balkan.bio
2025

6TH BALKAN CONFERENCE ON BIOSCIENCES

BOOK OF ABSTRACTS AND PROGRAM

ISBN 978-619-281-077-1

30-31 OCT 2025
PLOVDIV, BULGARIA

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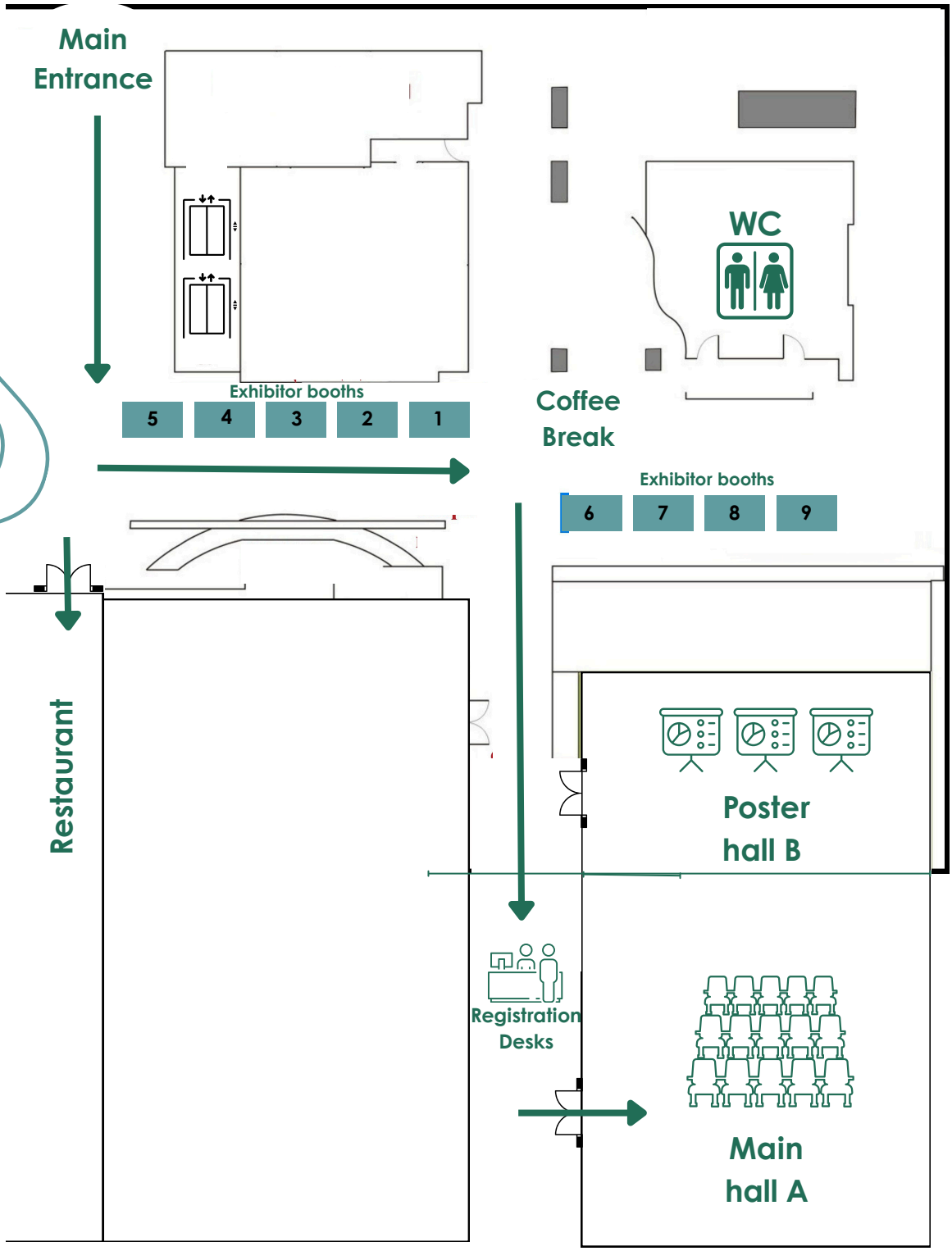
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Participants requested Social Dinner have SD sign

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PROGRAM

Day 1 - Thursday, 30th October

8:00 - 9:00 Registration

9:00 - 9:15 Opening

Session: Molecular Biology and Bioinformatics

Moderator: Prof. Galina Yahubyan

9:15 - 9:45 Keynote talk: Cancer epitranscriptomics: on the role of RNA methylation in tumour biology and therapeutics
Elena Martens, Erasmus University Medical Center, The Netherlands

9:45 - 10.15 Keynote talk: Mechanisms of Rare Diseases
Stefan Dimitrov, Institute of Molecular Biology, BAS, Bulgaria

10.15 - 11.15 Coffee Break and Poster Session 1 (Posters 1 – 43):
Grab a coffee and explore our exhibition booths and posters!

11.15 - 11.45 Keynote talk: Genomic approaches for sustainable and safe potato production: resistance to late blight and reduction of acrylamide formation
Yordan Muhovski, Walloon Agricultural Research Centre, Belgium

11.45 - 12.00 Peptide aptamers for blocking SARS-CoV2 proteins ORF6 and NSP13.
Elena Krachmarova, Institute of Molecular Biology, BAS, Bulgaria

12.00 - 12.15 A deep learning framework for binding free energy prediction.

Nevena Ilieva, Institute of Information and Communication Technologies, BAS, Bulgaria

12.15 - 12.30 Comprehensive In Silico Evaluation of BMPR2 SNPs Associated with Pulmonary Arterial Hypertension
Melih Gunay, Çanakkale Onsekiz Mart University, Turkey

12.30 - 12.45 Drought protective metabolites in *Capsicum annuum* and their biosynthetic control
Michael Wittenberg, Center of Plant Systems Biology and Biotechnology, Bulgaria

12.45 - 13.00 The impact of the glucagon-like peptide-1 receptor agonist semaglutide on endometrial physiology, embryo development and implantation
Apostol Apostolov, Celvia CC, Estonia

13.00 - 14.30 Group Photo and Lunch

Session: Microbiology and Biotechnology

Moderator: Prof. Velizar Gochev

14.30 - 15.00 Keynote talk: Ultrastructural morphology of the blood microbiota and possible mechanisms of their proliferation
Stefan Panaiotov, National Center of Infectious and Parasitic Diseases, Bulgaria

15.00 - 15.30 Keynote talk: Bioprocessing of plant in vitro systems: Speculation, reality and prospects
Atanas Pavlov, University of Food Technologies, Bulgaria

15.30 - 15.45 Characterization of antibiotic-resistant enterococci and beta-lactam resistance mechanisms in enterobacteria from groundwater in Ialomița County
Ilda Barbu, University of Bucharest, Romania

15.45 - 16.00 Postbiotics as an Alternative to Antibiotic Therapy: Potential for Biofilm Control in Staphylococcus aureus
Ivan Iliev, University of Plovdiv "Paisii Hilendarski", Bulgaria

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Valentina Ganeva, Sofia University "St. Kliment Ohridski", Bulgaria

17.15 - 17.30 Encapsulation of Beneficial Microorganisms in Polymeric Formulations for Enhanced Agricultural Biocontrol
Olya Stoilova, Institute of Polymers, BAS, Bulgaria

17.30 - 17.45 Innovative electrospun biohybrid polymer materials for sustainable agricultural applications
Mariya Spasova, Institute of Polymers, BAS, Bulgaria

17.45 - 18.00 Department “Biochemistry and microbiology” – 50 years of inspiration, traditions and future trends.
Velizar Gochev, University of Plovdiv "Paisii Hilendarski", Bulgaria

18.00 - 18.15 AI-Assisted Computational Laccase Engineering for Sustainable Biocatalysis: A Lab-Free Design Approach
Hyusein Yemendzhiev, Burgas State University "Prof. Asen Zlatarov", Bulgaria

18.15 - 18.30 Investigation of Microbiome Functionality with Phenotype Microarray system ODIN
Tania Rasheva, Diachim EOOD, Bulgaria

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Day 2 – Friday, 31st October

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Session: Biodiversity and Conservation

Moderator: Assoc. Prof. Gana Gecheva

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Nesho Chipev, Institute of Biodiversity and Ecosystem Research, BAS, Bulgaria

9.30 - 10.00 Keynote talk: Changing Ichthyofauna: Ecological, Anthropogenic, and Taxonomic Dimensions
László Antal, University of Debrecen, Hungary

10.00 - 10:15 Uncovering Hidden Diversity: Fieldwork and Taxonomic Discoveries of Fungus Gnats (Diptera: Mycetophilidae) in Northern Vietnam
Svetlozara Kazandzhieva, National Museum of Natural History, BAS, Bulgaria

10.15 - 11.15 Coffee Break and Poster Session 3 (Posters 85 – 128)

11.15 - 11.30 Bats and their bacterial and protozoan pathogens with zoonotic potential
Jan Zukal, Institute of Vertebrate Biology CAS, Czech Republic

11.30 - 11.45 Distribution and biological features of the species *Carcinus aestuarii* off the Bulgarian coast of the Black Sea
Sonya Uzunova, Institute of Fish Resources - Varna, Bulgaria

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Bartosz Bojarski, Bydgoszcz University of Science and Technology, Poland

12.00 - 12.30 Keynote talk: Role of fish as bioindicators of aquatic pollution.
Krisztián Nyeste, University of Debrecen, Hungary

12.30 - 13.45 Lunch

13.45 - 14.15 Keynote talk: Effects of adventive and invasive fish species on biodiversity and conservation
Dóra Somogyi, University of Debrecen, Hungary

14.15 - 14.30 Stress responses of Black Sea littoral species to multiple pollutants with special reference to microplastics
Albena Alexandrova, Institute of Neurobiology, BAS, Bulgaria

14.30 - 14.45 Urban and peri-urban forests as important wildlife habitats in urban residential canters
Hajri Haska, University Metropolitan Tirana, Albania

14.45 - 15.00 Widespread Exposure and Pathogenic Diversity of Lyssaviruses and Filoviruses in Eurasian Cave-Dwelling Bat Populations: Implications for Zoonotic Risk
Heliana Dundarova, Institute of Biodiversity and Ecosystem Research, BAS, Bulgaria

15.00 - 15.15 Drones revolutionize the monitoring of notoriously difficult to study raptors: breeding performance of Golden Eagles in Bulgaria
Ivaylo Angelov, National Museum of Natural History, BAS, Bulgaria

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Moderator: Prof. Evgeniya N. Ivanova

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Tibor Szekeres, Novogene Europe, Hungary

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Tzvetana Kerelska, Medical University - Sofia, Bulgaria

17.00 - 17.15 Genetic Diversity and Chemotypic Variation in Bulgarian Lavender (*Lavandula angustifolia* Mill.) Varieties and Accessions Revealed by SCoT Markers and Essential Oil Profiles
Mariya Zhelyazkova, Trakia University, Bulgaria

17.15 - 17.30 Integrated Genome-Wide Association and QTL Mapping Elucidate the Genetic Basis of Gray Mold Resistance in Tomato
Gabriele Adornato, Center of Plant Systems Biology and Biotechnology, Bulgaria

17.30 - 17.45 Impact of Diabetes mellitus induced in early life on rat spermatogenesis and fertility
Nina Atanassova, Institute of Experimental Morphology, Pathology and Anthropology, BAS, Bulgaria

17.45 - 18.00 SNP Discovery in Common Bean (*Phaseolus vulgaris* L.) Mutants Using High-Throughput Genotyping
Krasimir Mateev, Martisa Vegetable Crops Research Institute, Bulgaria

18.00 - 18.15 Resting state of the cells is a reflection of the activity of electrogenic transporters

Alexander Dimitrov, Institute of Biophysics and Biomedical Engineering, BAS, Bulgaria

18.15 - 18.30 Studying Biomechanical Properties and Interactions in Living and Phantom Tissue by Light Sheet Optical Microscopy (LSOM)
Stoyan Yordanov, Institute of Information and Communication Technologies, BAS, Bulgaria

18.30 - 18.45 Poster Award and Closing

ABSTRACTS

ORAL PRESENTATIONS

O1 Cancer epitranscriptomics: on the role of RNA methylation in tumour biology and therapeutics

Elena S. Martens-Uzunova[1]

[1]*Erasmus MC Cancer Institute, Department of Urology, Laboratory of Experimental Urology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands*

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Keywords: RNA, epitranscriptomics, ribosomes, methylation, cancer

Epitranscriptomics emerged rapidly as a new research field focused on the versatile chemical modifications of RNA and their regulatory roles. Over 150 known RNA modifications are currently known, and many have emerged as dynamic players in RNA biology, influencing transcript stability, splicing, translation, and turnover, thereby shaping the cellular phenotype in health and disease. Despite their early discovery, the functional implications of RNA methylation remained elusive for decades due to technical limitations in detection and mapping. Only with the advent of high-throughput sequencing technologies and specialized profiling methods it became possible to explore the epitranscriptomic landscape with molecular precision. These advances have revealed that aberrant RNA methylation patterns are not merely byproducts of disease but active contributors to tumorigenesis, influencing cancer cell survival, proliferation, and heterogeneity. This lecture will focus on RNA methylations - the most common type of RNA modifications in eukaryotic systems and how their dysregulation contributes to oncogenic processes, highlighting how the expanding field of epitranscriptomics is opening new frontiers in diagnosis and therapy.

O2 Mechanisms of rare diseases (from genome structural alterations to functional consequences)

Stefan Dimitrov[1,2]

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[2]*Institute of Advanced Biosciences, Grenoble, France*

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Keywords: rare diseases; Rett syndrome; Rahman syndrome; MeCP2; linker histone H1

Rare diseases are said to be rare when they affect one person in 2,000, i.e. more than 3 million French people and at least 30 million Europeans. There are 7-8,000 rare diseases identified to date and the vast majority is from unknown origin. More than 90% of rare diseases are without treatment. Rare diseases are a major threat for human health and understanding of the molecular etiology of rare diseases is of primary need.

Here, I will initially present shortly some of our data on the mechanism of Rett Syndrome (RS), a very severe neuro-developmental rare disease. The genetic cause of the disease was defined as loss of function of *methyl CpG binding protein 2* (MeCP2). Nevertheless, the precise mechanism of how loss of function of this protein causes this devastating disease remains obscure. In contrast to the existing dogma claiming that MeCP2 binds to CpG containing sequences, we observed that MeCP2 specifically recognizes and binds both *in vitro* and *in vivo* hydroxymethylated CA repeats. More over, we demonstrated a new function of MeCP2 as a long-range chromosome organiser, especially in chromatin domains associated with the nuclear lamina (LAD) which is the area at the inner face of the nuclear membrane. Therefore, MeCP2, previously described as transcriptional repressor, also organizes 3D chromatin architecture, and Rett Syndrome is, indeed, an epigenetic disease.

Next, I will describe a novel work flow for deciphering the molecular etiology of rare diseases and the application of this workflow on analyzing the Rahman Syndrome (RMNS) molecular origin. RMNS is a recently described developmental disorder caused by frameshift mutations in linker histone H1.4, that produce a truncated C-terminal domain (CTD) with reduced positive charge. By using Small Angle X-ray scattering (SAXS), Analytical Untracentrifugation (UAC) and cryo-electron microscopy (cryo-EM) we found that the mutation induces nucleosome arrays to adopt a more extended, flexible conformation exhibiting phase separation behavior similar to those lacking H1.4. Molecular dynamics simulations supported by FRET analysis indicate that the mutated CTD recognizes a shorter length of linker DNA, resulting in a more open nucleosome conformation. Correspondingly, the mutation substantially increases H1.4 mobility within cell nuclei. The combined data suggest that RS mutations alter gene expression during development by promoting a relaxed chromatin state. This suggestion was further supported by a series of experiments at genome-wide level by using a cohort of "omics" approaches including ATAC-seq, RNA-seq and ChIP.

O3 Genomic approaches for sustainable and safe potato production: resistance to late blight and reduction of acrylamide formation

Yordan Muhovski[1]

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Keywords: potato, late blight, *R* genes, genome editing, cold-induced sweetening

Potato (*Solanum tuberosum* L.) is a globally important food crop, yet it remains vulnerable to both late blight, caused by *Phytophthora infestans*, and postharvest quality losses associated with cold-induced sweetening (CIS). We present two parallel strategies that leverage modern genomics and genome editing to enhance potato resilience-through improved field resistance and reduced acrylamide formation during processing.

To assess late blight resistance, we employed Resistance gene enrichment sequencing (*RenSeq*) to profile *NB-LRR*-type resistance (*R*) genes across a panel of modern cultivated potato genotypes. *RenSeq* enabled high-resolution identification and comparative analysis of R gene complements, revealing both conserved and unique R gene repertoires within elite breeding lines. Candidate *R* genes were further analyzed for domain completeness, sequence variation, and potential resistance functionality. This approach facilitates the integration of naturally occurring *R* genes into breeding programs.

The second component focuses on mitigation of CIS by targeting vacuolar invertase (*StVInv1*), a key enzyme responsible for the hydrolysis of sucrose into glucose and fructose during cold storage. Elevated levels of reducing sugars predispose tubers to high acrylamide formation upon frying. Using CRISPR/Cas9-mediated editing of *StVInv1*, we have generated edited lines exhibiting significantly lower reducing sugars accumulation after cold storage, without detrimental effects on yield or tuber morphology. Molecular analyses confirmed frame-shift mutations and absence of off-target effects. Biochemical assays of stored tubers and processed chips demonstrated reduced acrylamide content, supporting the efficacy of *StVInv1* as a precision target.

Together, these approaches illustrate how functional genomics and genome editing can be applied to address both disease resistance and food safety traits in potato. Integrating these strategies into breeding pipelines offers a path toward more resilient cultivars, with benefits spanning from field performance to consumer health.

O4 Peptide aptamers for blocking SARS-CoV2 proteins ORF6 and NSP13

Elena Krachmarova[1], Elena Lilkova[2], Peicho Petkov[3], Kristina Malinova[1], Shina Pashova[4], Rossitsa Hristova[1], Miroslav Rangelov[5], Nadezhda Todorova[6], Anastas Gospodinov[1], Anastas Pashov[7], Nevena Ilieva[2], Leandar Litov[3], Genoveva Nacheva[1]

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Keywords: SARS-CoV-2 ORF6, NSP13 and NSP8, IFNγ, peptide aptamers, cyclotides

Nearly six years after its late-2019 emergence, SARS-CoV-2 remains a global health threat despite vaccines and antivirals. New variants evade immunity, underscoring the need to target essential viral proteins. Two promising targets are ORF6, which suppresses host defences, and the helicase NSP13, which drives replication and antagonizes interferon signalling.

ORF6 is the SARS-CoV-2's most toxic factor. We showed that it embeds in cytoplasmic membranes and, via its flexible C-terminus, sequesters the mRNA export factor RAE1, blocking nucleocytoplasmic transport, impairing S-phase progression, and causing RNA–DNA hybrid accumulation that compromises genomic stability. In silico modelling found that human interferon-γ (hIFNγ) binds ORF6's tail, preventing RAE1 interaction. In ORF6-overexpressing cells, hIFNγ relocates RAE1 to the nucleus, restores mRNA export, and reduces hybrids, highlighting hIFNγ's C-terminus as a potent ORF6 inhibitor.

NSP13 fuels viral RNA synthesis and blocks type I interferon by binding TBK1 and hindering IFNβ transcription. Within the Replication–Transcription Complex, NSP13 partners with NSP12, NSP7, and NSP8. Disrupting its interface with NSP8's N-terminal α-helix offered inhibition. Our molecular docking and dynamics simulations defined this interface and guided the design of an NSP8-derived peptidomimetic that binds NSP13. In NSP13-overexpressing cells, co-expression of this fragment partially restores interferon signalling, indicating NSP13 sequestration.

We are developing aptamers that mimic these inhibitors and selectively inhibit ORF6 and NSP13. For efficient delivery and stability, we graft aptamers onto cyclotide scaffolds. Cyclotides provide a compact, protease-resistant framework capable of traversing membranes, making them ideal carriers for therapeutic aptamers.

Acknowledgments: This work is supported by the Bulgarian Science Fund under Grant KP-06-N 71/3.

O5 A deep learning framework for binding free energy prediction from the protein-complex structure

Nevena Ilieva[1], Peicho Petkov[2], Veselina Ivanova[2], Elena Lilkova[1], Leandar Litov[2]

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Keywords: peptide-protein interactions; binding affinity; CNN; SARS-CoV-2; RBM

Protein–protein interactions (PPIs) are central to the regulation of most cellular processes, including signaling, immune response, and viral infection. Precise characterization of these interactions is essential for understanding molecular mechanisms and for the rational design of therapeutic agents. Despite advances in structural biology, experimental determination of binding affinities remains labor-intensive and limited in throughput. This has motivated the development of computational approaches, particularly those based on machine learning (ML), to predict binding sites and estimate interaction energies. However, current ML-based models often lack the accuracy and generalizability required for reliable binding energy estimation, leaving a critical gap in the computational toolkit for studying PPIs.

We present a novel computational framework centered on a convolutional neural network (CNN) trained to estimate the binding free energy of protein–protein complexes. The network operates on a tailored input representation that integrates both primary sequence information and three-dimensional structural features of the complex. These data are preprocessed and encoded into a format optimized for neural network input. This architecture enables accurate energy predictions while maintaining low computational complexity. As a proof of concept, we applied the model to assess the effects of the N501Y and E484K mutations in the receptor-binding motif (RBM) of the SARS-CoV-2 spike protein — mutations known to alter its interaction with the human ACE2 receptor. Our approach successfully captures the energetic consequences of these sequence variations, indicating its ability to resolve subtle changes in binding affinity. This framework provides a robust and scalable tool for binding energy estimation, with potential applications in protein engineering, mutation impact assessment, and rational drug design.

Acknowledgements: This research is partially funded by the Bulgarian National Science Fund under grant KP-06-N72/3/2023 AIDA.

O6 Comprehensive *In Silico* Evaluation of *BMPR2* SNPs Associated with Pulmonary Arterial Hypertension

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Keywords: *BMPR2*, PredictSNP2, Polymorphism, Pulmonary Hypertension

Pulmonary arterial hypertension (PAH) is a progressive vascular disease strongly linked to *BMPR2* mutations. This study used silico methods to analyze *BMPR2* missense variants, focusing on those with conflicting or pathogenic classifications. Variant data from gnomAD were assessed for allele frequency, molecular impact, and ClinVar annotations. Pathogenicity was predicted using VEP annotations and tools such as SIFT, PolyPhen, CADD, and PredictSNP2. Population-specific allele frequencies and phenotype-disease associations regarding PAH were evaluated. Of the 906 variants, 67.99% (n=616) were missense, 27.26% (n=247) synonymous, 1.66% (n=15) stop-gained, and 1.43% (n=13) frameshift. Among missense variants, 55.52% (n=342) had no ClinVar classification, 27.76% (n=171) were of uncertain significance, and 7.63% (n=47) had conflicting pathogenicity classifications. Synonymous variants were predominantly benign/likely benign (19.64%, n=121). Pathogenic missense variants (1.30%, n=8), including rs1085307150 (p.Arg10Trp), were associated with PAH or pulmonary disease in ClinVar. Allele frequencies were low, with most variants being rare. SIFT and PolyPhen analyses identified deleterious variants (e.g., rs759293027, SIFT score =0.01), while CADD scores highlighted high-impact variants (e.g., rs1336303188, PHRED=25). PredictSNP2 analysis of the 47 missense variants with conflicting classifications revealed 25 neutral and 22 deleterious predictions. Notably, variants like chr2:202556018 G>A (PredictSNP2: deleterious, CADD: 34.0, DANN: 0.9993, FATHMM: 0.9947) were consistently predicted as deleterious, while others, such as chr2:202555381 G>A (PredictSNP2: neutral, CADD:16.15, DANN:0.9272), showed mixed predictions. For the 8 pathogenic or likely pathogenic missense variants, specific results were unavailable than PredictSNP2; however, their ClinVar annotations confirm their clinical relevance to PAH. These 8 variants underscore the critical role of *BMPR2* in PAH pathogenesis. This analysis reveals a diverse spectrum of *BMPR2* variants, with missense variants predominating and a subset linked to PAH. The substantial proportion of *BMPR2* variants with uncertain or conflicting clinical significance highlights the pressing need for experimental validation through functional assays. Overall, these findings contribute to a deeper understanding of *BMPR2*'s pathogenic mechanisms in PAH and underscore the importance of implementing targeted genetic screening strategies in genetically predisposed populations.

O7 Drought protective metabolites in *Capsicum annuum* and their biosynthetic control

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Keywords: Pepper, drought, GWAS, metabolomics

Under climate change abiotic stresses such as drought are expected to increase, placing increasing pressure on food production. *Capsicum annuum* (pepper) is an important source of metabolites associated with human health and is particularly susceptible to abiotic stress. As such, in this work we aimed to improve the drought tolerance of *C. annuum* by screening a panel of 162 different natural accessions of *C. annuum* in a genome wide association study (GWAS) for phenotypic and metabolic traits under drought. In addition, a panel of 110 backcrossed inbred lines (BILs) from a cross between *C. annuum* and *C. chinense* were further screened to validate the GWAS results. This allowed for the identification of drought protective metabolites, such as some flavonoids and signature capsianosides that could reduce the loss of yield associated with drought. Moreover, the genetic regulation of these metabolites could by identified by overlapping the GWAS and QTL mapping results. Notably, a major quantitative trait locus (QTL) on chromosome 9, which presented a cluster of UDP-glucosyltransferases could reveal the capsianoside biosynthetic pathway. Moreover, under drought, capsianoside biosynthesis was additionally associated numerous clusters of diseases resistance R proteins, which may regulate their biosynthesis in response to stress. Thus, these findings provide new gene targets for biotechnologist and breeders alike to improve the metabolic composition and drought tolerance of *C. annuum.*

O8 The impact of the glucagon-like peptide-1 receptor agonist semaglutide on endometrial physiology, embryo development and implantation

Apostol Apostolov[1,2,3,4], Amruta D. S. Pathare[1], Darja Lavõgina[1,5,6], Cheng Zhao[2,3], Keiu Kask[1,7], Sanjiv Risal[2,3], Susana Ruiz-Durán[8,9], Merli Saare[1,7], Maire Peters[1,7], Ganesh Acharya[2,10,11], Fredrik Lanner[2,3], Andres Salumets[1,2,3,7], Alberto Sola-Leyva[1,2,3]

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Keywords: endometrium, embryo, semaglutide

Semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA), is used to treat type 2 diabetes, obesity, and PCOS. Emerging evidence suggests it may also support female fertility by restoring ovulation. However, its effects on implantation and early embryo development remain unclear, as does GLP-1 receptor (GLP-1R) expression in the endometrium and embryo.
GLP-1R expression was detected in endometrial tissue from fertile women, peaking in the mid-secretory phase when the endometrium is receptive to implantation. To explore functional effects, endometrial stromal cells (ESCs) and epithelial organoids (EEOs) were derived from biopsies and treated with semaglutide (0.039, 1.25, and 5 μM). EEOs showed increased intracellular cAMP production across all doses, confirming functional GLP-1R activation, while ESCs responded only at the highest concentration. Metabolic activity measured by resazurin assay in ESCs decreased in

a dose-dependent manner, and EEOs also showed reduced activity at 5 μM. Semaglutide impaired ESC decidualization, as shown by reduced prolactin secretion. Transcriptomic analysis revealed that semaglutide upregulated genes linked to endometrial receptivity in EEOs (e.g., PAEP, LIF, SPP1). Low doses had minimal effects, while 5 μM induced over 400 differentially expressed genes, primarily affecting mitochondrial and metabolic pathways in EEOs and downregulating proliferation-related genes in ESCs. Bulk RNA-seq of semaglutide-treated blastoids suggested upregulation of epiblast markers, however single-cell RNA-seq revealed lineage-specific changes, increased oxidative phosphorylation and reduced histone modifier expression in both epiblast and trophectoderm lineages. In summary, GLP-1R is expressed in both the endometrium and embryo model. Semaglutide activates receptor-specific signaling, influencing gene expression and cellular function in the endometrial epithelium and blastoids.

O9 Ultrastructural morphology of the blood microbiota and possible mechanisms of their proliferation

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Keywords: blood microbiota, electron microscopy, morphology, proliferation

Inroduction: The blood microbiome is still an enigma. The existence of blood microbiota in clinically healthy individuals was proven during the last 50 years. Indirect evidence from radiometric and sequencing analysis suggested the existence of living microbial forms in blood cells. The morphology and reproduction cycle of blood microbiota in healthy individuals are not sufficiently well studied. Aim of this study was to investigate the morphology and life cycle of blood microbiota in healthy individuals.

Methods: For the purpose of the study, we used freshly collected peripheral blood from healthy volunteers. Part of the samples were cultivated under stressful conditions. Blood samples were cultured at 43°C in the presence of vitamin K (1 mg/ml). Native and cultured blood preparations were examined by light and electron microscopy.

Results: In native blood, by light and electron microscopy methods, we have observed free-circulating microbiota that possess a well-defined cell wall and divide by budding or extrusion of progeny cells. TEM micrographs of stressed blood samples showed electron-dense and electron transparent bodies scattered in and between blood cells. We observed structures resembling L-forms of bacteria. Electron-dense bodies divide by forming simple fission or by forming chains of Gram-negative daughter cells or grow and then burst and release progeny cells 180 – 200 nm in size. A novel proliferation mechanism of the blood microbiota was observed, which we called "a cell within a cell" or the "Matryoshka" model.

Conclusions: The demonstrated rich diversity of eukaryotic and prokaryotic microbiota in blood by the next-generation sequencing technique and our microscopic results suggest different mechanisms of blood microbiota division in native and cultured blood. Our method for blood microbiome resuscitation can be applied for further understanding of the possible microbial contribution in diseases pathophysiology. Our results significantly enrich the current understanding of the presence and proliferation mechanisms of blood microbiota in healthy individuals.

Acknowledgments: The study was financed by Bulgarian National Science Fund within the framework of the VIHREN National Scientific Program, contract number KP-06-DV/10-21.12.2019 and KP-06-N73/5-05.12.2023.

O10 Bioprocessing of plant *in vitro* systems: Speculation, reality and prospects

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Keywords: plant cells, tissue and organ cultures, plant cellular agronomy, bioactive substances, bioprocess engineering of plant *in vitro* systems

Nowadays, because of their valuable pharmaceutical and nutraceutical properties, many plant species have been widely used as ingredients in food, pharmacy, and cosmetic industries. Most of the consumers preferred to use natural products of plant origin, following closely the growing interest to the concept of modern healthy lifestyle, based on prevention by consuming quality foods and natural nutraceutical supplements. Therefore, the market for natural plant products has expanded, and this trend will continue. However, the supply of plant bioactive substances is limited by climatic, environmental, and ecological reasons, which will lead to shortages of various plant products. These problems could be solved by using plant in vitro technologies. There are several important advantages of plant *in vitro* technologies, mainly the independence from climatic conditions and the sustainable production process under controlled conditions. For the successful industrial implementation of plant *in vitro* technology for bioactive substances production an integrated approach for process optimization (including selection of productive lines, media optimization, development of suitable elicitation procedures, etc.) must be applied. However, the scale-up of cultivation process in bioreactors still appears among the main problems during development and commercialization of these technologies. This problem remains unsolved, despite the researchers' efforts for more than 55 years in this area.

The lecture will be focused on integrated approaches for bioactive substances production in different plant *in vitro* systems. The main stress will be on bioreactor systems for plant cells and tissue culture cultivation – selection, optimization of design of apparatus an optimization of the environmental conditions of cultivation. Current status, speculations and future prospects as well as challenges of the commercialization of the products of plant cell and tissue cultures will be outlined. Finally, an example of product developed in our laboratory will be presented as eco-friendly alternative method for sustainable production of plant-derived ingredients.

The research presented in this lecture was funded by the Bulgarian National Science Fund (BNSF), grant number КП-06 Н66/5.

O11 Characterization of antibiotic-resistant enterococci and beta-lactam resistance mechanisms in enterobacteria from groundwater in Ialomița County

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Keywords: *Enterococcus* spp., Enterobacteriaceae, beta-lactam resistance

Background: Access to safe drinking wateris a fundamental human right, is emphasized by Goal in 6 of the 2030 Agenda. According to a 2023 WHO report, 18% of the global population lacks access to safe drinking water, and 12% lack adequate sanitation. The presence of antibiotic-resistant bacteria in environmental water sources, such as wells, represents a significant public health risk, particularly in rural areas with limited monitoring. **This study aimed** to characterize antimicrobial resistance in *Enterococcus spp.* and investigate beta-lactam resistance mechanisms in *Enterobacteriaceae* isolated from groundwater sources in Ialomița County.

Materials and methods: Groundwater samples were collected from local wells by the Public Health Directorate, adhering to established microbiological monitoring procedures. Isolates belonging to *Enterobacteriaceae* family *Enterococcus* spp. were cultured on selective/chromogenic media. Species identification and resistance gene detection (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{KPC}}$, *vanA, vanB, tet(M)*) were performed using multiplex PCR. Antibiotic susceptibility was assessed using disk-diffusion method.

Results: A total of 23 enterobacterial strains of interest were isolated established, of which 15 were resistant to ampicillin, 13 strains to gentamicin and amikacin, 10 strains to cefazolin and piperacillin. Gentamicin resistance was detected in samples from multiple locations, including Scânteia, Bărcănești, Cocora, Reviga, Sinești and Grindu. CTX-M was detected in 4 strains, KPC in one isolate and OXA-48 gene was absent in all tested strains. Among the 18 *Enterococcus*spp. isolates, four showed resistance, including one multidrug-resistant strain from Cocora (resistant to penicillin, ciprofloxacin0 and tetracycline). Vancomycin resistance was detected in one strain, while intermediate susceptibility to tetracycline and linezolid appeared in several others. No *vanA, vanB, or tet(M)* resistance genes were identified.

Conclusions: Antibiotic resistance is one of the major health threats. The presence of resistant bacterial strains in Ialomița County wells, used for drinking, raises concerns about prevention strategies and education in rural communities. Intensive agriculture and livestock activities create favourable conditions for groundwater contamination with faecal bacteria. Consequently, well water without microbiological treatment can act as a vector for dissemination of resistant bacteria, emphasizing the necessity of implementing water quality surveillance in rural areas.

O12 Postbiotics as an alternative to antibiotic therapy: potential for biofilm control in *Staphylococcus aureus*

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Keywords: postbiotics, *S. aureus,* antimicrobial resistance, Biofilm inhibition, *L. plantarum*

Staphylococcus aureus is a major etiological agent in chronic nasal infections and rhinosinusitis. It`s ability to form biofilms significantly impairs the efficacy of conventional antibiotic therapy, stressing the need for an alternative strategy focussed on disrupting biofilm formation and reducing the virulence of the pathogen. This study investigates the postbiotic potential of two *Lactiplantibacillus* strains—*L. plantarum* IZITR_24 and *L. paraplantarum* IZITR_13—isolated from traditional Bulgarian fermented vegetables. These strains exhibit favourable probiotic characteristics, including acid and bile tolerance, osmotic stress resilience, and co-aggregation with pathogens. Whole-genome sequencing by Nanopore ONT revealed that both strains possess genes encoding plantaricins PlnE/F and PlnJ/K, while *L. paraplantarum* additionally harbour pediocin. Antimicrobial susceptibility testing of nasal *S. aureus* isolates confirmed widespread multidrug resistance, with methicillin resistance and MAR indices above 0.2 in 70% of cases. The application of lyophilized, pH-neutralized, cell-free supernatants (CFS) from both strains demonstrated clear dose-dependent inhibition of biofilm formation. Based on the PROBIT analysis the minimal inhibitory concentration (MIC_{99}) for *L. plantarum* and *L. paraplantarum* was estimated to be 86.7±22.6 $mg.mL^{-1}$ and 143.3±33.2 $mg.mL^{-1}$, while their minimal biofilm-inhibitory concentrations (MBI_{99}) were calculated as 28.7 and 37.8 $mg.mL^{-1}$, respectively. Subinhibitory concentrations of 10 $mg.mL^{-1}$ significantly reduced biofilm biomass across all tested *S. aureus* strains. Fluorescence microscopy revealed an increased proportion of dead cells within the treated biofilms, while scanning electron microscopy demonstrated biofilm disruption, including decreased matrix density, morphological deformation of cells, and presence of fibrin-like aggregates. These findings support the potential of genomically characterized, food-derived *Lactiplantibacillus* strains as safe and effective sources of postbiotics in the control of *S. aureus* colonization and biofilm-associated infections, providing a promising complementary approach to antibiotic therapy.

O13 Extraction of biologically active compounds from yeast by combining pulsed electric field treatment and enzymatic hydrolysis

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Keywords: pulsed electric field, irreversible electropermeabilization, yeast, Alcalase, enzyme hydrolysis

Enzymatic hydrolysis using various commercial enzymes is among the most efficient methods for producing yeast extracts enriched in biologically active peptides. However, the cell envelope—comprising the plasma membrane and the cell wall—represents a major barrier that limits the access of externally applied enzymes to intracellular components, thereby hindering the hydrolysis process. This study investigates the effect of pulsed electric field (PEF) pretreatment on the enzymatic hydrolysis of baker's and spent brewer's yeast. PEF induces irreversible permeabilization of the plasma membrane and increases cell wall porosity, potentially facilitating enzyme access. Yeast cells were diluted in distilled water to a final concentration of 50 mg per gram dry cell weight (DCW) and subjected to monopolar rectangular pulses in a continuous-flow system. Incubation of electrically treated baker's yeast with Alcalase at concentrations of 0.2% and 0.5% resulted in significantly improved hydrolysis efficiency compared to non-pulsed controls. After 4 hours of incubation at 48 °C, the amount of released protein reached 163.7 ± 13 mg/g DCW. SDS-PAGE analysis revealed that the extracts predominantly contained peptides with molecular weights below 4.7 kDa. The phenolic content of the hydrolysates was comparable to that obtained after mechanical cell disruption. Moreover, the levels of free α-amino nitrogen and total antioxidant activity reached 218.2 ± 26 mg/g DCW and 53.4 ± 4.6 mg Trolox equivalents/g DCW, respectively—representing 3.2-fold and 2.65-fold increases over mechanically obtained lysates. Importantly, the combined PEF and enzymatic treatment did not cause substantial cell lysis, enabling easy separation of cells at the end of the process. Hydrolysates from PEF-pretreated cells stimulated the proliferation of the human keratinocyte cell line HaCat without requiring further purification. Comparable results obtained with spent brewer's yeast confirmed the efficacy of PEF as a mild and effective pretreatment strategy for enhancing enzymatic hydrolysis and producing extracts with high antioxidant activity. In summary, PEF pretreatment significantly improves the enzymatic hydrolysis of yeast, offering a promising approach for generating bioactive-rich extracts with potential applications in biotechnology and health-related fields.

O14 Encapsulation of beneficial microorganisms in polymeric formulations for enhanced agricultural biocontrol

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Keywords: Electrospinning, Biocontrol agents, Polymeric carriers, Microorganism encapsulation

In recent years, electrospinning and related "*green*" fabrication techniques have emerged as versatile, cost-effective approaches for producing polymeric materials with purposed properties. These methods enable the incorporation of functional agents into fibrous mats, beads, and coatings, yielding biohybrid formulations with tailored morphologies and functionalities for agricultural applications. This presentation focused on two complementary research strategies: (i) electrospun poly(3-hydroxybutyrate) (PHB)-based materials incorporating bioagents for plant protection, and (ii) chitosan-derived carriers, formulated through environmentally friendly processes for delivering beneficial microorganisms against phytopathogens. PHB-based electrospun fibers are engineered to host microbial bioagents with processing parameters, and post-treatments (e.g. dip-coating, electrospraying) optimized to control fiber diameter, surface morphology, and mechanical properties. Incorporation strategies ensure homogeneous dispersion or surface immobilization of microbial cells while preserving viability and biological functionality. *In vitro* assays demonstrate sustained cell viability, controlled release profiles, and effective antagonistic activity against phytopathogens. The combined insights into fabrication parameters, material-agent compatibility, and performance in both simulated environments and plant-based assays underscore the potential of these systems as eco-friendly alternatives to conventional agrochemicals.

Acknowledgements: This research was funded under Project BG-RRP-2.011-0005-C01 with financial support from the European Union - NextGenerationEU, Investment C2.I2 "Increasing the innovation capacity of the Bulgarian Academy of Sciences in the field of green and digital technologies".

O15 Innovative electrospun biohybrid polymer materials for sustainable agricultural applications

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Keywords: natural polymers, nanotechnology, biocontrol agents

Numerous pathogens can cause plant diseases, which are a global problem and generate considerable losses in agriculture. Eco-friendly, sustainable agriculture and products are crucial in order to solve these problems. Creating novel biocontrol agents to protect crops could drastically reduce dependence on conventional pesticides. Electrospinning is a versatile nanotechnology for producing polymer fibers with micro- and nanoscale diameters, exceptional lengths, and unique properties such as high specific surface area and fine porous structures. Electrospinning, alone or combined with other techniques, provides substantial advantages for creating materials containing biocontrol agents for agricultural applications. Biopolymer fibrous materials composed of cellulose derivatives, poly(3-hydroxybutyrate), poly(L-lactide), or chitosan were combined with beneficial bacteria. SEM was used to analyse the morphology of the biohybrid materials and encapsulated microorganisms, and ATR-FTIR spectroscopy was used to determine the chemical composition of their surfaces. Mechanical testing revealed favorable tensile strength and elastic modulus values. Viability experiments demonstrated that the polymer carriers maintained microbial viability during extended storage and supported normal microbial development. These findings highlight the potential of electrospun biohybrid materials as effective biocontrol formulations, offering promising applications for plant protection and growth promotion in sustainable agriculture.

Acknowledgements: This research was funded under the Project BG-RRP-2.011-0005-C01 with the financial support by the European Union - NextGenerationEU, Investment C2.I2 “Increasing the innovation capacity of the Bulgarian Academy of Sciences in the field of green and digital technologies”.

O16 AI-assisted computational laccase engineering for sustainable biocatalysis: a lab-free design approach

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Keywords: Laccase, AI-assisted enzyme design, AlphaFold3, sustainable biocatalysis.

Laccases are copper-containing oxidase enzymes widely recognized for their catalytic versatility and potential in green chemistry applications. Their ability to oxidize a wide range of phenolic and non-phenolic substrates makes them attractive biocatalysts for processes such as dye degradation, lignin valorization, and wastewater treatment. However, conventional enzyme engineering methods require extensive laboratory experimentation, often limiting rapid screening and innovation. This study presents a fully computational, lab-free workflow for the in silico design and evaluation of laccase enzymes using state-of-the-art artificial intelligence (AI) and molecular modeling tools. The laccase amino acid sequence was subjected to structural prediction using AlphaFold3, enabling high-confidence modeling of its 3D conformation. The predicted structures were further analyzed and refined to identify catalytically relevant regions and binding pockets.
To assess the interaction potential with industrially relevant phenolic substrates, molecular docking studies were performed using AutoDock Vina and SwissDock platforms. Binding energies and pose orientations were visualized using PyMOL and UCSF Chimera, providing insights into substrate affinity and spatial compatibility within the active site. Designed variants were compared to wild-type laccase models in terms of binding strength and active site exposure.
Our results demonstrate the efficacy of integrating AI-driven structural modeling with molecular docking to accelerate enzyme engineering, bypassing the need for immediate wet-lab validation. This lab-free approach offers a time-efficient and sustainable pathway for developing next-generation laccases tailored for green biocatalytic processes, with broad implications in environmental and industrial biotechnology.

