor muscle relaxant. Till date there are no published reports on the phytochemistry and pharmacological investigations on the secondary metabolites of *T. globiferus.* The present work aims to isolate, characterize and to carry out bioassay of the compounds obtained from *T. globiferus* from Guava tree (*Psidium guajava*). To accomplish the central goal of this work, methanolic extract of the leaves of the shrub was fractionated using hexanes, chloroform and n-butanol. The n-butanol fraction was further fractionated through normal phase column chromatographyto yield different fractions compounds using ethyl acetate/chloroform/methanol/water (5:4:1:0.1) as solvent system.

Detailed phytochemical as well as pharmacological investigations on different fractions, sub-fractions and isolated secondary metabolites from *T. globiferus* will be presented.

S 3-1

Plant/microbe interactions in a heavy metals bioremediation context; associations of Fabaceae with rhizobia as an example

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Heavy metals are not biodegradable elements that persist for years in the environment, hiding their toxic character which may be expressed to all living organisms. So far, many physic-chemical technics of the remediation the heavy metal contaminated areas have been developed, but in spite of many advantages they turn out to possess serious adverse effects i.e. significant interference with soil microbiota or alterations of soil physic-chemical properties. The green alternative for the clean environment is the usage of living organisms, like plants to remediate the polluted areas in a phytoremediation process. Plants possess many benefits as a tool for the remediation of contaminated fields, because they developed various adaptation traits for heavy metal oxidative stress like: metal accumulation in shoots or exclusion in roots. Enhanced accumulation as well as exclusion of toxic metals in plants may be performed with the usage of microorganisms which inhabit the rhizosphere. The aim of study is to characterize the role of microorganisms in the enhancement of phytoremediation, with a particular attention to leguminous symbiotic associations with rhizobia, and the mechanisms of heavy metal resistance in bacteria.

S 3-2

How might different research methods show metabolic diversity between C_4 plants of the NADP-ME subtype?

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Biochemical photosynthetic pathways are highly conserved amongst the plant species. Most of green plants are the C_3 type plants. An additional biochemical pathway, that allows for efficient concentration of CO_2 in leaves, exists in the C_4 type plants which represent some of the agriculturally most productive crops. In the C4 plants chloroplasts in mesophyll (M) cells contain grana, whereas bundle sheath (BS) chloroplasts exhibit various degrees of granal development depending on the plant species, age and growth conditions. In maize of the NADP-ME subtype, M chloroplasts are granal at all stages of their development, whereas BS counterparts are fully agranal. Other NADP-ME species, such as *Echinochloa crus-galli* and *Digitaria sanguinalis* exhibit variation in abundance of grana in BS chloroplasts. Little is known about the influence of light on the photochemical response of these species. It also remains largely unknown whether different mechanisms are required for adjustment of photosynthesis upon the induced changes in light quality and intensity. NADP-ME chloroplasts may be considered as an appropriate model to study the relationship between structure and function of thylakoid membranes components, affected by various environmental factors. Most data come from the experiments performed on *Z. mays*, and it is unknown whether other NADP-ME species show the same response to light. The aim of this study was to investigate whether the chloroplasts of *Zea mays*, *Digitaria sanguinalis*, and *E. crus-galli*, all NADP-ME-type C4 plants, possess the same mechanisms capable of inducing similar responses to light conditions, thereby maintaining high photosynthetic efficiency. The question refers also to the methods used in this experiments. We measured phosphorylation of PSII proteins, fluorescence in room temperature and in 77K, the ATP/ADP ratio, the activity of PSI and PSII complexes, CO_2 exchange, BN/SDS electrophoresis, as well as carotenoid contents and the activity of the enzymes superoxide dismutase and ascorbate peroxidase, the content of malondialdehyde and H_2O_2. Our interest was to understand why these species, grown under identical conditions, differ in their responses to light. For the first time, we report that three selected NADP-ME species cultivated under the same conditions differ in their photosynthetic and respiratory responses to light treatment. The most resistant to photoinhibition was the weed plant *E. crus-galli* which developed a strategy, allowing to maintain a high photosynthetic activity while protecting the thylakoid proteins from degradation mainly by PSII protein phosphorylation.

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S 3-3

The effects of diversified phosphorus nutrition on the growth of oat (*Avena sativa* L.)