O17 The new ecology: next steps

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Keywords: ecology, ecosystem, biodiversity, socio-ecological system, sustainability

At present, the term ecology is used much more frequently than at any time in the past. Ecology was originally defined by Ernst Haeckel as the "*the study of the relationship of organisms with their environment*" in the mid-19th century, when biology was a vastly different discipline than it is today. Since its original inception the science of ecology has undergone dynamic development and other definitions have been proposed. The second definition, which is perhaps the most commonly repeated, considers ecology as the study of the distribution and abundance of organisms. The third, and the latest definition focuses ecology on the study of ecosystems. As in every real science there are three approaches to the science of ecology – theoretical ecology, empirical ecology, and applied ecology. Here, I will briefly discuss all the three of them in their modern context.

Theoretically, current ecology is defined as the science dealing with the structure, dynamics and functions of nature including evolution, where structure involves the distribution and abundance of individual organisms, habitats and ecosystems; including growth, development, reproduction or renewal, the cycling of matter, flows of energy and information as well as the properties and niches of ecosystems in the landscape, ecoregion or in the whole Earth system. Modern ecological theory relies on three basic concepts: Ecosystems as Self Organizing Hierarchical Open (SOHO) systems; Role of biological diversity in ecosystem functioning; Ecosystem services. Theory accepts that ecosystems are not in equilibrium but are always far from an equilibrium state, that could be considered as "normal", continuously developing to adapt to changing conditions by an adaptive cycle in order to preserve resilience. Biological diversity has a significant role in ecosystem functioning and two forms of functional diversity have a key significance: Diversity of functional groups and Diversity of species within the functional group. These two forms of diversity of species and functional groups are critical for the adaptation of ecosystems to changes in the environment. At present, the concepts of the role of man in ecosystems has significantly changed: from Humans independent of ecosystems to Humans integral part of ecosystems. Mankind receives from ecosystems goods and services which are res-ponsible for the life support system on Earth. The human society and its surrounding ecosystems form self-organizing systems defined as Socio-ecological systems expected to follow a sustainable development pathway. Practically, these areas of ecology rarely talk much to each other and leaf the door open for dominating of applied ecology where the action is now. The mantra of applied ecologists is to do no harm to the environment while solving different real world problems. Thus, ecology is generally used as an ideological abracadabra, which contributes to the merging of various environmental ideas and is ungenial to ecology itself as it is a regular scientific discipline, one of the central branches of biology.

O18 Changing ichthyofauna: ecological, anthropogenic, and taxonomic dimensions

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Keywords: fish fauna dynamics, native species decline, species introduction, natural dispersal, taxonomic revision

Fish fauna is not a static component of aquatic ecosystems; rather, it is in constant flux due to a combination of ecological, anthropogenic, and scientific factors. One of the most pressing concerns is the decline or local extinction of native fish species, often driven by habitat loss, pollution, overexploitation, and biotic interactions with non-native species. These losses reduce functional diversity and threaten the ecological stability of freshwater and marine environments.

Simultaneously, the introduction and spread of alien species—through both deliberate stocking and accidental translocations—continue to reshape local fish assemblages. Human activities, such as aquaculture, the ornamental trade, and hydrotechnical developments, create pathways for these non-native species to establish, sometimes leading to invasive populations. Alongside intentional introductions, spontaneous range expansions—often facilitated by climate change or newly connected waterways—result in the natural immigration of species previously absent from the region.

A less frequently discussed, yet equally important driver of perceived change is taxonomic revision. Advances in molecular biology and systematics have led to the reclassification of many fish species, including the splitting of previously single species into multiple cryptic taxa or the merging of others. As a result, changes in species lists may not always reflect ecological shifts but rather improved scientific understanding.

Together, these processes—native species decline, human-mediated introductions, natural dispersal, and taxonomic redefinition—collectively contribute to the dynamic and often unpredictable nature of fish communities. Recognizing the interplay between these factors is crucial for effective biodiversity monitoring, conservation planning, and ecosystem management. A multidisciplinary approach that integrates ecology, taxonomy, and environmental policy is crucial for tracking, interpreting, and responding to ongoing changes in fish fauna.

O19 Uncovering hidden diversity: fieldwork and taxonomic discoveries of fungus gnats (Diptera: Mycetophilidae) in Northern Vietnam

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Keywords: taxonomy, fungus gnats, Vietnam, Oriental Region, Sciaroidea

The Oriental Region, characterized by its rich yet insufficiently studied ecosystems, remains one of the least explored biogeographical zones with respect to the diversity and taxonomy of fungus gnats (Diptera: Sciaroidea). This contribution presents new taxonomic data based on material collected during a 2023 expedition to Tam Dao National Park and Mount Fan Si Pan in northern Vietnam, conducted through a collaborative effort between the Bulgarian Academy of Sciences and Vietnamese research institutions. Specimens obtained from montane forest habitats revealed notable species richness, including the recently described *Chalastonepsia vumanhi* Kazandzhieva, 2025. Additional material indicates the presence of several undescribed taxa, underscoring the Oriental Region as a significant hotspot of sciaroid diversity. A subsequent expedition in October 2025, jointly organized by the Bulgarian Academy of Sciences, the Regional Natural History Museum – Plovdiv, and the Vietnamese Academy of Sciences, provided further insights into this fauna. The combined findings from both expeditions contribute to a deeper understanding of the region's species composition, distributional patterns, and the urgent need for continued documentation and conservation of invertebrate biodiversity in tropical Asia.

O20 Bats and their bacterial and protozoan pathogens with zoonotic potential

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Keywords: Leptospira, Babesia, Trypanosoma Non-tuberculous mycobacteria

Bats are well known for being reservoirs of many different pathogenic agents. The majority of recent scientific interest in this field has been focused on viruses, however bats can also host a wide range of bacteria, fungi, and protozoa. To address this, we examined Palearctic bats in Central and Eastern Europe, Caucasus and Siberia for the presence of two bacteria (Leptospira and non-tuberculous mycobacteria) and two protozoa (Babesia and Trypanosoma) during the last 15 years. A variety of samples were employed for the purpose of analysis, including blood, urine and droppings. Four bat species were newly found to be positive for pathogenic *Leptospira interrogans* but a potential novel species related to *L. weilii* was detected too. A high prevalence of trypanosomes (dominantly *Trypanosoma dionisii*) was observed, however, there was no effect on bat condition. In contrast, *Babesia vesperuginis* was found to have a significant effect on acid-base balance parameters during hibernation. Finally, a minimum of 23 sp., ssp. and complexes of non-tuberculous mycobacteria were identified in bat guano samples. The proportion of positive samples collected from caves was equivalent to that collected from attics, yet the infection load of the former was significantly higher. In conclusion, active surveillance of bat pathogens is imperative to understand threats to both chiropteran conservation and public health.

O21 Distribution and biological features of the species *Carcinus aestuarii* off the Bulgarian coast of the Black Sea

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Keywords: distribution and biology, Decapoda, Black Sea

Length-Weight relationships and condition factor provide important information about the biological characteristics and stock assessment of crustaceans. Having suffered a severe reduction due to intense eutrophication and oxygen depletion in the last century, decapod crustaceans are currently recovering their populations and are common as bycatch in bottom trawling. In order to establish the status of the populations of the decapod species *Carcinus aestuarii* off the Bulgarian coast of the Black Sea, length-weight relationships were studied. Carapace length (CL) and weight (W) were measured to the nearest 0.01 cm and 0.01 g, respectively. Crab samples were collected by diving and as a part of trawling activities. Results might serve for base of further conservation purposes or in the fishery practice.

O22 The influence of selected herbicides on common carp

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Keywords: fish, herbicide, toxicity

The aim of the study was to investigate the effects of two selected herbicide formulations—one based on glyphosate and the other on MCPA—on common carp. The haematological analysis of fish exposed to the glyphosate-based herbicide formulation revealed a decrease in RBC count and an increase in other erythrocyte parameters. These changes generally depended on the herbicide concentration and the duration of exposure. An increase in WBC count and percentage of immature neutrophils occurred, indicating the presence of inflammation. In the studied blood biochemical parameters, only minor and temporary changes were detected. The histopathological analysis revealed no alterations in the tested organs. Our analysis showed fluctuations in haematological parameters in fish exposed to the MCPA-based herbicide formulation during the treatment period. Plasma biochemical changes that were statistically significant subsided rapidly. No histopathological lesions in the analysed organs were identified. The results we obtained show that herbicides affect common carp. Blood parameters appear to be more sensitive markers than the microstructure of the examined organs.

O23 Role of fish as bioindicators of aquatic pollution

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Keywords: bioindicator, fish, aquatic toxicology, pollution monitoring, environmental assessment

Fish are among the most widely used bioindicators in aquatic environmental monitoring due to their ecological diversity, trophic position, and ability to integrate chemical exposure over space and time. Their importance extends beyond ecological assessment, as fish are also key components of human diets, making their contamination levels directly relevant for public health.

Fish accumulate pollutants such as trace elements and emerging contaminants (e.g., microplastics) through various uptake routes including gills, skin, and the digestive system. Accumulation patterns are influenced by species-specific traits such as habitat preference (benthic vs. pelagic), trophic level, age, and diet. Understanding these factors is crucial for accurately interpreting biomonitoring results and for assessing the magnification of pollutants across aquatic food webs.

Recent investigations in Hungary have focused on how age, feeding habits, and habitat use influence the accumulation and magnification of trace elements in freshwater fish species. Additionally, emerging research highlights the potential of fish tissues to serve as indicators of microplastic pollution, offering a promising tool for detecting and tracking this growing environmental concern.

This presentation provides an overview of the rationale behind using fish as bioindicators, summarizes key ecological and physiological factors affecting their contaminant loads, and discusses recent methodological developments. By integrating traditional trace element analyses with novel approaches such as microplastic detection, fish-based biomonitoring offers valuable insights for both environmental protection and human health risk assessment.

O24 Effects of adventive and invasive fish species on biodiversity and conservation

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Keywords: invasive fish species, biological invasions, aquatic ecosystems, climate change

The introduction and spread of adventive and invasive fish species represent a growing challenge to freshwater and marine ecosystems worldwide. These species, whether intentionally introduced for aquaculture, ornamental purposes, or unintentionally through ballast water or birds, can significantly alter native biodiversity and ecosystem functioning. Invasive fish often outcompete native species for resources, prey on endemic fauna, introduce novel pathogens, and alter habitat structures, leading to biodiversity loss and reduced ecosystem resilience.

Understanding the mechanisms of introduction is crucial for predicting and mitigating the spread of non-native fish. Pathways include deliberate human activities (e.g., fish farming, stocking), accidental releases, hydrological engineering (e.g., canals, reservoirs), and natural range shifts. Once established, non-native fish can impact native communities on multiple levels—altering food webs, modifying physical habitats, and affecting nutrient cycling and water quality. Sensitive or isolated habitats, such as bogs, are particularly vulnerable.

Climate change further complicates these dynamics by facilitating the expansion of warm-water tolerant non-native species into previously inhospitable areas. Shifting temperature regimes, altered precipitation patterns, and increasing frequency of extreme weather events can disrupt native assemblages, creating ecological opportunities for invaders. Consequently, the interplay between climate change and biological invasions is expected to intensify, requiring adaptive and forward-looking conservation strategies.

Addressing these challenges requires comprehensive research and dialogue on the ecological roles, thresholds, and long-term impacts of both adventive and invasive species. Only through an integrated approach—linking environmental science, policy, and public engagement—can we effectively safeguard aquatic biodiversity and ecosystem integrity in the face of growing biological invasions.

O25 Stress responses of Black Sea littoral species to multiple pollutants with special reference to microplastics

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Keywords: Black Sea, fish, invertebrates, microplastics, oxidative stress

This study investigates oxidative stress (OS) in Bulgarian Black Sea invertebrate and fish species from the northern (Varna Bay) and southern (Burgas Bay) littoral, focusing on MPs accumulated in them. The studied species include *Mytilus galloprovincialis* Lamarck, 1819; *Rapana venosa* (Valenciennes, 1846); *Bittium reticulatum* (da Costa, 1778); *Palaemon adspersus* Rathke, 1837; *Magallana gigas* (Thunberg, 1793); *Cerastoderma glaucum* (Bruguière, 1789); *Mya arenaria* Linnaeus, 1758, and *Mullus barbatus* Linnaeus, 1758; *Sprattus sprattus* (Linnaeus, 1758); *Mesogobius batrachocephalus* (Pallas, 1814). OS biomarkers were spectrometrically analyzed in fish liver and gills and in invertebrate soft tissues. MPs accumulation was quantified microscopically. MPs were detected in all species, with *P. adspersus* and *M. gigas* showing the highest MPs concentrations, while *B. reticulatum* had the lowest. *S. sprattus* exhibited the highest MPs in muscle tissue and was the only species with a significant positive correlation between MPs load and body size. *P. adspersus* and *S. sprattus* showed elevated lipid peroxidation and weakened antioxidant defenses. *B. reticulatum* displayed low MPs accumulation and strong antioxidant activity, whereas *M. gigas* maintained high antioxidant responses despite elevated MPs. Fish liver and gill tissues displayed varied OS profiles with species-specific stress mechanisms. The Specific Oxidative Stress (SOS) index emphasized cumulative stress effects, with *S. sprattus* being most vulnerable. These findings highlight MPs' pollution as a key feature of the Anthropocene, revealing complex cellular stress responses in Black Sea biota that may affect higher ecological levels. Therefore, identifying suitable stress indicators and conducting comprehensive biomonitoring are crucial to protect ecosystem health amid ongoing human pressures.

Acknowledgements: This work was supported by grant № КП-06-Н61/10 of the National Science Fund, Bulgaria.

O26 Urban and peri-urban forests as important wildlife habitats in urban residential canters

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Keywords: urban forests, wildlife, habitats, shelter, biodiversity

Urban and peri-urban forests-which include parks, greenbelts, street trees, and natural or created forests-are more than just green spaces for human recreation. They serve as important habitats for diverse wildlife, even in densely populated urban areas. Urban wildlife and biodiversity refer to the life of living things - plants, animals, insects, microorganisms -that exist within urban environments. Although cities are often seen as places of buildings and concrete and with little nature, when combined with green infrastructure they can support a high level of wildlife and biodiversity, if well thought out and managed. And of course, urban and peri-urban forest ecosystems, both within and near urban centers, are very important in this regard. First at all urban and peri-urban forests support a variety of species and serve as a shelter per wild live and biodiversity. These green spaces provide food, shelter, and breeding sites compared to places where natural habitats have been fragmented or lost due to urban development and construction. Thus, communities of birds as woodpeckers, songbirds, owls, insects as bees, butterflies, beetles, mammals as squirrels, foxes and even amphibians as triton, salamanders, frogs and reptiles as lizards, snakes, turtles, in areas with more vegetation or adjacent to water bodies, find shelter, food and breeding conditions in forested and shrubby territories of urban areas. Habitat's connectivity-urban forests can function as corridors that connect isolated habitat patches. Even small patches or rows of trees can be stepping stones that help species navigate urban landscapes. So, gene flow among wildlife populations, migration and seasonal movement, adaptation to climate and environmental changes are some of benefits provided from urban forests. Except others urban forestry provided and some ecological benefits by supporting wildlife contributing in pollination of urban gardens and crops, pest control by predatory birds and insects, nutrient cycling through decaying vegetation and animal activity. Urban forests, between correlations human- wildlife coexistence offer opportunities for education and awareness, helping city dwellers understand and appreciate local ecosystems. This can foster more sustainable behaviors and support for conservation efforts. Some of the challenges, despite their importance, are that urban forests face threats such as - the presence of invasive species, changes in land use and construction, pollution and waste disposal, conflicts between humans and wildlife, etc. Urban forests are not just attractive scenic spots — they are important ecological sites. Preserving and expanding these green spaces is essential for protecting urban biodiversity and ensuring a healthy and balanced urban environment, aspects that we will address in this presentation.

O27 Widespread exposure and pathogenic diversity of lyssaviruses and filoviruses in Eurasian cave-dwelling bat populations: implications for zoonotic risk

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Keywords: Bats, viruses, genotoxicity, One Health

Bats (Chiroptera) serve as natural reservoirs for RNA viruses, posing a potential zoonotic threat. This study investigated the prevalence and pathogenicity of lyssaviruses and filoviruses in cave-dwelling bat populations across Eurasia. In Bulgaria, surveys of *Miniopterus schreibersii* (Common bent-wing bat) winter and summer colonies detected high seroprevalence of lyssaviruses, alongside filoviruses. In Vietnam, targeted sampling from five bat species (*Hipposideros armiger, H. alongensis, H. poutensis, Taphozous maganoocndon,* and *Myotis pilosus*) identified IgG antibodies against *Lyssavirus* glycoproteins in all species except *T. maganoocndon*. Additionally, genotoxic stress analysis revealed the formation of micronuclei in the peripheral erythrocytes of all species except *M. pilosus*, indicating broader physiological impacts. Molecular screening via RT-PCR detection further confirmed the presence of lyssavirus RNA in 8.5% of samples from Bulgaria, Kazakhstan, and Vietnam. These findings underscore the critical importance of coordinated One Health strategies for monitoring emerging zoonoses in bat populations across Eurasia.

O28 Drones revolutionize the monitoring of notoriously difficult to study raptors: breeding performance of Golden Eagles in Bulgaria

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Keywords: monitoring, breeding success, drones, Golden Eagle, Bulgaria

Golden Eagles are secretive raptors that nest in remote locations, preferentially on cliffs. In Bulgaria, they have declined most likely due to poisoning and electrocution and low productivity has been suspected. In 2025, we conducted the first nationwide monitoring of the breeding performance of the Golden Eagle in the country. Aerial surveys using drones were employed to count the hatchlings and discern the reasons for breeding failure. We recorded 94 breeding territories as occupied, while 23 territories were vacant. Six other territories that were occupied during the former two years were not checked. We measured the breeding performance of 78 pairs (83%), while the current nests in the remaining 16 territories were not found. Of the 78 pairs, 29 (37%) did not start breeding, while 49 (63%) laid eggs. Eleven pairs failed during incubation, 35 hatched chicks, while for three it was not clear whether they failed during incubation or after hatching. Of the 35 pairs that hatched chicks, one failed at early stage and the remaining 34 had only a single chick, of which 26 are considered successfully fledged, as they were observed past the age of 50 days. The age of 130 territory-holding birds was identified, of which 109 (84%) were adults and 21 (16%) non-adults. Of 54 pairs, 35 (65%) consisted of two adults and 19 (35%) had

at least one non-adult member. Here we provide photos taken by drones of the current nests of the studied pairs. In many of the cases the photo-documentation with the drone enabled us to draw reliable conclusions, that would have otherwise required multiple visits, longer travel time to the observation points, more hours of observation and in many cases lower quality of the conclusions.

O29 Accelerating genomics research: Novogene's NGS solutions for Life Sciences and Medicine

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Keywords: Next-Generation Sequencing (NGS), Genomics, Bioinformatics, Biomedical and Biodiversity Research

As one of the world's leading providers of next-generation sequencing (NGS) and bioinformatics services, **Novogene** is dedicated to empowering researchers with high-quality, scalable, and cost-effective genomic solutions. From biodiversity and microbiology to biomedical research and bioinformatics, our mission is to support scientific discovery across disciplines.

In this talk, I will showcase how Novogene collaborates with academic and clinical researchers to advance multi-omics studies across a wide range of biological fields, including genetics, molecular biology, cell biology, and beyond.

I will also address key technical and logistical challenges that researchers often encounter, including:

- What is the most suitable NGS platform for my project?
- Which sequencing strategy should I choose?
- How should I prepare and process different sample types, including low-input material?
- How do I interpret complex multi-omics data?
- How can I ensure reproducibility and data quality across studies?

Through real-world examples and practical guidance, I will demonstrate how Novogene's integrated workflows from sample preparation and sequencing to advanced bioinformatics with **publication-ready figures** help researchers overcome these challenges and achieve reliable, high-impact results.

Whether you are working in human health, microbial ecosystems, plant genomics, or education in molecular life sciences, **Novogene is your trusted multi-omics partner** for advancing genomics-driven research in the Balkans and beyond.

O30 Bulgarian reference genome project, part of the Genome of Europe initiative

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Keywords: genome, personalized medicine, diagnostics, prevention, WGS

The Genome of Bulgaria project (GoBG) aims to build the first comprehensive reference genome of the current Bulgarian population using Whole Genome Sequencing (WGS). A representative sample of the population of Bulgaria (about 6500 healthy participants) from across the country will be collected. The data will help to estimate the frequencies of all genetic variations, both common and rare. This will serve as the basis for genetic epidemiological studies and as a reference database of the Bulgarian genome helping to elucidate the genetic contribution for all rare and common genetic disorders, such as cancer, diabetes, cardiovascular and neurodegenerative disorders.
In the long term, results of the project are expected to lead to a better understanding of the causes of genetic diseases, improved diagnostics and a personalized therapeutic approach, effective prevention and development of innovative solutions in medicine.
A pilot project for the first 1000 Bulgarian Genomes was supported by Ministry of Education and Science. Participants were recruited mainly from Sofia and Plovdiv.
The second phase of the GoBG continues as part of the Genome of Europe (GoE) project. It will include additional 2000 Bulgarian genomes. The GoE project will establish a comprehensive, pan-European reference database of at least 100,000 genomes to advance research, innovation, and personalized healthcare across Europe. This database, built through WGS of diverse national cohorts, will represent genetic diversity of the European population. It is a key component of the larger "1+ Million Genomes" initiative and will be crucial for personalized medicine, disease prevention, and healthcare advancements. Fostering research and innovation, the project will further unlock the potential of genomics for the benefit of all European citizens, improving healthcare.

Acknowledgements:
The GoBG, GoE and GDI projects are supported by Ministry of Education and Science in the frame of National Road Map of Research Infrastructures, 2020-2027, for consortium National University Complex of Biomedical and Translational Research (NUCBTR).
GoE project is co-funded by the EU Digital Europe Programme GA 101168231.
GDI project is co-funded by the EU Digital Europe Programme GA 101081813.

O31 Genetic diversity and chemotypic variation in Bulgarian lavender (*Lavandula angustifolia* Mill.) varieties and accessions revealed by SCoT markers and essential oil profiles

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Keywords: *Lavandula angustifolia*, SCoT markers, genetic diversity, essential oil, Bulgaria

Lavender (*Lavandula angustifolia* Mill.) is one of the most important essential oil-bearing crops cultivated in Bulgaria. In recent years, the mixing of different varieties and even species within commercial plantations has resulted in reduced essential oil quality and a decline in international market competitiveness. This study aimed to assess the genetic diversity and structure of Bulgarian lavender varieties and accessions, evaluate the extent of varietal mixing in commercial fields, and analyze the chemotypic variation in essential oil yield and composition.

Seven traditionally cultivated Bulgarian varieties-Hemus, Druzhba, Sevtopolis, Yubileina, Raya, Hebar, and Karlovo-were studied. Genetic diversity was assessed using 13 SCoT primers, while an extended analysis of 285 plants from 15 commercial fields across major production regions was performed using five SCoT primers to detect genetic admixture. Essential oil yield and chemical composition were evaluated both for individual genotypes and for composite samples from each field.

The molecular data showed clear genetic differentiation among varieties and accessions. At the same time, significant varietal mixing was detected in most production fields. Marker efficiency was evaluated through polymorphic information content, resolving power, effective multiplex ratio, and marker index. Principal Coordinate Analysis (PCoA), UPGMA clustering, and STRUCTURE analysis revealed detailed relationships between genotypes and confirmed the presence of admixed individuals. Nei's gene diversity and Shannon's Information Index indicated significant genetic variability.

Chemical analysis demonstrated that essential oil yield and composition varied significantly among different geographic regions and genotypes, highlighting the effects of both uncontrolled varietal mixing and the influence of genotype and environment on production traits.

This is the first large-scale, integrated study on the genetic structure and essential oil chemotypes in Bulgarian lavender. The results contribute to selection programs, field zoning, quality control, and the sustainable use of lavender genetic resources.

Acknowledgements: This study was funded by the National Science Fund grand number КП-06-М61/7 15.12.22 and the National Program „Young Scientists and Postdoctoral Students-2".

O32 Integrated genome-wide association and QTL mapping elucidate the genetic basis of gray mold resistance in tomato

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Keywords: Gray mold resistance, *Botrytis cinerea*, Tomato, Genome-wide association, Quantitative trait locus

Gray mold, caused by the necrotroph *Botrytis cinerea*, severely compromises the postharvest quality of ripe tomato fruit. To assess the genetic basis of resistance and its breeding potential, we combined genome-wide association studies (GWAS) in 152 diverse tomato lines with quantitative trait locus (QTL) mapping in 56 backcross inbred lines (BILs) segregating for fruit quality and shelf-life traits. Ripe fruits were inoculated and lesion diameter measured 5 days post-inoculation (dpi), revealing quantitatively inherited resistance with moderate to high narrow-sense heritability. Genomic selection (GS) models built on genotypic data achieved predictive accuracy for lesion diameter, highlighting GS as a viable strategy to accelerate resistance breeding. Moreover, additive genetic correlations reported in this study, suggest that higher soluble solids generally promote more aggressive fungal growth, suggesting a possible pleiotropic interplay between sugar content and gray mold susceptibility. GWAS uncovered multiple genomic regions associated with reduced lesion development, QTL mapping confirmed several of these loci and identified additional regions explaining a similar proportion of phenotypic variance. The mapped intervals harbor a range of putative defense-related genes. Notably, resistance alleles are not expected to compromise fruit shelf life or soluble solids (°Brix). These findings emphasize the polygenic nature of gray mold resistance in tomato and establish a foundation for marker-assisted selection, application of GS, and downstream functional validation of key defense genes in tomato.

O33 Impact of Diabetes mellitus induced in early life on rat spermatogenesis and fertility

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Keywords: Diabetes mellitus, testis, spermatogenesis, testosterone, Androgen receptor

A large number of studies indicate that Diabetes mellitus (DM) causes male infertility via action at multiple levels including altered spermatogenesis, degenerative changes in the testes, and reduced testosterone synthesis. We aimed to evaluate developmental effect of DM induced in early life on spermatogenesis and fertility. Diabetes was induced by single i.p. injection of streptozotocin on day 1 (neonatally, NDM) or day 10 (prepubertally, PDM) of male Wistar rats. Testes and sera were sampled on day 25 (puberty) and day 45 (post-puberty). Germ cells (GCs) and their sub types (spermatogonia, spermatocytes and spermatids); somatic cells - Sertoli cells (SCs) and Leydig cells (LCs) were counted. Androgen production by LCs (serum Testosterone levels) and action (Androgen receptor protein expression) were evaluated. Serum glucose was measured. On day 65 computer-assisted semen analysis was performed. Compared to control, glucose levels were elevated in higher extent in PDM than in NDM and the same tendency was found to the relative testis weight (testis/body weight). Total GCs number was slightly altered in puberty while in post-pubertal rats it was reduced only in PDM as a result of incomplete spermatogenesis and partial or complete loss of elongating spermatids (visualized by testicular Angiotensin Converting Enzyme). Hyperglycemia did not affect spermatogonial number while spermatocyte number was lower only in PDM. Spermatid number was elevated in NDM but reduced in PDM. SCs number was not significantly altered at both ages. Decreased number of LCs at puberty did not affect testosterone production due to higher ratio nuclear/cytoplasm volume in both DM groups. After puberty testosterone synthesis was diminished more pronounced in PDM. Protein expression of AR in SCs was altered in pubertal and post-pubertal PDM but not in NDM animals. Semen analysis showed reduced sperm concentration and motility in both groups. Profound elevation of round (undifferentiated) cells in semen samples was found in NDM. In conclusion, PDM affects spermatogenesis in a stronger extent compared to NDM. Germ cells are more vulnerable to DM at

the time of proliferative phase of spermatogonia (4.5-12 day) than the time of their mitotic arrest/quiescent period before day 4.5 in rat.

Acknowledgements: The study was supported by Grant No KP-06-N71/7 from the Bulgarian National Science Fund.

O34 SNP discovery in common bean (*Phaseolus vulgaris* L.) mutants using high-throughput genotyping

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Keywords: *Phaseolus vulgaris* L., induced mutagenesis, genotyping, SNPs

Common bean (*Phaseolus vulgaris* L.) is a globally important legume, valued for its adaptability to diverse agroecological conditions and its high nutritional content, particularly proteins and dietary fiber. To accelerate the development of improved varieties, integrating modern molecular techniques into mutation breeding programs presents a promising and powerful approach for enhancing desirable traits in a shorter time frame. The aim of this study was to identify genetic variability at the locus level by detecting single nucleotide polymorphisms (SNPs) in a diverse set of common bean mutant lines using high-throughput genotyping. A total of 118 common bean mutant lines were analyzed, comprising 86 accessions from Bulgaria, and 32 from North Macedonia. Genotyping of the above-mentioned collection was performed at CGIAR, specifically through the CGIAR Breeding Services and using the genotyping platform. Raw sequence reads were quality-checked using FastQC v0.11.9 and subsequently aligned to the reference genome using BWA v0.7.17. Reads were aligned to the initial genotypes and then to the selected *P. vulgaris* reference genome (GCF_000499845.2). After filtering by coverage depth and quality, 28,878 SNPs were detected, with the highest density observed on Chromosomes 2 and 8. Although phenotyping is still ongoing, the overall SNP distribution suggests that certain genomic regions may harbor loci or candidate genes associated with important agronomic and phenotypic traits. Future work will focus on the ongoing development of F_2 mapping populations for GWAS analyses. This approach aims to precisely identify candidate genes associated with drought tolerance, disease resistance, and enhanced productivity - traits that are vital for agriculture in Bulgaria and beyond, including Europe and other drought-prone areas worldwide. Preliminary results show that combining classical mutagenesis with high-throughput genotyping effectively reveals functional genetic variation in common bean, offering strong potential for speed breeding and molecular-assisted development of improved varieties.

Acknowledgements: ERA-NET CORE Organic (DIVERSILIENCE) (МОН—КП06ДО-02/7) and BUL/5/020 and MAK/5/010 funded by FAO/IAEA.

O35 Resting state of the cells is a reflection of the activity of electrogenic transporters

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Keywords: Na/K-ATPase, Ca-ATPase, SERCA, Na/Ca exchanger, K channel

Cell surface membrane resting state would depend from the interaction of conducting elements positioned on it. In biological membranes like cell surface membranes three types of current conductors could be defined. That would be channels, transporters and pumps. The channels would conduct some type of ions along its electrochemical gradient. Transporters would combine transport of a several substances thus mixing several electrochemical gradients into a single complex component. Pumps have a dissociation of ATP as an obligate part in definitions of their electrochemical gradients. The resting state would reflect their interactions. Channels and their currents are passive as a result in short time scales electrical membrane potential tends to adapt to current changes. However, in long time scales when currents are stable situation get reversed and ionic reversal potentials start to adapt to existing potential. Pumps are powerful and slow. The energy they transfer by a single cycle is carried by ATP and reflects the membrane potential of the inner mitochondrial membrane. It is several times bigger than physiological value for the surface membrane potential. Also, pumps have low turning rates of about 10/second while channels when open conduct with rates of about million times bigger. System composed of pumps and channels only, could not express physiological value of resting state as its attraction point. Only addition of electrogenic transporters to the system allows in principle balancing of the system around its physiological value. Even then a complex structure is required to meet the demand of the sources and the sinks. At the heart of such structures would be electrogenic transporters. And indeed the reversal potential of several ones are known to be close to the cell resting potential.

O36 Studying biomechanical properties and interactions in living and phantom tissue by light sheet optical microscopy (LSOM)

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Keywords: Brain Computer Interfaces (BCI), Tissue Engineering, Biomechanics, Light Sheet Optical Microscopy

Studying biomechanical interactions in living or phantom tissues is of high interest in fields such as BCI (Brain Computer Interfaces), Tissue Engineering, Cell Biology etc. It is known that the mechanical forces applied on various biological objects (cells/tissues/organs) play an important role in influencing their function and fate. This in turn plays crucial role in many important biological processes, such as cells/tissue/organ formation, growth, proliferation, differentiation and gene expression. Most of the biomechanical studies are done in 2D cell cultures. However, real life structures and formations occur in the 3D space and can behave drastically different compare to their respective 2D counterpart.

Therefore, new techniques and approaches are needed to study biological processes in 3D, in a way that the environment is as close as possible to the real one. For this purpose, we have developed a technique called Light Sheet Optical Microscopy (LSOM), to observe and measure the biomechanical response of phantom tissues based on hydrogels. This environment will mimic the biomechanical and diffusive properties of tissues in the brain, thus facilitating the study of biomechanical interactions of/in real brain tissues.

Of particular interest for us is the biomechanical behaviour of brain implants embedded in hydrogels as well as the viscoelastic properties of the medium itself. Being able to measure and tune these properties would allow for the manufacturing of useful tissue phantom models that can be used as working models of different tissues and a medium to grow 3D biological structures (e.g. organoids) for tissue engineering. Additionally, we will apply our technique as an elastography tool, i.e. to observe and extract the viscoelastic properties and responses of the developed various hydrogel media. This is an important step towards future applications, such as engineering of 3D tissues and organs.

POSTERS

P1 Molecular identification of *Ornithogalum* species using DNA barcoding

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Keywords: DNA barcoding, ITS, *Ornithogalum*, *Asparagaceae*

DNA barcoding is a rapid and reliable method for species identification that utilizes short, standardized DNA sequences. This approach involves sequencing a specific genomic region that varies between species but remains largely conserved within a species. The resulting sequences are compared against curated reference databases, such as the Barcode of Life Data Systems (BOLD) and GenBank, to achieve accurate species identification. DNA barcoding is especially valuable for detecting cryptic species, monitoring invasive species, and supporting taxonomic research. The integration of modern sequencing technologies with comprehensive reference libraries significantly enhances the precision, speed, and reliability of this method. Additionally, targeting the internal transcribed spacer (ITS) region improves discrimination among closely related species, facilitates subspecies differentiation, and aids in the detection of hybrids.

In this study, samples from 16 distinct *Ornithogalum* populations were investigated from diverse natural regions across Bulgaria. To characterize these populations, Sanger sequencing was performed on the ITS region as well as the chloroplast genes *trnL* and *rpoC1*. The variability of the Bulgarian population has not been studied until now. Our results showed significant levels of genetic variation of the species of genus *Ornithogalum*, not in all cases corresponding with morphological diversity. Future research is needed to clarify the taxonomic status of variability in the Bulgarian populations of *Ornithogalum*. The DNA barcoding techniques employed in this study enable reliable differentiation among species within the genus *Ornithogalum* and demonstrate strong potential for application in future analyses of additional taxa within the genus.

Acknowledgments: This study is supported by the Agricultural University - Plovdiv, grant 11-24

P2 Post-treatment elevation of circulating microRNAs in metastatic colorectal cancer responders: a pilot study

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Keywords: colorectal cancer, miRNA, miR-203a-3p

Circulating microRNAs (miRNAs) represent potential non-invasive biomarkers for assessing prognosis and treatment response in colorectal cancer. Our previous studies identified that serum miRNA-146a, miRNA-106a, miRNA-618, miRNA-15b-5p, miRNA-181b, and miRNA-203a-3p were significantly overexpressed in metastatic CRC patients before therapy. Notably, low levels of miRNA-618 and miRNA-203a-3p were associated with shorter overall survival. The present study aimed to evaluate post-treatment changes in these miRNA levels across different clinical responses (partial response, stable disease, and progressive disease) compared with pre-treatment levels. Six patients with metastatic colorectal cancer were evaluated for circulating miRNA levels before treatment and at response assessment. Serum miRNAs were isolated and analyzed by qRT-PCR using the $2^{-\Delta\Delta Ct}$ method. *KRAS*-mutated patients received bevacizumab/FOLFOX, while wild-type patients (*KRAS*/*NRAS*/*BRAF*) received cetuximab/FOLFIRI as first-line therapy. Response was assessed every 2-4 months. miR-203a-3p, miRNA-106a and miR-181b showed higher expression levels in patients achieving partial response (PR) or stable disease ≥6 months (SD) compared to baseline and progression levels. Patients with PR/SD demonstrated a 2.4-3.2-fold or greater increase in these miRNAs. When disease progression occurred in these patients, miRNAs showed lower levels but remained elevated compared to pre-treatment baseline. miRNA-203a-3p maintained consistently high expression in all patients with PR and SD, suggesting distinct roles in treatment response versus prognosis. Circulating miR-203a-3p, miRNA-106a, and miR-181b may serve as dynamic biomarkers for monitoring treatment response in metastatic CRC. Elevated levels correlate with favorable treatment outcomes, suggesting potential utility for real-time treatment monitoring. These preliminary findings warrant validation in larger cohorts to establish their clinical applicability.

Acknowledgements: This study is financed by grants from European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0009-C02.

P3 Analysis of UTR-localized transposon content in disease-associated genes, mRNAs, and long noncoding RNAs using the TR Viewer software

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Keywords: transposons, transposon fragments, TR Viewer software, visualization, disease-associated genes

The impact of transposon insertions on the human genome, transcriptome, and proteome has been extensively studied, revealing both beneficial and detrimental effects. Transposable elements (TEs) integrated within the 5' and 3' untranslated regions (UTRs) of mRNAs and long noncoding RNAs (lncRNAs) can influence gene expression levels, alternative splicing, and the spatio-temporal regulation of transcription. Dysregulation of transcription in TE-containing genes has been linked to increased susceptibility to cancer, autoimmune disorders, neurological conditions, and other diseases.

We have developed an original software tool, **TR Viewer**, for the visualization and statistical analysis of transposon insertions in genes and transcripts. Using TR Viewer, we conducted an initial analysis of UTR regions in both protein-coding and noncoding transcripts from disease-associated genes. The results reveal notable differences between disease-associated and general human genes, as well as between coding and noncoding transcripts. Specifically, we observed non-random patterns in TE family distribution (with enrichment of specific TE families), fragment size, and strand orientation relative to the associated gene. The discovered trends could help reveal potential molecular mechanisms underlying transcriptional dysregulation and its potential roles in disease susceptibility.

P4 Enhancing tomato yield and fruit size through gamma irradiation-induced mutagenesis

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Keywords: *Solanum lycopersicum* L., induced mutagenesis, productivity, fruit size, genome size

This study investigates the genetic basis of enhanced yield and fruit size in tomato (*Solanum lycopersicum* L.) following gamma irradiation-induced mutagenesis using several doses of ^{137}Cs ranged from 80 Gy to 350 Gy. Sixteen M_8 advanced mutant lines, developed from the variety 'Hektor', were grown under field conditions.

Plants from the M_5-M_7 generation were evaluated over three years for yield and yield-related traits (fruit number and weight). Several mutant lines demonstrated significantly higher fruit weight and total yield compared to the control, with fruit weight increases of up to 46% in the top-performing lines. For example, mutant line M_8-8 showed the highest average fruit weight while lines M_8-3 and M_8-10 exhibited both high fruit weight and overall yield consistently over multiple years. These differences highlight the potential of induced mutagenesis to generate beneficial genetic variation.

The genomic size of several mutant tomato lines was analyzed through flow cytometry. Genome size (2C DNA content) ranged from 1.79 to 1.86 pg among the mutants, compared to 1.89 ± 0.2 pg in the initial variety. These results provide insights into the potential genomic changes associated with these mutant tomato lines.

Mutant lines are currently under investigation to identify causative alterations in the genome. This ongoing research aims to deepen the understanding of the genetic basis of improved traits. Characterizing the underlying mutations responsible for the observed phenotypic changes accelerates and supports future breeding outcomes.

Acknowledgements:
ERA-NET CORE Organic (DIVERSILIENCE), funded by the EU and the Bulgarian NSF, project number КП06-ДО-2/7;
Project BUL/5/020, funded by the FAO/IAEA;

P5 Metatranscriptomic profiling of viruses in *Petunia hybrid* from Bulgarian commercial markets

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Keywords: virome, RNA sequencing, petunia

Petunia hybrida is widely cultivated in Bulgaria for ornamental landscaping and gardening. However, this broad cultivation under varying environmental conditions increases the plant's susceptibility to a diverse array of viral and viroid pathogens. Conventional virus detection methods—such as electron microscopy, serological assays, and nucleic acid-based techniques—have been widely used, but often lack the sensitivity required to detect low-abundance or previously uncharacterized viruses. In contrast, high throughput sequencing (HTS) offers a powerful, unbiased platform capable of identifying a wide range of viral agents without prior sequence information.

Recognizing the growing commercial and ecological importance of virome research in ornamental species, we conducted a metatranscriptomic analysis of *Petunia hybrid* plants sourced from commercial nurseries in the Plovdiv region, South-East Bulgaria. The objective was to characterize the viral composition and diversity present in both symptomatic and asymptomatic plants. Viral sequences were detected in all analyzed samples, underscoring that visible symptoms are not a reliable indicator of infection. The viromes were predominantly composed of petunia vein clearing virus (PVCV), cucumber mosaic virus (CMV), and tomato aspermy virus (TAV), along with sequences associated with fungal viruses and bacteriophages.

Our findings provide new understanding of how viruses interact with petunias, show that hidden infections can spread viruses, and highlight the potential risks these viruses pose to the ornamental plant trade.

Acknowledgments: This research was funded by the Bulgarian National Science Fund under Grant No. КП-06-Н 76/10 from 2023.

P6 Identification and characterization of ABI2 molecular partners, involved in the ABA-modulated flowering pathway

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Keywords: abscisic acid, flowering regulation, interacting partners, plant development, transcription factors

The plant hormone abscisic acid (ABA) is an important regulator of plant growth and development and plays a crucial role in responses to both biotic and abiotic stresses. Recent studies have shown that ABA influences the floral transition in plants. For instance, ABA-activated SnRK2 kinases negatively regulate flowering time through activation of ABI5-FLC module which delays floral transition [2]. Conversely, in the absence of ABA, PP2C phosphatases such as ABI1 and ABI2 inhibit SnRK2 activity, thereby initiating floral transition. However, the involvement of the PP2Cs in the regulation of flowering time largely remains unknown.

A recent study identified ABI2, a major PP2Cs in the ABA signaling cascade as a key component that fine-tunes ABA-mediated floral transition. CRISPR/Cas9-mediated loss-of-function *ABI2* mutants exhibited delayed flowering compared to the WT plants. In contrast, lines overexpressing *ABI2* displayed early flowering under normal growth conditions. Taken together, these results suggest that ABI2 rheostatically controls ABA signaling and flowering time.

Through Y2H screening, using Arabidopsis cDNA library, we identified several interesting ABI2 interacting partners. Notably prominent among these were one MYB- and one MADS-box transcription factors (TFs), which are presumed to take part in the flowering pathway as their homologs have already been shown to regulate flowering time. To test whether these TFs interfere with flowering time, knockout mutants of these genes were ordered from NASC stocks and tested their flowering phenotypes. Compared to WT, mutants of both TFs show early flowering, whereas *abi2-2* consistently shows late flowering phenotype. These results were further supported by molecular studies, elucidating the involvement of these TFs in the regulation of *FT* and *FLC* transcript levels, which are typical markers for induction and delaying floral transition, respectively.

P7 Cyclical dynamics of tRNA-derived fragments in endometrial tissue and uterine fluid extracellular vesicles

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Keywords: healthy endometrium, small RNA sequencing, tRNA-derived fragments, tRNA-Valine

The human endometrium undergoes cyclical changes critical for fertility, yet the molecular mechanisms that govern its receptivity are not fully understood. Transfer RNA-derived fragments (tRFs), a recently identified class of small non-coding RNAs, have emerged as potential regulators in reproductive biology, though their specific roles within the female reproductive system remain largely unclear.

In this study, we conducted small RNA sequencing on paired samples of endometrial tissue and uterine fluid extracellular vesicles (UF-EVs) collected across four distinct phases of the menstrual cycle: mid-proliferative, early-secretory, mid-secretory, and late-secretory (Research Ethics Committee of the University of Tartu, Estonia, protocol code No. 330M-8). Our analysis reveals that while microRNAs are the predominant small RNA species in both tissue and UF-EVs, tRFs represent a considerable and dynamically regulated subset—particularly within the endometrial tissue.

tRF abundance in endometrial tissue increases progressively through the cycle, peaking during the mid-secretory phase, which aligns with the window of implantation. Conversely, tRF levels in UF-EVs remain relatively low and consistent throughout the cycle. Most of the detected tRFs originate from cytosolic tRNAs, with fragments derived from tRNA-Lysine and tRNA-Valine being

particularly abundant. Notably, 5′-tRFs from tRNA-Valine are significantly upregulated in both tissue and UF-EVs during the early to mid-secretory phases, indicating a possible role in endometrial remodeling and intercellular communication.

Differential expression analyses highlight phase- and compartment-specific regulation of tRF subtypes, including the selective enrichment of certain isodecoder variants within UF-EVs. These findings offer new insights into the dynamic small RNA profile of the endometrium and suggest a functional role for tRFs in modulating endometrial physiology and receptivity.

Acknowledgements: This research was funded by European Union-NextGeneration EU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, grant number BG-RRP-2.004-0001-C01.

P8 Jacalin facilitates Au porphyrin cytotoxicity on prostate cancer cells PC3

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Keywords: Au porphyrin, complex, docetaxel, jacalin, prostate cancer

One of the leading causes for death among men is prostate cancer. In order to reduce the severe side effects of classical anticancer drugs for treating this type of cancer, our study focuses on seeking new compounds and their combinations. We investigated and compared the classical anticancer drug docetaxel and non-classical compounds, like Au porphyrin and the plant lectin jacalin, in different combinations on the prostate cancer cell line (PC3). Jacalin specifically recognises the tumour-associated Thomsen– Friedenreich antigen. The present study shows the interaction of jacalin with Au porphyrin, registered by the conformational changes in the protein due to the binding. From the titration curve, we calculated the affinity of 1.8±0.39 μM for the jacalin-Au porphyrin interactions. After treating of PC3 cells with docetaxel, Au porphyrin, jacalin, and combinations, a decrease in cell viability was registered. Interestingly, we found that the jacalin-Au porphyrin complex is more cytotoxic than the docetaxel-Au-porphyrin complex. Our results indicate the effects of the three compounds as well as the effect of their combinations on the treatment of PC3 cells. Interestingly, a low concentration of jacalin (3 μM) facilitated the cell cytotoxicity of Au-porphyrin. More experiments are planned in our future study.

P9 Betulinic Acid Derivatives on Cytokine Secretion by PBMCs

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Keywords: Betulinic acid salts, peripheral blood mononuclear cells, cytokines

Background: Betulinic acid (BA) has demonstrated potential for various biological activities, including anti-inflammatory, anticancer, and antiviral effects. In our recent study, we demonstrated that the modification of BA into amino acid ethyl ester salts ([AAOEt][BA]) improves its water solubility in some cases but may influence their immunomodulatory activity by affecting cytokine production.
This study aimed to compare the effects of BA and its derivatives on pro-inflammatory cytokine secretion by peripheral blood mononuclear cells (PBMCs) from healthy donors.
Materials and Methods: PBMCs were isolated from whole blood donated by healthy volunteers and cultured *in vitro* with lipopolysaccharide (LPS), betulinic acid (BA), [ProOEt][BA], [ValOEt][BA] or [LysOEt][BA]$_2$ at final concentrations of 2.5μM, 5μM, 10μM, and 20μM for 24 hours. The cytotoxicity was assessed using the MTT assay, and the half maximal inhibitory concentration (IC_{50}) values were determined. TNF-α, IL-6, and IL-18 levels were measured by enzyme-linked immunosorbent assay (ELISA). The data are presented as medians with interquartile range (IQR). A *p*-value less than 0.05 was considered significant.
Results: All tested compounds significantly reduced TNF-alpha and IL-6 secretion compared to LPS, even at the lowest concentration of 2.5μM. TNF-alpha levels decreased to 34.04 (21.2-46.9)pg/mL with BA, 54.8 (37.9-71.9)pg/mL with [ProOEt][BA], 71.2 (62.9-83.1) pg/mL with [ValOEt][BA] and 50.0 (30.7-56.9) pg/mL with [LysOEt][BA]$_2$ versus 318.7 (156.9-526) pg/mL with LPS. IL-6 levels followed a similar trend. In contrast, IL-18 secretion increased significantly after treatment with BA and all [AAOEt][BA] derivatives at lower concentrations (2.5–5μM) compared to untreated cells and was higher than LPS treatment. [ValOEt][BA] at 10μM (close to IC_{50} = 10.57μM) induced the highest IL-18 level of 494 (428.3–582.4) pg/mL.
Conclusion: The studied betulinic acid derivatives [ProOEt][BA], [ValOEt][BA] and [LysOEt][BA]$_2$ induced lower level of pro-inflamatory cytokines as TNF-a and IL-6 production of healthy PBMCs but enhanced IL-18 secretion, suggesting potential inflammasome activation.

Acknowledgments: This research has been supported by the National Science Fund of Bulgaria, contract No. КП-06-Н69/2.

P10 An AI-Powered Knowledge Base for Scientific Abstracts: A Case Study on Environmental DNA (eDNA) in Biomonitoring

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Keywords: AI, LLM models, RAG, eDNA, biomonitoring

Environmental DNA (eDNA) refers to genetic material shed by organisms into their environment, such as water, soil, or air. As a non-invasive biomonitoring method, eDNA has revolutionized biodiversity assessment by enabling detection of species presence without direct observation or capture. This approach is especially critical for tracking invasive, elusive or endangered species and monitoring ecosystem changes due to climate or anthropogenic pressures.

Over the past decade, a growing body of scientific literature has explored eDNA applications, resulting in a fragmented but rich landscape of domain-specific knowledge. Navigating this information is increasingly challenging for researchers and policymakers. To address this, we developed BioTrace, an AI-powered knowledge base designed to support conversational exploration of scientific abstracts focused on eDNA in biodiversity monitoring.

BioTrace leverages a Retrieval-Augmented Generation (RAG) architecture, integrating the mistral-saba-24b large language model via the Groq API for ultra-fast, low-latency inference. Scientific abstracts are indexed using a vector store, and retrieved passages are reranked using the all-MiniLM-L6-v2 model to improve answer relevance. Users can query the system in natural language and receive grounded, context-aware responses that synthesize findings across multiple studies. So far, the knowledge base includes more than 4000 abstracts regarding eDNA studies.

This work demonstrates the potential of large language models (LLMs) to distil scientific literature into accessible, structured knowledge. BioTrace empowers users with real-time, interpretable insights into eDNA research, serving as a blueprint for future AI-based tools in ecological and environmental sciences.

Acknowledgments: This study is financed by the European Union—NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project BG-RRP-2.004-0001-C01.

P11 Fungal community dynamics in compost: site- and phase-dependent patterns revealed by ITS amplicon sequencing

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Keywords: Eukaryotic microbiome, Next-generation sequencing, Composting, Thermophilic and mesophilic phases, Site-specific microbial communities

Composting is a dynamic biological process driven by microbial communities that transform organic waste into stable, nutrient-rich material. While bacterial succession during composting is well-documented, fungal communities, key players in organic matter degradation, remain underexplored, particularly in relation to composting phase and site-specific conditions. In this study, we investigated the diversity and structure of eukaryotic microbiomes in compost samples collected from two Bulgarian composting facilities (Harmanli and Yasno pole) during thermophilic and mesophilic phases. High-throughput sequencing of the internal transcribed spacer (ITS) region was employed to profile fungal communities across four representative samples: A1 (thermophilic, Harmanli), A2 (mesophilic, Harmanli), B1 (mesophilic, Yasno pole), and B2 (thermophilic, Yasno pole). Sequencing yielded over 310,000 high-quality reads, allowing for the identification of taxon-assigned, unclassified, and unique tags, along with operational taxonomic units (OTUs) clustered at 97% similarity. OUT richness and taxonomic resolution were highest in sample B2, suggesting that the thermophilic phase at the Yasno pole site supported the most diverse fungal assemblages. In contrast, the lowest diversity was recorded in A1, indicating thermophilic conditions at Harmanli may favor a narrower set of thermotolerant taxa. Unclassified tag analysis revealed the presence of potentially novel fungal lineages, particularly in mesophilic samples. These findings demonstrate that both composting phase and geographic site strongly influence the composition and richness of fungal communities. These insights contribute to a better understanding of fungal metabolism during composting and highlight the importance of site-specific management practices to optimize microbial-driven waste transformation.

P12 Modulation of Sorghum-associated fungal communities by *Trichoderma* bioinoculants: insights from ITS amplicon sequencing

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Keywords: *Sorghum bicolor*, *Trichoderma*, bioinoculant, ITS amplicon sequencing, pathogenic fungi suppression

Sorghum (*Sorghum bicolor* (L.) Moench) is an essential cereal crop, particularly well-suited to dryland farming. However, its growth and yield are often threatened by fungal pathogens in the soil. This study explores how bioinoculants based on *Trichoderma* influence fungal communities and their ecological roles in different plant compartments—namely, the soil, roots, seeds, and stems. Using high-throughput ITS sequencing and robust statistical tools, we observed that treatment with the bioinoculant led to a noticeable drop in fungal richness and evenness, especially in the seeds and stems, while encouraging the growth of certain beneficial fungal groups. Community composition varied significantly between treated and untreated plants (PERMANOVA, $p < 0.01$), with the most distinct changes seen in the aerial parts. Biomarker analysis (LEfSe) pointed to *Trichoderma* and *Mortierella* as characteristic of treated samples, while *Fusarium*, *Alternaria*, and *Penicillium* were more common in the controls. Functional profiling through FUNGuild revealed that fungi favoured by the treatment were predominantly decomposers or plant symbionts, whereas those prevalent in untreated samples included many pathogens. These findings indicate that applying *Trichoderma*-based bioinoculants can shift the sorghum microbiome in favour of fungi that support plant health, suppress harmful microbes, and enhance nutrient turnover. This work highlights the potential of microbial treatments to transform both the root zone and the inner plant microbiota, thereby contributing to more resilient and productive cropping systems.

P13 Effect of Cordycepin and Novobiocin on replication fork speed in tumor cell lines treated with 5-fluorouracil

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Keywords: POLθ, DNA damage, 5-FU, NVB

Chemotherapeutic agents induce replication stress (RS) through DNA damage, including double-strand breaks (DSBs), leading to quiescence, senescence, or cell death. Tumors deficient in homologous recombination (HR) rely on alternative repair pathways involving DNA Polymerase theta (POLθ). This study examined POLθ's role in DSB repair under RS in colon cancer cell lines with different mismatch repair (MMR) status (HT29: MMR-proficient; HCT116: MMR-deficient). Cells were treated with 5-FU alone or combined with POLθ inhibitors (Novobiocin [NVB], ART558) or Cordycepin. Cytotoxicity, replication fork dynamics, and DSBs were assessed. The 5-FU + NVB combination increased cytotoxicity compared to 5-FU alone. In HCT116 cells, POLθ inhibitors slowed replication forks, while Cordycepin co-treatment restored fork speed. In HT29, 5-FU alone caused more fork slowing than combinations. 5-FU + NVB enhanced DSB formation in HT29 at 24 h and in HCT116 at 48 h. These findings suggest POLθ involvement in RS response varies with MMR status and may trigger distinct repair pathways.

Acknowledgements: This research was supported by the Bulgarian National Science Fund, Grant KP-06-H61/2, 2022.

P14 Altered DNA replication dynamics in a cellular model of Rahman syndrome

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Keywords: DNA replication, H1.4 histone, Rahman syndrome

Rahman syndrome is a rare genetic disorder caused by frameshift mutations in the C-terminal domain of the histone variant H1.4, a key regulator of chromatin structure. While the transcriptional consequences of this syndrome are under investigation, its impact on DNA replication remains largely unknown. In this study, we examined how Rahman-associated H1.4 mutations affect DNA replication and the cellular response to replication stress. Using mouse embryonic fibroblasts (MEFs) carrying the Rahman mutation, we performed cell cycle profiling, EdU incorporation assays, and DNA fibre labelling to monitor replication fork dynamics. Our preliminary data indicate abnormal DNA replication profiles in mutant MEFs, including altered S-phase distribution and reduced replication fork progression. These findings suggest that the mutation impairs replication timing and fork stability. Further experiments are underway to explore the molecular mechanisms underlying these defects, with a focus on chromatin organization and stress recovery pathways.

Acknowledgements: This work was supported by КП-06-ПН-71/6.

P15 Assessing the efficiency of 23S rRNA metabarcoding in characterising phytoplankton community structure using mock communities

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Keywords: metabarcoding, eDNA, phytoplankton, mock communities

Molecular tools combined with bioinformatics are increasingly applied in biodiversity studies and biomonitoring. Metabarcoding of environmental DNA has proven effective for characterizing phytoplankton community structure and dynamics in aquatic ecosystems. However, accurate taxonomic identification is hindered by incompleteness of reference barcode libraries and the difficulties in selecting a universally suitable barcode for both prokaryotic and eukaryotic microalgae. The presented research is part of a study that aims to assess the applicability and success of metabarcoding in characterizing phytoplankton communities by testing it on artificial mock communities of known composition. DNA was extracted from 11 phytoplankton strains from Thonon Culture Collection (4 Cyanobacteria, 4 Bacillariophyta, and 3 Chlorophyta). The 23S rRNA barcode region was amplified with primers targeting cyanobacterial and chloroplast ribosomes. Three mock communities were set up by combining amplicons in different ratios: (1) an equimolar mix (30 ng per strain), (2) a mix with varying DNA concentrations (1–50 ng), and (3) a mock with three abundant (50 ng), four low-abundance (1 ng), and four rare (0.1 ng) taxa. Each mock was sequenced in triplicate using Illumina technology. Reads were processed using DADA2, and taxonomy was assigned with Phytool, a reference library dedicated to microalgae. Ten of the eleven strains were detected in expected proportions, including all three rare taxa in Mock 3, demonstrating sensitivity to low-abundance species. All but 2 unexpected taxa were excluded by applying pipeline filtering thresholds, highlighting the importance of cutoffs in distinguishing true rare taxa from potential false positives. Half of the detected taxa were assigned to the species level, but only two matched the mock input. Others were misassigned, likely due to barcode limitations or strain misidentification. The high number of unassigned sequences reflects the limited coverage of the Phytool library for freshwater phytoplankton. Overall, 23S rRNA metabarcoding with DADA2-Phytool pipeline is a sensitive and effective method for phytoplankton community profiling. However, improving library coverage and marker selection

are essential in enhancing taxonomic resolution and reliability in ecological studies.

Acknowledgments: This work has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101079234 (BIOLAWEB project).

P16 Thermodynamic and spectral characterization of a type 2 diabetes drug: GLP-1 receptor agonist interaction with albumin

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Keywords: GLP-1 receptor agonist, thermodynamic analysis, spectral characterization, albumin interaction, type 2 diabetes

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) represent a significant advancement in the treatment of type 2 diabetes mellitus (T2DM), offering benefits beyond blood glucose lowering, including weight loss and cardiovascular protection. In this study, we present the thermodynamic and spectral characterization of a representative GLP-1 agonist, focusing on its structural integrity, stability, and interactions with albumin under physiological and stress conditions.
Thermodynamic analysis results obtained via differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC) demonstrate high thermal stability. ITC further revealed strong binding not only between the GLP-1 agonist and its receptor but also significant interaction with human serum albumin (HSA), which may influence the drug's pharmacokinetics and bioavailability.
Spectral characterization using ultraviolet-visible (UV-Vis) and fluorescence spectroscopy showed characteristic absorption and emission profiles, including changes upon binding to albumin, suggesting conformational modifications. These results emphasize the structural resilience of GLP-1 agonists and highlight the importance of albumin interaction for the delivery and stability of the drug, supporting the further development of effective and long-acting therapies for T2DM.

P17 The effect of petasin on lipid ordering of biomimetic systems, modeling mast cell plasma membranes

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Keywords: large unilamellar vesicles, lipid ordering, mast cell model membranes, petasin

Mast cells are multifunctional immune cells with a critical role in the development of inflammatory and allergic reactions by releasing various mediators. The plant extracts of *Petasites hybridus*, containing petasin as the main active substance, represent a new class of antiallergic drugs since they block leukotriene synthesis. A new mechanistic perspective for a better understanding of their pharmacological effects would be to study the interaction with lipids in biological membranes, which consist of raft-like (liquid-ordered phase, L_o) and non-raft (liquid-disordered phase, L_d) domains with distinct compositions and properties. Considering the importance of membrane structure and lipid molecular dynamics in the immune response of mast cells, we studied the effect of petasin on the membrane structural organization. For this purpose, large unilamellar vesicles (LUVs) in L_o/L_d phase coexistence that mimic the lipid composition of mast cell membranes in normal and pathological conditions (activated mast cells), as well as vesicles in the "pure" L_d and L_o lipid phases, were prepared. We studied the structural effect of petasin on the model membranes in different phase states by fluorescence spectroscopy of Laurdan-labeled LUVs. Petasin rigidified membranes in L_d phase and mast cell model membranes in L_o/L_d coexistence, while in L_o phase showed fluidizing effect. Furthermore, larger influence on lipid ordering was observed in more disordered membranes. The sesquiterpene ester petasin changes the membrane lipid order at the hydrophobic-hydrophilic level of the bilayer which could be of utmost importance for membrane-bound receptors, enzymes, and ion channels.

Acknowledgments: This study was financially supported by Grant KP-06-M81/3/04.12.2024. The authors thank the support provided via SICTB DO1-361/14.12.2023.

P18 The synthetic proteasome inhibitor MG132 affects DNA damage response and inflammation response in peripheral blood mononuclear cells after *in vitro* γ-irradiation

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Keywords: MG132, DNA damage and repair, inflammation response, PBMCs

MG132 is a tripeptide aldehyde derivative of leucine that reversibly binds and inhibits the proteolytic activity of the 26S proteasome without affecting its ATPase or isopeptidase functions. The aim of the present study was to analyze the modulatory effects of the proteasome inhibitor MG132 on the repair and inflammatory responses in irradiated human peripheral blood mononuclear cells (PBMCs).

To assess the levels of DNA double-strand breaks, a neutral comet test was performed. The inflammatory response was estimated by analyzing the levels of a panel of pro- and anti-inflammatory cytokines, as well as the transcription factor NF-κB and its negative regulator, the kinase IκBα, using ELISA assays.

Significant effects on the kinetics of DNA repair were observed between 10 and 60 min post-irradiation and complete DNA repair was achieved in MG132-treated cells within 60 min after irradiation. The effects of MG132 on the inflammatory response were examined by measuring the secretion levels of cytokines IL-6, IL-8, IL-10 and TNFα. The results indicated that MG132 suppressed the secretion of all analyzed cytokines, both when the proteasome inhibitor was applied alone or in a combination with radiation. While the levels of total NF-κB remained unchanged, the levels of phosphorylated forms of NF-κB and IκBα were significantly reduced.

P19 Magnetoliposomes for biomedical applications

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Keywords: magnetic nanoparticles, lipid, magnetoliposomes, biomedicine, nanocarriers

Advances in nanotechnology have enabled the development of competing carriers for effective therapies. Combining liposomes (as promising drug delivery carriers) with magnetic nanoparticles (MNPs) (as targets) allows the formation of magnetoliposomes, which can be guided by a magnetic field and tracked by magnetic resonance imaging at the same time. Magnetoliposomes could significantly improve the effectiveness of tracking and treatment methods due to the possibility of selectively directing magnetoliposomes to the target site and retaining the drug in the diseased tissue by applying a permanent magnet there. To study the mechanism of magnetic particle-liposome interaction and the effect of MNPs on the liposome stability, the single-component 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) vesicles were prepared. The interaction of MNPs with liposomes let to an insignificant reduction of POPC vesicle size and a slight increase of POPC zeta potential, measured by dynamic light scattering and zeta potential analysis. Furthermore, to investigate the degree of lipid order in the lipid bilayer, a low fluidizing effect of magnetic particles was established by using Laurdan fluorescence spectroscopy. Based on the experimental results, new fundamental knowledge about the effect of MNPs on model lipid membranes will be acquired with a focus on the application of magnetoliposomes in pharmacology and clinical practice.

Acknowledgements: This work was supported by the Bulgarian National Science Fund under the project KP-06-N88/6/2024 NanoLB; COST action CA23132 Magnetic Particle Imaging for next-generation theranostics and medical research (NexMPI); the Bulgarian Ministry of Education and Science via D01-352/2023 and DO1-361/2023, which are part of the National Roadmap for Scientific Infrastructures 2020-2027.

P20 Effect of palmitoyl-oxovaleroyl-phosphocholine on lipid order and phospholipase A_2 action in model membranes

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Keywords: oxidized lipids, lipid order, phospholipase A_2 action

Oxidized phospholipids (OxPLs) that arise from lipid oxidation during oxidative stress, and secretory phospholipase A_2 (sPLA$_2$) enzyme, which hydrolyse cell membrane phospholipids, are key players in inflammation and immune responses. While the fundamental mechanisms of OxPL and sPLA$_2$ activity are understood, a complete picture of their molecular mechanisms and their effects on membrane physical properties and morphology remains lacking. Since sPLA$_2$ activity depends on the lipid composition and physicochemical properties of the plasma membrane, the presence of OxPLs may directly or indirectly influence the enzyme's action. To investigate this, we examined the effect of the oxidized lipid palmitoyl-oxovaleroyl-phosphocholine (POVPC) on the membrane organization of large unilamellar vesicles, composed of palmitoyl-oleoyl phosphatidylcholine/sphingomyelin/cholesterol mixtures (a model system that mimics lipid rafts in cell membranes), using Laurdan fluorescence spectroscopy. Also, giant unilamellar vesicles were studied via fluorescence microscopy to visualize how the presence of POVPC modulates sPLA$_2$ action. Our results demonstrated that POVPC increases the lipid order in a concentration-dependent manner. Additionally, an enhancement of sPLA$_2$ action was observed with increasing POVPC levels. These findings elucidate a potential mechanism by which POVPC modulates membrane organization and enzymatic function, providing novel insights into the molecular basis of inflammation and its regulation in human health.

Acknowledgments: The work was supported by Grant KP-06-N88/6/2024 NanoLB (BNSF), D01-361/2023 (SICTB) and CA22153/2024 EuroCurvoBioNet (COST Action).

P21 PFAS-induced changes in lipid order in human skin cells reveal membrane related mechanisms of toxicity

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Keywords: PFAS**,** lipid order, HaCaT cell line, biomimetic membranes

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that readily accumulate at biological interfaces, including epithelial tissues. Despite growing concerns over their health impacts, the effects of PFAS on structural changes of plasma membranes remain poorly understood. In this study, we investigated how three legacy PFAS—perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and the shorter-chain perfluorohexanoic acid (PFHxA)—influence lipid order in biomimetic and human skin keratinocytes (HaCaT cell line) membranes. Using generalised polarisation (GP) parameter of the membrane-sensitive probe Laurdan, we quantified PFAS-induced changes in membrane lipid packing. Our results revealed compound-specific effects. These alterations in lipid order occurred at sub-cytotoxic concentrations and preceded measurable oxidative stress or loss of cell viability, suggesting that PFAS may exert their toxic effects through primary membrane disruption. Our findings demonstrate that PFAS modulate membrane organisation in a chain length- and head group-dependent manner and support a model in which differential membrane effects contribute to compound-specific and tissue-specific toxicological outcomes.

Acknowledgments: The authors thank the Bulgarian Ministry of Education and Science, project KP-06-H88/6/2024 and COST Action CA22153 EuroCurvoBioNet. The authors thank the Bulgarian Ministry of Education and Science for support—the Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB) D01-361/2023 (Spectroscopy facilities) and the National Center for Biomedical Photonics D01-352/2023 (Malvern Panalytical Zetasizer Nano) - part of the Bulgarian National Roadmap for Scientific Infrastructures 2020–2027.

P22 *In Silico* analysis of *ISM1* missense variants in the Turkish population: Allele frequencies and functional implications

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Keywords: *ISM1*, PredictSNP2, SNP, SIFT, CADD

Isthmin 1 (*ISM1*) is a secreted matricellular protein implicated in angiogenesis, metabolic homeostasis, and innate immunity. Three naturally occurring missense variants—rs77255807 (T>C; S102P), rs3747933 (C>G; P193R) and rs117461286 (C>T; R352W)—map to conserved *ISM1* exons, yet their allele frequencies and potential functional impacts in the Turkish population remain uncharacterized. This study aims to estimate the allele frequencies of three *ISM1* missense variants (rs77255807, rs3747933, rs117461286) in the Turkish population and evaluate their functional impacts using *in silico* prediction tools to guide targeted genotyping and functional studies. In this study, minor allele frequencies were estimated *in silico* by computing weighted averages of 1000 Genomes Project subpopulation data reflecting the Turkish population's genetic admixture based on historical migration patterns. Functional impact was assessed using six prediction tools: SIFT, PolyPhen-2, REVEL, MetaLR, Mutation Assessor, and CADD. *In silico* results showed that C-allele frequency for rs77255807 (S102P) was 0.0382 (3.82 %); SIFT (0.61), PolyPhen-2 (0.203), REVEL (0.142), MetaLR (0.02), Mutation Assessor (0.25) predicted benign/low impact, whereas CADD = 21 suggests possible deleteriousness. G-allele frequency for rs3747933 (P193R) was 0.0035 (0.35 %); all predictors (SIFT 0.95; PolyPhen-2 0.04; REVEL 0.08; MetaLR 0.01; Mutation Assessor 0.15) indicated benign change, and CADD = 12 remained below pathogenic thresholds. Additionally, T-allele frequency for rs117461286 (R352W) was 0.0257 (2.57%); mixed benign-to-borderline scores (SIFT 0.12; PolyPhen-2 0.45; REVEL 0.31; MetaLR 0.15; Mutation Assessor 1.2), with CADD=18 indicating moderate risk. In conclusion, these integrated population-genetic and functional annotations provide a quantitative framework for targeted genotyping and downstream functional assays of *ISM1* variants in Turkish cohorts to elucidate their roles in vascular and metabolic regulation.

Acknowledgements: This work was supported by Çanakkale Onsekiz Mart University the Scientific Research Coordination Unit, Project number: TSA-2025-5039.

P23 Identification of key candidate genes in prostate adenocarcinoma using bioinformatics and machine learning approaches

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Keywords: Prostate adenocarcinoma, Bioinformatics, Machine learning, Candidate genes, Gene expression

Prostate adenocarcinoma (PAC) is one of the most common cancers in men and is associated with high mortality rates, particularly in advanced stages. This study aims to identify molecular biomarkers that can distinguish between early- and late-stage PAC and to contribute to early diagnosis and personalized treatment strategies through an integrative bioinformatics and machine learning approach. Gene expression profiles were obtained from The Cancer Genome Atlas (TCGA) and categorized based on tumor stage. Differentially expressed genes (DEGs) were identified using Student's t-test combined with Benjamini-Hochberg False Discovery Rate (FDR) correction (adjusted $p \leq 0.05$). The identified DEGs were subjected to various bioinformatics analyses. Functional enrichment was performed using KEGG, Reactome, and WikiPathways databases to explore relevant biological pathways, molecular functions, and cellular components. Furthermore, a protein–protein interaction (PPI) network was constructed to identify hub genes that play central roles in PAC-related molecular mechanisms. Following bioinformatics characterization, machine learning algorithms were applied to develop classification models that differentiate disease stages. For feature selection, Random Forest-based Recursive Feature Elimination (RFE) was used to iteratively remove less informative genes and retain the most predictive gene subset. These selected genes were then used to train supervised learning models, including Random Forest (RF), Support Vector Machines (SVM), AdaBoost, and Decision Trees (C5). Model performance was evaluated using Receiver Operating Characteristic (ROC) curves and Area Under the Curve (AUC) metrics, with the Random Forest model achieving the highest accuracy (AUC = 94.7%). Among the selected genes, GABRA1, GABRB2, and CHRNA4 emerged as key candidates. Functional analyses indicated that these genes are involved in neurotransmission processes mediated by GABA (gamma-aminobutyric acid) and nicotinic acetylcholine receptors, including synaptic signaling, membrane potential regulation, and intercellular communication. Overall, this study highlights the value of integrating bioinformatics and machine learning in the discovery of diagnostic biomarkers and provides a foundation for future clinical validation and therapeutic development in PAC.

P24 Therapeutic paths in breast cancer: novel pyrimidine-derived compounds with potential application in breast cancer treatment

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Keywords: breast cancer, drug toxicity, novel pyrimidine-derived compounds

Breast cancer remains a leading cause of mortality worldwide, with treatment hindered by drug toxicity, resistance, and limited long-term efficacy. In this study, we address these challenges by exploring novel pyrimidine-derived compounds similar to kinase inhibitors, with potential anticancer activity. Using breast cell lines with varying metastatic potential (MCF-10A, MCF-12F, MCF-7, and MDA-MB-231), we evaluated cytotoxicity via MTT assays and anti-migratory effects through wound healing assays. Changes in membrane structural organisation were monitored via Laurdan fluorescence spectroscopy and morphological investigations on cytoskeleton organization of the studied cell lines were performed by fluorescent labelling of actin filaments. Our results demonstrate that the pyrimidine-derived compounds have cytotoxic effects and selective effect was quantified by calculating the Selectivity Index (SI). Several pyrimidine derivatives showed significant antiproliferative and anti-migratory activities, accompanied by measurable alterations in membrane fluidity. Protein kinase inhibitors exert their anticancer effects by blocking key signalling pathways involved in cell proliferation, survival, and migration, primarily through inhibition of ATP binding to kinase domains. Considering the observed effects of the pyrimidine derivatives on cell viability, migration, and membrane dynamics, a potential mechanism of action may involve interference with kinase-mediated signalling cascades. Overall, this study highlights the promise of novel pyrimidine-based compounds, as strategies to improve breast cancer treatment.

Acknowledgements: This study was financially supported by Grant KP-06-M81/3/04.12.2024, KP-06-COST/30 and National Research Program "Young scientists and postdoctoral students-2", project "Interaction of conventional cytostatics with selected plant extracts on breast cancer models". The authors thank the support provided via SICTB DO1-361/14.12.2023.

P25 Biocrops: genetic and metabolomic insights into seaweed biostimulant priming for stress tolerance and nutritionally superior crops

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Keywords: biostimulant, GWAS, abiotic stress, gene editing

In recent years, freshwater resources are becoming scarcer due to climate change, overconsumption and depletion. The water shortage is causing significant damage to the agricultural sector, affecting the quality and yield of various crops. Seaweed biostimulants, such as SuperFifty (SF) derived from *Ascophyllum nodosum*, are eco-friendly products used for promoting plant stress tolerance through molecular priming. Preliminary research shows the ability of SF to boost stress tolerance and improve marketable yield in major crops, including tomato, pepper, maize, raspberry, and strawberry.

One of the main objectives of BIOCROPS is to study the relationship between SF biostimulant treatment and drought stress using GWAS panels of tomato and pepper (>300 lines), and to associate the SF-induced molecular priming with particular traits related to fruit quality and yield. Comprehensive transcriptomic and metabolite profiling (GC/UHPLC-MS, ICP-MS) will help identify genes, pathways, and metabolites that are regulated during biostimulant-induced stress protection and crop enhancement. Using CRISPR-Cas9 genome editing and/or RNAi/VIGS, we will validate selected candidate genes to confirm their functional roles.

BIOCROPS aims to decode the genetic and metabolic networks driving biostimulant effectiveness, advancing sustainable agriculture by delivering stress-tolerant, nutritionally enriched crops.

P26 Gold nanoparticle and oligoglycine interactions with human serum proteins: insights from microcalorimetry, dynamic light scattering, atomic force microscopy, and capillary electrophoresis

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Keywords: blood proteome; gold nanoparticles; oligoglycines

Gold nanoparticles are increasingly finding medical applications as drug-delivery agents. This study examines the interactions of human blood serum with gold nanoparticles as well as with oligoglycines at different concentrations. Gold nanoparticles are initially produced by pulsed laser ablation of a metal target, immersed in water and irradiated by a focused laser beam, characterised later using Atomic Force Microscopy. The effects of blood serum interactions with gold nanoparticles and oligoglycines were quantified by analysing protein denaturation profiles obtained by Differential Scanning Microcalorimeter after deconvolution by the main blood serum components. The parameters of the observed denaturation transitions before and after interaction were compared. Changes in the serum protein profiles were further assessed by capillary electrophoresis, revealing alterations in protein composition and distribution. Additionally, the properties of the gold nanoparticles upon interaction with oligoglycines are investigated by means of Dynamic Light Scattering with non-invasive backscattering and zeta potential measurement. Both techniques indicate a stable surface modification of the gold nanoparticles by the self-assembled oligoglycine structures. This study shows that gold nanoparticles and oligoglycines interact with human blood serum, causing stable surface modifications and changes in protein structure, supporting their potential for drug delivery.

P27 Morphological and genomic characterisation of novel bacteriophages active against multidrug resistant *Escherichia coli* strains

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Keywords: bacteriophage, *Escherichia coli,* antibiotic resistance, host specificity

Background. This study investigates the potential use of bacteriophages as a targeted biological control agent against resistant *E. coli* strains isolated from clinical and environmental sources. In this purpose, we have performed the morphological and genomic characterization of novel bacteriophages isolated from surface water, evaluated the lytic capacity and established their host specificity in order to formulate potential applications for treating or preventing *E. coli* infections and controlling faecal pollution of water sources.
Methods. Four bacteriophages were isolated from Dâmbovița River (surface water) and one from the influent of a wastewater treatment plant (WWTP) and further purified and enriched before storage in SM Buffer. Total DNA was obtained using PEG precipitation followed by phenol chloroform isoamyl alcohol isolation method. The morphology was investigated by TEM. Bacteriophage genomes were sequenced using the MinION (Oxford Nanopore Technology platform), assembled with Unicycler and annotated with PHASTEST and Bakta softwares. Lytic activity was assessed through spot assays and plaque morphology evaluation, using a panel of 30 multidrug-resistant (MDR) *E. coli* isolates from clinical and environmental sources.
Results. The TEM images revealed a morphology characteristic of the former Siphoviridae and Myoviridae families. The genome sequencing affiliated the studied phages to the *Kagunavirus* and *Kayfunavirus* genera. Bioinformatic analyses revealed the presence of lytic enzymes encoding genes (holins, spanins) and the absence of lysogeny specific genes, which support their potential use in therapy. The observed genomic similarity (80–90%) to known phages indicates that these isolates may belong to poorly characterized lineages. Host range analysis revealed effective lytic activity against up to 47% of the tested *E. coli* strains, with variable plaque morphologies indicating differences in phage-host interactions.
Conclusions. Considering wastewater as a sentinel source of circulating MDR clones, the isolated phages here may target clinically important strains beyond the local context, highlighting their relevance for international phage therapy initiatives.

P28 Advanced stochastic approaches for multidimensional integrals connected to computational biology

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Keywords: Computational Biology, Monte Carlo, Bayesian inference, Uncertainty

This work investigates the development and application of optimized Monte Carlo algorithms for the efficient evaluation of multidimensional integrals arising in computational biology. High-dimensional integrals frequently emerge in biological modeling for example in Bayesian inference for genetic networks and uncertainty quantification in biochemical systems. Traditional numerical integration techniques often struggle with the curse of dimensionality, leading to slow convergence and high computational cost. Building upon recent studies by Shaowei Lin, who highlighted the critical challenge of numerically integrating functions in spaces of up to thirty dimensions, this study focuses on improving the performance of Monte Carlo methods in such contexts. The proposed approach incorporates variance reduction strategies and adaptive sampling schemes, specifically tailored to the structure of biological integrands, which often exhibit localized features and high variability. Numerical experiments are conducted on a set of benchmark integrals with dimensionality ranging from three to thirty. The results demonstrate that the optimized Monte Carlo algorithm significantly outperforms standard crude Monte Carlo in terms of accuracy and convergence rate, particularly in higher-dimensional settings. The robustness and scalability of the method are also examined, providing insight into its potential for broader applications in systems biology and related fields. Overall, the findings confirm the effectiveness of the proposed algorithm in tackling complex integrals in computational biology, offering a valuable computational tool for researchers working with biologically motivated stochastic models and high-dimensional data.

P29 Structure-based drug modeling using intelligent algorithms and molecular data

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Keywords: Molecular Docking, Structure–Activity Relationship (SAR), Computational Drug Design, Receptor–Ligand Interaction, Drug Discovery

The development of computational methods and algorithms in recent decades has significantly expanded the possibilities for modeling complex biomolecular interactions. The present study presents an algorithmic approach for building a predictive model based on machine learning for analyzing the structure-activity relationship (SAR) of a series of bioactive compounds. A system for predicting the biological activity of new compounds is built by applying molecular docking to a receptor model, which is used as input for training the model, and then correlating the docking results to experimental biological data. In the proposed approach, molecular modeling, bioinformatics, and machine learning are combined into a single framework that evaluates potential drug molecules before experimental validation. These results demonstrate that computational sciences can be used to accelerate the design and optimization processes for drug candidates.

P30 Modeling the electrochemical behavior of materials regarding the hydrogen evolution reaction using machine learning

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Keywords: modeling, machine learning, microbial electrolysis, cathode materials, electrochemical techniques

The development of efficient cathode materials plays an important role in improving hydrogen production processes through microbial electrolysis (MES). MES is a bioelectrochemical process in which microorganisms degrade organic substances and, with the help of an externally applied voltage, hydrogen or other valuable substances (methane, acetate) are produced. Machine learning (ML) has an increasing role in the study of the electrochemical behavior of cathode materials, especially in the context of microbial electrolyzers. In this type of systems, ML can be used to analyze, model and predict electrochemical properties and behavior of cathodes, as well as for process optimization, which is of utmost importance. Machine learning methods can also be used to develop predictive models that successfully identify structural features of materials in relation to their assessment of their corrosion resistance, catalytic properties with respect to HER. As a result, new electrodes can be virtually tested. Key parameters of the hydrogen evolution reaction (HER) in MEC are the potential at which hydrogen evolution begins (Ve) and the rate of its evolution (Vh). The electrocatalytic properties of a given material can be analyzed by various electrochemical techniques such as linear voltammetry, chronopotentiometry, impedance spectroscopy, etc. In order to predict the electrochemical behavior, machine learning regression models are trained on experimental data based on electrochemical tests, physicochemical properties, operating conditions, etc. The focus of this study is modeling the electrochemical behavior of cathode materials with respect to the hydrogen evolution reaction (HER) in a microbial electrolyzer, with the aim of better understanding and optimizing their performance. Through this approach, new electrode materials, such as potential cathodes in a microbial electrolyzer, can be developed and analyzed more quickly and efficiently.

P31 Advanced techniques for molecular structures comparison in 3D

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Keywords: RMSD, Hausdorff distance, Manhattan distance, molecular similarity, 3D structure comparison

Bioinformatics researchers often use the root mean square deviation (RMSD) to determine the similarity between two data sets. The purpose of this study is to present an approach to calculate this metric by applying Manhattan distance in order to improve the accuracy of RMSD calculations. Results were compared with existing methods for determining the similarity between two three-dimensional structures, and the new method demonstrated much better performance. Furthermore, the Hausdorff distance is presented here as another method of assessing the similarity between molecular structures. It provides a complement to RMSD, allowing for a more accurate assessment of structural differences that are localized, and provides an alternative perspective on structural coincidence. By using RMSD and Hausdorff distance in combination, molecular structure is analysed more accurately by combining global and local similarity assessment. We developed a web-based C# tool to calculate and minimize RMSD in Manhattan space for bioinformatics research.

P32 Antifungal-Resistant candida in the city: environmental hotspots in urban river water

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Keywords: antifungal resistance, urban wastewater

Urban waterways increasingly serve as reservoirs for microbial contaminants, including opportunistic fungal pathogens. In this study, we investigated the presence of yeast species in water samples collected downstream of the Glina wastewater treatment plant, along the Dâmbovița River in Bucharest, Romania. Using ChromAgar Candida Plus, we selectively cultured presumptive *Candida* sp. colonies, followed by species-level identification via whole-genome sequencing on the Illumina MiSeq platform.

Species identification was performed using KmerFinder, revealing a diverse fungal community composed of both clinically relevant and environmental yeasts. Among the isolates, we identified *C. parapsilosis* (CA_II), *C. glabrata* (CGLAB), *C. tropicalis* (C3D, C7D), and *C. krusei* (C2D, synonym *Pichia kudriavzevii*), as well as two isolates of *Wickerhamiella sorbophila* (C8D, CG_I), a non-pathogenic yeast with potential biotechnological applications.

Notably, *C. parapsilosis* (CA_II) exhibited resistance to micafungin, as confirmed by E-test, suggesting potential antifungal resistance in environmental isolates. *C. krusei*, intrinsically resistant to fluconazole, and *C. glabrata*, often associated with reduced azole susceptibility, were also recovered, further highlighting the clinical significance of these findings. The detection of such species in treated wastewater-contaminated river water suggests that environmental reservoirs may contribute to the dissemination and persistence of antifungal-resistant *Candida* species outside healthcare settings.

Our findings emphasize the importance of environmental surveillance in the One Health framework and raise concerns about the underexplored role of urban aquatic systems in harbouring and possibly transmitting resistant fungal pathogens.

Acknowledgements: This work was supported by contract no.760238/28.12.2023, code CF 162/31.07.2023, funded by the EU's Next Generation EU instrument through the National Recovery and Resilience Plan of Romania - Pillar III-C9-I8. The authors gratefully acknowledge the support of Microbial Resource Research Infrastructure – European Research Infrastructure Consortium (MIRRI-ERIC) developed through MCUB (Microbial Collection University of Bucharest).

P33 Detection of Hepatitis E virus in black Rats (*Rattus rattus*) from pig farms in Plovdiv province, Bulgaria, using One-Step RT-PCR

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Keywords: hepatitis E virus, *Rocahepevirus*, *Paslahepevirus*, black rat, swine farms, one-step RT-PCR, Nested PCR

Hepatitis E virus (HEV) is an emerging zoonotic pathogen of increasing public health concern. Among the known HEV variants, rat HEV (genotype C1, *Rocahepevirus* genus) is commonly found in rodents, particularly rats, while HEV genotype 3 (HEV-3, *Paslahepevirus* genus), a zoonotic strain, is widespread in domestic and wild pigs and frequently detected in humans. Recent reports have identified rat HEV (HEV-C1) as a cause of hepatitis in humans, but its global prevalence remains underestimated due to limited data on its transmission and genetic diversity.
In this study, a total of 28 Black rats (*Rattus rattus*) were captured from pig farms in Plovdiv Province, Bulgaria. Liver tissues were collected, and total RNA was extracted using the Trizol reagent method. HEV RNA was detected using a broad-spectrum one-step reverse transcription PCR (RT-PCR) with primers HEV-cs and HEV-cas (Johne et al., 2010), followed by nested PCR with HEV-csn and HEV-casn. HEV RNA fragments of approximately 335 nucleotides were successfully amplified in 15 of the 28 samples, indicating a prevalence of 53%.
These findings highlight a substantial presence of HEV-like RNA in rat populations near pig farms, suggesting a potential role of rats in the epidemiology of HEV. Further genomic and epidemiological studies are essential to characterize the circulating HEV strains in rodents and to assess their zoonotic potential and role in interspecies transmission.

Acknowledgments: This work is funded by the Plovdiv University research fund, project №MUPD25-BF-004.

P34 Colostrum: the first source of beneficial microflora

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Keywords: human milk, colostrum, microbiome, infant, biological functions

Human milk harbors a diverse and intricate microbiome that is specifically structured rather than randomly composed, consisting of well-organized microbial communities and interactions. Various bacterial genera, including *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Cutibacterium* (previously known as *Propionibacterium*), *Lactobacillus*, *Lactococcus*, and *Bifidobacterium*, have been identified using both traditional culture techniques and modern molecular methods. These microbial populations in colostrum and mature milk are transferred to the infant and represent some of the earliest contributors to the establishment of the gut microbiota. Nevertheless, the exact role and impact of human milk microorganisms in shaping the infant gut microbiome remain to be fully understood. Changes in the human milk microbiota can influence infant gut colonization, metabolic processes, immune system function, neuroendocrine development, as well as the health of the mammary gland. For our study, we collected samples from healthy lactating women and examined the relationship of the colostrum microbiome with various other factors that could potentially influence its composition, such as mode of delivery, gestational week, and others. We found no significant differences in microbiome composition and mode of delivery. The composition of the colostrum microbiome is greatly influenced by the mother's diet.