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Oat plants (*Avena sativa* L. cv. Arab) were grown for 1–3 weeks on nutrient media with inorganic phosphate (+P, control), reduced Pi content (0.1 P), phytic acid (PA), as organic P source, and without phosphorus (–P) in standard conditions or in a split-root culture system. Pi deficiency in nutrient medium decreased shoot growth but increased root elongation, whereas 0.1 P and PA plants showed similar growth to control. The growth on –P medium significantly decreased phosphorus content in tissues, but only a slight Pi decrease was observed in plants grown on 0.1 P and PA media, or in various split-root system conditions. –P plants showed increased activity of acid phosphatases (APase) in root exudates when compared to +P, 0.1 P and PA plant samples. APase distribution differed between root tissues – the highest activity was found in the vascular tissues and epidermis in root cross sections. Intracellular APase activity in –P plants was less affected by Pi deficiency, however, a higher enzyme activity was observed in shoot extracts than in roots. APase activity in 0.1P and PA plants was similar to control. Protein extracts from tissues were run on native PAGE to determine which isoform(s) may be affected by Pi deficiency. Generally, lower Pi (0.1 P) was not stressful to oat plants for up to 3 weeks. Oat plants grew equally well both on medium with Pi and where Pi was replaced with phytate. The studied oat plants activated not only extracellular APases, but also to a lesser extand – intracellular enzymes (rather *via* non-local signals) to acquire Pi from external/internal sources under Pi deficiency.

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S 4-1

New data for reconstructing of the environment and climate in vicinity of the Wigry National Park at the end of the last interglacial

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The Wigry National Park is located in the northeastern part of Poland, within the transition zone between oceanic and continental climate. This is very important, because some species occur here at their ecological tolerance limit, so their abundance, regeneration and pollen production are constrained by climate. Therefore, the main goal of the research is to determine how the global changes of climate that occurred during the Late Glacial and Holocene influenced the succession of vegetation in the study region.

Palaeoecological research were conducted on the Lake Suchar Wielki sediments in the Wigry National Park, with the pollen analysis as the primary research method. The method is based on the qualitative and quantitative analysis of the composition of sporomorphs (pollen and spores) preserved in the fossils state. As a supplementary method providing information on climate change derived from the reaction of aquatic communities, the subfossil Cladocera analysis was used.

At the current stage of research, pollen profile allowed for determining two periods of environmental changes during Late Glacial – the Allerød interstadial and the Younger Dryas stadial. These periods abounded in climate phenomena that considerably influenced vegetation. On the one hand, the reconstructed development of the interstadial terrestrial environment revealed the dominance of the boreal pine and birch forests. On the other hand, the Younger Dryas – the last cold climate fluctuation of the Late Glacial – showed a significant increase in the acreage of open communities.

S 4-2

Changes in natural renewal of trees and tree planting in secondary succession of the oak-hornbeam community on the basis of 27-year experimental study

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Changes in the species composition and structure of natural renewal of trees and tree planting in the process of anthropogenically forced secondary succession in the secondary community of oak-hornbeam forest from the *Tilio-Carpinetum* circle have been analyzed. Development of regenerations and forestations was studied on a potential habitat of typical oak-hornbeam forest after felling the pine stand in 1989 growing previously in the area of study. The study was performed for 27 years (1989–2016) in representative patches of 200 m^2 each. In 1989 these patches were reforested with deciduous species aged from 2 to 7 years. The spontaneous processes of growth and development of the young generation of trees and bushes and development of artificial planting were analyzed in the years 1994, 1996, 2006 and 2016, taking into account the number of trees and bushes, their sizes, changes in spatial structure, species composition and developmental phase structure.

In the secondary community of *Tilio-Carpinetum*, over the years 1994–2016, the total number of trees growing from seeds and planted increased by 28.4% (from 234 to 327), by 36.2% increased the number of trees growing from seeds (from 203 to 318) and by 14.8% increased the number of deciduous trees and bushes (from 144 to 169). In the whole area studied, the number of trees growing from seeds increased over 3 times. In the period of 1994–2016, a significant reduction in the number of light-seed tree species (from 9.4% to 1.8%) and a small increase in the number of coniferous species (from 44% to 48%) was observed. The number of oak-hornbeam species varied from 43% to 38%. The trees from natural regeneration and planting play an important role of protection of the habitat and initiation of transformations related to the changes in the composition of falling litter. On the basis of the 27-year observation it can be concluded that the floristic composition of trees and bushes has been developing towards the species composition of the natural oak-hornbeam forest community.

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