Acknowledgements: This research was funded by the Bulgarian National Science Fund, project number KP-06-M81/6 (КП-06-М81/6).

P35 Green biocontrol: chitosan gel-beads encapsulation of *Bacillus subtilis* against soilborne phytopathogens

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Keywords: *Bacillus subtilis*, chitosan gel beads, encapsulation, biocontrol, phytopathogens

Developing sustainable plant protection technologies is essential to reducing reliance on synthetic pesticides. This study presents an eco-friendly biocontrol strategy using *Bacillus subtilis* encapsulated in chitosan gel beads to suppress phytopathogenic fungi. Chitosan, a natural biopolymer with intrinsic antimicrobial properties, was used to produce gel beads via ionotropic gelation, employing varying molecular weights to assess impacts on structure, water retention, and bacterial viability.

Scanning electron microscopy revealed a porous microstructure conducive to microbial encapsulation and controlled release. Encapsulated *B. subtilis* remained viable after long-term storage and successfully germinated upon rehydration, confirming retained functionality. Antifungal efficacy was evaluated against two major soilborne plant pathogens: *Fusarium avenaceum* and *Rhizoctonia solani*. Co-culture assays demonstrated that encapsulated *B. subtilis* significantly inhibited fungal growth, whereas control beads without bacteria had no effect. Chitosan molecular weight influenced bead porosity and water retention, with minimal impact on bacterial growth kinetics. All formulations supported gradual microbial release and reduced fungal proliferation, offering durable protection in soil environments.

In summary, chitosan gel beads provide a stable, biodegradable delivery system that maintains the antifungal activity of *B. subtilis*. This technology represents a practical, low-impact alternative to chemical fungicides and supports the transition toward sustainable plant disease management.

Acknowledgements: This study was funded by the European Union – NextGenerationEU, Investment C2.I2 “Increasing the innovation capacity of the Bulgarian Academy of Sciences in the field of green and digital technologies” under the Grant BG-RRP-2.011-0005-C01 “Design of Innovative Polymeric Materials as Biocontrol Agents with Application in Eco-Agriculture” (AGROBIOMAT).

P36 Electrospun poly(3-hydroxybutyrate) carriers for immobilization of antifungal yeasts

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Keywords: electrospinning, poly(3-hydroxybutyrate), chitosan oligosaccharide, hydroxyethyl cellulose; yeasts

Plant pathogens pose a serious threat to global food security, driving interest in biocontrol agents such as yeasts as sustainable alternatives to synthetic pesticides. Previous research has demonstrated the successful incorporation of beneficial bacteria into poly(3-hydroxybutyrate) (PHB) biopolymer matrices coated with cellulose derivatives, ensuring long-term viability and growth.

This study explores the immobilization of novel yeast strains with known antifungal properties using electrospun PHB fibers coated with chitosan oligosaccharide (COS) and 2-hydroxyethyl cellulose (HEC). Scanning electron microscopy and water contact angle measurements were used to characterize the materials, revealing improved surface wettability. Microbiological assays confirmed sustained yeast viability and effective adhesion to the modified PHB surfaces. The COS and HEC coatings significantly enhanced the hydrophilicity of the PHB fibers, which facilitated yeast adhesion and prolonged viability. Additionally, the yeasts demonstrated the ability to metabolize COS and HEC as sole carbon sources, underscoring their enzymatic versatility and potential contribution to biomass degradation.

These findings highlight the promise of PHB-based fibrous materials as eco-friendly, biologically compatible platforms for delivering viable yeast biocontrol agents in plant protection applications.

Acknowledgements: This research was funded under the Project BG-RRP-2.011-0005-C01 with the financial support by the European Union - NextGenerationEU, Investment C2.I2 “Increasing the innovation capacity of the Bulgarian Academy of Sciences in the field of green and digital technologies”.

P37 Diversity of *Enterococcus faecalis* bacteriophages isolated from wastewater in Bulgaria

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Keywords: bacteriophages, *Enterococcus faecalis*, phage therapy, antibiotic resistance

The increasing emergence of antibiotic-resistant bacteria is considered a "silent epidemic", globally, requiring urgent action in the development of new effective treatments for severe bacterial diseases. One of the most promising solutions nowadays is the **phage therapy** – application of bacteriophages as antibacterial agents. Thus, the isolation and full characterization of new potentially therapeutic phages is a critical step in the development of new and effective phage-based therapeutics. The aim of this study is to investigate the diversity of bacteriophages, infecting *Enterococcus faecalis* - a Gram-positive bacterium, recognized as leading causative agent of severe human infections like bacteremia, urinary tract infection (UTI), endocarditis, etc. The objects were three bacteriophages, isolated from wastewater in Bulgaria (vB_SEF_8, vB_SEF_13 and vB_SEF_15). Double agar overlay plaque assay was used for studying the diversity in plaques' morphology, host range, stability at storage temperature (4°C) for 9 months and sensitivity to different pH (2.0 to 13.0) and temperatures (28°C to 95°C). RFLP analyses was used for establishing the molecular diversity between the phage isolates. All phages formed clear plaques on *E. faecalis* lawn (d, mm – 0.68 to 3.21). Twenty-nine *E. faecalis* strains were used as host in host range analyses. vB_SEF_8 showed the greatest host range infecting 41% of the tested strains (n=12), followed by vB_SEF_13 – 21% (n=6) and vB_SEF_15 – 7% (n=2). All phages remained viable in wide pH range (4.00 to 10.5) and up to 80°C after incubation. The storage of crude phage lysates at 4°C for nine months resulted in slight decrease in phages titers (up to 2 $\log_{10}$ units). RFLP analyses with restriction enzyme Hind*III* revealed that the three phages differed significantly as they formed different restriction patterns. Our experiments revealed diversity among the isolated *E. faecalis* phages and are solid bases for continuation of phage research in Bulgaria.

Acknowledgements: This study was financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008", scientific group 3.2.4.

P38 Distribution of MDR *Enterococcus faecalis* in wastewater and their susceptibility to bacteriophages isolated in Bulgaria

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Keywords: bacteriophages, *Enterococcus faecalis*, MDR, wastewater

Wastewater is a habitat of diverse bacteria, especially originating from human and animals' intestine. *Enterococcus faecalis* is a normal part of mammals' enteric microbiota and thus its persistence in wastewater is expected. However, this bacterium is considered as one of the leading causative agents in difficult-to-treat hospital-acquired infections. Moreover, it possesses plastic genetic material with a large capacity to receive and transmit genes for antibiotic resistance which is a prerequisite for the development of MDR in some strains. Thus, the aim of this study is the establishment of phenotypic antibiotic resistance of *E. faecalis* strains isolated from wastewater in Bulgaria and the evaluation of the potential of three *E. faecalis* phages, previously isolated in Bulgaria, to destroy the potential MDR strains. Nine wastewater samples, collected for a period of three months, were used as sources for *E. faecalis* isolation. A total of 37 bacterial strains were isolated using Slanetz and Bartley selective agar medium. The strains were identified via MALDI-ToF mass spectrometry as *E. faecalis* (n=13), *E. faecium* (n=21) and *E. hirae* (n=3). All strains were tested for phenotypic antibiotic susceptibility to 13 antibiotics according EUCAST. MDR was detected in 32% of the isolates: *E. faecium* strains (n=8), *E. faecalis* strains (n=2, WeS3 and WeS10) and *E. hirae* strains (n=2). Three *E. faecalis* bacteriophages, part of our laboratory phage collection, were tested for their capacity to destroy the two MDR *E. faecalis* strains. The results showed that *E. faecalis* WeS3 was destroyed by phage vB_SEF_8 and *E. faecalis* WeS10 by phage vB_SEF_13. These results cleary show that our bacteriophages can be considered as potential agents against multydrug resistant *E. faecalis*. Our findings are good bases for continuation and deepening of the phage potential as antimicrobial agents in Bulgaria.

Acknowledgements: This study was financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008", scientific group 3.2.4.

P39 Physiological, kinetic and technological characteristics of the N_2-fixing *Azotobacter vinelandii* NBPMCC 1619

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Keywords: *Azotobacter*, N_2-fixing capacity, bioinoculant

The ability of *Azotobacter* spp. to fix N_2 non-symbiotically has been extensively studied. In recent years, *Azotobacter* spp. has become an essential bioinoculant component and an environmentally friendly alternative to chemical fertilizers due to its N_2-fix potential and production of secondary metabolites (especially phytohormones) and exopolysaccharides. The *Azotobacter* inoculants formulation and their high-scale production comprise an essential approach to increasing crop production under quality-assured and economically acceptable conditions. The objectives of this study were to establish the physiological, kinetic, and technological characteristics of *the Azotobacter vinelandii* NBPMCC 1619 strain and to optimize the biochemical and technological (fermentation and processing) parameters of the cultivation process for the production of bioinoculants to improve plant and soil health. *Azotobacter vinelandii* NBPMCC 1619 was subjected to batch cultivation on four different nutrient media and under three regimens of pO_2 saturation. The growth and N_2-fix capacity were monitored for a period of 72 h, and the kinetic parameters of the process (generation time [g], (maximal)specific growth rate [μ and μ^{max}], and growth yield [Y]) were calculated. The viability of the batch cultures was tested after storage for 6 months at 4 °C and subsequent freeze drying. Among all tested conditions, the *Azotobacter vinelandii* NBPMCC 1619 strain showed the highest nitrogen-fixation capacity (19.0–24.4 µg/ml N) when grown on nitrogen-free nutrient media, outperforming media containing organic, inorganic, or combined nitrogen sources. On the same N-free medium, the strain showed g = 6.59 h, $\mu = 0.105\ h^{-1}$, $\mu^{max} \approx 0.134\ h^{-1}$, and $Y \approx 3.47*10^8$ CFU/g glucose used**.** When subjected to low and ultralow temperatures preservation, the *A. vinelandii* NBPMCC 1619 cultures significantly kept their viability ($1.7*10^9$ to $3.4*10^8$ for the cultures stored at 4 °C for 6 months and $3.4*10^8$ to $5*10^7$ for the freeze-dried cultures). The kinetic characterization of *A. vinelandii* NBPMKK 1619 in batch culture under selected optimal physicochemical conditions allowed standardization of the cultivation process for scaling up active biomass production. The proposed efficient regime of post-fermentation processing and storage ensures good survival of the bioinoculant.

Acknowledgments: This work is financially supported by SU Science Fund project No 80-10-20/21.05.2025.

P40 Comparative analysis of metabolism of prebiotic and dietary carbohydrates in *Lactobacillus species* from breastmilk microbiome

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Keywords: Breastmilk, prebiotics, synbiotics, dietary carbohydrates, glycosyl hydrolases

This study explores the metabolic diversity of *Lactobacillus* strains isolated from human breastmilk, with an emphasis on their capacity to utilize different types of dietary prebiotics. A comparative species-level approach highlights the relevance of microbial functional diversity in shaping the infant gut microbiome. Eight strains were isolated and identified, including representatives of *L. rhamnosus*, *L. brevis*, and *L. plantarum*.

To investigate carbohydrate utilization, bacterial growth and acidification were assessed in modified MRS media supplemented with selected prebiotic substrates.

Building on these findings, a subset of three representative strains (1 *L. rhamnosus* and 2 *L. plantarum*) was subjected to in-depth phenotypic profiling using the Biolog PreBioM™ system, which assesses utilization of a broad panel of prebiotic and dietary carbohydrates. While all three strains primarily used fermentation-based metabolism, the *L. rhamnosus* strain exhibited a detectable color shift in the redox dye, suggesting a capacity for oxidative respiration. Strain- and species-specific differences in prebiotic preferences were noted and guided the selection of substrates for enzymatic assays.

All eight strains underwent enzymatic activity assays targeting key glycoside hydrolases involved in the degradation of lactulose, cellobiose, and lactose. The results confirmed inter- and intra-species variation in enzymatic activity, with specific strains exhibiting elevated β-galactosidase and β-glucosidase functions.

Overall, the outcomes reveal the functional variability among *Lactobacillus* strains isolated from breastmilk and emphasize the need for detailed strain-level profiling when selecting compatible probiotic-prebiotic combinations. The findings contribute to the development of targeted synbiotic strategies, especially for shaping the infant gut microbiome, and provide a foundation for future genomic and functional investigations into host-adapted *Lactobacillus* species.

Acknowledgments: This research was funded by the Bulgarian National Science Fund, project number KP-06-M81/6 (КП-06-М81/6).

P41 Utilization of industrial waste - a cheap resource for microbial production of lactic acid

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Keywords: DDGS, wood chips, lactic acid

In recent years, there has been growing interest in producing lactic acid (LA) from second-generation sources, such as agricultural and industrial waste. This study explored the ability of two strains of Lactiplantibacillus plantarum—Lf53 and 2HS—to produce LA using waste materials. The researchers tested two types of pretreated waste: distiller's dried grains with solubles (DDGS) and wood chips, and compared the results to those using lactose as the carbon source. Both bacterial strains successfully utilized the sugars from these wastes. The fermentation process yielded about 20 g/L of LA from 23 g/L of reducing sugars in DDGS, which was comparable to the 22 g/L of LA produced from 22 g/L of lactose monohydrate. The hydrolysate derived from wood chips contained 10 g/L of reducing sugars (RS), and its fermentation led to the production of 5 g/L of lactic acid (LA). This yield is half of what was obtained from fermenting 11 g/L of lactose monohydrate.

Acknowledgements: The authors express their gratitude to Project PVU–63 from 16.12.2024 г. /BG-RRP-2.017-0047-C01 for the financial support.

P42 Production of lactic acid through microbial fermentation of whey - from waste to products with high added value

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Keywords: whey, *Lactiplantibacillus plantarum,* lactic acid

Recently, there has been growing interest in producing lactic acid (LA) from second-generation sources as industrial waste. In this study, several *Lactobacillus* strains were evaluated, and two *Lactiplantibacillus plantarum* strains—Lf53 and 2HS—were chosen to assess their ability to produce LA from waste-derived materials. Cheese whey was tested as a substrate for LA production. Their performance was compared to that of lactose, used as a standard carbon source. Both strains were able to effectively utilize sugars from all the waste material. Approximately 22 g/L of LA was produced from 21 g/L of whey lactose, closely matching the 22 g/L of LA yielded from 22 g/L of lactose monohydrate in the control setup.

Acknowledgements: The authors express their gratitude to Project PVU–63 from 16.12.2024 г. /BG-RRP-2.017-0047-C01 for the financial support.

P43 Modeling the fermentation process of the lactic acid production from different agro-industrial waste materials

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Keywords: lactic acid production, *L. plantarum*, mathematical modeling, parameter identification

Lactic acid production from different agro-industrial waste materials by *Lactiplantibacillus plantarum* was studied. A mathematical model of the fermentation process includes a set of coupled differential equations describing cell growth, substrate consumption, and product formation. Distiller's dried grains with solubles (DDGS), spent coffee grounds (SCG), wood chips, and cheese whey were used as substrates after pretreatment, and the results were compared with those with lactose as a carbon source. A mathematical model based on the Compertz and Luedeking – Piret equations was proposed and tested, showing very good agreement with experimental data.

Acknowledgements: The authors express their gratitude to Project PVU–63 from 16.12.2024 г. /BG-RRP-2.017-0047-C01 for the financial support.

P44 HPLC analysis of sugar content in agricultural waste hydrolysates – a step towards the sustainable synthesis of bioactive compounds

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Keywords: hydrolysates, wastes, sugars, HPLC, sustainability

Agricultural and industrial wastes have become the primary focus of research in recent years as a potential feedstock for the production of various bioactive compounds, for example, lactic acid (LA). This study aims to determine the content of monosaccharides – glucose, xylose, arabinose, and mannose in acid hydrolysates of agricultural wastes. The following materials were subjected to acid hydrolysis: dried distilled grains with soluble (DDGS); grain leftovers (GL); sunflower seed press cake (SSPC); spent coffee grounds (SCG); and sunflower seed husks (SSH).

The purpose of this work is to facilitate the future implementation of the results as part of technology for a more profound conversion of agricultural waste. In the future, sugars and other valuable components derived from waste could replace the costly ingredients in fermentation broths. This could impact the operational expenses of bioactive compounds production via fermentation.

P45 Evaluation of three culture media for micropropagation of *Prunus mahaleb* (L.) rootstock using the PlantForm™ TIS bioreactor

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Keywords: *Prunus mahaleb* L.*, t*emporary immersion systems*,* PlantForm™ TIS bioreactor

Prunus mahaleb (L.) is a commonly used cherry rootstock for developing standard cherry trees with excellent compatibility, vigorous canopy development, and sustained fruiting performance, even in calcareous soils with elevated limestone content. Temporary immersion systems (TIS) have demonstrated positive effects on the growth and multiplication of various woody species, and plants derived from TIS have exhibited excellent performance during *ex vitro* acclimatization.
This study aims to investigate the efficiency of three culture media on the micropropagation of a mahaleb cherry genotype using the PlantForm™ TIS bioreactor with the same aeration and medium supply regime. *In vitro* cultivated shoots were grown in liquid proliferation media, including Murashige and Skoog (MS), McCown Woody Plant Medium (WPM), and DKW/Juglans medium, supplemented with 1 mg/L BAP (6-benzylaminopurine), 0.01 mg/L NAA (α-naphthaleneacetic acid) and 30 g/L sucrose.
The number of shoots was similar in MS (3.4 shoots/explant) and DKW (4 shoots/explant) but lower in WPM (1.46 shoots/explant), where the proliferated explants also produced more callus. The average shoot length was 19.29 mm in DKW, 18.3 mm in MS, and 14.34 mm in WPM. The number of leaves per explant was 29 in DKW, 22.7 in MS, and 16.14 in WPM. These findings highlight the superior performance of DKW and MS media over WPM for the micropropagation of *P. mahaleb* using TIS bioreactors.

Acknowledgments: This study was financially supported by COST Action CA21157 and the Bulgarian National Science Fund (project КП-06-КОСТ/17 (12.08.2024) “Optimizing micropropagation of valuable tree species through innovative PlantForm™ TIS bioreactors”).

P46 Detection of Phytoplasma in Sweet Cherry and Peach Orchards in the Plovdiv Region, Bulgaria

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Keywords: Cherry, Peach, Phytoplasma, nested PCR detection

Phytoplasmas are associated with significant economic losses in fruit crops worldwide. The ecology of phytoplasma-related diseases is complex, typically involving one or more insect vectors and, in some cases, alternative plant hosts. Both the concentration of phytoplasmas within infected plants and the expression of disease symptoms can vary markedly between seasons, with symptom remission frequently observed.
In this study, a total of 225 samples were collected from the shoots, leaves, and flowers of 46 sweet cherry trees (*Prunus avium*) and 29 peach trees (*Prunus persica*). Nested PCR, a widely adopted method for phytoplasma detection and molecular diagnostics, was used to analyze the samples. Genomic DNA was extracted from phloem tissues, including shoot bark, leaf midribs, stalks, and petioles. The results revealed that 46 samples from both cherry and peach trees tested positive for phytoplasma DNA using the universal primer pairs P1/P7 and R16F2n/R16R2. Two of the amplified products were subsequently characterized by Sanger sequencing, and the resulting sequences were submitted to GenBank for annotation.

Acknowledgments: This study is supported by the Bulgarian National Science Fund under Grant No KP-06-N 36/10 from 2019, “Actual phytopathological challenges in fruit tree species in Bulgaria: Unexplored and invasive align phytopathogens with potential risk for biodiversity and biosecurity”.

P47 Biofilm formation by *Enterococcus* isolates from Serbian traditional goat cheese

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Keywords: *Enterococcus* sp., biofilm formation, traditional food

This study explores the biofilm-forming potential of *Enterococcus* isolates derived from Serbian traditional goat cheese. In the absence of added starter cultures, the microbiological profile of this cheese is inherently shaped by the autochthonous microbiota originating from raw materials and the production environment. Among the 71 tested *Enterococcus* isolates, 15 demonstrated the ability to form biofilms, a trait essential for their colonization, persistence, and contribution to the maintenance of factory-specific microbial signatures. Specifically, the isolates exhibited a range of biofilm-forming capacities, from weak to strong. These findings emphasize the adaptive capacity of enterococci to withstand environmental fluctuations and their integral role in shaping the sensory and microbial characteristics of traditional cheese.

P48 Antibacterial efficacy of Si-Cu nanocomposites against gram-negative bacteria

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Keywords: sol-gel, nanocomposites, antibacterial properties, Gram-negative bacteria

The rise of antibiotic-resistant bacteria poses a significant global health challenge, particularly with respect to Gram-negative pathogens such as *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, which are responsible for a wide range of infections. Conventional antibiotics are becoming increasingly ineffective against these resistant strains, necessitating the exploration of alternative antimicrobial strategies. In recent years, nanotechnology has gained attention as a promising approach for the development of novel antimicrobial agents. Among various nanomaterials, metal-based nanocomposites, particularly those incorporating silicon and copper, have shown considerable promise due to their unique synergistic properties. Silicon is known for its biocompatibility, while copper exhibits potent antimicrobial activity against a broad spectrum of bacteria. In this study, we investigated the antibacterial efficacy of silicon-copper (Si-Cu) nanocomposites against Gram-negative bacterial strains, including *E. coli*, *Salmonella typhimurium and Pseudomonas aeruginosa*. The nanocomposites were synthesized using a controlled chemical "sol-gel" method and characterized by electron microscopy and X-ray diffraction. Antibacterial activity was assessed through Diffusion assays on solid medium and Minimum bactericidal concentration (MBC) measurements in liquid medium. The results demonstrated a significant inhibition of bacterial growth, suggesting that the synergistic effect of silicon's biocompatibility and copper's antimicrobial activity enhances the overall effectiveness of the nanomaterial. These findings highlight the potential of Si-Cu nanocomposites as alternative agents in combating Gram-negative bacterial infections, particularly in the context of rising antibiotic resistance.

Acknowledgments: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project SUMMIT BG-RRP-2.004-0008-C01 № 4120, contract 70-123-661/16.04.2024.

P49 Influence of silicon on the micropropagation of peach rootstock GF677

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Keywords: tissue culture, in vitro, woody plants

The rootstock GF 677 (*Prunus amygdalus* × *Prunus persica*) is the most commonly used rootstock for peach in Europe, propagated almost exclusively by tissue culture. It is tolerant to Fe deficiency and especially suited to soils with poor fertility, low water availability, and high $CaCO_3$ content. In the recent years many studies have shown that silicon (Si) has a positive role in improving various aspects of plant micropropagation (organogenesis, somatic embryogenesis), cryopreservation, and production of secondary metabolites. The aim of the study was to improve the micropropagation of the GF 677 rootstock by applying silicon (Si) as an additional component of the nutrient medium. The plantlets were cultivated in vitro on Murashige and Skoog (MS) nutrient medium, supplemented with 5 µM 6-benzylaminopurine (BAP), 0.01 µM indol-3-butyric acid (IBA), 30 g/l sucrose, 6.5 g/l Phyto agar (Duchefa, The Netherlands). Silicon was added to the culture medium as calcium ($CaSiO_3$) or potassium (K_2SiO_3) silicate (1 or 2 mg/l). Control plants were cultivated on a silicon-free medium. At the end of 3 passages of 4 weeks each, some biometric indicators, fresh and dry biomass were recorded. The results obtained showed that the addition of 1 mg/l potassium silicate to the culture medium stimulated the accumulation of biomass and the development of new shoots, although the latter was not statistically proven. Incorporation of Si into tissue culture media might have favourable effect on micropropagation of the peach rootstock GF677, but source and concentration must be thoroughly tested.

Acknowledgments: This study is based upon work from COST Action CA21157 "European Network for Innovative Woody Plant Cloning", supported by COST (European Cooperation in Science and Technology) and is financially supported by the Bulgarian National Science Fund (projects KP-06-COST/17 "CopyFruitTree: Innovative approaches for woody fruit species cloning").

P50 Effect of LED light on in vitro cultivation of Persian walnut (*Juglans regia* L.)

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Keywords: micropropagation, tissue culture, light, recalcitrant, woody species

The Persian walnut (*Juglans regia* L.) is one of the most valuable nuts cultivated in the world due to its valuable nutritional composition and health benefits. The micropropagation of walnut trees is difficult and present some complications, such as establishment of culture, irregular and low multiplication or rooting rates, and high number of losses during ex vitro acclimatization of plantlets to soil. The present work aims to focus on opportunities to improve the in vitro cultivation of walnut by LED light. The plants studied were cultivated on DKW medium supplemented with BAP (0, 2.5 or 5 µM) under a Philips GreenPower LEDs research module with four spectral regions: white (W), red (R), blue (B) and mixed (W:R:B:far-red = 1:1:1:1). The control plantlets were grown under fluorescent lamps (FL) and the PPFD (Photosynthetic photon flux density) of all treatments was set at 87 ± 7.5 µmol m^{-2} s^{-1}. The effect of different light sources on some growth parameters of plantlets was monitored. Mixed light (WBR) stimulated the development of explants and the accumulation of biomass. Excellent results were also obtained with plants cultivated under white light (W) on a nutrient medium with the higher concentration of BAP (5 µM). The red light (R) had a beneficial effect on stem length.

Acknowledgments: This study is based upon work from COST Action CA21157 "European Network for Innovative Woody Plant Cloning", supported by COST (European Cooperation in Science and Technology) and financially supported by the Bulgarian National Science Fund (projects KP-06-COST/17 "CopyFruitTree: Innovative approaches for woody fruit species cloning").

P51 A look at the identification, characterization and incidence of cherry leaf roll virus in cherry in South Bulgaria

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Keywords: sweet cherry, sour cherry, CLRV, detection, spread

Cherry leaf roll virus (*Nepovirus, Secoviridae*) has been known by wide host range including numerous woody and herbaceous plant species. That suggests the existence of a great diversity of variants and isolates of the virus. Among the fruit tree species cherry leaf roll virus (CLRV) is an economically important pathogen for sweet cherry and walnut. In Bulgaria, CLRV was studied in walnut, but research on the virus in sweet and sour cherries are limited. The current study aimed to ascertain the occurrence of CLRV in cherry orchards in the South Central and Southeast regions, and the characteristics of the virus isolates spread in cherry in South Bulgaria. Symptoms observation and sampling were carried out in sweet and sour cherry crops located in Plovdiv, Pazardzhik, Stara Zagora, and Burgas provinces. A total of 230 samples were tested for the cherry strain of CLRV by Enzyme-linked immunosorbent assay (ELISA). Reverse transcription – polymerase chain reaction (RT-PCR) was carried out using two primer pairs corresponding, respectively, to the coat protein (CP) gene and 3' end of untranslated region (3`UTR). Biological tests were conducted on woody and herbaceous plants. CLRV was detected in 15.2% of tested sweet cherry samples by ELISA whereas by RT-PCR with the primer pair corresponding to CP genomic region, the virus was identified in 25% of the samples. In the sample of sour cherry ELISA failed to detect CLRV, but the virus was identified by RT-PCR in 8.7% of the tested samples. Isolates of CLRV were successfully transmitted by grafting on the peach indicator GF 305, sweet cherry cultivar 'Sam' and cherry rootstock Gisela 5, and by mechanical inoculation on the herbaceous species *Chenopodium quinoa*, *C. amaranticolor* and *Nicotiana benthamiana*.

Acknowledgements: This study is supported by the Bulgarian National Science Fund under Grant No KP-06-N 36/10 from 2019, "Actual phytopathological challenges in fruit tree species in Bulgaria: Unexplored and invasive align phytopathogens with potential risk for biodiversity and biosecurity".

P52 ‘Spasena’ – a new Bulgarian peach cultivar resistant to plum pox virus

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Keywords: peach, Sharka disease**,** field resistance

Plum pox virus (PPV), the casual agent of Sharka disease, is the most devastating viral pathogen on stone fruits (*Prunus* sp.) worldwide. Efforts to find PPV-resistant *Prunus* genotypes began in the 1930s, immediately after the virus was discovered. So far some success has been achieved in the breeding of plum and apricot varieties resistant to the virus, but it is believed that there are no natural sources of resistance to PPV within the peach (*Prunus persica* L. Batsch) genotypes. In this report, we present the results of a long-term study on the response of the peach cultivar ‘Spasena’ to PPV under high PPV natural infection pressure. The cultivar ‘Spasena’ is a clingstone peach cultivar, result of the breeding program aiming the establishment of peach cultivars resistant to biotic and abiotic stress factors. The research was conducted during 2013 – 2025, in an experimental orchard of Fruit Growing Institute – Plovdiv. The observations for presence/absence of Sharka symptoms on the leaves and fruits were assessed annually. All trees included in the study were analyzed for PPV by enzyme-linked immunosorbent assay (ELISA) and reverse transcription – polymerase chain reaction (RT-PCR). The data from a twelve-year study showed that Sharka symptoms appeared sporadically every few (3-4) years on single leaves of three ‘Spasena’ trees of all 31 surveyed trees, and no symptoms were detected on the fruits. During the growing seasons in which no visual Sharka symptoms were observed on the leaves of the studied trees, ELISA failed to detect the virus and it was only identified by RT-PCR. That suggests the virus is a low titer in the PPV-positive peach trees and its replication and distribution in plant tissues are hindered by plant defense mechanisms. Based on the results obtained, the new Bulgarian peach cultivar ‘Spassena’ can be determined as resistant to PPV under field conditions. As to our knowledge this is the first report of a peach (*P. persica*) genotype resistant to PPV.

P53 Recovery of Human adenoviruses from wastewater samples in cell culture model

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Keywords: wastewater, Human Adenoviruses, cell cultures

Viruses are omnipresent and persistent in wastewater, which poses a risk to human health. Not all detectable by molecular methods viruses are infectious. The recovery in cell cultutre of potentially pathogenic viruses from wastewater is of concern since it can pose risks to human health and could be the sources of infection in waterborne disease outbreaks. To prevent wastewater transmission and decrease the threat to human health, there is a need to better understand the critical role of wastewater as a potential source of viruses. Also the spread of viruses in the human population can be traced since the predominant virus strains circulating in the human population usually are isolated from wastewater. Several studies have estimated that more than 90% of the human population worldwide is seropositive for one or more of the Human Adenoviruses serotypes. Human Adenoviruses occur at significantly higher frequencies in wastewaters than other enteric viruses. In our work wastewater samples periodically collected from different cities in Bulgaria were tested through real-time polymerase chain reaction for the presence of viral pathogens that attack humans. The samples, in which a large amount of human adenovirus DNA was detected, were used to infect the VERO E6 cell line. The infected cell line was cultured for 5 days. After a freeze-thaw cycle, the culture fluid was used for the next viral passage. Cytopathic effect was observed only at the third viral passage (after two consecutive blind passages) and was limited to small areas of the cell monolayer. At the fifth or sixth passage, the virus multiplied in sufficient numbers for the cytopathic effect to cover almost the entire monolayer. Virus replication in cell culture was confirmed by real-time PCR. The human adenovirus isolated from the first sample collected in the city of Sofia in February 2024 was sent for whole genomic sequencing, which identified it as species *Mastadenovirus adami* (virus name *human adenovirus 31*).

P54 Evaluation of the disinfection effectiveness of the plasma sources ß-device and Surfaguide on the liquid fraction of digestate from WWTP "Kubratovo"

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Keywords: digester, disinfection, wastewater utilization, plasma, pathogens

One of the waste products in wastewater treatment plants (WWTPs) is water released from the dewatering of stabilized sludges from digesters. Often, it is returned to the plant's inlet for retreatment because it contains organics, nitrogen, phosphorus, pathogens, etc. In the context of the circular economy and the sustainable management of water resources, the liquid phase of digestate could be used for agricultural purposes after processing. A new and promising method for the inactivation and removal of pathogens is plasma treatment. It generates highly reactive particles, including oxygen and hydroxyl radicals, ozone, high-energy electrons and UV radiation. The present study aimed to evaluate the effect of treatment by two types of plasma sources (β-device and Surfaguide) on the water quality from digesters at the WWTP "Kubratovo", in terms of chemical parameters and the elimination of microorganisms.

The Surfaguide treatment showed a high effectiveness of disinfection (100% for total coliforms, *E. coli*, *Clostridium sp.*; 99.9% for faecal coliforms; 99.8% for total coliforms and 98.2% for *Salmonella sp.*). The ß-device eliminated with a high percentage *E. coli* and *Clostridium sp*., but for the remaining microbial groups it had lower effectiveness. The liquid phase of the digestate contained high concentrations of organics and ammonium ions before and after treatment with the plasma sources. The water had a twofold increase in phosphate concentration after plasma treatment. The obtained data showed that the treatment of water from digesters with Surfaguide is a promising disinfection method. Using the plasma treated water of digestate reduces the need for synthetic fertilizers and impact on water sources used for agricultural irrigation.

P55 Plasma as a promising disinfection tool in wastewater treatment technologies: case study at WWTP "Ravda"

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Keywords: digester, wastewater, pathogens, plasma treatment

The sustainable utilization of biodegradable waste is a key environmental and energy priority, requiring innovative approaches to enhance the safety and effectiveness of the involved processes. Microorganisms, including pathogens, are present in wastewater that is produced when excess sludge is treated in wastewater treatment plants (WWTPs). The object of this study is the liquid phase of digestate, separated after the anaerobic stabilization of excess sludge at the WWTP "Ravda", which treats wastewater from the settlements and resorts of Ravda, Sunny Beach, Aheloy, Nessebar, etc. The aim of the study was to assess the disinfection effectiveness of liquid digestate after anaerobic sludge stabilization at the WWTP "Ravda", treated with two plasma sources (ß-device and surfaguide). The following physiological and taxonomic groups of microorganisms were analyzed: aerobic heterotrophs (AH), anaerobic heterotrophs (AnH), faecal and total coliforms, *E. coli*, *Salmonella sp*., and *Clostridium sp*.

The results showed that surfaguide achieved significantly higher effectiveness compared to the ß-device in the inactivation of pathogens and other groups of microorganisms. Effectiveness of bacterial elimination was higher for surfaguide and lower for ß-device - for the amount of AH, the reduction reached 99.18% with surfaguide versus 16.32% with the ß-device, and for AnH – 99.84% and 68.98%, respectively. Complete elimination (100%) of faecal and total coliforms, and of *Salmonella sp*. was achieved only for the surfaguide plasma source. The study showed that surfaguide treatment riches significantly higher disinfection effectiveness and potential for application in digestate recovery processes, while also ensuring sanitary safety of the liquid digestate which can be used for agriculture.

P56 Genome exploration and enzymatic activity of a *Pseudomonas fluorescens* strain isolated from wastewater

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Keywords: *Pseudomonas fluorescens*, ONT, genome sequencing, enzymes, bioremediation

A bacterial strain AXL was isolated from wastewater and taxonomically identified as *Pseudomonas fluorescens* using Oxford Nanopore sequencing technology. The complete genome of strain AXL was sequenced and annotated, revealing approximately 5,600 predicted genes, including around 1,300 putative enzymes. A more detailed analysis of the enzymatic repertoire and the strain's metabolic potential is currently underway, with a focus on its applicability in environmental biotechnologies.

Phenotypically, the strain exhibited saccharolytic activity and the ability to grow on media containing aliphatic alcohols as sole carbon and energy sources. Enzymatic profiling confirmed extracellular production of phospholipase C and lipase. Culture conditions were optimized to enhance phospholipase C secretion, with maximal activity detected during the early stationary phase (12–14 hours) in soy-casein medium supplemented with 0.5% xylose. Notably, high phospholipase activity was sustained in the culture medium for up to 20 hours, suggesting enzyme stability and resistance to proteolytic degradation. The strain demonstrated psychrotolerant growth at 4°C, with temperature-dependent phospholipase synthesis.

Taken together, these results position *P. fluorescens* AXL as a promising candidate for advanced biotechnological applications, particularly in enzymatic biotransformation and cold-adapted bioremediation of hydrocarbon-contaminated wastewater environments.

Acknowledgements: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project BG-RRP-2.004-0001-C01.

P57 Biosorption of tetracycline from model aqueous solutions on *Ganoderma resinaceum* biomass: effect of process parameters, kinetic and equilibrium studies

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Keywords: biosorption, *Ganoderma resinaceum*, tetracycline

Biosorption of tetracycline from model solutions was investigated using *Ganoderma resinaceum* biomass as biosorbent pretreated with sodium hypochlorite. The study was conducted in a batch system, evaluating the effects of initial pH, initial tetracycline concentration, and contact time. To describe the kinetics of the process, pseudo-first-order and pseudo-second-order models were applied to the experimental data. The results showed that the biosorption kinetics followed the pseudo-second-order model proposed by Ho. For equilibrium modeling, both Langmuir and Freundlich isotherms were employed. The equilibrium data were better described by the Langmuir model, with a calculated maximum biosorption capacity of 14.2 mg/g under the following conditions: initial pH 5.0, contact time of 120 minutes, biosorbent dosage of 1 g/L, and tetracycline concentration range from 4 to 20 mg/L.

P58 Profiling of endogenous phytohormones in shoots of transgenic *Llccs* lines of *Viola cornuta*

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Keywords: genetic transformation**,** cytokinins, auxin, jasmonic acid, ABA

Capsanthin/capsorubin synthase (CCS) is an enzyme of the carotenoid biosynthetic pathway that catalyzes the synthesis of the red plant pigments, namely capsanthin and casporubin, in fruits and flowers of plants. Transgenic lines of *Viola cornuta* cv. "Lutea Splendens" were regenerated after genetic transformation with *Agrobacterium tumefaciens* harbouring the empty pWBVec10a vector (*Llccs*$^{-}$) or the pWBVec10a/CaMV 35S::Llccs::TNos vector (*Llccs*$^{+}$) carrying the capsanthin/capsorubin synthase gene (*Llccs*) from the tiger lily (*Lilium lancifolium*). Due to the lower shoot multiplication and rooting ability of *Llccs*$^{-}$ and *Llccs*$^{+}$ transgenic lines, compared to the non-transformed control plants, we wanted to investigate the phytohormone profiles of the regenerated shoots. The resulting phytohormone analyses showed that the manipulation of carotenoid synthesis significantly affected the endogenous phytohormone status in the shoots of *V. cornuta*. The results show that the total content of cytokinins and their precursors is more than 30% higher in transgenic shoots (*Llccs*$^{-}$ and *Llccs*$^{+}$) than in non-transformed shoots. A significant change in total auxin content (45% increase) and auxin catabolic products (1.4-fold) was only observed in *Lccs*$^{+}$transgenic shoots compared to *Llccs*$^{-}$ and the non-transformed shoots. In addition, a significant increase in the content of jasmonic acid (by 68.5%) and gibberellic acid (8.9-fold) was observed in the shoots of the transgenic *Llccs*$^{+}$ lines. In contrast, a significant decrease in ABA content (65.7%) was observed in the *Llccs*$^{+}$ transgenic lines compared to the non-transformed and *Llccs*$^{-}$transformed shoots. Our results suggest that manipulation of the carotenoid biosynthetic pathway by metabolic engineering of capsanthin/capsorubin synthesis may lead to significant alternations in the endogenous phytohormone profile, which may further affect plant growth and regeneration, especially in photosynthetic tissues such as shoots.

P59 Species identification of yeast and moulds in yellow cheese

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Keywords: *Cladosporium*, *Rhodotorula*, *Torulaspora*, yellow cheese

Yellow cheese is traditional dairy product prepared from cow's or sheep's milk. Due to the production technology, cheese pieces are exposed for a prolonged period to open chambers to dry and ripen. Without taking proper measures these chambers provide favourable conditions for the development of yeast and moulds. Although general procedures for strict hygiene can avoid or remove contamination with yeast and moulds, their species identification may reveal their ecology, origin, growth conditions and suggest improvements in the prevention routines.

In the present study two samples were analysed either directly from discolouration spots on the surface of the product (case 1) or after cultivation on selective Yeast Extract Glucose Chloramphenicol (YGC) Agar (case 2). After PCR amplification of part of the ribosomal genes cluster, comprising both intergenic spacers, they were sequenced with the di-deoxy dye termination procedure with universal primers ITS1 and ITS4. The obtained sequences were then searched with BLAST against the NCBI database. In Case 1, microscopic observation showed that red spots on the surface of the product were caused by yeast, while dark brown spots contained hyphae of a mould. The causative agents were identfied as *Rhodotorula glutinis* and *Cladosporium cladosporoides*, respectively. In Case 2, the sampled colonies on YGC agar were determined to belong to the yeast species *Torulaspora delbrueckii* and the mould *Penicillium* sp. *Rhodotorula* species have been identified in dairy products, which is attributed to their ability to grow rapidly under refrigerated conditions. Similarly, the identification of *Cladosporium cladosporoides* among spoilage moulds of cheese is derived from the psychrophilic nature of this species. Representatives of *Torulaspora* have been reported as part of the yeast microflora of artisinal cheese. *Penicillium* sp. is reported as common contaminant of air and cheese-making equipment, being resistant to sanitizers.

The applied method of sequencing the intergenic spacer regions in yeast and mould genomes is a widely applied method for their species identification and in this study it resulted in equivocal identificatioon of yeast and moulds contaminating yellow cheese.

P60 Optimization of key medium components and growth parameters for high density growth of probiotic lactic acid bacteria strains

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Keywords: lactic acid bacteria, growth parameters, high cell density

Lactic acid bacteria (LAB) are amongst the most common microorganisms used in the production of probiotic products such as foods and supplements. Their beneficial role in the human microbiome and through it, the well-being of the whole organism, has been studied and pointed out by scientists and nutrition experts for decades. Industrial production of probiotics is a complex process, affected by multiple factors. In this study, the effect of key parameters (inoculum concentration, pH, agitation) and medium components (glucose and yeast extract concentrations) have been studied with the objective of optimizing the production of cultures with high cell density of LAB strains with a promising probiotic potential - two strains *Limosilactobacillus fermentum* N 2 and TC 3-11, *Lactobacillus delbrueckii* subsp. *lactis* VG 2 and *Weissella confusa* NN 1. An increase of the optical density of cultures of all four microorganisms was achieved, exhibiting species-specific and strain-specific preferences of optimal growth conditions. Three of the strains, except *W. confusa* NN 1, also showed an increase of the cell density (CFU/ml) after the optimization of the parameters, with the most pronounced increase observed for the strain *L. fermentum* TC 3-11. Two out of the four tested strains - *L. fermentum* N 2 and *L. delbrueckii* subsp. *lactis* VG 2, were cultivated in their exhibited optimal parameters in a scale experiment in the laboratory bioreactor for evaluation of culture growth dynamics and strain-specific cultivation characteristics.

Acknowledgements: This work was supported by SU-project 80-10-79 / 27.05.2025. The support of the Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG), project BG16RFPR002-1.014-0001, funded by the Program "Research, Innovation and Digitization for Smart Transformation" 2021-2027, co-funded by the EU, is greatly acknowledged.

P61 Hydrolase enzyme production by filamentous fungi through solid-state fermentation of spent coffee grounds

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Keywords: hydrolase enzymes, filamentous fungi, spent coffee grounds, solid-state fermentation

The sustainable utilization of agro-industrial residues through microbial biotechnology offers a promising path for both waste valorization and the production of high-value biomolecules. In this study were investigated and optimized the solid-state fermentation (SSF) of the mold fungi strains *Trichoderma reesei* M7 and *Aspergillus awamori* K1, focusing on the enzymatic production of carboxymethyl cellulase (CMCase) and xylanase using spent coffee grounds (SCG) as the main substrate. The research explores the effects of key fermentation parameters, including initial moisture content, zeolite supplementation, and enzyme extraction conditions, on enzymatic activity and total soluble protein yield. Results demonstrate that the optimal moisture content varies between the two fungi strains and has a significant impact on the levels of enzyme production. Furthermore, the incorporation of zeolite as a structural matrix enhances the substrate's porosity and has a positive impact on enzyme yield and protein content. These findings underscore the potential of SCG as a viable low-cost substrate for enzyme production and highlight the value of process optimization in microbial bioconversion. The study contributes to the growing field of circular bioeconomy by integrating waste valorization strategies with microbial enzyme biotechnology.

Acknowledgements: This work was supported by SU-project 80-10-79 / 27.05.2025. The support of the Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG), project BG16RFPR002-1.014-0001, funded by the Program "Research, Innovation and Digitization for Smart Transformation" 2021-2027, co-funded by the EU, is greatly acknowledged.

P62 Evaluation of biological activity of cosmetic formulations with propolis for skin healthcare

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Keywords: propolis, biological activity, skin healthcare

In response to the high demand for sustainable and bio-based cosmetics, at the end of the twentieth century a new interdisciplinary field focusing on developing innovative formulations with natural ingredients emerged. These unique products rely on the topical application of biologically active ingredients that have direct impact on the skin, aiming to improve its function. Propolis, a mixture of beeswax and saliva, plays a key role as a provider of the aforementioned substances in this study. The variety of compounds and phytonutrients included in propolis elucidate its extensive array of acknowledged biological activities, therefore affirming its suitability for personal care products. The aim of the current study was to prepare propolis-containing model cosmetic formulations and assess their antibacterial activity against five skin pathogens - *Cutibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Malassezia furfur* and *Candida albicans*. The most effective formulations were additionally tested to determine the minimum inhibitory concentrations of active components, as well as the time of contact needed to achieve pathogen inhibition. The successful development of model formulations efficient in treating dermatological conditions provides a solid foundation for improvement of personal care products, continually requested by customers from the increasingly expanding cosmetic industry.

Acknowledgements: This work was supported by SU-project 80-10-79 / 27.05.2025. The support of the Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG), project BG16RFPR002-1.014-0001, funded by the Program "Research, Innovation and Digitization for Smart Transformation" 2021-2027, co-funded by the EU, is greatly acknowledged.

P63 Inhibition effect of green-synthesized ZnO/Pr powders on selected Gram(+) and Gram(-) bacteria

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Keywords: antibacterial activity, green chemistry, doped oxide, nanoparticles

Inhibition effect of green-synthesized ZnO/Pr powders was tested on six Gram (-) and five Gram (+) bacterial species. The ZnO nanoparticles were produced by green synthesis with addition of Lavender or Thyme oils. The PXRD, SEM, EDS and XPS analyses were performed. The using of lavender essential oil leads to increased antimicrobial efficiency of the doped particles in comparison to that obtained with thyme oil. The non-doped particles exhibited higher antibacterial activity than the doped ones, due to the effect of the dopant, which changes the size and shape of particles. MICs were higher in bacteria that have pathogenic potential.

Acknowledgments: Thanks to the project with the Bulgarian National Science Fund, KP-06-N69/8 (КП-06-Н69/8), "Novel polymer-hybrid materials containing (bio)synthesized metal oxide particles with improved photocatalytic and antimicrobial potential" for the financial support and technical support from project PERIMED BG05M2OP001-1.002-0005.

P64 Study of the microbiological status of bottled natural mineral, spring and table waters for the period 2015 - 2024

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Keywords: bottled water, monitoring, *Pseudomonas aeruginosa*.

Mineral and spring waters are good examples of natural aquatic habitats, as these aquifers originate from underground water sources and are protected from human intervention.
This widespread use of water requires meeting certain sanitary and hygienic requirements set out in national and European regulations, thus ensuring its quality and safety. Compliance with these requirements is controlled through annual monitoring. The present study is in this regard.
The aim is to track the microbiological monitoring of bottled natural mineral, spring and table waters (including 19 liter gallons), Bulgarian production for the 2015 – 2024 period.
A total of 696 samples of bottled water were tested according to the requirements of the Regulation on the requirements for bottled natural mineral, spring and table waters intended for drinking purposes. The microbiological parameters by which the samples were tested are set out in national legislation. The methods used are ISO standards.
During the monitored period, the indicator *Pseudomonas aeruginosa* showed the highest non-standardity. Of all 45 analyses that did not meet the requirements of the Regulation, 33 were non-standard for the indicator *Pseudomonas aeruginosa* – 73.3%.
The high prevalence of *P. aeruginosa* in bottled water can be explained by its ability to form biofilms and its resistance to chlorine disinfection. These characteristics allow it to be preserved for a long time in pipe bottling systems in the production of bottled water.
P. aeruginosa is one of the most important opportunistic pathogens. Therefore, its occurrence in natural mineral and spring waters should be limited as much as possible.
Bottled water should be safe and healthy for consumption, as it comes from the water source without the need for treatment or disinfection.
European and national legislation does not allow the purification of natural mineral and spring waters to remove microorganisms.
The quality of natural waters is guaranteed by strict control of the entire process from ensuring water protection at the source, through the distribution channels to the end user, as the waters are given a bottling concession. Frequent quality tests and analyses based on HACCP and good hygiene practice standards verify the safety of the extracted groundwater.

P65 Effect of temperature on the pigment profile and antioxidant activity of *Scenedesmus incrassatulus* Bohlin (Chlorophyta) under intensive cultivation in a bioreactor

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Keywords: microalgae, bioreactor, temperature, pigments, antioxidant activity

Representatives of the genus *Scenedesmus*, including the species *Scenedesmus incrassatulus* Bohlin (Chlorophyta), synthesize a variety of photosynthetic pigments, including chlorophylls (*a* and *b*) and carotenoids such as β-carotene, lutein, violaxanthin, zeaxanthin, echinenone, etc. It has been established that environmental factors such as temperature significantly influence the biosynthesis and accumulation of these pigments. The aim of the present study is to evaluate the effect of temperature on the pigment profile and antioxidant activity of *S. incrassatulus* strain 3140 under intensive cultivation in an Airlift-type bioreactor. Cultivation was carried out under two temperature regimes: 14 °C and 25 °C. The results show that the higher temperature (25 °C) leads to a significant increase in biomass, as well as to an approximately tenfold rise in total carotenoid content. A direct proportional relationship was observed between carotenoid concentration and antioxidant activity of the extracts, evaluated through radical scavenging capacity (DPPH and CUPRAC methods). At 25 °C, the antioxidant activity was three to five times higher compared to that at 14 °C, which highlights the importance of temperature as a critical factor for optimizing the biotechnological production of bioactive compounds from microalgae.

Acknowledgments: This participation is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01, DUECOS.

P66 Impact of increasing imazamox residues on wheat physiology and soil microbial dynamics

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Keywords: imazamox, AHAS activity, non-target crop, Biolog EcoPlate, Gini coefficient, Lorenz curve, microbial communities

Imazamox is a widely used selective herbicide from the imidazolinone family, known for its high effectiveness against broad spectrum of weeds. However, under certain unfavorable conditions, imazamox degradation in soil may be slow and incomplete. Prolonged persistence of the herbicide can adversely affect both non-target crops and soil biota. This study aimed to evaluate the effects of simulated increasing soil doses of imazamox – from 100 to 500 µg/kg – on wheat and soil microbial activity in a pot experiment. The analysis included selected soil parameters, leaf enzyme activity, and protein content. Microbial metabolic activity was assessed using the Biolog EcoPlate method. The results revealed significant inhibition of wheat growth correlated with increasing herbicide concentrations. The highest doses of 400 and 500 µg/kg exhibited a lethal effect on wheat plants. Additionally, imazamox exposure suppressed enzyme (acetohydroxyacid synthases, AHAS) activity and reduced protein content in leaf tissue. Microbial metabolic activity was also markedly affected by imazamox residues, as indicated by the Gini coefficient and corresponding Lorenz curves based on Biolog data. Preserving soil fertility and microbial functionality requires the sustainable use of agricultural chemicals. This can be achieved through a holistic approach that integrates various soil parameters to provide practical insights for soil management.

P67 Adjustment of culture media for *in vitro* cultivation of the endemic species *Brassica jordanoffii*

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Keywords: calcium, zink, plant growth, endemic species

In vitro cultivation of endemic plants is a well known tool in *ex situ* conservation. It might not only provide rapid propagation of the plants, but it is a first step that benefits medium- and long-term conservation when combined with other methods and approaches for plant conservation.
Brassica jordanoffii O.E. Schulz (Brassicaceae) is a local endemic species, distributed in open habitats on marble substrates in Northern Pirin in Bulgaria. Due to its endemic nature and vulnerability, according to the Red Data Book of Bulgaria, measures for *ex situ* conservation are needed. The aim of the study was to optimize the in vitro medium by evaluating the effects of Ca^{2+} and Zn^{2+} ions added to the cultivation medium in different concentrations with or without activated charcoal. Rhizogenesis as well as shoot and leaf number were assessed.
The plants were cultivated on a modified double-strength B5 medium supplemented with phosphorus and different concentrations of Ca^{2+} and Zn^{2+} – 1, 5, 10, 25, and 75 mg/l – with or without 0.5 g/l activated charcoal.
Zn^{2+} increased root number at concentrations from 1 to 10 mg/l, but slightly decreased their number at higher concentrations (25 mg/l and 75 mg/l). Leaf number gradually decreased with increasing concentrations of Zn^{2+}, as did the number of adventitious shoots, except at the concentration of 5 mg/l. Supplementation of the medium with activated charcoal inhibited rhizogenesis; however, Zn^{2+} mitigated this inhibitory effect, most notably at the concentration of 10 mg/l.
Ca^{2+} also stimulated rhizogenesis at lower concentrations (1, 5, and 10 mg/l) and alleviated the inhibitory effect of activated charcoal. However, Ca^{2+} increased shoot number at higher concentrations (25 and 75 mg/l) and also stimulated their development in the variants supplemented with activated charcoal. The addition of Ca^{2+} slightly decreased leaf number.
In conclusion, Zn^{2+} and Ca^{2+} stimulated rhizogenesis. However, they didn't significantly influence leaf development. As far as shoot number is concerned, Zn^{2+} decreased their number (except at 25 mg/l), whereas Ca^{2+} increased it.

Acknowledgements: This research was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science (Project КП-06-Н81/10 from 09.12.2024).

P68 Dynamics of growth and phenolic compounds accumulation by in vitro plant cell suspension culture of *Passiflora caerulea* L.

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Keywords: blue passionflower, medicinal plant, suspension culture, secondary metabolites

Cultivation of plant cells in liquid nutrient medium under controlled conditions is a highly efficient and innovative technology for obtaining and producing targeted biologically active metabolites. This study focuses on the cultivation of a cell suspension culture from *Passiflora caerulea* L., for production of phenolic compounds. Traditional methods for cultivation and propagation of *Passiflora* have some difficulties. The plant has been recognized for its medicinal properties, but its full biological potential has not been systematically studied yet. We report for the first time a initiation of stable growing cell suspension culture of *Passiflora caerulea* L., cultivated in flasks. The dynamics of biomass accumulation, changes in conductivity and pH of the culture medium have been reported. High-performance liquid chromatography (HPLC) has been used to analyze the phenolic compounds produced in cell suspension. The major compounds were found to be (-)-Epicatechin, Rosmarinic acid, Rutin and Ferulic acid. The results show that the cell suspension culture of *Passiflora caerulea* L., could be a prospective producer of phenolic compounds.

Acknowledgements: This work was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science, under the contract No. KP-06-M86/3; BG-175467353-2024-10-0027-C01.

P69 Accumulation of exopolysaccharides by cell suspension culture of black chokeberry

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Keywords: suspension culture, *Aronia melanocarpa,* exopolysaccharides

Black chokeberry (*Aronia melanocarpa*) has been used for food and juice production from centuries. Recently, plant cell suspension culture of the plant has been developed and proposed as perspective model for in vitro production of dietary supplements by cellular agriculture approach. In this study, the dynamics of exopolysaccharides accumulation by *Aronia melanocarpa* cell suspension culture have been investigated during cultivation in liquid medium, supplemented with different growth regulators. The cultivation on WP medium, supplemented with combination of picloram and kinetin lead to significant increase in fresh biomass accumulation, but almost twice fold decrease in production of exopolysaccharides by Aronia cell suspension, when compared to cultivation on the same medium, but supplemented with indole-3-acetic acid (IAA) and 2-isopentenyladenine (2-iP). This is important finding, because it was demonstrated that the exchange of plant growth regulators can be used to stimulate the accumulation of exopolysaccharides by Aronia cells, and thus, can be applied as effective strategy to increase the nutritional value of the produced biomass.

Acknowledgements: This work was supported by the Bulgarian National Science Fund, Bulgarian Ministry of Education and Science, under the contract KP-06-H66/14; BG-175467353-2022-04-0214-C01.

P70 Evaluation antioxidant potential and total phenolic content of *Leucodon sciuroides* (Hedw.) Schwager

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Keywords: antioxidant activity, CUPRAC, DPPH, *Leucodon sciuroides* (Hedw.) Schwager.

Bryophytes have been attracting increasing attention as potential sources of natural bioactive compounds due to their diverse ecological adaptations and secondary metabolite profiles. In this study, the antioxidant potential and total phenolic content of the moss species *Leucodon sciuroides* (Hedw.) Schwägr. were evaluated. Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the cupric ion reducing antioxidant capacity (CUPRAC) method, while phenolic content was determined by total phenolic content analysis
The DPPH assay was applied at eight different concentrations ranging from 0.0078125 to 1 mg/mL. Scavenging activity increased in a concentration-dependent manner, reaching a maximum inhibition of 40.48% at 1 mg/mL. The EC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals, was calculated as 5.00 mg/mL, indicating a moderate level of antioxidant capacity.
In the CUPRAC method, absorbance values expressed in terms of ascorbic acid equivalents (AAE, mM) increased with rising concentrations. At concentrations of 1.0, 2.5, 5.0, 7.5, and 10.0 mg/mL, the AAE values were determined as 0.258±0.01, 0.540±0.02, 0.722±0.02, 0.889±0.03, and 0.940±0.07, respectively. These results demonstrate the extract's ability to reduce Cu (II) to Cu (I), thus confirming its antioxidant power.
The total phenolic content was determined in terms of gallic acid equivalents. As a result of the analysis, the total phenolic content of *L. sciuroides* was found to be 51.18±4.28 mg GAE/g extract. This indicates that phenolic compounds contribute significantly to the antioxidant activity observed.
In conclusion, *L. sciuroides* exhibits measurable antioxidant activity in terms of both radical scavenging and reducing power and contains a notable amount of phenolic compounds. These findings provide a scientific basis for the evaluation of mosses as natural antioxidant sources for potential use in pharmaceutical or nutraceutical applications.

P71 Development of a novel transformation system for *Rhodotorula mucilaginosa,* using endogenous promoters and codon-optimized selectable markers

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Keywords: yeast transformation, *Rhodotorula mucilaginosa*, endogenous promoter

Rhodotorula is a genus of oleaginous yeasts within the division *Basidiomycota*, recognized for its ability to grow on diverse substrates under minimal culturing conditions. This metabolic versatility has positioned *Rhodotorula* species as promising candidates for the sustainable production of value-added compounds, including single-cell oils and mannan-oligosaccharides with immunomodulatory properties. To further exploit their biotechnological potential, genetic engineering approaches have been applied to enhance their biosynthetic capacities. In this study, we report the cloning and functional validation of novel endogenous promoters from *Rhodotorula mucilaginosa* and their implementation in a newly developed yeast transformation system. To increase transformation efficiency, antibiotic resistance genes were codon-optimized based on the codon usage preferences determined in *R. mucilaginosa*. This optimized system provides a valuable tool for genetic manipulation in *Rhodotorula* species and supports future metabolic engineering strategies.

P72 Hybrid β-barrel pore-forming proteins from *Bacillus thuringiensis*

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Keywords: hybrid proteins, parasporin2, cry23Aa1, A1470 protein

Bacillus thuringiensis is a Gram-positive bacterium intensively studied for its pesticidal proteins produced during sporulation. Recent research has uncovered the production of several non-three-domain β-barrel pore-forming proteins by this species. Although these proteins lack insecticidal activity, their notable anticancer properties have positioned them as promising candidates for anticancer drug development. Despite their potential, the molecular mechanisms underlying their function remain poorly understood. In this study, we report the rational design and construction of hybrid genes between parasporin-2Aa1 and cry23Aa, as well as between parasporin-2Aa1 and its non-cytolytic homolog, the 26 kDa protein (A1470). This approach, previously successful in structure–function studies of Cry proteins, was applied here to investigate functional domains of parasporins. Hybrid genes were assembled using the Gibson assembly method into a pQE60 plasmid backbone and expressed in an *Escherichia coli* system. Proteins were purified and characterised further.

P73 The K77 capsular polysaccharides as determinants of virulence and resistance in hypermucoid clinical isolate *Acinetobacter baumannii* 10593

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Keywords: *Acinetobacter baumannii*, capsular polysaccharides, *galU* gene, virulence; resistance

Acinetobacter baumannii is an opportunistic pathogen and a major cause of hospital-acquired infections worldwide, with intrinsic antibiotic resistance and remarkable desiccation tolerance. Important virulence factor is the polysaccharide capsule which protects the bacteria from environmental stressors, including antimicrobials and the host immune response. With limited treatment options, capsular polysaccharides have become promising vaccine targets. To investigate the biological role and function of the capsule, the clinical isolate *A. baumannii* 10593, characterized by a hypermucoid phenotype, was selected from the laboratory's collection. The capsule biosynthesis genes are clustered in the K locus. Following whole genome sequencing of the selected isolate, the KAPTIVE tool was used to analyse the K locus type, which was identified as KL77. Subsequently, the *galU* gene, involved in the biosynthesis of simple sugars, was selected for mutagenesis, and a corresponding knockout strain was successfully generated. The outcome of the selected gene deletion was assessed by comparing biofilm production between the wild type and the mutant strain using fluorescence microscopy, with the mutant showing a higher ability to form biofilm. The constructed deletion mutant was further employed to investigate the role of the capsule in several key aspects of *A. baumannii* pathogenicity, including antimicrobial resistance and tolerance to disinfectants. The knockout strain showed increased susceptibility to all tested disinfectants (benzalkonium chloride, benzethonium chloride, and chlorhexidine digluconate) and most antibiotics used in this study (meropenem, ciprofloxacin, gentamicin, amikacin, tobramycin, and colistin). Adhesion to extracellular matrix proteins (collagen type I and fibronectin) was examined and it was demonstrated that the mutant was able to adhere more strongly to these proteins compared to the wild type. Furthermore, LDH cytotoxicity assay using human keratinocyte line (HaCaT) showed that the mutant strain was less cytotoxic and therefore less virulent towards host cells than the wild type strain. Based on these findings, the K77 capsular polysaccharides play a significant role in the pathogenicity of *A. baumannii* 10593, as they enhance resistance to antimicrobials and disinfectants as well as cytotoxicity, whereas their disruption promotes bacterial adhesion.

P74 Green cell factories" as a sustainable biotechnological platform for production of wealth promising bioactive products

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Keywords: Biotechnology, plant *in vitro* systems, plants, secondary metabolites

Plants are distinguished by a wide range of chemical substances, especially the so-called specialized metabolites (SMs), which have beneficial biological properties for humans. Medicinal plant therapies containing a complex of natural molecules have always attracted interest due to their potential for synergistic therapeutic effects, due to this multicomponent nature.
However, variation in the composition of metabolites in the naturally-grown plant material, due to changes in the environment and habitat of the plant, poses a major challenge for the production of standardized herbal preparations.
The intensive use of plants and plant-based natural products creates a huge discrepancy between their demand and availability. Furthermore, plant SMs are usually present in low (< 0.5%) quantities and collecting sufficient plant material to meet current needs can be impractical and environmentally destructive.
The goal of the project is to develop a methodology of producing a standardized biotechnologically derived extract based on plant *in vitro* systems with potential anti-rheumatic activity, as well as to demonstrate the applicability of the extract for the production of dietary supplements.
The technology could be applied in the biotechnology, food, cosmetic and pharmaceutical industries. It is intended for individuals with symptoms of rheumatoid arthritis, supporting the inclusion of medicinal plants or nutritional supplements in their anti-inflammatory therapy.

Acknowledgements: This research received funding from Bulgaria's Recovery and Resilience Plan, a part of the European Union's NextGenerationEU instrument for the implementation of an investment under C2I2, "Enhancing the innovation capacity of the Bulgarian Academy of Sciences (BAS) in the field of green and digital technologies" (contract number BG-RRP-2.017-0046-C01).

P75 HRV in young healthy subjects after exposure to orthostatic posture

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Keywords: heart rate variability, anxiety, depression

Heart rate variability (HRV) metrics are markers of autonomic nervous system activity.
Objectives: assessment of HRV in the field of time and spectral domain at rest and during orthostatic stimulation
Material and methods: short-time HRV recordings (5 minutes) were performed using an emWave Pro device, in resident doctors, at rest (session A) and in orthostatism (session B); all were evaluated according to the Hospital Anxiety and Depression Scale. The HRV parameters in time domain were: standard deviation of the NN intervals (SDANN), the root mean square of the mean squared differences of successive NN intervals (rMSSD), and the spectral HRV parameter: total spectral power (TP), high-, low- and very low-frequency spectrum (HF, LF, VLF) and relative values of HF and LF, according to the formulas: nHF= HF/LF+HF and nLF= LF/HF+LF. The statistical analysis noticed the differences between sessions (p) and between sexes (p`).
Results: general lot (GL) characteristics: 17 females and 7 males; average age was 27.04 years (sd= 1.97); mean anxiety score 7.04 (std = 3.97), mean depression score 3.13 (std = 2.27). The subjects were grouped according to anxiety and depression score: A0-7 (anxiety score less than 7)- 15 subjects (7 males and 8 females), and A8-21 (anxiety score 8-21)- 9 subjects (9 females), D0-7 subgroup (depression score 0-7) = 22 subjects (7 M and 15 F) and D8-21 subgroup (depression score 8-21) = 2 subjects (2 F). TP, LF, HF decreased with age in both genders without differences between sexes only in A session (p=0.0413, p=0.0432, respectively p=0.0490); VLF decreased with age only in B session (p=0.0573). HR increased in B vs A, in both sexes, on the GL (p= 0.0002), in A0-7 (p= 0.0015), in D0-7 (p= 0.0004), in A8-21 (p= 0.0325); nLF increased in B, with a larger increase in males, observed on GL (p= 0.0165; p`= 0.0072), in A0-7 (p`= 0.0150), in D0-7 (p= 0.0275, p`= 0.0056); nHF had an inverse evolution to that of nLF. LF/HF increased in B, with a greater increase in male subjects (p= 0.0227, p`= 0.0217), in A0-7 (p=0.0122, p`= 0.0434), in D0-7 (p= 0.0042, p`= 0.0062).

P76 Influence of the thermal treatment on antimicrobial performance of sol-gel derived $TiO_2/TeO_2/CuO$ nanopowders

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Keywords: sol-gel, thermal treatment, antimicrobial activity

This study aims to contribute to the development of metal-based antimicrobial materials by examining the relationship between synthesis conditions, elemental composition, and functional properties of sol-gel obtained $TiO_2/TeO_2/CuO$ powders. The combination of the synthesis method and the controlled thermal treatment offers a versatile approach for designing nanomaterials with potential applications in healthcare, sanitation, and antimicrobial surface coatings.
The investigated sample is with nominal composition $80TiO_2/10TeO_2/10CuO$. After the drying of the gel, the sample has undergone thermal treatment at 400 °C and 600 °C for 2h exposure time. The powders were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), UV-Vis spectroscopy and infrared spectroscopy (IR). The morphology of the investigated samples has been verified applying SEM and EDX mappings. Antimicrobial activity against *Escherichia coli*, was assessed using minimum inhibitory concentration (MIC) assays and spot tests. Both antibiotic resistance and hospital-acquired illnesses are frequently linked to the representative Gram-negative bacteria which additionally motivated our research. The obtained results showed strong antibacterial activity towards the selected bacterial strain which makes the synthesized materials promising antibacterial agents.

Acknowledgements: This work was supported by project BG05SFPR001-3.004-0004-C01, Interdisciplinary training of doctoral students to develop knowledge and competencies in various branches of modern research for healthy life and clean technologies (INTERDOC), funded within procedure "Support for development of doctoral projects" of Executive agency "Programme EDUCATION" , co-funded by the European Union.

P77 Biodegradation of synthetic dyes in fuel cells

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Keywords: synthetic dyes, biodegradation, fuel cells

The present work is dedicated to microbial and catalytic degradation of synthetic dyes in fuel cells. Methylene blue and Congo red are used as model solution of dyes. A screening for an appropriate catalyst for dyes degradation is also conducted among ZnO, TiO_2, ZrO_2 incorporated on a matrix of activated carbon and sole activated carbon. The TiO_2 is proved to be suitable for the process. The catalyst is characterized by means of SEM, EDS, XRD and BET analysis. Electrodes are fabricated based on TiO_2 on a stainless steel mesh with Teflonized carbon black. The electrodes are double-sidedly coated and are with a size of 10 cm^2 with a nickel mesh current conductor. 35% (PTFE) Teflonized carbon black from Vulcan XC-72R was used as a barrier layer. The catalyst is 40 mg/cm^2.

A test for cytotoxicity is conducted using the strain *Pseudomonas putida 1046.* It shows tolerance to the dyes up to concentrations of 250 mg/l and to the used catalyst. *Pseudomonas putida 1046* decolors Methylene blue and Congo red with concentration of 250 mg/l for 42 h up to 96% and 92% respectively.

Comparison of the dyes decolorization rate of the bacterial, catalytical and combination of bacterial and catalytical degradation in a fuel cell and in flasks is given.

Acknowledgements: The authors express their gratitude to Project PVU–63 from 16.12.2024 г. /BG-RRP-2.017-0047-C01 and KP-06-N67/6 from 12 December 2022.for the financial support.

P78 Comparing the effects of two types of plant biostimulants on soil microbial community activity using data from the biolog ecoplate method

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Keywords: biostimulants, microbial activity, Biolog EcoPlate method

Biostimulants appear as a promising alternative to chemical fertilizers, serving as a tool for transitioning toward eco-friendlier and sustainable agriculture. However, their effects depend on various climatic, edaphic, and biological factors and are not consistently predictable. Some studies suggest that biostimulants' impact may be linked to changes in the metabolic activity and structure of soil microbial communities – the crucial component responsible for nutrient turnover in soil. The aim of the current study was to evaluate the activity and structure of microbial communities in control soil and in soil treated with two different types of biostimulants: seaweed-based (PStim) and microbial (MbPB) using a greenhouse experiment. Microbial community assessment was conducted using the Biolog EcoPlate method, and relative utilization of substrates and soil respiration were estimated. The results from the study did not indicate changes in soil chemical composition after biostimulants application. In general, both treated and untreated variants exhibited relatively high metabolic activity, expressed as average well-color development (AWCD), and specific substrate utilization per guild. In general, the functional indexes indicated higher microbial biodiversity for biostimulant-treated variants. The observed effects of biostimulants may be associated with selective stimulation of specific species within the microbial communities, leading to differences in metabolic activity. Further research into the impact of biostimulants on soil microbial community metabolic activity could help elucidate some of the potential mechanisms underlying their influence on soil fertility and overall health.

P79 A biological activity of *Cicerbita alpina* (L.) leave extracts

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Keywords: *Cicerbita alpina,* biological activity, methanol leaf extract potential

Cicerbita alpina Wallr. is a perennial plant, common in the alpine parts of the mountains of the Republic of Bulgaria. The plant is used in the traditional cuisine of some countries such as Italy and Scandinavia, as well as in traditional folk medicine of many countries for the treatment of various diseases, including infectious diseases. The aim of the study was to investigate the anti-inflammatory and antimicrobial activity of the leave extracts from *Cicerbita alpina*, growing on Vitosha Mountain, Republic of Bulgaria, using the haemolytic complement method and the disk diffusion method. The study showed that methanol extracts had higher antihaemolytic activity than chloroform extracts. The methanol extracts showed bactericidal activity against most Gram-positive bacterial strains and a bacteriostatic effect to the Gram-negative bacteria - *A. calcoaceticus* and *E. cloacae.* No antibacterial activity of the chloroform extracts towards the tested microorganisms was recorded. In conclusion, the leave methanol extracts showed better anti-inflammatory and antimicrobial activity compared to chloroform extracts, which is probably due to the polar compounds contained in them.

P80 Monitoring of the *ex vitro* regeneration potential in different lighting conditions of *Achillea thracica* Velen. plants after *in vitro* cultivation

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Keywords: *Achillea thracica, ex situ* conservation, regeneration potential

Achillea thracica Velen. is a Bulgarian endemic species, distributed throughout the territory of Manole village, Plovdiv, the Republic of Bulgaria. Yarrow (*Achillea* sp. L., Asteraceae) have been used in the traditional folk medicine for treatment of wide range of human ailments, including different inflammations, for its hemostatic effect, etc. The aim of the study was to estimate the photosynthetic potential of, primary metabolism and stress in *ex vitro* adapted plants grown under different light conditions – blue-red artificial light and white sun light, for 8 weeks by measuring chlorophyll fluorescence, plastid pigments, reducing sugars, stress markers (MDA and H_2O_2) content, and activity of antioxidant enzymes. The research showed that plants grown under blue-red light had more active PS2 reaction centers (RC) per leaf cross-section, but displayed a tendency for lower absorbed light flux per RC and quantum yield of end electron acceptors reduction compared to sun-light-grown plants. No difference in quantum yield of primary photochemical reaction between the light variants was found. However, tendency for higher quantum yield of electron transport and perfromance index of the photosynthetic light reactions as a whole was registered in blue-red-light-grown plants. Moreover, those plants exhibited higher concentrations of plastid pigments and increase, though insignificant, in the reducing sugars content. Nonetheless, higher levels of the stress markers and antioxidant enzymes activity were also characteristic for that growth condition. These observations indicate higher primary metabolism performance along with development of stress in plants grown under blue-red light compared to those under white light.

In conclusion, the blue-red light regime we tested elicited an ambiguous response in the yarrow plants, therefore future research is needed to find fully optimal conditions for *ex vitro* adaptation of *Achillea thracica*.

P81 Fungal microbiome profiling in pulmonary sarcoidosis patients as revealed by target sequencing of biopsy, bronchoalveolar lavage fluid (BALF), and blood samples

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Keywords: sarcoidosis, fungiome, BALF - bronchoalveolar lavage fluid, ITS – target sequencing, biopsy

Sarcoidosis is a granulomatous inflammatory disease of unknown etiology. Recent research has turned attention to the pulmonary fungiome as a potential contributor to its pathogenesis. Our aim was to profile the fungal biodiversity by sequencing of internal transcribed spacer (ITS) in lung biopsy, BALF, and blood samples from patients with sarcoidosis. Samples were collected from 15 sarcoidose patients according to standard clinical procedures. Additionally, a portion of each blood sample was grown on BHI medium with vitamin K (1 mg/mL) at 43 °C for 24 hours. Both cultured and non-cultured fractions were further analyzed. Total DNA was extracted and subjected to ITS-targeted sequencing. QIIME2 was used for taxonomic classification. Statistical analyses and visualizations were conducted in R and Python. A total of 2.06 million reads were obtained across all samples, with an average of approximately 31,000 reads per sample. Analysis revealed distinct fungal community structures across the different sample types. BALF samples were dominated by *Penicillium* (12%) and *Candida* (6%), with smaller contributions from *Blumeria* (5%), *Amphinema* (4%) and *Rozellomycota* (3%). In biopsy, a strong presence of Penicilium (12%), *Amphinema* (10%) and *Homiactis* (5%) was observed. All taxa presented in control were excluded. Cultured blood samples were enriched in *Saccharomyces* (12%), *Blumeria* (16%), *Komagataella* (7%), and *Zoopagomycota* (10%). **Non-cultured blood samples** displayed a distinct community structure, with elevated levels of environmental and slow-growing fungi such as *Pseudoanungitea* (8%), *Salinomyces* (7%), Rozellomycota (4%), *Ochrolechia* (3%), and *Thermomyces* (3%). Washout samples had the highest relative abundance of *Penicillium* (18%) and were uniquely enriched with *Nigrospora* (8%). The findings suggest multiple environmental and endogenous origins of the blood and airway fungal communities. The prominence of opportunistic genera such as *Candida*, *Saccharomyces*, and *Penicillium*, may play a role in the pathogenesis of sarcoidosis. Future studies

should explore the temporal dynamics of the diversity of pulmonary and blood fungiomes to clarify their contribution to disease onset, persistence, or resolution.

Acknowledgments: This study was supported by the BNSF: grant numbers KP-06-DV/10-21.12.2019 and KP-06-H73/5 5.12.2023

P82 Application of a zero-inflated Poisson regression model of spatial distribution of viral families in bat communities across Asia

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Keywords: bat viral zoonoses, vector-borne infections, zero-inflated Poisson regression model

Bats are recognized as significant reservoirs for diverse viruses, many of which have zoonotic potential. Understanding these viruses' distribution and family-level associations across different bat species is critical for predicting and mitigating potential outbreaks. The Database of Bat-associated Viruses - DBatVir database the database of zoonotic and vector-borne viruses. A zero-inflated Poisson (ZIP) regression, which is a statistical model used when count data contains an excess of zero values compared to what a standard Poisson distribution would predict. A ZIP model combines two processes to generate the observed count data: The zero-generating process models the probability of observing a zero count, often using a logistic regression model. This process accounts for the excess zeros that don't fit the Poisson data. The count process models the non zero process using a standard Poisson distribution. Sparse data often has a high proportion of zero values. ZIP models explicitly account for this excess, making them more appropriate than standard Poisson regression. Understanding the distribution of these viruses at the family level across different bat species is essential for anticipating and preventing future outbreaks. This model effectively addressed overdispersion and excess zeros by distinguishing between true absence and low-frequency occurrences of viruses. By implementing relevant covariates from Asian bat communities, ZIP modelling allows for a more accurate and biologically meaningful interpretation of spatial and ecological patterns in viral distribution and potential zoonotic risk.

Acknowledgements: This work was supported by the Bulgarian National Science Fund: grant number KP-06-PN-51/8

P83 Pharmacological activities and chemical composition of extracts from Antarctic yeast *Dioszegia* sp. AL$_{105}$ and *Bannozyma* sp. AL$_{104}$

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Keywords: Antarctic yeast, biomass, metabolite, antineoplastic cytotoxicity

Antarctica is the world's largest open laboratory providing highly extreme living conditions for all inhabitants. The microorganisms have developed their own adaptation mechnisms being able to manage the disadvantageous adverse environmental conditions. The Antarctic yeasts have been extensively studied in recent years in regard to biodiversity and biotechnology potential. In the present study two yeast strains were identified and examined. Both species are rare in Antarctica, understudied and new for the Bulgarian Antarctic collection. *Dioszegia* sp. AL$_{105}$ was isolated from penguin feathers while *Bannozyma* sp. AL$_{104}$ was obtained from an Antarctic soil sample. Cell growth of the species was observed under submerged culture conditions. Good cell growth was observed in both species with the Antarctic strain *Bannozyma* sp. AL$_{104}$ showing an optimum of approximately 8 g/L accumulated biomass. Efforts were focused on evaluating the biological activity of cell extracts from both yeast species on malignant cell lines. Our study is the first one using the Antarctic yeasts *Dioszegia* sp. AL$_{105}$ and *Bannozyma* sp. AL$_{104}$. For this purpose, two

types of malignant T-cell lymphoma (leukemic HuT-78 and cutaneous MJ) cells and two types of bladder carcinoma (invasive CAL-29 and non-invasive T-24) cells were used. The IC_{50} of all extracts ranged from 113 µg/mL to 278 µg/mL. The extracts did not show antimicrobial activity against the tested opportunistic bacteria *Staphilococcus aureus* and *E.coli*. The yeast extracts were also studied for antioxidant activity. Significant neutralization activity and scavenging of hydrogen peroxide and •OH radicals as well as NO• radicals was recorded. Metabolites in the studied extracts were identified. Among them various types of carotenoids, amino acids, organic acids, di- and triglycerides and other were detected. The heat map of metabolite distribution gave a more specific idea for their presence in the individual types of extracts.

Acknowledgments: We thank the staff of the Bulgarian Antarctic Expedition for their logistic support.
This research was funded by the Bulgarian Ministry of Education and Science through the National Centre for Polar Studies, and Sofia University "St. Kliment Ohridski" in the frames of the National Program for Polar Studies 2022-2025 (grant numbers 70-25-40/23.01.2023).

P84 Effect of the carbon source on the biosynthesis of an extracellular polysaccharide by the Antarctic yeast strain *Rhodotorula mucilaginosa* AL$_{109}$

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Keywords: Antarctic yeasts, *Rhodotorula mucilaginosa*, submerged cultivation, exopolysaccharides

Despite the efforts of scientists, the Antarctic desert remains one of the least explored territories on the planet. The extreme conditions in which Antarctic microorganisms live include low temperatures, strong winds, strong UV radiation, multiple freeze-thaw cycles, combined with long dark and light periods. One of the adaptations that helps them to overcome adverse environmental conditions is related to the synthesis of specific metabolites. Microbial exopolysaccharide (EPS) macromolecules that are synthesized and secreted have been shown to possess unique composition and functional properties. In the present study, we investigated the cell growth characteristics and biosynthetic exopolysaccharide capability of a newly isolated Antarctic yeast producer *Rhodotorula mucilaginosa* AL$_{109}$. The strain identification was confirmed by investigation of ITS1-5.8S-ITS4 regions of rRNA and the sequence was deposited in the NCBI. Two main carbon sources in the culture medium and their influence on the biomass accumulation, in terms of dynamics of the fermentation process and biopolymer accumulation were studied. The optimal conditions for the extracellular polymer biosynthesis were determined. All characteristic phases of submerged cultivation were observed. In the experiment with glucose, the entry into stationary phase was after 96 h, while in the medium with sucrose it was recorded after 72 h. The optimal accumulation of EPSs, when using glucose as a main carbon source in the culture medium, was 1.9 g/L after 96 h,

while when using sucrose 2.0 g/L after 96 h were obtained. The cell growth with both carbon sources was similar and reached values above 9 g/L. The self-regulating process with respect to pH was also monitored. The quantities of the synthesized biopolymer were sufficient to transfer the process to a bioreactor cultivation system and to seek practical applications of the resulting target product, after studying its functional physicochemical and biological properties.

Acknowledgments: We are greatly thankful to the staff of the Bulgarian Antarctic Expedition for their logistic support. Funding: This research was funded by the Bulgarian Ministry of Education and Science through the National Centre for Polar Studies and the Sofia University St. Kliment Ohridski in the frames of the National Program for Polar Studies 2022-2025 (grant number: 70-25-103/23.06.2025).

P85 The study of consumer behavior in the context of separate waste collection, and the application of behavioral economics solutions

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Keywords: waste management, waste sorting, consumer behavior, nudge, behavioral economics

Separate waste collection is one of the most sensitive elements of individual behavior in the big picture of environmental protection. At the same time, despite the popularity of the "green" wave spreading in all walks of life, we are struggling to meet our country's separate waste collection goals. Consumer behavior plays an important role in constructing approaches to tackle the problem of separate waste collection. The conventional approach implies putting financial incentives, regulations and rules, convenience, and affordability first to achieve the goal of maximizing utility. But this behavior is direct and indirect, i.e. consumers are not always aware of the consequences of their decisions. On the one hand, there is the problem of environmental degradation, which is a reaction to the impact of everyday consumer behaviour. On the other side, it is also difficult to predict consumer behavior due to not always making rational decisions, these circumstances require the use of different tools from the mainstream approach. Actual behavior in terms of environmental protection, sustainability, and more specifically for waste separation may differ from the position stated by individuals, since the action is not a single one, but requires building new habits, which significantly complicates this process. In this respect, it is necessary to take these positions into account when developing policies to change the behaviour of the Bulgarian consumer. Behavioral economics in this sense is helpful to complement policies on the challenges we face in waste management.

This paper explores the gaps in behavioral analysis. It relates the suggestions of behavioral economics for environmental issues and the potential of popular "nudges" to the problem of separate waste collection. Based on a questionnaire survey, behavioral patterns and barriers are identified, a literature review of behavioral economics potential is conducted and, as a result, simple, low-cost, and unobtrusive solutions are proposed.

Acknowledgements: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N65/13.

P86 Contamination of agricultural land with micro- and nanoplastics

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Keywords: soil pollution, plastic pollution, food security, health risk, bioaccumulation

The use of plastic products in modern agriculture is an increasingly common phenomenon worldwide, as it contributes to increasing yields, reducing the use of water and herbicides and leading to a controlled increase in crop quality at low costs, but with high risk to looming environmental crisis. Most agricultural plastic products are single-use and often remain in the environment long after their intended use. Soils are one of the main receptors of agricultural plastics and contain greater amounts of microplastics than the oceans, as presented by irrefutable evidence in a comprehensive FAO expert report from December 2021. This new FAO report is a call for urgent action by countries and the sector to better manage plastics in food systems before and after they reach the end of their life, promote circular approaches and implement sustainable innovative and environmentally friendly solutions to limit conventional single-use plastics. Microplastics can enter agricultural lands not only through direct application of plastic materials (for mulching, etc.), but also through sludge from sewage treatment plants, composts, etc. From the soil, they easily pass into living organisms, where they can cause disruptions in biological functions, physiological processes, reproduction, as well as lead to the accumulation of pollutants in biomass, and through the food chain inevitably increase the risk to human health.
In this context, it is of great importance to clarify the potentially toxic effects of microplastics on soil and plants, as well as their ability to enter and accumulate in various tissues and organs.

Acknowledgements: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N86/12.

P87 Digital genebank for improving *ex situ* collections' management

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Keywords: plant biodiversity, conservation, information system, genebank quality standard.

Each country is responsible for preserving its gene pool as a national treasure – a resource with biological and economical value, initial material for the creation of varieties that meet the requirements of current and future generations. The National Genebank, located in Sadovo is the main center for the long-term seed conservation of Bulgaria and as such is an active partner in the international system for the preservation of plant biodiversity. The Institute has been nominated by the European Program on Plant Genetic Resources (ECPGR) as a responsible national research organization for participation in the European Electronic Catalogue (EURISCO) and in the construction of the virtual European Genebank (AEGIS). The study focuses on the development and implementation of innovative technologies for the management of the process of seed storage and monitoring in the genebank. The intelligent search in ontologies improves access to information about the conserved accessions. The results increase the functionality and security of the management system, which have significant scientific and public contribution. The innovation approach ensures better control and more efficient use of resources, which is essential for the conservation of biological diversity, the utilization of the gene pool and the sustainable development of agriculture in the face of climate change. The digital genebank creates the open access to collections, free used by the scientific community, breeding companies and all other users. The study supports the establishment of the quality standard for *ex situ* storage and the development of professional capacity, in accordance with the Operational Guide for the Bulgarian Genebank, approved by the ECPGR Management Committee (2023).

P88 The role of local varieties in the context of biodiversity conservation

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Keywords: genebank, collection missions, germplasm exchange, passport database, valorisation.

Last decades a huge number of old and traditional varieties have been replaced by new commercial varieties. Under these circumstances, an approach for biodiversity conservation by using two strategies: *on farm* and *ex situ* preservation, represents a research priority. International collaboration between genebanks has become crucial in ensuring the global conservation and sustainable use of genetic resources in the framework of maintaining compatibility and interoperability in the European area. The Green Deal promotes ecological practices that have positive impact on protection of bio resources and support agricultural systems in the climate change. The purpose of the study is to improve documentation and visibility in the national genebanks of Bulgaria and Slovakia due qualitatively exchange of experience and establishing joint research plan based on their long-standing mission of storage and use of plant diversity. By expeditions for local accessions in the rural areas and international germplasm exchange using Standard Material Transfer Agreement the diversity of the National collections is increased, following the priorities of EU Biodiversity Strategy 2030. The results help valorisation of plant gene pool through open access to *ex situ* collections for promoting the role of local varieties in the biodiversity conservation and meeting the farmers' needs. The Bulgarian and Slovakian genebank collections are documented by FAO/Bioversity descriptors and published with in the European catalogue EURISCO (http://eurisco.ecpgr.org).

P89 New data on the Paleogene flora from Ustren (E Rhodopes, Bulgaria)

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Keywords: Bulgaria, Early Oligocene, Paleoflora, Rhodope Mts., Ustren

Five new taxa have been registered for the Paleogene flora from Ustren (Eastern Rhodopes). These are *Dodonaea pteleaefolia* Heer, *Hovenia* sp., *Laurophyllum acutimontanum* Mai, *Myrica lignitum* (Unger) Sap., and *Neolitsea palaeosericea* Takht.
The stratigraphic distribution of each of them is related to the Oligocene on the territory of Bulgaria. Only the discovery of *Dodonaea pteleaefolia* expands the previously known stratigraphic range of the species - Upper Oligocene, now including the Lower Oligocene.
A revision of this species on the territory of Bulgaria has also been made, in accordance with the new understanding of the taxonomy of the modern species *D. viscosa* Jacq., which is accepted as the nearest living relative of the fossil species.
Both the *D. viscosa* and the nearest living relatives of the other fossil taxa are distributed in the subtropical and tropical zones of the Earth. This is a prerequisite to assume that the climatic conditions in the Eastern Rhodopes region during the Early Oligocene were like the modern subtropical to tropical ones.

P90 Railway network as a pathway for the introduction and spread of alien plant species in Southern Bulgaria

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Keywords: invasive plants, railway, alien species

The railway network is one of the pathways for the introduction and spread of alien plant species. The areas around the stations are some of their first habitats, from where they subsequently migrate and enter semi-natural and natural habitats.
The purpose of the present study is to identify the plant species distributed around 37 railway stations in southern Bulgaria and to assess the proportion of alien and invasive species.
As a result of the field study, 399 species of Magnoliophyta were identified. Of these, the families with the largest number of species were: Asteraceae (65 species), Poaceae (43), Fabaceae (35), Brassicaceae (19), Rosaceae (18) and Amaranthaceae (16). From a phytogeographical point of view, the group of species with the largest share were: the Euro-Asian (16.9%) and the Euro-Mediterranean species (15.1%). Alien (adventive) species were 43 and represented 11.6% of all identified taxa, ranking third in number of representatives. Higher number of alien species was found at the following stations: (1) stations with intensive traffic; (2) sites with abandoned wagons and unmaintained terrain. Most of the alien species were of North-American origin (31%), followed by those originating from South America (10%).
The high share of aliens confirmed that the railway network was one of the main pathways for the introduction of those species in the Bulgarian flora. The results of this study are the basis for monitoring and elaboration of control measures for the alien plants associated with this pathway.

P91 From sediment to shell: a multi-matrix approach to pollutant monitoring in the Black Sea

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Keywords: Black Sea, pollution, mussels, biomarkers

This study presents the first comprehensive assessment of heavy metals, PAHs, PCBs, and pesticides in sediments, waters, and mussels from the Bulgarian Black Sea. Iron, zinc, PCB-28, and several PAHs were found in water; arsenic, copper, iron, and zinc in sediments; and multiple metals and PAHs in mussels. While various contaminants were detected, no pesticides were found, and levels of regulated substances in mussels remained below EU safety thresholds (Regulation 2023/915). Although not posing immediate health risks, the findings indicate chronic environmental exposure, highlighting the need for future assessments to evaluate potential sublethal effects on mussels.

Acknowledgements: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01.

P92 Mediterranean mussel (*Mytilus galloprovincialis*) as a bioindicator for microplastic accumulation in the Bulgarian Black Sea

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Keywords: Black Sea, microplastics, mussels, biomarkers

Plastic pollution is a significant environmental issue in many seas worldwide. The Black Sea is no exception and suffers from pollution originating mainly from land-based sources. Environmental issues are exacerbated by the fact that the sea is semi-enclosed, which leads to the accumulation of plastic over time. The sea also receives plastic waste from three major transboundary rivers – the Danube, Don, and Dnieper. Additionally, plastic debris of all sizes – macro, meso, micro, and nano-plastics – can carry various anthropogenic toxicants, such as heavy metals and persistent organic pollutants (e.g., pesticides, pharmaceuticals, etc.), over long distances, and they can also leach hazardous substances like bisphenol A and phthalates. The negative effects of microplastics on aquatic organisms – including marine fish, turtles, birds, and mammals – resulting from plastic ingestion or entanglement, are already well documented globally. However, there is currently insufficient information available for the Black Sea regarding the accumulation of priority pollutants in various matrices (biota, water, and sediments) and the effects of pollution on biomarkers at different biological levels (cellular, tissue, and organismal), particularly in the context of the Marine Strategy Framework Directive (MSFD). Comprehensive studies on the ecological status of the Black Sea in Bulgaria are extremely scarce, especially those that include analysis of microplastic particle (MP) content in commercially important mussel species, surface waters, and sediments. The scientific hypothesis we have formulated is that the Black Sea and mussels are contaminated with MPs. Therefore, the main goal of this project is, for the first time in Bulgaria, to investigate and assess the quantity and composition of MPs in commercially important mussels, waters, and sediments from the Black Sea area, their negative impact on specific biomarkers, and the risk to human health. Emerging contaminants such as microplastics require innovative analysis techniques. The mussel samples will be sent to an accredited laboratory for external analyses, and quantum cascade laser spectroscopy will be used. The Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System brings unprecedented speed of analysis and ease of use to analytical challenges such as this. The 8700 LDIR system's fully automated microplastics workflows are ideally suited to the analysis of microplastic particles in environmental samples. By processing samples in minutes or hours, the 8700 LDIR allows a higher sample throughout with minimal operator intervention, with reduced potential errors, and gives the results fast.

Acknowledgements: This study is financed by the Department of Scientific Research of University of Plovdiv, project MUPD25BF001 (МУПД25-БФ-001).

P93 Shells of stress: lysosomal integrity as a biomarker in wild and farmed Mediterranean mussels

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Keywords: Black Sea, pollution, mussels, biomarkers

This study assessed environmental stress in Mytilus galloprovincialis by evaluating lysosomal membrane stability via the Neutral Red Retention Time (NRRT) assay in hemocytes from wild and farmed populations across winter and spring. Both groups showed significantly reduced NRRT values, indicating lysosomal damage and exposure to stressors. Notably, wild mussels consistently exhibited lower NRRT values than farmed ones, suggesting higher stress levels, though the underlying causes remain unclear.

Acknowledgements: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01.

P94 Gills under threat: biomarker evidence of pollution in a Bulgarian river sanctuary

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Keywords: gills, histology, fish, protected area

This study investigates the impact of pollution on freshwater fish from the Veleka River, a protected area near the Bulgarian Black Sea. Using selected bioindicator species, histopathological biomarkers revealed circulatory, degenerative, and proliferative lesions in fish gills. Complementary chemical analyses detected priority pollutants in water samples, confirming environmental stress in this freshwater ecosystem.

Acknowledgements: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01.

P95 Addressing male infertility: the role of tobacco, alcohol, and therapeutic interventions

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Keywords: male infertility, supplements, sperm parameters

Male infertility is a significant global health concern, contributing to nearly 50% of all infertility cases in couples worldwide, according to various studies. The World Health Organisation (WHO) estimates that approximately 7% of men globally suffer from infertility, with lifestyle factors playing a major role in declining reproductive outcomes. Among these, smoking and alcohol consumption are two prevalent and modifiable risk factors. This study examines the effectiveness of treating male infertility with supplements and explores clinical insights into how tobacco and alcohol adversely affect male reproductive health. Harmful chemicals introduced through smoking increase oxidative stress and damage sperm DNA, while excessive alcohol consumption disrupts the endocrine system, negatively affecting spermatogenesis and potentially leading to testicular degeneration. The combined use of these substances may have additive or synergistic detrimental effects on sperm count and quality. Our results show that men who smoke and consume alcohol exhibit poorer sperm parameters and respond less effectively to treatment, suggesting that addressing these habits is critical to improving therapeutic success in male infertility. Our data also outlines current treatment approaches and evaluates their effectiveness in patients dealing with infertility. Understanding the impact of these modifiable behaviours is crucial for improving fertility outcomes and enhancing the effectiveness of clinical treatments in the future.

Acknowledgements: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01.

P96 Urban green area and biodiversity

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Keywords: biodiversity, urban green spaces, ecosystem sustainability, Albania, biodiversity indices

Biodiversity, once considered an abstract concept, has evolved into a well-defined framework encompassing the variety of life forms on Earth. The Convention on Biological Diversity (CBD) defines it as "the variability among living organisms from all sources—including terrestrial, marine, and other aquatic ecosystems—and the ecological complexes of which they are part; this includes diversity within species, between species, and of ecosystems." In June 1992, approximately 154 countries signed the CBD in Rio de Janeiro, which entered into force in December 1993, underscoring the global commitment to biodiversity conservation.

Biodiversity is categorized into three interconnected levels: genetic diversity (variation within species), species diversity (variety and abundance of species), and ecosystem diversity (diversity of habitats and ecological processes). While quantifying biodiversity was challenging in the past, modern indices such as Shannon, Simpson, and Sørensen's Coefficient now facilitate the assessment of biodiversity across regions and habitats.

Recent scientific consensus emphasizes the critical role of biodiversity in ecosystem sustainability and the support of life forms on Earth. Wetlands are recognized as particularly biodiverse habitats. Urban areas, traditionally dominated by infrastructure, now demonstrate increased biodiversity when equipped with green spaces. These urban green spaces—featuring trees, shrubs, grass, flowers, forests, and water bodies—provide essential resources such as food, shelter, and breeding grounds for various species, thereby enhancing urban biodiversity.

This paper examines the evolution of biodiversity concepts and definitions, with a focus on urban habitats in Albania and beyond, highlighting the importance of green infrastructure in fostering biodiversity.

P97 Traditional and molecular studies on a recently introduced non-indigenous diatom species in Europe, *Discostella asterocostata* (B.Q.Lin, S.Q.Xie and S.X.Cai) Houk and Klee

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Keywords: metabarcoding, centric diatoms, phytoplankton

The species *Discostella asterocostata* was first described in China in 1985 as *Cyclotella asterocostata*, and later reported from various Asian regions. In 2004, based on marginal fultoportulae position, it was reassigned to the genus *Discostella*. By 2012, it had been detected in North America, where it became increasingly frequent. In Europe, its earliest known occurrence was in 2016 in the Mura River (Croatia). Light microscopy revealed its presence in 2021 in the Serbian Sava and Tisza rivers and the Ráckevei-Soroksári Danube in Hungary. Molecular data had already detected it in low abundance in Hungarian oxbow lakes in 2019 and in the main Danube channel in 2021. In 2023, the species was found microscopically in the Meuse River in France and Belgium. Morphometric and genetic analyses revealed no significant differences from Asian populations, confirming a high degree of similarity. The species showed a clear preference for warmer, low-nutrient waters, with positive correlation to temperature and negative to organic carbon and nitrogen. These findings suggest its potential spread in Europe may be driven by climate change. Our results stress the importance of combining microscopy with molecular techniques to detect non-indigenous taxa. The emergence of *D. asterocostata* in Europe raises ecological concerns about its role in freshwater ecosystems and potential impacts on native phytoplankton, underlining the need for continued monitoring and research into river ecosystem responses to global change. The research was carried out within the framework of the Széchenyi Plan Plus programme with the support of the RRF 2.3.1 21 2022 00008 project.

P98 Above and Below the Halocline: Stratification-Driven Contrasts in Coastal Benthic Life

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Keywords: benthic ecology, benthic-pelagic-coupling, stratification, functional groups

This study compares the structure, diversity, and functioning of macrobenthic invertebrate communities between two adjacent depth transects (10 m: upper; 40 m: lower) sampled monthly for 13 months along the southern coast of İzmit Bay (Sea of Marmara) between August 2020 and September 2021. Despite their spatial proximity, the two layers hosted markedly different benthic assemblages. The upper layer, located above the halocline, was characterized by significantly higher taxonomic richness, abundance, and biomass, with consistently high frequencies of Polychaeta (89%), Bivalvia (80%), and Crustacea (64%). In contrast, the lower layer, situated below the halocline, exhibited sparse and unstable communities, frequent zero-fauna occurrences, and significantly reduced biomass, particularly between May and July 2021—coinciding with a regional mucilage event. Functional group composition was more variable in upper layer, while lower transect assemblages were dominated by a few tolerant taxa such as *Corbula gibba*. SIMPER and ANOSIM analyses revealed a significant separation in taxonomic structure between depths ($R = 0.54$, $p = 0.0001$), with Myida, Lucinida, and Amphipoda contributing most to dissimilarity. These differences were linked to contrasting environmental regimes: the upper transect experienced fluctuating but oxygenated and phytoplankton-rich conditions, whereas the lower transect was characterized by persistent stratification, low dissolved oxygen, and reduced organic matter quality. The data suggest that even small-scale vertical differences in water column structure can cascade into substantial ecological divergence at the seafloor. Our findings underscore the importance of considering vertical heterogeneity when assessing benthic ecosystem health in stratified coastal systems, especially under increasing pressure from eutrophication and climate-induced stratification.

P99 Litterfall stocks - part of a research in the "Bulgarian Network for Long-Term Ecosystem Research - LTER BG"

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Keywords: litterfall, stocks, annual dynamic

The "Bulgarian Network for Long-Term Ecosystem Research LTER BG" is a part of the "European Network for Long-Term Ecosystem Research". One of the indicators that must be monitored is the amount of litterfall. It is measured once at every 5 years.
Forest litter is an important part of the ecosystem, as it provides the connection between plant species and the soil. It returns into turnover many macro- and micronutrients.
Litterfall quantities were studied in five sample plots in beech and spruce forests in the Balkan Mountains, located in sites of the LTER BG network.
The litter was collected monthly through square 0,25 m^2 catchers installed above ground. It was fractionated into leaves, branches, cupules, seeds, etc. and dried for 48 hours at a temperature of 85°C for leaves and 105°C for other fractions.
The annual dynamics of deciduous forest shows, as expected, the highest amounts of litterfall during the autumn months, with the leaf fraction dominating, followed by the cupulas and seeds. The average annual amount for beech forests was 67.4 $g.m^{-2}$. In spruce forests, the average stocks were 92.5 $g.m^{-2}$, with the largest percentage being needles, followed by cones and branches.
Further studies can continue with the study of the chemical composition of the litterfall in order to clarify the cycling of elements.

P100 Study of the possibilities for cultivated growing white mussels *Mya arenaria*, *Chamelea gallina* and *Donax trunculus* through development of environmentally friendly technology in the water area of Tsarevo town

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Keywords: *Mya arenaria*, *Chamelea gallina*, *Donax trunculus*, cultivated growing

The present study is aimed at the conservation of resource species of molluscs from the coastal zone of the Black Sea. In order to reduce the anthropogenic pressure on resource species of mussels, a multifunctional bottom technology for semi-extensive cultivation of white sand mussels has been developed in the littoral zone of the city of Tsarevo, Southern Bulgaria.
The advantages of the installation are exceptional flexibility, the possibility of positioning on any type of bottom habitat and the ability to adjust the distance of the collector platforms from the bottom. There are no connecting ropes and/or buoys on the sea surface and they do not affect the traditional coastal fishing and marine tourism. In the defined bottom layers, the impact of storm waves is negligible and the amount of nutrient substrate is high. The presence of this distance is also a prerequisite for strongly limiting the influence of the rapana (*Rapana venosa*). The growth indicators of the cultivated mollusks, the chemical composition and the qualities of the cultivated species have been established.

Acknowledgements: The study was implemented with the support of project No. BG-RRP-2.004-0006 "Development of scientific research and innovation at Thrace University in the service of health and sustainable well-being"

P101 Addition to the mycota of Vrana park (Sofia region)

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Keywords: Bulgaria, *Diaporthales*, fungal diversity, *Xylariales*

This study contributes to the diversity of fungi with minute ascomata on the territory of Vrana park. Data on plant-substrata were collected in the field. Main collection points were shown on a map, and geographic locations were taken with the help of Garmin Etrex 10. Fungal micromorphology was studied in water mounts under light microscope. During the field work in 2018, and 2022–2025, *Melanomma subdispersum* on dry twigs of *Betula pendula* and *Leptosphaeria vitalbae* on dead stems of *Clematis vitalba* were found as new for the Bulgarian mycota. Forty fungal species with minute fruit-bodies were recorded in the park on dead or living leaves, bark, small twigs from broadleaf trees or dead stems of climbers. Twelve of them were recorded also for the first time in Sofia region, and one new host-plant of a xylarialean fungus was discovered. Additionally, *Cantharellus cibarius* was found on mossy meadow, *Russula virescens* was found under American oaks, and *Suillus luteus* was collected near pine-trees. *Apiognomonia errabunda*, *Armillaria mellea*, *Ascochyta quercuum*, *Asteromella quercifolii*, *Gnomonia geranii-macrorrhizi*, *Hypospilina pustula*, and *Xylaria polymorpha* were confirmed from the study area. The territory of Vrana park helds rich diversity of microscopic fungi and mushrooms, not reported in the previous works, conducted from 2007-2008 and during 2017-2018. Among the most typical ascomycete members with minute fruit-bodies found in the park were nineteen diaporthalean species, eight xylarialean species, seven dothideomycetes, four discomycetes, and three hypocrealean species. With the exception of a small group of dying pine-trees located in the southern part, all planted forest areas in Vrana park are in good condition. This work was held within the project 'Phylogeny, taxonomy and sustainable use of fungi'.

P102 New hypocrealean fungus in Bulgaria

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Keywords: boletes, fungicolous fungi, *Hypocreales*, *Sepedonium*

This study was aimed to contribute to the fungicolous fungi of Bulgaria. During routine field work in June-July 2018, two localities on boletoid fungi with basidiomata infected by one *Sepedonium* species, previously not described from our country, producing golden yellowish and white spore masses on mushroom caps and stems, were found. The specimens firstly were recorded on *Boletus* sp., in Forebalkan, at alt. ca 485 m, and on *Hortiboletus bubalinus*, in Sofia region, at alt. ca 560 m. Later, the same *Sepedonium* fungus was collected during the summer of 2021-2022 in its original locality in Sofia region (Sofia city, and Iztok quarter). One collection, containing two infected fruit-bodies of *H. bubalinus*, was recorded subsequently in the autumn of 2022 under *Tilia* tree in Sofia city. It was documented macroscopically with both golden yellowish and white *Sepedonium* synanamorphs. In the summer of 2024-2025, a new boletaceous host, *Xerocomus subtomentosus* was discovered from Forebalkan, at alt. ca 460 m from the turkey oak forest infected by the same *Sepedonium* species. The aleurioconidia from selected collections were examined morphologically in water mounts under Boeco BM-117 and Olympus BX-41 LM. Comparisons of our data on both fungal morphs with the known published information revealed *Sepedonium microspermum*, a new record for Bulgarian mycota. *Sepedonium microspermum* posesses globose aleurioconidia sized usually 11–15 µm in diameter, and ovoid to cuneiform, hyaline phialoconidia, ca 9–16 ×5–8 µm in our collections. It was commonly known to infect basidiomata of *Xerocomellus chrysenteron* and related taxa in Europe and North America. This work was held within the project 'Phylogeny, taxonomy and sustainable use of fungi'.

P103 Multi-omics analysis highlights genetic regions impacting tomato fruit quality under drought stress

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Keywords: Abiotic stress, Tomato, GWAS, WRKY46,

Water scarcity poses a major challenge to tomato cultivation, as water stress triggers a cascade of physiological disturbances that compromise plant development, yield and fruit quality. This research explored physiological and molecular adaptations to water stress in a diverse GWAS panel of 152 tomato accessions. Concurrently, metabolic alterations in red – ripe fruit were characterized under well-watered and water stressed conditions using GC-MS and LC–MS analysis. Genome wide association mapping using more than 100 000 single nucleotide polymorphisms pinpointed strong association between metabolic traits and candidate genes, most notably *WRKY46* and *cytochrome P450 monooxygenase*. Furthermore, this metabolite-GWAS identified loci controlling adaptive metabolic pathways, linking a promoter SNP in the SlWRKY46 transcription factor to stigmasterol levels. Transcript analysis confirmed more than 2 fold induction in fruit pericarp for both genes under water stress compared to control when mined with Tomato Expression Atlas database. To further explore the specificity of *WRKY46* effect on steroidal glycoalcaloid pathway (SGA) biosynthesis, we designed tomato hairy root system using the tomato cultivar "Money Maker" through *Agrobacterium rhizogenes* mediated transformation with the binary vector pK7WG2D which offers rapid, high-throughput transformation and steroidal metabolite accumulation. It was shown an increased accumulation of cholesterol and the SGA intermediates, dehydrotomatidine, dehydrotomatine and α-tomatin for *WRKY46*-OE compared to *GUS*-OE hairy root lines. This observation demonstrates that the aforementioned transcription factor activates both cholesterol and downstream SGA metabolic pathways. This finding reveal that *WRKY46* is a positive regulator of the sterol/SGA metabolic network, a role consistent with the initial stigmasterol association found in stressed fruit and could acts as a novel regulatory axis with a clear dual function in stress adaptation.

P104 The flora of Dervishka Mogila Nature monument, SE Bulgaria

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Keywords: flora, plant diversity, protected site, Sakar Mountain

Dervishka Mogila mount (690 m) is part of Sakar Mountain – a low mountain and hilly area located in Southeast Bulgaria. The peak with surrounding area of 30 ha are declared as a protected territory – "Skalnite obrazuvania v m. Dervishka Mogila" ("Rock formations in Dervishka Mogila locality") with category III (natural monument). It is also included in "Sakar" Natura 2000 site (BG0000212). The purpose of the study is floristic survey and floristic analysis of the protected area. We report the preliminary results of the inventory and mapping of vascular plant species in the region of Dervishka Mogila peak, including species of conservation importance (e.g. *Alkanna tinctoria* Tausch, *Tulipa australis* Link), endemics (e.g. *Armeria rumelica* Boiss., *Moehringia grisebachii* Janka) and relicts (e.g. *Aconogonon alpinum* (All.) Schur, *Carpinus orientalis* Mill.). The occurrence of non-native and invasive alien higher plant species is discussed.
We further report the results of the floral analysis (incl. taxonomic and ecological structure, and floristic elements), performed on the basis of the full list of vascular plants identified in Dervishka Mogila Natural monument.

P105 The Impact of potassium chloride (KCl) on germination and seedling growth of *Aegilops neglecta* Req. ex Bertol.: Implications for salinity tolerance

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Keywords: *Aegilops neglecta* Req. ex Bertol., KCl, salinity, germination, seedling growth, salt stress

Soil salinity poses a significant threat to global agriculture, impacting crop productivity and land sustainability. Understanding the mechanisms of salt tolerance in wild relatives of crops holds the key to developing salt-resilient varieties. This study investigates the effects of potassium chloride (KCl), a common salt contributing to soil salinity, on the germination and seedling growth of *Aegilops neglecta* Req. ex Bertol, a wild relative of wheat. Seeds were subjected to varying concentrations of KCl (0 mM, 50 mM, 100 mM, 150 mM, 200 mM, 250 mM and 300 mM) in controlled laboratory conditions. Germination percentage, germination rate, seedling length (shoot and root), and seedling dry weight were assessed. The results revealed a concentration-dependent inhibitory effect of KCl on all measured parameters. This study demonstrates that KCl significantly inhibits the germination and seedling growth in the studied genotupes of *A. neglecta* Req. ex Bertol. The observed reductions in germination percentage, germination rate, root length, shoot length, and biomass accumulation are indicative of the stressful effects of KCl on plant development. However, *A. neglecta* Req. ex Bertol. exhibits some degree of tolerance to KCl stress, suggesting the presence of underlying salt tolerance mechanisms. Based on the integrated analysis of tolerance and susceptibility indices, genotype BGR43687 was identified as tolerant to salinity stress, suggesting it potential utilization as valuable sources for salt tolerance genes in wheat breeding programs.

P106 Application of Geographical Information System (GIS) tools for analysis of *Aegilops* collection in the National genebank of Bulgaria

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Keywords: *Aegilops*, Diva-GIS, diversity, collection, habitat suitability mapping

GIS mapping is an invaluable tool for preliminary diversity analysis, identification of gaps in collections, assessment of diversity loss and prediction of distribution. Despite its potential, it is less used in the context of PGR management in Bulgaria. Therefore, the aim of this study was to analyse the *Aegilops* collection of the National Genebank of Bulgaria using the DIVA GIS tools. The study used data on Bulgarian populations of *Aegilops* sp. collected and stored in the genebank from 1984 to 2024. The analysis of the passport data showed that of the 805 specimens of 9 *Aegilops* species collected, only 253 of 7 taxa were stored in the basic collection of the genebank. The geo-referenced diversity distribution map showed that *Aegilops cylindrica* Host. is the most widespread species in the country, followed by *Aegilops triuncialis* L., *Aegilops biuncialis* Vis., *Aegilops neglecta* Req. ex Bertol. and *Aegilops geniculata* Roth. The species richness analysis identified three subregions in the Haskovo region with the highest diversity of *Aegilops* species: Svilengrad, Ivaylovgrad and Stambolovo. The reserve analysis identified three hotspots of species diversity in the eastern part of the Haskovo region and in the central and eastern parts of the Pazardzhik region, which are considered high priority areas for future collections, surveys and *in situ* conservation. The predicted habitat suitability map, based on historical climate data from 1950 to 2000, indicated the presence of a highly suitable area for *Aegilops* species covering 12 provinces.

P107 What to find and realize in the new scientific monograph "Honeybee mortality in Bulgaria – visible and invisible causes and consequences"

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Keywords: honey bee colony losses, reasons, rates

The scientific monograph done by a team of authors consisting of Evgenia N. Ivanova, Plamen Petrov, Teodora Staykova, Teodora Popova, Vesela Mitkovska, Ivan Stoyanov, Penka Vasileva, Tsenka Chasovnikarova, Stella Stoyanova, Elenka Georgieva summarizes the results of the work done on a research project supported by the Bulgarian Scientific Research Fund by a contract No KP-06-N-51-12 and is dedicated to the identified problems related to the loss of honey bee colonies in Bulgaria. It is based on complex and interdisciplinary approaches and developed in several chapters, sequentially examining the questions of: 1) the identified causes of honey bee mortality worldwide; 2) the extent of honey bee colony losses in Bulgaria over a period of five last years; 3) the significant role of pesticides and their toxicity in the identified negative trend; 4) DNA damage and morphological abnormalities in spermatozoa of drones from *A. mellifera* populations with registered different levels of honey bee colony losses; 5) Histopathological changes in the reproductive system of drones under the influence of pesticide pollution in the environment.

The scientific monograph presents original results and analyses proving that pesticides used in agriculture for plant protection and in beekeeping – against *Varroa destructor* have multilayer toxic potential at the molecular, cellular and organismal level, expressed in the induction of DNA damage, chromosomal aberrations, disturbances in the course of mitotic division, morphological abnormalities in spermatozoa and histopathological changes in the gonads of drones.

The authors provide recommendations to beekeepers and farmers regarding possible actions and measures to prevent the losses of honey bee colonies in Bulgaria and reduce damage to nature and agriculture in a following period.

P108 Some aspects regarding the complex phenotypical profile of the Bulgarian population

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Keywords: descriptive and behavioral characteristics, traist of personality

This study characterized and analyzed the phenotypic diversity in a representative sample of the Bulgarian population, focusing on a complex of selected descriptive and behavioral characteristics and the potential dependencies between them.
The investigation included 945 individuals differing in gender and age (mean 32.3 years).
A total of 29 monogenic, polygenic and multifactorial traits (descriptive, immunological, with genetic predisposition and basic personality characteristics) were object of the investigation.
For data collecting a specific survey was used regarding the selected descriptive traits, blood type (AB0 and Rh), and traits with genetic predisposition. The "Big 5" questionnaire – "Markers for the big five factors", in its adapted version for the Bulgarian population was used to study the basic personality characteristics.
Specific statistically significant relationships were established between most of the studied descriptive, immunological and multifactorial traits, on the one hand, and the basic characteristics of the personality, on the other hand, which allows the grounding of a complex phenotypic profile for the studied Bulgarian population.
The multifaceted design of the study and the achieved results, established trends, and the prepared phenotypic profile of the studied Bulgarian population can be successfully used in the field of education and for professional counseling.

P109 Rare planktonic diatoms in the Black Sea – *Leptocylindrus mediterraneus* and *Chaetoceros tortissimus*

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Keywords: Black Sea, Bulgarian marine waters, non-native diatom species, *Leptocylindrus mediterraneus*, *Chaetoceros tortissimus*

The present study aims to analyze the distribution in Bulgarian marine waters of two rare, non-native planktonic diatom species: *Leptocylindrus mediterraneus* (H. Peragallo) Hasle, 1975 and *Chaetoceros tortissimus* Gran, 1900. Between 2020 and 2024, a total of 162 phytoplankton samples were collected using research vessels or from the shore. Quantitative samples were obtained with a 5 L Niskin bottles. For qualitative analysis, a plankton net with a 20 µm mesh size was used. The samples were fixed onboard with 2% formalin. Analyses were conducted using an Olympus BX41 light microscope at the Institute of Fish Resources, Varna. The challenges facing marine ecosystems due to climate change and anthropogenic pressures are of increasing importance for marine biodiversity. Sea surface temperatures (SST) in the Black Sea exhibit a consistent rising trend. Anthropogenic influence has been a persistent factor affecting the marine ecology since the 1970s. Both non-native planktonic diatom species were found in relatively low abundances. Over three years 2020, 2023, and 2024 the species *L. mediterraneus* was regularly detected in the waters of Varna Bay during September and October. *Ch. tortissimus* was recorded in August 2020 and 2021 in the coastal marine waters at Station Shabla and Station Kamchia.

P110 Horological survey of *Abies* x *borisii-regis* Mattf. (King Boris Fir) in Bulgaria

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Keywords: *Abies x borisii-regis* Mattf., horology, ecology

Abstract: The present study summarizes and enriches the available information on the localities of *Abies* x *borisii-regis* Mattf. in Bulgaria. The information available in the three authorized herbariums in Bulgaria was analyzed: Institute of Biodiversity and Ecosystem Research - Sofia (SOM), Faculty of Biology of Sofia University (SO) and Agricultural University - Plovdiv (SOA), as well as from specialized databases. Personal data from newly established or confirmed localities of the species in Bulgaria were added to the available information. The results of the study summarize and supplement the information on the horology and ecology of *Abies* x *borisii-regis* Mattf. in Bulgaria. They also add new scientific information on King Boris Fir for which studies in Bulgaria are extremely few, especially in the field of ecological studies of the species.

P111 Effects of protected areas and linear elements on bat and moth diversity in intensive agricultural landscapes in Serbia

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Keywords: Chiroptera, Lepidoptera, agroecosystems, acoustic monitoring, light traps

Agricultural intensification has altered landscapes and driven biodiversity loss, notably impacting bat and moth populations through habitat fragmentation. However, heterogeneous agricultural mosaics, including forested areas and linear elements, can provide key refuges and foraging sites that help sustain their diversity. The aim of this study was to evaluate the influence of linear elements and protected areas adjacent to agricultural landscapes in Vojvodina (Serbia) on the diversity of bats (Chiroptera) and moths (Lepidoptera). The study was conducted within an intensively used agricultural landscape. To assess the importance of protected areas and linear elements, we used passive acoustic sampling for bats and LED light trap sampling for moths. We placed bat detectors and light traps at three distances from the protected area boundaries: directly at the edge, 1.5 km and 3 km away. Sampling occurred in three site types: within linear elements, in adjacent fields, and in fields without nearby linear features, allowing for a comparative diversity analysis across different landscape contexts. The Shannon Diversity Index was calculated to represent the diversity of studied groups at particular sites and was used to compare diversity across different distances from protected areas and between site types. Distance from protected area boundaries had a significant effect on bat diversity, with the highest diversity index values closest to the protected areas. In contrast, moth diversity remained relatively consistent regardless of proximity to the protected areas. However, linear elements significantly affected moth species richness, with more species found in these sites compared to agricultural fields, while bat diversity showed no significant variation between linear elements and agricultural fields. Bats and moths respond sensitively to landscape structure, making them valuable indicators of habitat alteration. These findings highlight the distinct roles of protected areas and linear elements in maintaining their biodiversity within agricultural landscapes.

P112 Fungal diversity of bat skin from caves of Eastern Serbia: contribution to the understanding of subterranean biodiversity

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Keywords: Bats, Fungi, Opportunistic pathogens, Serbia, Skin microbiota, White-Nose Syndrome

Subterranean ecosystems, such as caves, harbor unique and understudied microbial communities, including diverse fungal assemblages associated with cave-dwelling bats. Following the growing attention to fungal threats in bats, including the White-nose syndrome (WNS) caused by fungus *Pseudogymnoascus destructans*, we investigated the wing and nose skin mycobiota of bats in Eastern Serbia with the aim of detecting the presence of potentially pathogenic fungi. Skin swab samples were collected from 11 individuals of bats belonging to the following species: *Miniopterus schreibersii* (seven individuals), *Myotis capaccinii* (three individuals), and *Rhinolophus euryale* (one individual). Samples were collected during spring, summer and autumn in two caves in Eastern Serbia (Bogovinska and Sesalačka cave), inoculated on Sabouraud dextrose agar and incubated at 5 °C in darkness for two months. All of the tested samples were negative for WNS causative agent based on morphological characteristics and ITS sequencing. However, results revealed a diverse species of psychrophilic and psychrotolerant fungi associated with bat skin. ITS-based identification confirmed several genera, including the following species: *Debaryomyces hansenii*, *Cladosporium cladosporioides*, *Microascus atrogriseus*, *Chaetomium subaffine*, *Coprinellus xanthothrix*, and *Malbranchea chinensis*. Additional isolates of *Aspergillus* sp., *Mucor* sp., and *Penicillium* spp. could not be resolved to the species level using ITS data alone. Five of the eleven identified taxa are known opportunistic pathogens in humans and animals. These findings suggest that, even in the absence of *P. destructans*, bat skin may host fungal species of potential ecological and public health significance. Further species-level identification and functional characterization are warranted to assess their pathogenic potential and role in host–microbiota interactions.

Acknowledgments: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Contract nr. 451-03-136/2025-03/ 200178

P113 Comparative analysis of morphological traits and body condition in *Myotis blythii* and *Myotis myotis* from the Central Balkans

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Keywords: Bats, Body condition index, Morphological traits, *Myotis blythii*, *Myotis myotis*

Differences in body mass, forearm length, and body condition index (BCI) reflect species-specific ecological adaptations and can contribute to more accurate species identification based on morphological traits and assessment of individual bats' physiological condition. Due to their morphological similarity, distinguishing between *Myotis blythii* and *Myotis myotis* in the field remains a challenge, particularly in sympatry regions such as the Central Balkans. The aim of this study was to evaluate these morphological traits and the body condition of individuals of both species roosting in cave shelters across the Central Balkans. We measured the forearm length (mm) and body mass (g) of 80 adult individuals (females and males) belonging to the species *Myotis blythii* (43 individuals) and *Myotis myotis* (37 individuals). The fieldwork was carried out in ten caves in Bosnia and Herzegovina, Montenegro, and Serbia. The average forearm length of *M. myotis* (60.60 ± 2.54 mm) was significantly greater than that of *M. blythii* (57.66 ± 2.08 mm) ($F = 31.32$, $p < 0.001$). Similarly, the average body mass of *M. myotis* (25.57 ± 3.16 g) was significantly greater than that of *M. blythii* (23.55 ± 3.05 g) ($F = 8.36$, $p = 0.005$). Body condition index, assessed via residuals from the mass-size regression, did not differ significantly between the two species. Sex-based differences in body mass within each species were not statistically significant. No statistically significant differences in body condition were found between sampling sites or in major geographical regions. Despite occupying the same roosting habitats in the Central Balkans, *Myotis myotis* and *M. blythii* differ significantly in forearm length and body mass, highlighting clear morphological divergence useful for field identification. The lack of significant differences in body condition index between species, sexes, and locations indicates comparable physiological status and resource availability in shared habitats.

Acknowledgments: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Contract nr. 451-03-136/2025-03/ 200178 and 451-03-137/2025-03/ 200178

P114 Bioaccumulation of some heavy metals in soil, plants and insects

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Keywords: plants, insects, heavy metals

The study in this scientific paper aimed to analyze the content of potentially toxic elements — heavy metals lead, cadmium, and zinc in soil, plant samples, and insect samples from three different areas: an uninhabited area, an industrial zone, and an urbanized area.
Using inductively coupled plasma mass spectrometry (ICP-MS), it was found that the levels of heavy metals in soil, plants, and insects from the three studied areas were higher for lead, cadmium, and zinc in the industrial zone — soil: Pb - 850, Cd - 21.3, Zn - 996 (mg/kg); plants: Pb - 191, Cd - 7.79, Zn - 298 (mg/kg); insects: Pb - 24.5, Cd - 1.32, Zn - 160 (mg/kg).
The results for each individual sample are presented as the mean value of three parallel analytical measurements with the corresponding relative standard deviation (RSD%) in percentage. Values below the limit of detection are given as < the respective limit of detection (LOQ).
Based on the obtained data, the degree of bioaccumulation of lead, cadmium, and zinc was traced using the bioaccumulation factor (BAF) through the pathway: soil, plants, and insects. A BAF < 1 was found for plants, and a bioaccumulation process for cadmium and zinc was observed in two insect species (BAF > 2).

P115 Filarial infection in South Caucasian bats and their ectoparasites

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Keywords: vector-borne parasites, bat blood parasites, Filarioidea nematodes

Recent research on chiropteran parasites suggests a high prevalence and diversity, and extensive spatial distribution of filarial species; however, ecological and phylogenetic studies are still in their infancy. We sampled blood from 78 bat specimens, collected 1181 ectoparasites at summer colonies in Armenia and Georgia, and used nested-PCR targeting the cytochrome c oxidase subunit I gene to detect and genotype filarial parasites. The overall prevalence of filarial DNA was 17.9 % in blood samples of *Myotis blythii*, *Myotis emarginatus*, *Miniopterus schreibersii*, and *Rhinolophus ferrumequinum*, and 8.5 % in ectoparasites, including two mite species (*Eyndhovenia euryalis* and *Spinturnix myoti*) and two bat flies (*Nycteribia kolenatii* and *Penicillidia dufouri*). The prevalence of microfilarial infection was significantly higher in mites (13.8 %) than in bat flies (4.1 %). Bats with ectoparasites positive for filarial DNA had a significantly higher total number of ectoparasites. Phylogenetic analysis placed the 18 sequences obtained into different closely-related clades of onchocercid nematodes, with four different species recorded: two belonging to the genus *Litomosa* and two to a newly observed genus of the family Onchocercidae. Additionally, two new species of these parasites, one *Litomosa* sp. and one Onchocercid sp., were genetically recognised. As predicted, diversity of filarial parasites reflects the diversity of bat hosts in the Caucasus.

P116 Notes on morphological and taxonomical characteristics of *Caenis luctuosa* (Burmeister, 1839) (Ephemeroptera: Caenidae) - a new mayfly species for the fauna of Republic of North Macedonia

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Keywords: Freshwater, rivers, aquatic insects, mayflies'diversity, Balkans

Although mayflies (Ephemeroptera, Insecta) play essential role in the freshwater ecosystems as bioindicators in biomonitoring activities on European scale, their distribution across Balkan ecoregions is still fragmentary and more or less outdated. These gaps raised the research motivation to focus dipper on the current regional species diversity and the richness across semi-mountainous and mountainous rivers from the territory of Republic of North Macedonia. Thus, in the last few years we are step by step filling the gap as within the present research we report the first country records of *Caenis luctuosa* (Burmeister, 1839). Up to now, four species within the Caenidae family are known - *Caenis horaria* (Linnaeus, 1758), *Caenis macrura* Stephens, 1835, *Caenis pseudorivulorum* Keffermüller, 1960 and *Caenis strugaensis* Ikonomov, 1961). The larval stadium of *C. luctuosa* was collected from various river and the natural lake littoral zone localities, mainly in semi-mountainous regions that belong to hydrofaunistical Ecoregion 6 (Hellenic Western Balkans). These findings are part of the water assessment monitoring programs based on freshwater macroinvertebrates. We also present the illustrated description of larval morphological characteristics of *C. luctuosa* (for comparative determination features with related Caenidae species updating the mayfly keys). The results have also been confirmed with the DNA barcoding. Further notes on its zoogeography and ecology is given.

Acknowledgements: National program „Young scientists and post-doctoral students-2"- BAS, Module „Post-doctoral students", Project title: Mayfly fauna (Insecta: Ephemeroptera) as a hotspot zone in the biodiversity of the Balkan Peninsula: taxonomical, ecological and conservation approaches.

P117 Pesticide-induced DNA damage and morphological abnormalities in *Apis mellifera* drone spermatozoa correlate with colony mortality

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Keywords: *Apis mellifera*, sperm morphology, DNA damage, pesticide exposure, colony losses

Environmental stressors, particularly anthropogenic pesticide exposure, are well-established contributors to the compromised reproductive fitness of *Apis mellifera* drones, a factor implicated in broader colony decline. This investigation aimed to elucidate the link between pesticide-induced DNA damage, subsequent morphological aberrations in drone spermatozoa, and observed increases in colony mortality rates. Semen samples were collected from drones across six apiaries, exhibiting a wide range of annual colony losses (0.8% to 63.5%). Comprehensive chromatographic analyses identified residues of 24 distinct pesticides in both bee samples and apicultural food stores. Spermatozoa DNA integrity was quantitatively assessed using the comet assay, with damage severity determined via tail intensity percentage (TI%) and Olive tail moment (OTM). Furthermore, sperm morphology was evaluated through Bio-Diff–stained smears, with a minimum of 200 cells systematically analyzed per replicate.

The findings reveal a statistically significant incidence of morphological abnormalities—specifically impacting the acrosome, head, and flagellum—in drones originating from colonies experiencing elevated mortality rates. Correspondingly, comet assay results indicated the most pronounced DNA damage in spermatozoa collected from apiaries characterized by the highest recorded levels of colony loss. Linear regression modeling demonstrated robust positive correlations between the prevalence of sperm abnormalities, quantifiable DNA damage parameters, and increased colony mortality. Notably, the most compelling correlations were observed between colony loss and comet assay indicators, specifically TI% ($R^2 = 0.991$) and OTM ($R^2 = 0.963$). Analogous strong associations were established between DNA damage and the proportion of morphologically aberrant spermatozoa. Collectively, these data substantiate a direct causal relationship among pesticide-induced cytogenotoxic stress, impaired drone reproductive health, and heightened colony mortality. Consequently, comet assay parameters and detailed sperm

morphological assessments emerge as efficacious biomarkers for reproductive and environmental risk assessment within *Apis mellifera* populations.

Acknowledgments: This study was supported by the National Research Fund of Bulgaria by the contract KP-06-H51-12/2021, "Complex assessment of genetic and environmental factors related to the losses of honey bees (*Apis mellifera* L.) in Bulgaria."

P118 Environmental genotoxic stress in cave-dwelling *Miniopterus schreibersii* populations in Bulgaria

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Keywords: bats, cadmium, lead, micronuclei, polychromatic erythrocytes

Bats are recognized as efficacious biomonitors in environmental risk assessment, owing to their role as apex insectivores and their susceptibility to anthropogenic habitat alterations. Their longevity and tendency to bioaccumulate contaminants make them valuable indicators of widespread pollution and ecosystem disturbance. *Miniopterus schreibersii*, a common bat species in Bulgaria with wide-ranging foraging habits, is believed to accumulate a diverse, potentially toxic metal profile in its guano due to its varied prey diet. Bat guano serves as a non-invasive proxy for assessing environmental conditions and wildlife exposure to contaminants. The frequency of micronuclei (MN) is a highly integrative biomarker, capable of detecting genotoxic damage from various mechanisms, particularly relevant in evaluating the effects of environmental contaminants. This study investigated cadmium and lead concentrations in *M. schreibersii* guano and the frequencies of micronuclei (MNs) and polychromatic erythrocytes (PCEs) in the peripheral blood of bats from three Bulgarian caves. The aim was to assess environmental genotoxic stress within these populations. A causal relationship was established between guano lead and cadmium concentration, reflecting exposure, and observed MN frequencies, representing a genotoxic effect. This correlation confirms that cadmium and lead levels in guano are indicative of systemic exposure and potential DNA damage in bats. The detected MN and PCE frequencies signify genotoxic and cytotoxic stress responses in these cave-dwelling insectivorous bats, likely attributable to the mutagenic potential of their surrounding cave environment. Consequently, MN and PCE analyses in bat erythrocytes offer a robust methodology for assessing the health impacts of environmental contamination on these crucial mammals and, by extension, on the ecosystems they inhabit.

Acknowledgments: This study was supported by the National Research Fund of Bulgaria by the contract KP-06-H71/5, "Study of the role of *Miniopterus schreibersii* (long-winged bat) as an "umbrella species" for determining the zoonotic potential of cave-dwelling bat species in Bulgaria."

P119 *Tolypothrix distorta* Kützing ex Bornet & Flahault (Cyanobacteria) as a producer of cyanotoxins

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Keywords: Cyanobacteria, cyanotoxins, *Tolypothrix*

Cyanotoxins produced by some species of Cyanobacteria can be a real threat to human and animal health and pose a serious ecological risk. Therefore, it is important to know the cyanobacteria that produce cyanotoxins. Little is known about the representatives of the genus *Tolypothrix* that produce cyanotoxins, and what toxins they produce. Two strains of *Toplypothrix distorta* were tested for cyanotoxin production using an ELISA assay. Methanol extracts of the *Toplypothrix distorta* strains (for detection of intracellular toxins) and the medium in which they were cultured (for detection of extracellular toxins) were examined. The tests conducted showed the presence of both hepatotoxins and neurotoxins in the samples from both *Toplypothrix distorta* strains. In the cyanobacterial growth medium of the *Toplypothrix distorta* strain CCALA 194, we detected only hepatotoxins – microcystins/nodularins with a concentration of 0.2 ng/mL and cylindrospermopsin with a concentration of 0.07 ng/mL. In the extract from the same strain, we detected 0.05 ng/mL of cylindrospermopsin and saxitoxins with a concentration of 0.15 ng/mL. The analyses showed that *Tolypothrix distorta* strain SAG 1482-2 produces microcystins/nodularins in the growth medium and in the extract (0.13 ng/mL and 0.5 ng/mL, respectively). Cylindrospermopsin (0.3 ng/mL) and saxitoxins (0.3 ng/mL) were detected in the extract obtained from the strain. So far, *Tolypothrix distorta* has been reported as a producer of saxitoxins. As a result of the present studies, we can conclude that the two strains of *Tolypothrix distorta* (CCALA 194 and SAG 1482-2) are producers of microcystins/nodularins, cylindrospermopsin and saxitoxins. These data enrich the knowledge about the toxic potential of the genus *Tolypothrix* and in particular the species *Tolypothrix distorta*.

P120 *In vitro* cytotoxic effects of extracts from *Tolypothrix* strains (Cyanobacteria) on human carcinoma and normal cell lines

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Keywords: Cyanobacteria, *Tolypothrix*, cytotoxicity, carcinoma cells, fibroblasts

Assessment of the biological activity of cyanobacterial extracts with the aim to evaluate their practical application is important and relevant issue. Effects exerted by extracts (non-polar and polar fraction) obtained from five *Tolypothrix* strains were evaluated using three human carcinoma (Caco-2, HeLa and HT-29) and one normal (fibroblast; HFFC) cell line. The Neutral red assay was applied to evaluate the cytotoxic effects. The cells responded differently to treatment with non-polar and polar *Tolypothrix* extracts depending on the cell line, the type of extract, the applied dose (50 µg/mL, 100 µg/mL and 200 µg/mL) and the exposure time (24, 48 and 72 h).

The viability of cancer cells was affected as early as 24 h after treatment with non-polar extracts at a concentration of 200 µg/mL. The percentage of cell growth inhibition was statistically significant (I) in Caco-2 cells treated with extract from strains *T. tenuis* PACC 5497 (23%) and *T. distorta* SAG 1482-2 (26%); (II) in HeLa cells treated with extract from strains *T. tenuis* PACC 8648 (23%), *T. distorta* CCALA 194 (30%) and *T. distorta* SAG 1482-2 (18%); and (III) in HT-29 cells treated with extracts from strains *T. tenuis* PACC 5497 (24%), *T.* distorta SAG 1482-2 (27%) and *T. tenuis* PACC 8648 (29%). This inhibition rate almost doubled at the same concentration at 48 and 72 h, indicating a dose- and time-dependent effect. Such effect was not detected in fibroblasts after 24 and 48 h exposure to the same extract concentrations. Thus, a selective anticancer effect has been established.

Polar extracts of *Tolypothrix* strains induced statistically significant cytotoxic effect in almost all cell lines treated for 72 h with 200 µg/mL. The inhibitory activity varied between different extracts and cell lines within 5–40%. Lower doses of 50 µg/mL and 100 µg/mL had a stimulatory effect or no effect and this was observed at all three exposure times. There was no clear anticancer effect of polar extracts. For some samples, there was a weakly pronounced trend. The stimulatory effect found at lower concentrations of polar extracts is a common and well-known phenomenon for toxic compounds.

P121 *In vitro* and *in silico* evaluation of the cytotoxic and anticancer potential of arachidonic acid

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Keywords: arachidonic acid, anticancer activity, molecular docking, COX-2, ALOX5

Arachidonic acid ($C_{20:4\ (n-6)}$) is an ω-6 fatty acid involved in many important functions in the body, including immune function, brain function, growth, muscle repair, and anti-inflammatory effects. However, the anticancer potential of arachidonic acid (ARA) has not been sufficiently studied. The aim of our study was to evaluate the anticancer activity of ARA by assessing its effect on three carcinomas and one normal cell line *in vitro* at varying exposure times (24 h, 48 h and 72 h) and concentrations (1.2 µg/mL, 6 µg/mL and 30 µg/mL). Data showed a clear dose- and time-dependent cytotoxic effect observed in all carcinoma cell lines, supported by high statistical significance. In normal human fibroblasts, the cytotoxic effect induced by ARA treatment was significantly weaker, indicating a specific anticancer activity. The most sensitive to the action of ARA were the HT-29 cells with an IC_{50} value of 3.61 µg/mL, followed by HeLa cells with an IC_{50} value of 4.02 µg/mL, CaCo-2 cells with an IC_{50} value of 6.39 µg/mL and human fibroblasts with an IC_{50} value of 27.4 µg/mL. The key mechanisms of biological activity involving ARA are several and they are related to its target molecules and receptors, such as COX-2 and ALOX5. The binding affinity of ARA to COX-2 and ALOX5 was calculated. Optimization of the 3D structures was performed and the docking site was determined. The interaction energy was also calculated. Analysis of the results showed the most likely positions and configurations for these interactions. The use of arachidonic acid may provide multiple health benefits.

P122 Morphological characteristics and pigment composition of *Tolypothrix* strains (Cyanobacteria)

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Keywords: Cyanobacteria, *Tolypothrix*, morphology, pigments, variability

Cyanobacteria are prokaryotic, photosynthetic organisms with a long evolutionary history, cosmopolitan distribution and great biodiversity. They are the source of a number of biologically active substances, carrying both benefits and risks to humans and animals. The simple morphology and high level of variability make their correct identification problematic. Therefore, the refinement and detailing of identification criteria is important. The present study presents data on the morphology and pigment composition of five *Tolypothrix* strains, which have not been studied in this direction so far. Cell biometric measurements were performed on a minimum of 50 cells. Since Cyanobacteria are a potential source of phycobiliproteins, in addition to the other classification criteria, the qualitative and quantitative composition of the main antenna pigments – phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC), chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids – was added. The amount of PE detected in the different strains of *Tolypothrix* varied between 56 µg/mL (strain *Tolypothrix sp*. PACC 5501) and 140 µg/mL (strain *T. tenuis* PACC 5497). This strain also produced the highest amount of APC – 70.35 µg/mL. PC production was highest in strain *T. distorta* SAG 1482-2 – 69.95 µg/mL, which also produced high levels of PE (71 µg/mL) and APC (64.6 µg/mL), and lowest in strain *Tolypothrix sp*. PACC 5501 – 21.2 µg/mL. On the other hand, strain *Tolypothrix sp*. PACC 5501 produced the highest amount of chlorophyll-a (3.7 µg/mL) and chlorophyll-b (2.49 µg/mL), and had the highest value for the total chlorophyll indicator – 6.2 µg/mL. The measured amount of carotenoids ranged from 1.6 µg/mL (*T. distorta* strain CCALA 194) to 4.02 µg/mL (*T. distorta* strain SAG 1482-2). Data obtained in the study identified the strains *T. tenuis* PACC 5497 and *T. distorta* CCALA 194 as suitable for the biotechnological production of PE and APC, and the strain *T. distorta* SAG 1482-2 for the production of PE, PC and APC.

P123 Megakaryocytes from rat bone marrow, volume density and structural features

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Keywords: bone marrow, megakaryocytes, rats

The bone marrow is a hematopoietic organ that is important for the proper functioning of the organism. Therefore, knowledge of the structure and function of healthy bone marrow is extremely important for the diagnosis of various pathological conditions. Megakaryocytes are polyploid cells that are involved in the formation of platelets and their distribution in the circulation.
The aim of this study was to determine the average volume density and size of megakaryocytes, the grouping of megakaryocytes and the degree of lobulation of the nucleus of megakaryocytes in healthy rat bone marrow. Five examined groups of bone marrow stained with hematoxylin & eosin were analyzed. The test results were determined using the Image J program and statistically evaluated using the ANOVA program.
The results of the average size of megakaryocytes obtained in our study were similar between the groups and the values were approximately (157.00 μm). The average megakaryocyte volume density varied slightly between groups and changed with the degree of cell maturation. Clustering of megakaryocytes was observed in groups of 2 or more cells. A polylobulated nucleus was present in each group, but with differences in the degree of nuclear lobulation. From the results, it can be concluded that the clustering of cells and polylobular nuclei are also characteristic of normal conditions and not only of pathological conditions, as has been shown in some studies.
To date, there is little literature data that has dealt extensively with the analysis of rat megakaryocytes, and there is almost no data on megakaryocyte volume density. Of all the parameters analyzed, the information on megakaryocyte grouping is the most interesting. While in humans, megakaryocyte grouping has mostly been associated with polycythemia, our study has shown that the grouping of 2 to 3 megakaryocytes in rats may not be related to a pathological condition, but also prompted us to think about what causes the tendency of megakaryocytes to group and whether the cause is similar or different from that of human megakaryocytes.

P124 Nucleoplasmic bridges as a biomarker for genomic instability and predictors for cancer risk

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Keywords: nucleoplasmic bridges, biomarkers, genomic instability, cancer risk

Introduction: Chromosome and nuclear alterations, especially nucleoplasmic bridges (NPBs) are excellent biomarker to detect short- and long-term genotoxic effects. For that reasons these abnormalities can be reasonably used as biomarkers to evaluate an individual's cancer risk. With Cytokinesis-Blocked Micro Nucleus (CBMN) we could measure early biological effects of ionizing radiation and different genotoxic chemicals or genotoxic damage on human cells.
Aims of the study: This study aimed to determine the presence of (NPBs) in peripheral binuclear blood lymphocytes, as a potential bio-dosimeter for genotoxicity on humans exposed to ionizing rays (IR).
Material and methods: Blood samples were collected in vitro from healthcare workers exposed to IR a strong clastogenic agent and a potent inducer of NPBs. CBMN assay has proven to be a reliable and useful assey in the field of cytogenetics and human biomonitoring.
Results: The presence of nuclear anomalies observed as a NPBs confirmed the genotoxic effects two or more years after an exposure of human population to IR. The healthcare workers with long time experience or longer exposer on IR and also smokers have a high frequency or presence of NPBs than the control group, young and health populations, non-exposed to IR.
Conclusion: NPBs can confirm existence of a possible influence of on genetic damage induced by ionizing radiation and smoking, also can be used as biomarkers to evaluate genomic instability as predictors for individual's cancer risk.

P125 Effect of microplastic exposure on hematological parameters in mice

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Keywords: polystyrene, microplastics, mice, blood, toxicokinetic

Microplastics (MPs), defined as plastic particles smaller than 5 mm, are an emerging class of environmental contaminants. Widely distributed in air, water, and soil, they can enter biological systems mainly through ingestion and inhalation. The first detection of MPs in human blood was recently reported, confirming their ability to cross the epithelial barriers and circulate systemically. These findings raise significant concerns about the potential physiological impacts of MPs' exposure in humans. Therefore, this study aimed to evaluate the effects of subchronic oral exposure to polystyrene microplastics (PS-MPs) on hematological parameters in a mouse model. Male Swiss albino mice were randomly assigned to a control group or an experimental group that received 1 μm PS-MPs suspended in drinking water at a dose of 0.01 mg/day for four weeks. Blood samples were collected weekly and analyzed for complete blood count (CBC) parameters to assess potential alterations in hematological profiles. Exposure to PS-MPs resulted in a progressive decline in several hematological parameters. Notably, reductions in granulocyte count (Gran#), monocyte count (Mon#), monocyte percentage (Mon%), and mean platelet volume (MPV) were observed after the first week. On the fourth week, decreases in red cell distribution width (RDW), platelet count (PLT), and plateletcrit (PCT) were recorded. In contrast, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were significantly elevated, suggesting alterations in erythrocyte morphology and hemoglobin content. The findings of this study demonstrated that subchronic oral exposure to PS-MPs can induce significant alterations in hematological parameters in mice. The observed shifts in leukocytes and erythrocytes suggest impaired hematopoiesis, potential inflammatory responses, and disruptions in blood homeostasis. These results highlight the capacity of MPs to affect systemic physiology and underscore the need for further research into their short- and long-term health impacts, particularly concerning immune and hematological function in both animals and humans.

Acknowledgements: This work was supported by Grant № КР-06-Н81/2, National Science Fund.

P126 Promising new serum biomarkers for diagnosis of colorectal carcinoma

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Keywords: colorectal cancer, mucin-1, galectin-9, lysozyme, biomarkers

Colorectal carcinoma (CRC) is one of the leading oncological diseases in terms of incidence and mortality. The gold standard for screening and diagnosis is colonoscopy with histological examination, but this is an invasive method. Therefore, serum biomarkers are sought to support the non-invasive diagnosis, prognosis and monitoring of colon cancer. In this regard, the aim of the current study was measurement and comparative analysis of serum levels of other potential biomarkers in patients with CRC and healthy individuals. Twenty patients aged between 45 and 80 years with stage IV of CRC undergoing chemotherapy, and 20 healthy volunteers aged between 40 and 72 years, were included in the study. Serum levels of mucin-1, galectin-9 and lysozyme were measured by ELISA method using a SpectraMax i3x spectrophotometer. In patients with CRC, serum levels of mucin-1 (mean 4.02 ng/mL) were significantly higher as compared with healthy individuals (mean 0.55 ng/mL, $p = 0.0008$). Despite variability in serum galectin-9 levels among individual CRC patients, galectin-9 concentrations in this group were significantly elevated in comparison with healthy controls (mean 68.52 ng/mL vs. 16.13 ng/mL, $p = 0.0009$). Similar results were obtained for the levels of lysozyme. CRC patients showed increased levels (mean 176.62 ng/mL) compared to the control group (126.45 ng/mL, $p = 0.0016$). Our results demonstrate that mucin-1, galectin-9 and lysozyme could be used as biomarkers to improve early detection of CRC or determine the prognostic risk and response to treatment. The use of an appropriate combination of several biomarkers in the routine clinical practice will allow for high accuracy in the diagnosis of CRC and personalized treatment management.

P127 Immunomodulatory properties of extracts from *Tolypothrix* strains (Cyanobacteria)

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Keywords: *Tolypothrix*, peripheral blood leukocytes, immunomodulatory activity

Extracts from different cyanobacterial species represent a valuable source of biologically active compounds. It has recently been shown that the non-polar extract fractions obtained from five *Tolypothrix* strains (*T. tenuis* PACC 5497, *T. tenuis* PACC 8648, *T. distorta* CCALA 194, *T. distorta* SAG 1482-2, *Tolypothrix* sp. PACC 5501) demonstrate significant anti-inflammatory potential, which was defined in mouse macrophage model RAW264.7. Therefore, the present study aimed to evaluate the immunomodulatory activity of *Tolypothrix* extracts using human immune cells. Peripheral blood leukocytes were obtained from patients with inflammatory conditions and were treated *ex vivo* with 100 μg/mL *Tolypothrix* extracts for 48 h. The cells were then analyzed by flow cytometry and the percentages of main immune cell populations were determined. The levels of cytokines secreted by human leukocytes after treatment with cyanobacterial extracts were determined by ELISA. Immunophenotypic analyses demonstrated significantly increased T, NK and B lymphocyte populations in the cultures treated with *Tolypothrix* extracts compared to the control untreated cells. Particularly, elevated percentages of T cells did not correlate with increased expression of the activation marker CD25 suggesting that the observed effect was not due to uncontrolled activation, and the treatment with cyanobacterial extract is associated with an immunomodulatory effect. This was confirmed by the cytokine analyses that showed lower content of IL-2, IL-6, TNF-α and IFN-γ in the culture supernatant of treated cells compared to the untreated controls. In addition, the production of IL-10 was not affected except for the cells treated with *T. distorta* SAG 1482-2. Overall, these data indicate that non-polar *Tolypothrix* extracts exert immunoregulatory activity based on reduction of proinflammatory cytokine levels, sustaining the levels of main immune cells populations and the anti-inflammatory response.

P128 Anti-collagen type II antibody responses in ACPA-positive rheumatoid arthritis and osteoarthritis patients from Bulgaria

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Keywords: collagen type II, citrullination, osteoarthritis, rheumatoid arthritis

Collagen type II (COL2) is the main extracellular matrix protein in hyaline cartilage. Therefore, the pathogenesis of diseases that affect the joints could be associated with an immune attack against this molecule. Anti-COL2 antibody reactivity has been detected in a subgroup of patients with rheumatoid arthritis (RA), and three major epitopes for collagen-specific humoral immunity have been identified (denoted as the C1, U1, and J1 epitopes).

In the present study, blood plasma from 38 RA patients who were positive for anti-citrullinated protein antibodies (ACPA) and 30 osteoarthritis (OA) patients was analyzed by ELISA to determine the levels of anti-COL2 antibodies, as well as antibodies specific for the C1, citrullinated C1, U1, and J1 epitopes of COL2.

The results demonstrated significantly higher anti-COL2 reactivity in OA patients compared to ACPA-positive RA patients. The levels of antibodies specific for the C1, citrullinated C1, U1, and J1 epitopes were also markedly higher in OA patients. In both the RA and OA groups, a strong correlation was observed between the titers of anti-COL2 protein antibodies and anti-C1/citrullinated C1 epitope antibodies, anti-U1 epitope and anti-C1/citrullinated C1 epitope antibodies, and between the levels of anti-J1 and anti-C1/citrullinated C1 epitope antibodies. The analyzed antibody titers did not correlate with the age of the patients. A moderate correlation was found between the levels of anti-COL2 protein antibodies and anti-citrullinated C1 epitope antibodies in RA patients, but not in OA patients, confirming the specificity of citrullination for RA-associated humoral immunity.

In conclusion, it was demonstrated that anti-COL2 protein and anti-COL2 epitope (C1, U1, J1) antibody reactivity is present not only in autoimmune arthritis but also in degenerative joint disease such as OA. Interestingly, humoral responses against COL2 epitopes identified in RA were stronger in OA patients, suggesting a role for this type of immunity in OA pathogenesis.

P129 Leukogram variability as a tool for assessing the conservation-relevant health impact of microfilarial infection in bats

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Keywords: Bats**,** Eosinophilia, Immune reaction, Lymphocyte subpopulations, Wildlife health

Bats can carry zoonotic pathogens without obvious physiological stress, likely due to their unique immune regulation. Although filarial infections occur in bats, their haematological responses are not well understood. Investigating leukogram alterations in infected individuals can offer insights into host–parasite dynamics and serve as a valuable tool for conservation-oriented health assessment. We hypothesised that bats with circulating microfilariae exhibit elevated eosinophil counts, and to test this, we analysed total leukocyte counts and leukogram profiles in four bat species sampled during summer. The first group included 16 microfilaria-free individuals of the Long-fingered bat (*Myotis capaccinii*) from Bogovinska cave. The second group was mixed population of 11 microfilaria-positive individuals – six Schreiber's bent-wing bats (*Miniopterus schreibersii*), two Long-fingered bats (*Myotis capaccinii*), two Greater mouse-eared bats (*Myotis myotis*), and one Mediterranean horseshoe bat (*Rhinolophus euryale*), from Sesalačka cave. Romanowsky-stained blood smears were examined microscopically to determine the total leucocyte count at 40×HPF (average count×2000). The leukogram, including neutrophils, monocytes, eosinophils, basophils, and lymphocytes (subclassified as total, large, small, large granular, and small granular), was assessed at 100×HPF. Statistical analysis was performed using Mann-Whitney U test for independent samples (MedCalc® software). No significant differences were observed in total leucocyte, neutrophil, or overall lymphocyte counts. However, eosinophil count was twice as high in bats with microfilariae ($U = 45$, $z = 2.14$, $p = 0.032$). Additionally, small lymphocyte counts were approximately 30% higher ($U = 33$, $z = 2.71$, $p = 0.007$), while large lymphocyte counts were nearly three times lower ($U = 18$, $z = 3.45$, $p < 0.001$). Monocyte counts were reduced by half ($U = 37$, $z = 2.56$, $p = 0.010$) in bats with microfilariae. Leukogram changes in bats with microfilariae, including increased eosinophils and altered lymphocyte and monocyte profiles, reflect a typical immune response. These findings highlight the potential of haematological markers for monitoring the impact of parasitic infections on bat populations,

especially in vulnerable species or disturbed habitats.

Acknowledgments: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract numbers: 451-456/2024-03/3314, 451-03-136/2025-03/200178, and 451-03-136/2025-03/200143).

P130 Wing skin depigmentation in bats: histological and ultrastructural study

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Keywords: bats, depigmentation, melanin, wing skin

Bats, like all mammals, have pigmented skin due to melanin originating from melanocytes at the base of the epidermis. Depigmentation, partial or complete, can be caused by a reduction and/or dysfunction of melanocytes. Although depigmentation associated with genetic alterations and fungal infections is well documented, its occurrence beyond these contexts has received limited scientific attention. This research aimed to observe tissue and ultrastructural differences in the wing skin of bats with depigmentation, in comparison to normally pigmented areas. Pairwise wing skin punch biopsies (Ø 3 mm) were collected from the plagiopatagium of eight individuals (*Miniopterus schreibersii* N=4 and *Myotis capaccinii* N=4), one taken from the depigmented area and another from the normally pigmented region (serving as a control). Skin samples were fixed using two protocols: in 4% formaldehyde for light microscopy to examine tissue structure, and in 2.5% glutaraldehyde for electron microscopy to analyze tissue ultrastructure. Structural skin features were visualized using different histological stainings and PCNA immunohistochemistry. Aside from reduced pigmentation, the location and appearance of melanin granules remained unaltered. Also, depigmented tissues show increased proliferation of both epidermal and dermal cells, with the epidermis comprising 2–4 cell layers. Epidermal thickness increases to 15–26 µm compared to ~9 µm in *M. schreibersii* and ~8 µm in *M. capaccinii* controls. In the dermis, proliferative changes include more fibroblasts and a lack of large blood vessels, muscles, and hair structures relative to pigmented controls. A positive reaction to PCNA was observed in depigmented regions rich in proliferative cells, where the presence of this protein, known for its roles in cell proliferation and genome protection, indicates active reparative processes in the epidermis. Depigmented tissues lacked signs of fungal infection, including necrosis, inflammatory infiltration, and hyphal presence. Increased epidermal layers and reduced melanin content during proliferation may reflect an adaptive mechanism where thickened epidermis serves as additional skin protection upon melanin reduction. Although the origin of depigmentation remains unclear, aside from melanin-related alterations, the observed histological and ultrastructural changes in the skin highlight complex adaptive responses.

Acknowledgments: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract numbers: 451-456/2024-03/3314, 451-03-136/2025-03/200178).

P131 Evaluation of key traits in green pepper male sterile lines (*Capsicum annum* L.)

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Keywords: backcross, ms-8, pepper plant, F_1 hybrids

Male sterility is a key tool in hybrid breeding of pepper (*Capsicum annuum* L.), as it facilitates efficient F_1 seed production and enables the development of F1 hybrids with high heterosis potential. One well-characterized source of such sterility is the ms-8 system, which is governed by nuclear nature and is widely used for the creation of new F_1 hybrids. Despite its broad application, the phenotypic expression of sterility, as well as the dissimilarities between sterile and fertile plants across different genetic backgrounds, remain insufficiently investigated.

The study was conducted during two consecutive years at Maritsa VCRI, Plovdiv, Bulgaria. It aimed to assess the quantitative characters of mother line with ms-8, stabilized advanced male sterile lines ($BCP_2 \times BCP_2$) developed by it through recurrent backcrossing, and their respective father parents, in order to evaluate phenotypic proximity between parental forms and their progenies. Traits related to plant architecture, bud and fruit morphology as well as sterility : fertility ratio were evaluated. Some ($BCP_2 \times BCP_2$) lines outperformed the mother and/or respective father component by two or more fruit traits, which highlighting their breeding value. Significant differences between fertile and sterile plants, buds, fruits were established within the mother line and new original $BCP_2 \times BCP_2$ green pepper sterile lines. The ratio of fertile to sterile plants in the backcrossing lines did not show significant deviations from the theoretically expected ratio 1:1. The observed dissimilarities between sterile and fertile plants provide insight into potential phenotypic indicators of sterility and are important for the breeding of stable and productive mother lines with various fruit shape and usage. The findings contribute to a deeper understanding and more effective use of the ms-8 system in F_1 hybrid breeding.

P132 Influence of drought stress on morphological and yield traits of Bulgarian green pepper cultivars (*Capsicum annuum* L.)

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Keywords: abiotic stress, *Capsicum annuum* L., irrigation

Climate change - especially the increase in average temperatures and the decrease in water resources - requires in-depth study of vegetable crops in order to increase their tolerance to abiotic stress. Pepper (*Capsicum annuum* L.) is a sensitive crop to water deficit, which makes the identification and evaluation of tolerant genotypes essential. The aim of the present study was to investigate the response of Bulgarian pepper cultivars with different fruit shape intended for early production. The experiment was conducted at the Maritsa Vegetable Crops Research Institute Plovdiv, Bulgaria. Plants were grown under two irrigation regimes: optimal -100% irrigation and reduced by half - 50% irrigation. Data were collected on putative traits on flowers, plants and fruits - including plant height, number of branches at first order, fruit length and width, fruit wall thickness and number of loci. Preliminary results show significant variability in the response to drought stress among the cultivars tested. Changes in overall plant growth and fruit characteristics were detected. Some of the genotypes evaluated showed relatively stable indicators, indicating increased drought tolerance. These results highlight the importance of native Bulgarian gene plasma as a valuable source of drought tolerance in future breeding programs.

P133 Potential for selection of pepper genotypes with increased tolerance to drought stress by their male gametophyte response and productivity

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Keywords: pepper, drought stress, biplot, pollen viability

In the last decade, water scarcity has been a major environmental constraint on plant yield and quality. The effect of drought on sweet pepper is expressed in reduced pollen viability and lack of fertilization, which leads to reduced productivity. The aim of this study was to evaluate eight pepper genotypes under water stress conditions, through their productivity and the response of their male gametophyte. Plants were grown under two irrigation regimes - a well-watered control treatment without stress (NS) and a 50% deficit irrigation - drought stress (DS). Reproduction and yield data were collected during the reproductive stages on three plants from three replicates.

Reducing the irrigation regime had a negative effect on the male gametophyte, reducing pollen viability and pollen tube elongation in all plants studied. BiPlot analysis showed a good correlation between pollen response and productivity components - mean productivity (MP), geometric mean productivity (GMP), harmonic mean productivity (HARM), stress tolerance index (STI) in pepper plants. This defines this methodology as an effective tool for increasing drought tolerance in pepper.

P134 Investigation of the possibilities for the application of fluorescence spectroscopy in combination with cytological methods to support the breeding process in pepper

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Keywords: fluorescence spectroscopy, cytological examination, male sterile line, pepper

An advantage of conducting a study with fluorescence spectroscopy is the small depth of penetration into the plant material or conducting the analysis without violating the integrity of the sample. The technique associated with fluorescence spectroscopy, which was applied in this study, is in vivo. It was chosen because the optical properties of pepper and their spectra also change depending on temperature, pressure, external electric and magnetic fields, etc., which allows obtaining essential information about the sample under study. The studies were conducted during the growing season on stems and leaves of sterile and fertile plants from a selected male-sterile line. When analysing the leaves and stems of sterile and fertile plants, the differences in intensity and emission signal are close, but clearly distinguishable. A clear difference is observed between a sterile and fertile plant, which is proportionally comparable to the results of the cytological study. This gives reason to assume that fluorescence spectroscopy is applicable in primary screening for male sterility during the growing season in a greenhouse. Based on the performed analyses and the overall evaluation of the obtained results, a long-term plan for the development of scientific research will be prepared, which will be aimed at: New results related to the combination of cytological and optoelectronic methods.

P135 Gene expression related to immunity and angiogenesis in colorectal tumors and their metastases assessed by nanostring technology

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Keywords: mRNA, Colorectal Tumor, Immunity, Angiogenesis, Nanostring technology

Introduction: The immune system plays a vital role in recognizing and eliminating tumor cells. Disruptions in immune function, along with abnormal regulation of angiogenesis, are closely associated with cancer development and metastasis. During the metastatic process, tumor cells and the immune system engage in complex bidirectional interactions, both within the tumor microenvironment and throughout the body. The aim of this study was to investigate alterations in the expression of genes associated with immune response and angiogenesis throughout the metastatic progression of colorectal cancer.
Materials and Methods: We employed NanoString technology to measure mRNA expression levels of 20 selected genes related to immunity and angiogenesis. The analysis was performed on tissue samples from primary colorectal tumors and their corresponding metastatic lesions.
Results: Our analysis revealed a more than twofold increase in gene expression in metastases compared to primary tumors for several genes. Notably, ***HIF1A***, which encodes the alpha subunit of the hypoxia-inducible factor-1 transcription complex—a key regulator of cellular adaptation to hypoxia—showed a 4.8-fold increase. ***THBS1***, known to inhibit T cell and dendritic cell activation, increased 4.7-fold. ***KLF10***, implicated in cell cycle regulation, rose 2.7-fold, while ***STAT6***, a transcription factor involved in cytokine signaling, increased 2.4-fold. Additionally, ***AMD1***, a key enzyme in polyamine biosynthesis, and ***ITM2B***, an integral membrane protein, showed 2.2- and 2.1-fold increases, respectively.
Discussion: NanoString technology enables comprehensive mRNA profiling in both primary tumors and metastatic sites, offering insights into the molecular mechanisms driving metastasis. By comparing gene expression patterns across different stages of cancer progression, we can identify critical molecular targets that may serve as prognostic indicators for tumor angiogenesis and metastatic potential.

Acknowledgments: Grant BG-RRP-2.004-0004-C01.

P136 MicroRNA profiling in search of potential biomarkers and therapeutic targets in metastatic melanoma

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Keywords: miRNA, Metastatic melanoma, FFPE Biomarker, Nanostring technology

Introduction: Metastatic melanoma is a deadly aggressive cancer. MicroRNAs (miRNAs) are small RNAs that regulate gene expression and impact tumor growth. This study examines 50 miRNAs to understand their role in melanoma and find potential biomarkers for diagnosis and treatment.
Materials and Methods: The analysis was conducted using formalin-fixed, paraffin-embedded (FFPE) tissue samples from patients diagnosed with metastatic melanoma (n=6), compared to healthy skin tissue (n=3). RNA extraction was followed by miRNA tag ligation reaction and quantitative analysis of microRNA expression by digital counting using the Nanostring platform. Data normalization and fold change (FC) calculation were performed using nSolver Analysis Software v4.0 (NanoString Technologies).
Results: In this study, we detected dysregulated expression in 17/50 miRNAs – 7 were up-regulated and 10 were down-regulated. The highest expression without statistical significance was detected for miR-29b-3p (FC=4,6 on average) and miR-4286 (average FC=4,1), followed by miR-150-5p, miR-4488, miR-1915-3p, miR-1268a and miR-30b-5p. Ten miRNAs showed statistically significant decreased expression (FC<2) in melanoma compared to normal skin: miR-16-5p, miR-4455, miR-548ar-5p, miR-548aa, miR-548n, miR-942-5p, miR-145-5p, miR-125b-5p, miR-30e-5p, and miR-4536-5p.
Discussion: Despite advances in the diagnosis and treatment of melanoma, the search for reliable molecular markers remains crucial for improving early detection and prognosis. The use of FFPE samples and the Nanostring technology enabled reliable quantitative analysis of microRNAs in clinical specimens with limited material. Notably, miR-4455 and miR-16-5p exhibited markedly decreased expression in melanoma samples, which highlight their tumor-suppressor role in metastatic melanoma. These results support the potential of these microRNAs as diagnostic and therapeutic biomarkers. These findings shed new light on the molecular mechanisms underlying the disease and open the door to new diagnostic and therapeutic opportunities. The presented data provide a foundation for future research and potential clinical applications in the diagnosis and treatment of metastatic melanoma.

Acknowledgments: Grant BG-RRP-2.004-0004-C01.

P137 Distinct nuclear expression of eNOS and iNOS in brown adipose tissue under hyperinsulinemic conditions

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Keywords: Brown adipose tissue, nitric oxide synthase, hyperinsulinemia

Nitric oxide (NO) is involved in adipose tissue biology in many ways: it affects adipogenesis, thermogenesis, insulin-stimulated glucose uptake and lipolysis. The enzymes responsible for NO production in adipose tissue are mainly endothelial (eNOS) and inducible NO synthase (iNOS). The intracellular localization of the NOS isoforms was investigated in brown adipose tissue (BAT) of hyperinsulinemic rats. To induce hyperinsulinaemia, adult male rats of Wistar strain were treated with high dose of insulin (4IU/kg body mass, intraperitoneally) for one or three days. The animals were sacrificed and the interscapular portion of BAT was isolated and routinely prepared for light microscopy (LM) and transmission electron microscopy (TEM). Chronic insulin administration led to an increase in tissue mass through adipocytes hypertrophy, adipogenesis and angiogenesis, as well as an increase of blood flow. Ultrastructural analysis revealed prominent mitochondriogenesis and changes in the nuclear shape and amount of heterochromatin. Immunohistochemistry and immunofluorescence demonstrated two eNOS and iNOS positive cell types in the tissue: brown adipocytes and endothelial cells. Additionally, immunohistochemical analysis detected cytoplasmic and nuclear positivity for eNOS while iNOS expression was restricted to the cytoplasm. eNOS positivity was found in brown adipocytes cell membrane, cytoplasm and nuclei, while endothelial cells showed only nuclear immunopositivity. In addition, immunofluorescence analysis confirmed that positive nuclear eNOS reaction correlates with the observed nuclear changes through treatment: the most abundant type of nucleus was also the most positive. These results show that hyperinsulinemia, alters NOSs expression in BAT, by increasing eNOS while suppressing iNOS, which may represent an adaptive mechanism. Althought we found increased eNOS positivity, its active phosphorylated form, p-eNOS, was not detected. Understanding the balance between NOS isoforms in BAT could open new avenues for treating metabolic diseases like obesity and diabetes, characterized by hiperinsulinemic conditions.

Acknowledgments: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract numbers: 451-456/2024-03/3314, 451-03-136/2025-03/200178).

P138 Repurposing SARS-CoV-2 NSP13-targeted compounds as G-quadruplex ligands in triple-negative breast cancer: functional insights from MDA-MB-231 cell model

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Keywords: G-4, heterocyclic ligands, small molecules, triple-negative breast cancer (TNBC)

Recent advances in antiviral drug discovery have generated libraries of small molecules with high specificity for nucleic acid-interacting proteins. In this study, we explore the repurposing potential of 13 drug-like compounds (OTO1-13), originally developed to target the SARS-CoV-2 NSP13 helicase, as candidate ligands for guanine-quadruplex (G4) DNA structures. The human MDA-MB-231 cell line was employed as an *in vitro* model to evaluate the cytotoxic, genotoxic, and membrane-modulating effects of these compounds. Cell viability was assessed by the WST-1 assay, and morphological changes were monitored via phase-contrast microscopy. Genotoxicity was evaluated through the Comet assay, while alterations in membrane structural organization were investigated using the Laurdan fluorescence spectroscopy. Cell cycle distribution was analyzed by FACS. Several compounds, including OT05, OT08, and OT13, exhibited significant cytotoxicity in the micromolar range. Notably, OT08, OT09, and OT12 induced increased DNA strand breaks, while OT03-OT06 and OT10-OT13 disrupted cell cycle progression. Changes in membrane lipid order detected by Laurdan fluorescence suggested possible interactions with membrane-associated processes, potentially linked to G4 stabilization. In addition, LDH and ROS assays confirmed increased cellular stress and cytotoxicity in response to several of the candidate G4 ligands. Collectively, these findings support the potential of structurally optimized NSP13-targeted compounds to be repurposed as G-quadruplex ligands, offering a novel therapeutic strategy for targeting transcriptionally active genomic regions in aggressive breast cancer subtypes such as TNBC.

Acknowledgements: This research is funded by the Bulgarian Science Fund, grant number KP-06-N59/1 (КП-06 Н59/1/ 2021).

P139 Targeting G-quadruplex DNA structures: a novel therapeutic strategy for hormone rereptor-positive breast cancer through repurposed SARS-CoV-2 NSP13 inhibitors

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Keywords: guanine-quadruplex, heterocyclic ligands, repurposing, MCF-7cells

The targeted stabilization of guanine-quadruplex (G4) DNA structures has emerged as a highly promising anticancer strategy, offering a unique approach to modulate oncogene expression and disrupt critical cancer cell pathways. In this study, we investigate the therapeutic potential of 13 drug-like compounds (OTO1-13), developed initially as potent inhibitors of the SARS-CoV-2 NSP13 helicase, as novel G4-stabilizing ligands for the treatment of hormone receptor-positive (HR+) breast cancer. Using the well-characterized MCF-7 cell line, a model of ERα+/PR+ luminal breast cancer, we evaluated the cytotoxicity profiling and morphological aalterations by WST-1 viability assays and phase-contrast microscopy. Further, reactive oxygen species (ROS) generation were measured using the H2DCF-DA fluorogenic probe, and membrane dynamics analysis was conducted using Laurdan fluorescence spectroscopy. Our approach enabled the identification of the compounds demonstrating selective G4-binding capability, as confirmed through biophysical validation, potent cytotoxic activity against HR+ breast cancer cells, and a dual mechanism of action involving both G4 stabilization and membrane modulation. Notably, the most promising candidates exhibited a significant induction of cancer cell death, alterations in cell morphology, enhanced ROS generation, and an altered membrane fluidity, suggesting potential effects on membrane-associated signaling pathways. These findings not only validate the successful repurposing of antiviral compounds as anticancer agents but also highlight the therapeutic potential of G4-targeting strategies in hormone-responsive breast malignancies. The dual activity on nucleic acid structures and membrane dynamics positions these novel compounds as promising candidates for further development in the treatment of treatment-resistant HR+ breast cancer.

Acknowledgements: This research is funded by the Bulgarian Science Fund, grant number KP-06-N59/1 (КП-06 Н59/1/ 2021).

P140 Multiplex nanopore sequencing of CXCL10 and CXCR3 genes in healthy and type 1 diabetes cohorts: evaluation of basecalling and barcoding strategies

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Keywords: ONT, amplicon sequencing, multiplexing

Oxford Nanopore Technologies provides a relatively cheap sequencing platform accessible for low budget scientific projects. Despite the flexibility of the platform, its application for target-sequencing in diploid organisms remains limited to very few examples in the literature. Here we offer a simple approach for multiplex sequencing of the whole genome sequences of two human genes, CXCL10 and CXCR3, using custom barcode system in a panel of healthy people and patients with type 1 diabetes, totaling sixty individuals. The target regions were amplified and barcoded in two consecutive PCRs. The products were pooled in equimolar concentrations and sequenced on a R10.4.1 flow cell. For the data analysis were used standard bioinformatic tools for ONT data processing, which can be executed on an average PC configuration. After the sequencing we tested two basecalling approaches and compared the variant calling results of both. The first approach used a computationally more demanding basecalling model, *dna_r10.4.1_e8.2_400bps_sup@v5.0.0*, and the second, a less demanding model *dna_r10.4.1_e8.2_400bps_sup@v4.3.0,* implemented in a duplex basecalling. To avoid wrong classification, the sequencing reads were classified to a particular barcode population only if the oligos on both ends of the amplicon were recognised. The reads produced with the *sup@v5.0.0* had a better classification of around 53% versus 37% for the duplex *sup@4.3.0*. For the latter data we used a custom script to rescue unclassified duplex reads whose both parental reads were classified to a particular barcode population. The comparison of the variant calling results showed a higher on average quality (Q 59.7) for the variants called in the *sup@v5.0.0* data set compared to those called in *sup@4.3.0* Q37.6 for the other.

P141 mRNA expression of immune related genes in endometriosis across different sample types

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Keywords: endometriosis, gene expression, biomarkers, non-invasive diagnostics, NanoString

Introduction: Endometriosis is a chronic, estrogen-dependent inflammatory disorder marked by endometrium-like tissue outside the uterus. Affecting 6–10% of reproductive-aged women, it is associated with pelvic pain, infertility, and impaired quality of life. Current diagnostics are mainly invasive, highlighting the need for reliable, non-invasive biomarkers.
Objective: To assess expression of 48 immune-related genes across multiple biological samples from a patient with confirmed endometriosis and matched controls, to identify potential diagnostic and therapeutic targets.
Materials and Methods: Samples (venous blood, endometrial tissue, menstrual blood pre- and postoperatively, and endometriotic lesion) were collected from a Bulgarian patient with surgically confirmed disease (#ENZIAN: O2, T1.). From six matched controls, venous blood, endometrial tissue, and menstrual blood samples were obtained (n = 3 per type). RNA was extracted with RNAzol® RT and analyzed using NanoString nCounter® Elements XT. Data were normalized via nSolver Analysis Software; genes with $\log_2FC \geq |1|$ or < -1 were considered differentially expressed.
Results: Dysregulation was observed in eutopic endometrium (ADM, PTGS2, TNFAIP3, TP53) and lesion tissue (THBS1, PPFIA2, CYR61). In preoperative menstrual blood, 20/48 genes mirrored lesion profiles. AMD1, ASPA, CHGB, EXOC2, HIF1A, HIST1H1D, NDUFV3, POLR1B, PROSER1, TDG, ZBTB4, and ZSCAN32 showed similar patterns to lesion samples and were dysregulated versus postoperative and control samples, indicating a disease-specific signature. Venous blood showed weaker correlation and limited diagnostic value.

Conclusion: Identified genes highlight diagnostic and therapeutic potential, while menstrual blood shows promise as a non-invasive source for endometriosis diagnosis and monitoring, warranting validation in larger cohorts.

Acknowledgments: Grant BG-RRP-2.004-0004-C01.

P142 Genetic polymorphisms in genes, related to coagulation and hormonal pathways in Bulgarian women with endometriosis

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Keywords: Endometriosis, Genetic polymorphisms, ESR1, Estrogen signalling, Coagulation

Background: The underlying causes and mechanisms of endometriosis remain unclear. As a chronic, inflammatory, and estrogen-dependent condition, endometriosis has also been associated with a hypercoagulable state in affected individuals.
Objective: This study aimed to assess the influence of genetic polymorphisms in coagulation-related genes (FII, FV, and PAI-1) and in genes involved in estrogen synthesis and signaling (CYP19A1 and ESR1) on the susceptibility to endometriosis in a Bulgarian patient cohort.
Materials and Methods: We analyzed the frequency of seven specific gene polymorphisms in 30 Bulgarian women diagnosed with endometriosis. Their genetic profiles were compared to those of two control groups: healthy pregnant women (n = 221) and population data from the Ensembl genome database. Statistical analysis was performed using the chi-square test to determine significance.
Results: Our findings indicated a higher frequency of heterozygosity for the FII G20210A mutation among patients compared to controls (10% vs. 4.5%), and a higher prevalence of the PAI-1 4G/4G genotype in patients (35% vs. 23%), though neither reached statistical significance. Notably, a significantly increased incidence of the homozygous G/G genotype for the ESR1 -351 A>G (XbaI) polymorphism was observed in patients versus controls (22% vs. 9%, $p < 0.05$). No significant differences were found in the distribution of CYP19A1 alleles or genotypes between the groups.
Conclusion: Polymorphisms in the ESR1 gene may contribute to altered gene expression and play a role in the development and progression of endometriosis. Further research involving a larger patient cohort and gene expression analysis is recommended to clarify these associations.

P143 Assessment of genetic diversity in oil-bearing *Rosa* genotypes using SCoT markers

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Keywords: genetic diversity, *Rosa alba*, *R. centifolia*, *R. damascena*, *R. gallica*, SCoT markers

The genus *Rosa* contains a wide range of species and cultivars with high ornamental, medicinal, and industrial value. In Bulgaria, the cultivation of oil-bearing roses has major economic importance, with *Rosa damascena* recognised as a national symbol and part of country's cultural heritage. The Institute of Roses and Aromatic Plants (IRAP) in Kazanlak is a key national supplier of essential oil rose planting material, maintaining a valuable genetic repository of oil-bearing and dog roses that supports research and breeding efforts.

This study evaluates the genetic diversity of 38 rose genotypes, most of which are preserved in the IRAP's collection. The analysed materials include a newly identified *Rosa* accession from an old plantation, three oil-bearing rose species (*R. alba* L., *R. gallica* L., *R. centifolia* L.), the Russian variety 'Raduga', locally improved Population 5 from *R. damascena* Mill., and four Bulgarian-bred cultivars ('Svejen', 'Eleina', 'Janina', and 'Iskra'). To assess the genetic diversity and relationship, the Start Codon Targeted (SCoT) molecular marker system based on short ATG start codon, was employed. Among thirty SCoT primers initially screened, a set of least ten highly polymorphic and reproducible primers was selected for further analysis. Genetic diversity indices, Nei's genetic distance, UPGMA clustering, and population structure were evaluated using GenAlEx 6.5, POPGENE 1.32, MEGA 12 and STRUCTURE 2.3.4.

Our study provides new data on the genetic variability and relationships among the examined rose genotypes, thereby extending the baseline for future breeding strategies, targeted germplasm conservation, and the long-term preservation of Bulgaria's rose genetic heritage.

Acknowledgments: The study was supported by the Bulgarian Ministry of Education and Science: the National Science Fund grant number КП-06-Н56/1 10.11.2021 and the National Program „Young Scientists and Postdoctoral Students-2".

P144 Racing against time: CAR as a fast-track marker for ICU outcomes

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Keywords: COVID-19; inflammation; prognosis; sepsis.

Background: The C-reactive protein-to-albumin ratio (CAR) has emerged as a promising marker that reflects both inflammation and nutritional status. While it has been explored in various clinical settings, its prognostic value in critically ill patients with COVID-19-related sepsis remains underreported. CRP rises rapidly in response to systemic inflammation, whereas albumin decreases in severe illness, reflecting poor nutritional and metabolic status—making their ratio a sensitive indicator of disease severity.
Methods: This retrospective observational study included 133 patients with COVID-19 sepsis admitted to the intensive care unit (ICU). CAR levels were recorded within the first 24 hours of admission. The association between CAR and 28-day mortality was evaluated using receiver operating characteristic (ROC) curve analysis and logistic regression modeling.
Results: Among the biomarkers analyzed, CAR showed the highest predictive accuracy for 28-day mortality (cut-off value: 5.5; AUC = 0.665). Multivariate analysis confirmed CAR as an independent predictor of early mortality ($p = 0.015$).
Conclusion: CAR is a practical and accessible marker that may aid in early risk assessment in patients with COVID-19 sepsis. Incorporating CAR into routine ICU evaluation could help guide timely clinical decisions and support more personalized treatment strategies.

P145 The Immunity Tug-of-War: neutrophil-to-lymphocyte ratio in the ICU arena

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Keywords: COVID-19; inflammation; neutrophil-to-lymphocyte ratio; prognosis; sepsis.

Background: The neutrophil-to-lymphocyte ratio (NLR) has emerged as a simple and accessible marker of systemic inflammation. Neutrophils drive the initial inflammatory response, while lymphocytes mediate adaptive immunity, making their ratio (NLR) a sensitive indicator of immune imbalance in critically ill patients. Although its utility has been explored in various clinical settings, the prognostic value of NLR in patients with COVID-19-related sepsis remains insufficiently characterized.
Methods: This retrospective study analyzed data from 133 ICU patients diagnosed with COVID-19 sepsis. NLR values were collected within 24 hours of admission. The relationship between NLR and 28-day mortality was assessed through analysis of receiver operating characteristic (ROC) curves and multivariate logistic regression.
Results: Elevated NLR levels were significantly linked to poor outcomes. With a cut-off value of 25, NLR demonstrated strong predictive accuracy for early mortality (AUC = 0.663). Among the biomarkers assessed, NLR consistently emerged as one of the most reliable indicators of patient prognosis.
Conclusion: NLR is a valuable, cost-effective tool for early risk stratification in critically ill patients with COVID-19 sepsis. As a reflection of the dynamic balance between pro-inflammatory and adaptive immune responses, NLR offers meaningful insight into the severity of immune dysregulation. Its simplicity, availability, and prognostic strength make it a practical addition to routine ICU assessments. With further validation, NLR could become a standard supportive marker across a broad spectrum of critical care settings.

P146 Study of forearm anthropometrical indices and their relationship with hand grip strength in young Bulgarian people

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Keywords: forearm anthropometry, hand strength**,** secular changes, students

Introduction: Hand strength is one of the important indicators for assessing the functional capabilities of the upper limb. In an anthropological context, this indicator is important in studying the evolution of the human hand and its adaptation to changing conditions of life, labor and sport.
Aim of this study is to investigate the hand grip strength in Bulgarian university students, and to assess secular changes in grip strength over the past 60 years.
Material and Methods: The study sample included 272 male and female Bulgarian students aged 18–24 years. Of each person the strength (kg) of both hands, body weight (kg), forearm length and circumference (cm), muscular forearm circumference (cm), forearm skinfold thickness (mm) were measured. The strength-to-weight ratio and muscle-mass forearm ratio was also calculated using established formulas. Grip strengths were measured using a standard manual handle Russian dynamometer. Anthropometric measurements were conducted using the Martin-Saller method (1959), and skinfold thickness was assessed using a GPM caliper (Switzerland). To evaluate secular changes in grip strength, historical data from nationwide samples of Bulgarian youth aged 18–24, reported by Yanev et al. (1965) and Slanchev et al. (1993), were used for comparison. Statistical analysis was performed using STATISTICA 12.0 software.
Results: The significantly higher average values for all investigated indicators in male participants compared to females were found. A strong correlation was found in men between grip strength (both hands), forearm muscular circumference and strength-to-weight ratio. In women, a strong positive correlation was observed only between right-hand grip strength and the strength-to-weight ratio, with weaker correlations between grip strength and forearm anthropometric characteristics. Regarding secular trends, a consistent decline in right-hand grip strength was observed in both sexes over the past 60 years, with an average decrease of 6.2 kg.
Conclusion: the findings reflect distinct changes in hand function among young Bulgarians, likely due to adaptation to contemporary lifestyle factors and the predominance of cognitive over manual labor.

P147 Nerve growth factor levels in peripheral blood of children with autism spectrum disorder: a meta-analysis

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Keywords: Nerve growth factor, peripheral blood, autism spectrum disorder, meta-analysis

This study aims to evaluate the levels of nerve growth factor (NGF) in the peripheral blood (serum/ plasma) of children with autism spectrum disorder (ASD) through a comprehensive meta-analysis. The present work strictly follows the guidelines proposed by the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-analysis). Initially, we performed an online search through the PubMed and Web of Science databases using a structured query with no time-period limit. The published studies were evaluated for eligibility based on the following inclusion criteria: 1) provided quantitative information about the NGF levels in peripheral blood of human probands; 2) reported sample sizes, arithmetic means, and standard deviations (SDs) from the applied quantitative analyses; 3) reported sample sizes and statistical outcomes, in case means and SDs were not available; 4) comparison between ASD and typically developing children (TDC).
The initial search identified 101 original articles from the PubMed repository and 41 from the Web of Science records. After examining the titles and abstracts, 13 articles in line with the purpose of this study were further considered for full-text screening. Among them, five were excluded either due to not examining NGF (three), being focused on cerebrospinal fluid (one), or examining anti-NGF antibodies (one). We were able to extract all the information required from seven out of the remaining eight articles.
We evaluated the NGF levels in the peripheral blood of 270 children with ASD and 212 TDC, examined in seven studies. The applied random effects model demonstrated that the levels of NGF in ASD children were significantly higher than those in TDC (Hedges' $g = 0.450$; 95% CI [0.230; 0.600]; p-value < 0.001). In addition to its markedly higher levels in ASD children, the NGF showed no significant between-study heterogeneity ($Q(d.f. 6) = 8.75$, p-value $= 0.188$, $tau^2 = 0.029$ [0.000; 0.416], $I^2 = 31.4\%$ [0.0%; 70.8%]).

Acknowledgements: This work received support from the Science Fund at the "Paisii Hilendarski" University of Plovdiv – Grant No. СП25-БФ-004

P148 Decoding ferroptosis in ischemic brain injury

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Keywords: ischemic stroke, iron, ferroptosis

Stroke is the second leading cause of death worldwide and accounts for over 50% of all neurological disabilities. Bulgaria ranks third in stroke incidence, with nearly 10% of all strokes occurring in young and middle-aged patients, with a male predominance among the affected. About 40,000 patients are diagnosed with ischemic stroke annually in the country, with 8,000 death cases, and over 70% remaining with varying degrees of disability.

Ferroptosis is a newly characterized form of non-apoptotic cell death driven by iron-dependent lipid peroxidation due to pharmacological or pathological perturbation of antioxidant systems. Lipid peroxidation can kill cells directly by damaging biomembranes or indirectly through metabolic products. Iron is among the essential nutrients for cell growth. On the other hand, iron overload is one of the most significant causes and typical hallmarks of ferroptosis. Excess iron compromises mitochondria by limiting oxidative phosphorylation and the antioxidant response. In ischemic stroke, reduced blood flow to the brain leads to iron accumulation and oxidative stress, both of which exacerbate brain damage during and after ischemic events.

Studying these pathways will shed light on the causal role of ferroptosis in the pathogenesis of the disease. This will help in the development of a panel of biomarkers for prognosis and prediction of the severity of the patient's outcome.

Acknowledgements: This study is financed by Medical University of Plovdiv, project № HO-01/2025.

P149 Prognostic significance of the long non-coding RNAs lnc-IRF2-3 and lnc-KIAA1755-4 in chronic lymphocytic leukemia

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Keywords: chronic lymphocytic leukemia, long non-coding RNA, lnc-IRF2-3, lnc-KIAA1755-4, expression, prognosis

Aberrant expression of long non-coding RNAs (lncRNAs) has been reported in many cancers, including B-cell malignancies. By modulating the expression of various genes, lncRNAs are involved in the pathogenesis, disease progression and treatment outcome. The aim of this study was to analyze the expression patterns of lnc-IRF2-3 and lnc-KIAA1755-4 in chronic lymphocytic leukemia (CLL), and to evaluate their association with clinico-biological characteristics of patients (pts) at diagnosis and survival. The expression of the investigated lncRNAs was measured in peripheral blood mononuclear cells of 112 previously untreated CLL pts by qRT-PCR; median expression levels were used as a cut-off to discriminate between high- and low-expressing cases. High lnc-IRF2-3 levels were associated with high leukocyte and lymphocyte counts, high β2-microglobulin, advanced Binet stage, unfavorable cytogenetics, CD38-positivity and *IGHV*-unmutated status. Regarding lnc-KIAA1755-4, its high expression was associated with high leukocyte count, lymphocyte count, β2-microglobulin, lactate dehydrogenase and low hemoglobin, as well as with *IGHV*-unmutated status. Expression of both lncRNAs was higher in pts with intermediate, high and very high CLL-IPI scores in comparison to pts with low CLL-IPI before the initiation of treatment. In addition, we observed shorter time to first treatment (TTFT) and overall survival (OS) of pts expressing high levels of lnc-IRF2-3 in comparison to low-expressing pts. The same association with shorter TTFT and OS was found regarding high expression of lnc-KIAA1755-4. Moreover, the shortest TTFT and OS were detected in pts with concomitant high expression of both lncRNAs. In conclusion, our results show that elevated expression of lnc-IRF2-3 and lnc-KIAA1755-4 at diagnosis predicts adverse prognosis in CLL. The causes of their upregulation, as well as their mechanisms of action in CLL cells remain to be elucidated.

P150 Interaction of green-synthesized silver nanoparticles with plasma proteins albumin, fibrinogen, and immunoglobulin G

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Keywords: silver nanoparticles, green synthesis, plasma proteins, fluorescence spectroscopy, thermodynamics

Green-synthesized silver nanoparticles (AgNPs), created using microbial polymer-based reducing agents, are gaining interest for biomedical applications because of their environmentally friendly nature. This study investigates the interaction of AgNPs with key human plasma proteins—albumin, fibrinogen, and immunoglobulin G (IgG)—which play crucial roles in transport, clotting, and the immune response. AgNPs were specificly characterized, confirming their stability and uniformity. Their interactions with proteins were assessed using fluorescence spectroscopy, isothermal titration calorimetry (ITC), and differential scanning calorimetry (DSC). Fluorescence quenching revealed strong, concentration-dependent binding, particularly with albumin, accompanied by structural changes. ITC indicated spontaneous, enthalpy-driven interactions. DSC analysis revealed alterations in protein thermal stability, indicating conformational changes following AgNP binding. The findings suggest the formation of a protein corona that may influence nanoparticle behavior in circulation. Together, DSC, ITC, and fluorescence offer complementary insights into these biophysical interactions. In summary, this study highlights the importance of understanding nanoparticle–protein interactions in optimizing the design and safety of AgNPs for medical applications.

Acknowledgments: The investigation was financed from the EU – Next Generation EU, through the NPRS in the Republic of Bulgaria, project "SUMMIT BG-RRP-2.004-0008-C01" 3.4., project 4120, contract 70-123-661/16.04.2025

P151 Development of hydrogel-based brain phantom

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Keywords: Brain phantom, hydrogel, viscoelasticity, diffusion

Mechanically realistic brain phantoms are promising for diagnostic and surgical training as well as for probing the viscoelastic coupling between brain implanted electrodes and neural tissue, predicting how circulation, head motion, and respiration affect device displacement. Moreover, convective and diffusive transport of products of inflammatory cell activity in tissue creates biochemical gradients that govern both cell behavior and electrode performance.

We have developed Gellan-gum hydrogel formulations (0.1–1.0 % w/v) using three cross-linking methods: Ca^{2+}, Phosphate Buffer Saline (PBS), and pH-mediated gelation. Ca^{2+} produced gels at low polymer loads but their shear moduli were too stiff ($G' > 8$ kPa). PBS cross-linking formed networks at 1 % Gellan and—when doubled—allowed 0.5 % gels that were more compliant than Ca^{2+} variants, revealing a promise for 3D culture in neural applications. The lowest G' values were observed upon pH-mediated gelation of a 0.4% solution at the gum's isoelectric point (pH ≈ 2.9) resulting in gels whose $G' \approx 1$ kPa falls within the range reported for human neural tissue.

To evaluate transport properties, we performed diffusion assays using 5 kDa and 40 kDa blue dextrans. Rectangular hydrogels containing parallel cylindrical channels (1.27 mm diameter) were loaded with 40 mg/mL dextran solutions. Experiments were conducted at 20°C under saturated humidity ambient and documented by bright-field optical microscopy. Concentration profiles extracted from time-lapse images were fitted to one-dimensional diffusion models—an Error Function solution and an Error Function with Drift—to yield apparent diffusion coefficients: $D_5 = 17.5 \times 10^{-7}$ cm²/s and $D_{40} = 4 \times 10^{-7}$ cm²/s, in line with free-solution values.

The Gellan gum–based hydrogel platform offers a cost-effective, reproducible surrogate that is promising to capture both the viscoelastic and diffusional hallmarks of brain tissue. Its tuneable mechanics and transport properties make it ideally suited for calibration of imaging modalities, validation of implant–tissue finite-element models, and development of neural tissue–engineering constructs. Ongoing work on engineered tortuosity will further enhance its physiological realism and elucidate how inflammatory gradients impact long-term electrode stability.

P152 Cardiovascular risk assessment in participants of the 32nd Bulgarian Antarctic expedition

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Keywords: Antarctic expedition, Cardiovascular risk, Biochemical parameters, Stress test

Bulgarian Antarctic expeditions involve leading scientists at middle age and relatively limited physical activity, which are prerequisites for the occurrence of cardiac problems. Considering the remoteness from specialized medical care during the stay in Antarctica, the control of the participants' cardiovascular disease (CVD) risk is essential.

This study aimed to assess the CVD risk of participants in the 32nd Bulgarian Antarctic expedition. The study involved 29 (6 women and 23 men) participants in the 32nd Bulgarian Antarctic expedition. The average age ranged from 45.5 ± 11.31 years for women to 43.0 ± 8.00 years for men. All participants underwent anthropometric, physiological, and biochemical assessments before and after the expedition. The CVD risk was calculated using the SCORE2 algorithm. Before the departure, all participants successfully passed the veloergometric stress test.

The results indicated that before departure, a significant part of the participants had elevated BMI, with 3 women and 16 men classified as overweight or obese. Post-expedition, there were no statistically significant changes in the average values of body weight, BMI, or fat percentage. Lipid profiles revealed elevated LDL cholesterol levels in 17 participants, of whom 12 showed improvement after the expedition. HDL cholesterol demonstrated minimal changes, while post-expedition, the serum ferritin significantly decreased among men, likely due to diet-related factors during the expedition. Serum cortisol, a stress marker, was elevated in some participants, both before and after the expedition, likely due to physical and psychological challenges. According to the calculated CVD risk, four men exhibited very high risk, which post-expedition decreased in three cases but increased for one of them.

The results emphasize the importance of pre-emptive measures to reduce risk, including managing weight, diet, and physical activity. It is recommended that participants pass a stress test before departure. Individual strategies for nutrition and physical activity during expeditions are also recommended.

Acknowledgements: This work was supported by grant ЦРАС-13/18.02.2025 of the National Sports Academy, Bulgaria

P153 Chronic stress-induced behavioural changes in juvenile rats

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Keywords: Chronic stress, adolescence, behavior, memory, rats.

Adolescence is characterized by a significant reorganization of brain activity, which, on the one hand, precedes changes in behavior and social activity, but on the other hand, makes key brain structures vulnerable to external factors. Chronic stress in early life can impact the normal brain development process and lead to an increased inflammatory response to environmental factors later in life. The present study aimed to characterize the effects of chronic stress on normal exploratory activity, anxiety-like behavior in novel environments, and memory in Wistar rats exposed to social defeat during adolescence.

Social defeat stress (SS) was induced via chronic (20 days) exposure to the Resident-Intruder Paradigm of adolescent (21-35 postnatal days) male Wistar rats. Unstressed controls and SS groups were subjected to behavioral tests: Open field (OFT), Elevated plus maze (EPM) for evaluation of anxiety-like behavior and total motor activity; Novel Object Recognition Test (NORT) for study of working memory, and biochemical assessment of carbonylated proteins in blood plasma and hippocampus.

The data showed that exposure to chronic SS provoked anxiety-like behavior in the adolescent rats both in OF and EPM expressed by a drastic decrease in total motor activity, a reduced number of re-entries into the aversive central area and an increased stay in the protected corner zones OFT, as well as a reduced time spent in the open arms of the EPM. Chronic SS during the period of adolescence impaired normal working memory in NORT, and elevated levels of the impaired carbonylated proteins in blood plasma and hippocampus.

These data highlight the importance of social factors in the development of cognitive functions during adolescence and are preliminary results for further investigation of treatment with potential adaptogens.

Acknowledgements: This work was supported by the Burgas State University "Prof. Dr. Assen Zlatarov", Burgas, Bulgaria, contract № 522/2025.

P154 Alternative biotechnological applications of sunflower meal and sunflower seed hulls

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Keywords: sunflower meal, sunflower seed hulls, waste, alternative applications

Among the global problems of last years is the generation of huge amounts of waste. When unmanaged properly, this waste poses a potential global hazard for the ecology, economy, and social activities, impacting on climate change, soil and water quality, and public health. Therefore, there is an urgent need to establish sustainable management practices that will mitigate environmental impacts and improve resource efficiency in the long term. Sunflower oil production generates various by-products such as hulls, flakes, expelled cakes, or extracted meal.
Sunflower seed hulls and meal are among the most abundant by-products of the food industry. They are an example of waste and, at the same time, a plentiful biomass that cannot be utilized directly in human and animal diets due to their hard digestability and low nutritional value. They are known to contain carbohydrates, lipids, and proteins, and constituents such as vitamins, minerals, and especially phenolics contributing to their antioxidant capacity. Numerous benefits can be retrieved from such co-products. All the beneficial substances in sunflower meal and hulls and the urgent need for change in our daily life impose establishing the potential of these by-products. This is the reason why in recent years there is such a serious interest in their utilization and valorization towards the concept of a circular bio-based economy and process sustainability. Sunflower meal and seed hulls are reported to find alternative biotechnological applications. For example, sunflower meal is an excellent source of protein applicable in the food industry while sunflower seed hulls are characterized by their rich content of antioxidants. Sunflower seed hulls can also be used for the production of bio-oils and biofuel, as a corrosion inhibitor, concrete stabilizer, and as an additive to cultivation media. Moreover, sunflower seed hulls are a tool for interesting environmental benefits as this crop is able to adsorb various hazardous substances like chloroquine, phenol, naphtenic acids, pesticides, simazine, chlorpyrifos, trifluralin, chlorfen-vinphos, metal ions such as copper, nickel, and dyes like astrazon red, reactive red 195, and reactive blue 49. In addition, sunflower seed hulls are applicable as a bioinsecticide and fertilizer.

Acknowledgements: The authors would like to thank Bulgarian National Science Fund, Grant № КП-06-М87/3 for the support.

P154 Characterizing endothelin isoforms, uric acid, and systemic inflammation in progressive chronic kidney disease

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Keywords: Chronic kidney disease, Endothelin, uric acid

Introduction: Chronic kidney disease (CKD) is a progressive condition that results in impaired renal function and systemic inflammation. Endothelin-1 (ET-1) has been demonstrated to exert a deleterious effect on renal injury; however, the roles of endothelin-2 and -3, along with their interaction with uric acid (UA) and inflammatory markers, remain less well understood. The objective of this study was to examine the relationships between all three endothelin isoforms, UA levels, and a variety of inflammatory and biochemical markers across different CKD stages.
Methods: The present cross-sectional study included 76 CKD patients categorized by eGFR. We assessed renal function (SCr, eGFR), mineral metabolism, systemic inflammation (hs-CRP, IL-6, WBC), complement components, and plasma levels of ET-1, ET-2, and ET-3.
Results: As anticipated, advanced CKD stages showed lower eGFR and higher SCr, UA, and PTH levels**. Uric acid levels were significantly elevated in severe CKD** (Group 3, median 350.5 µmol/L) compared to normal renal function (Group 1, median 206 µmol/L; *p*=0.018). Notably, **ET-2 significantly increased with worsening renal function** (Group 1 median 23.75 pg/mL vs. Group 3 median 24.49 pg/mL, *p*=0.030). Hs-CRP levels were significantly elevated in CKD groups. Strong correlations were observed between age and eGFR (*rho* = -0.294, *p*=0.010), age and UA (*rho* = 0.254, p=0.028), and age and ET-3 (rho = -0.238, p=0.040).
Conclusion: This study confirms that CKD is associated with worsening renal function and elevated levels of UA, as well as inflammatory markers. The finding that ET-2 significantly increases with the progression of CKD suggests its potential role in the pathogenesis of the disease. These results highlight the complex interrelationships between renal function, uric acid levels, inflammatory processes, and endothelin isoforms in patients with CKD.

P156 Antihyperalgesic effect of an Angiotensin 1-7 structural analog in rats

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Keywords: Angiotensin 1-7, Peptide analog, Antihyperalgesia

Angiotensin 1-7 is a key endogenous heptapeptide with an active role in the Renin-Angiotensin System. It exerts vasodilatory, antiproliferative, antioxidant, anti-inflammatory, and peripheral antinociceptive effects, binding to a specific receptor (MasR). Previous data have shown that the antinociceptive effect of Angiotensin 1-7 is independent of the opioidergic system, representing a significant positive aspect in terms of its analgesic potential and stimulating the synthesis of new peptide analogs with improved characteristics. The present study aimed to analyze the antihyperalgesic effect of Ang-P2, a newly synthesized analog of Angiotensin 1-7, administered peripherally at different doses in rats.

The experiment was carried out on adult male Wistar rats weighing ~200 g. Hyperalgesia was induced by an intraplantar injection of Carrageenan (100µl, 1% solution). Ang-P2 was administered intraplantarly (into the same paw) at doses of 5, 2.5, and 1.25µg in a volume of 10µl, 10 minutes before the Carrageenan injection and then daily (for 7 days), 10 minutes before the measurement of pain sensitivity. The measurements of mechanical pain threshold were made via electronic Von Frey analgesimeter (Ugo Basile), and the mean values were further statistically analyzed. Indomethacin at a dose of 800µg/100µl was used as a reference drug.

The results showed that Carrageenan caused a significant and long-lasting decrease in the pain threshold (local hyperalgesia). Ang-P2 at a dose of 2.5µg/10µl significantly increases the pain threshold during the first 4 days of the treatment. This antihyperalgesic effect was comparable to that of the reference drug Indomethacin. We can conclude that the local administration of this newly synthesized Angiotensin 1-7 analog has a significant and dose-dependent antihyperalgesic effect. Further studies are planned to elucidate its mechanism of action.

Acknowledgements: This work was supported by the Sofia University "St. Kliment Ohridski", №80-10-87/27.05.2025

P157 Chronic angiotensin-converting enzyme type 2 inhibition suppressed its expression in the brain and promoted inflammation

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Keywords: *ACE2* expression, Brain cortex, Hippocampus, TNF-α

Angiotensin-converting enzyme type 2 (ACE2) is a key regulator of the balance in the Renin-Angiotensin System (RAS). It can convert the biologically active octapeptide Angiotensin II (Ang II) into Angiotensin 1-7, which binds to the Mas receptors and exerts effects opposite to Ang II. ACE2 is widely distributed in all organs and systems, including the brain. In addition to its regulatory functions, ACE2 has been identified as a membrane receptor for the entry of coronavirus 2 (SARS-COV-2) into cells.

We hypothesized that chronic inhibition of ACE2 would alter its gene expression and disrupt the integrity of the RAS. To test this, the selective ACE2 antagonist MLN-4760 was administered intraperitoneally at a dose of 1 mg/kg daily for 14 days in male ICR mice. After the end of treatment, their brains were dissected, and the prefrontal cortex and hippocampus were isolated according to their anatomical coordinates. Expression levels of mRNA transcripts were determined by quantitative RT-PCR analysis. For this purpose, a total RNA was extracted from the brain tissue samples. Reverse transcription reactions were performed and the cDNA products from each sample were then subjected to Real-Time PCR analysis. The serum levels of TNF-α were analysed by an ELISA.

Our results show that chronic ACE2 inhibition led to a fivefold reduction in *ACE2* gene expression in brain regions involved in memory formation and behavioral control. Furthermore, this treatment significantly increased the level of the blood inflammatory TNF-α. This preliminary study provided data on the potential of chronic ACE2 inhibition, which can mimic its binding by specific viruses, induce changes in the brain RAS, and may modulate cognitive functions.

Acknowledgements: This work was supported by grant № KP-06-H71/9 of the Bulgarian National Science Fund, Ministry of Education and Science, Bulgaria.

P158 Transformation of spent coffee grounds, grape seeds and sunflower seed hulls to valuable products

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Keywords: spent coffee grounds, grape seeds, sunflower seed hulls, waste

Nowadays increasing attention is drawn to the effective use of waste biomass as a renewable resource of non-energy and energy related oils with wide applications in the food, cosmetics, and pharmaceutical industries, and for production of biofuels. Moreover, biomass storage is associated with problems such as dry matter losses and compositional changes. In recent years, the biorefinery concept has been identified as the most promising route for exploiting to the full the potential of biomass by maximizing its conversion into high value products with high content of important compounds like unsaturated fatty acids and antioxidants.

Spent coffee grounds (SCGs) are among the principal bio-wastes in the production of instant coffee and coffee brewing. In EU alone, from each kg of coffee 0.91 kg of solid waste is produced. Grape seeds, which represent about (20-25) % of the biomass generated by the wine industry, are considered of great interest since they are an excellent sustainable source for seed oil production. Sunflower seed hulls account for about 20-30% of sunflower seeds and are usually removed before oil extraction. They are an abundant and inexpensive by-product that is subsequently stored outdoors (occupying considerable space), landfilled, or incinerated.

One of the most widely used approaches for oil recovery is the conventional *n*-hexane Soxhlet extraction of waste biomass.

In our work the yields achieved applying *n*-hexane Soxhlet extraction of SCGs, grape seed oil, and sunflower seed hulls were10.4, (12.28 ±0.35), and 2.2119 % w/w, respectively.

In general, the bio-oils recovered by those waste biomasses are excellent candidates to be incorporated into foods, cosmetics, nutraceuticals due to their biological activities and in the biofuels production, as well.

Acknowledgements: The authors would like to thank Bulgarian National Science Fund, Grant № КП-06-М87/3 for the support.

P159 Phytochemical and nutritional characteristics of apricot, plum-apricot, and plum fruits

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Keywords: bound and free phenolics, proteins, carbohydrates, dietary fibres, mineral composition

Fruits have long been acknowledged as important sources of nutrition. The "Stendesto" is the sole successful hybrid of plum and apricot that has been registered in Bulgaria. Despite the global popularity of Prunus spp., information regarding its characteristics remains quite limited. The "Stendesto" is derived from the "Modesto" apricot and the "Stanley" plum. This study assessed the total phenolic content, total flavonoid content, total monomeric anthocyanins, and antioxidant capacity (using ABTS, DPPH, FRAP, and CUPRAC assays) to reveal the biological activity of the three fruits under investigation. Furthermore, the contents of protein, lipid, and carbohydrates were analyzed. Data concerning the micro- and macroelements found in the three fruits were also included. The results indicated that the antioxidant potential of the fruit extracts containing free phenolic compounds exhibited higher values across all samples studied compared to those containing bound compounds. The fruits of the "Stendesto" hybrid displayed characteristics that are more akin to those of the "Stanley" plum rather than the "Modesto" apricot. The "Stendesto" hybrid is distinguished by having the highest levels of free phenolic compounds, total flavonoids, and total monomeric anthocyanins. This research is regarded as one of the pioneering studies on plum-apricot hybrids in Bulgaria and aims to enhance the current scientific knowledge base, facilitating future comparisons.

P160 Investigating the role of demographics in cryptosporidiosis distribution within Pleven's population

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Keywords: *Cryptosporidium*, oocysts, diagnosis, basic demographic indicators, Pleven district

Cryptosporidiosis is a protozoan disease that manifests in two distinct forms: as an asymptomatic infection or as acute gastroenteritis. The disease is currently regarded as an opportunistic parasitosis, which is indicative of AIDS.

The objective of the present study is to establish the distribution of cryptosporidiosis among the population of the district of Pleven. A study among target groups of examined persons according to the basic demographic indicators, namely gender, age, ethnic origin and type of settlement, has been conducted.

The following section will outline the materials and methods employed in this study. The examination of the main target group of 1,133 persons from the district with the application of three main methods of diagnostics showed a total of 86 persons to be positive for cryptosporidiosis.

The results of the study are as follows: The statistical analysis and the application of the three main diagnostic methods indicate that gender, ethnic origin and place of residence are not significant factors in the distribution of cryptosporidiosis. Long-term studies have indicated a weak tendency for the distribution of cryptosporidiosis, with individuals of Bulgarian ethnic origin and residents of towns and cities demonstrating a higher prevalence. The present study found no difference in terms of frequency of parasitic infection between male and female participants in the district of Pleven.

Cramer's ratios have been demonstrated to exhibit a correlation between age and the frequency of parasitic infection with *Cryptosporidium* spp. The largest relative share of individuals excreting oocysts is that of children between 3 and 7 years of age.

P161 Algae diversity of the Izubra River (Mt. Golija), Serbia

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Keywords: algae, diversity, the Izubra River, Serbia

The Izubra River is a tributary of the Studenica River, situated in the Golija Mountain region in southwestern Serbia. Due to its high number of endangered, rare, and endemic species, Golija Mountain is protected at the first, second, and third levels. The algal flora of the Izubra River is poorly known, making it an ideal location for a study focused on assessing algae diversity.
The phytobenthos samples were collected from three sites along the river in June 2024 by brushing the stones. All samples were fixed with formaldehyde to a final concentration of 4% and deposited in the Herbarium of the University of Belgrade (BEO), Department of Algology and Micology – Algae Wet Collection. The qualitative analysis of the samples was performed using a Carl Zeiss AxioImage M.1 microscope with an AxioCam MRc5 camera and AxioVision 4.9 software.
A total of 120 algal taxa were recorded, belonging to 5 divisions: Heterokontophyta (94), Cyanobacteria (15), Chlorophyta (5), Rhodophyta (4), and Charophyta (2). Two freshwater red algae, *Lemanea fluviatilis,* and *Hildenbrandia rivularis*, which are strictly protected in Serbia, were observed. The majority of recorded taxa belong to Bacillariophyceae, with *Navicula* and *Nitzschia* being the most diverse genera. Additionally, *Encyonema reichardtii* was recorded for the first time in the diatom flora of Serbia. Based on species composition, the Izubra River can be characterized as oligotrophic.

Acknowledgments: This work was financially supported by PE "Srbija šume", Belgrade, FE "Šumarstvo" Raška. "Wetland Monitoring Project of the Golija Nature Park".

P162 Evaluating the use of saline diatom indices for bioassessment through metabarcoding and microscopy in two inland saline lakes (Serbia)

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Keywords: diatoms, saline lakes, metabarcoding, microscopy, biomonitoring

Diatoms are widely recognized as effective bioindicators of ecological status in aquatic ecosystems, routinely used in biomonitoring. Traditional microscopy-based identification is time-consuming and requires taxonomic expertise, prompting the use of DNA metabarcoding as a faster and complementary or alternative method for bioassessment. This study evaluates the applicability of *rbcL*-based metabarcoding alongside microscopy for the assessment of two Serbian saline lakes—Plava Banja and Pečena Slatina—located in the Pannonian Plain. These lakes host specialized diatom communities dominated by halophilic and halotolerant taxa. To assess their ecological status/potential, two salinity-specific diatom indices were applied: the Diatom Index for Soda Pans (DISP) and the Trait-Based Index (TBI). In Pečena Slatina, both indices yielded comparable values between microscopy and metabarcoding, suggesting methodological consistency. In contrast, results from Plava Banja showed only partial agreement. In both lakes, index values were positioned near the upper end of the scale, indicating good ecological status/potential. However, microscopy consistently included a significantly higher proportion of taxa in index calculations—exceeding 79% in Plava Banja and 90% in Pečena Slatina—compared to 51–65% for metabarcoding. This discrepancy reflects current limitations in reference libraries for saline environments and incomplete trait annotation in molecular datasets, particularly the lack of species-level resolution and ecological or biovolume data. These constraints reduce the robustness of index-based assessments using metabarcoding. While metabarcoding shows promise, its application in saline ecosystems remains limited until gaps in reference libraries and trait coverage are addressed.

Acknowledgments: This work has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101079234 (BIOLAWEB project) and Alexander von Humboldt Foundation, and was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-136/2025-03/200178, No. 451-03-137/2025-03/200178 and No. 451-03-1041/2024-03/2/3322).

P163 Thermal analysis of human bronchoalveolar lavage fluid: impact of sequential lung washing via differential scanning calorimetry

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Keywords: bronchoalveolar lavage, differential scanning calorimetry, surfactant, thermal analysis

Background: Bronchoalveolar lavage (BAL) is a widely used method for sampling lung epithelial lining fluid. Sequential BAL fractions target different lung compartments, but their impact on the biophysical properties of surfactant and soluble proteins remains underexplored. Differential scanning calorimetry (DSC) provides a sensitive method for assessing thermal transitions in biological fluids, offering insight into the stability of proteins and lipids.
Objective: To assess the effects of sequential BAL washing on the thermal properties of lung fluid and compare profiles between right and left lung samples using DSC.
Methods: BAL fluid was obtained via bronchoscopy from the right middle lobe and left lingula of patients without localized lung disease. Each lung was lavaged with ten 1-liter aliquots of saline. Supernatants were centrifuged to remove cells, concentrated, and normalized for protein content. DSC was used to measure melting temperatures (Tm) and enthalpy changes (ΔH) across lavage fractions and lung sides.
Results: Early lavage fractions showed higher ΔH and broader thermal transitions, indicating more diverse protein-lipid content. Later fractions displayed simpler profiles, suggesting depletion of the biomolecule. Subtle but consistent thermal differences were observed between right and left lung samples.
Conclusion: Sequential BAL significantly alters the thermal profile of lung fluid, emphasizing the need for standardization in sample collection. DSC proves to be a valuable technique for evaluating surfactant structure and protein dynamics in pulmonary research.

P164 Health benefits of *Ginkgo biloba* seeds without the risk of their uses

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Keywords: plant extracts, *Ginkgo biloba*, ginkgotoxin, UHPLC-MS

Introduction: *Ginkgo biloba* seeds are rich in flavonoids and the unique terpenoids ginkgolides and bilobalide, which are known to have beneficial effects on cognitive function. Unlike the commonly used leaves, however, the seeds contain significant amounts of ginkgotoxin (4′-O-methylpyridoxine), a compound that acts as an antivitamin B6. At high concentrations, ginkgotoxin is neurotoxic and may cause seizures, respiratory distress, and loss of consciousness, posing a major obstacle to the safe use of Ginkgo seed-based products.
Aim: This study aims to develop a simple yet effective method to reduce ginkgotoxin levels in extracts obtained from *Ginkgo biloba* seeds, while retaining their beneficial bioactive components.
Materials and Methods: A multi-step liquid–liquid extraction protocol was applied using a series of polar and non-polar solvents. After each extraction step, samples were collected and analyzed via ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). The concentrations of flavonoids, ginkgolides, bilobalide, and ginkgotoxin were quantified at each stage to assess the efficiency of the process.
Results: Compared to a single-step extraction using 70% methanol, the multi-step approach significantly reduced ginkgotoxin levels to near-undetectable concentrations. Importantly, most of the key bioactive compounds—including flavonoids and terpene lactones—were retained in the final extract at levels suitable for functional use.
Conclusion: The results demonstrate that multi-step extraction techniques offer an effective strategy for minimizing ginkgotoxin in *Ginkgo biloba* seed extracts while preserving their beneficial phytochemical profile. This approach provides a promising basis for the development of safe and functional Ginkgo-based products.

P165 Evaluation of spring and winter wheat (*Triticum aestivum* L.) varieties under irrigated and drought stress conditions

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Keywords: wheat, drought, photosynthesis, antioxidants

Drought is one of the major important abiotic stress factors that constrain crop yields worldwide, and depending on the season it can seriously limit wheat production. The increasing world population and the changing climate require enhanced wheat production despite environments where drought affects yield and end-use quality. Characterizing genotypes for adaptive traits could increase their selection for better performance under stress. The unfavorable conditions (soil moisture deficit or late fore crop) coupled with mild winters (a frequent event in Europe in the last decades) makes appropriate early spring sowing of other wheat types, such as spring or facultative spring wheat cultivars so that the land could be efficiently utilized. In this study morphological features and functional efficiency of the photosynthetic apparatus were compared in spring and winter wheat varieties by measuring leaf area, plant biomass, total chlorophyll content, chlorophyll *a* fluorescence and thermoluminescence. The level of stress was detected with the help of antioxidant enzyme activities. We also paid special attention to substances that may play a role in leaf cell defense (polyamines, salicylic acid), and secondary metabolic products. The study also demonstrated the prospects of applied metabolomics for wheat classification, phenotyping and potential use in plant breeding and crop improvement.

Acknowledgements: This work was supported by Bulgarian-Hungarian project IC-HU/02/2024-2025 between IPPG-BAS and HUN-REN ATK.

P166 Manipulating the light spectrum to increase the biomass production, physiological plasticity and nutritional quality of *Eruca sativa* L.

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Keywords: antioxidants, *Eruca sativa*, LED, photosynthesis

Light plays a crucial role in driving photosynthesis, regulating plant growth and phytochemical biosynthesis. Therefore, through precise management of the irradiance and wavelength in controlled environments it is possible to control the development of plants, their photosynthetic performance and even the production of different antioxidants. The extensive progress in light-emitting diodes (LEDs) technology in recent years provides an opportunity to positively influence plant growth and biomass accumulation and to optimize biochemical composition and nutritional quality. This study aimed to assess how different light spectra affect the growth, photosynthesis and biochemical properties of *Eruca sativa*. Therefore two LED lighting modes - red:blue (RB, 1:1) and red:green:blue (RGB, 2:1:2) were compared to the conventional white light fluorescent tubes (WL). Plant biomass, photosynthetic performance, several antioxidants, polyamines and nitrates contents were analyzed across different treatments. Our results showed that the plant growth was strongly affected by the light quality. The presence of green light in the spectrum led to the production of smaller plants with lower leaf area, and correspondingly less biomass. RB and WL light mode produced plants with higher biomass and bigger leaves. Both LED light treatments, RB and RGB, enhanced photosynthesis. The afterglow thermoluminescence band varied according to leaves development, being higher in RB and WL as a consequence of their faster growth. RB spectral mode enhanced the total antioxidant capacity and pigments levels. RB light also increased the content of flavonoids, polyphenols and ascorbate. This effect under RB was combined with significant decrease in nitrate accumulation in the leaves. The RB light generated also modifications in polyamines. The results of our experiments pointed out this spectral mode as the most efficient and indicated that RB 1:1 light in accurate way meets the *Eruca sativa* lighting requirements and induces fine physiological and metabolic adjusments for the needful quality of the produced plants. Together with the high efficacy of the LED lighting, this should provide useful information to farmers and food producers.

Acknowledgements: The authors are grateful to Bulgarian-Hungarian project IC-HU/02-2024-2025.

P167 Photosynthetic performance of two wheat varieties with different drought tolerance under drought stress

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Keywords: wheat, drought, growth, photosynthesis

Drought is one of the most important environmental factors decreasing growth and productivity of plants. At early stages of development plants are extremely sensitive to soil water availability. For crop plants such as wheat overcoming water deficit at seedlings stage is crucial for plant survival and obtaining sufficient and quality production. Drought tolerance is a major economic consideration to yield. Many biological processes are affected by low water deficit. Photosynthesis which is a fundamental process for plants survival is majorly influenced. Plants undergo several physiological modifications during drought to maximize water use while maintaining essential pathways. The aim of this work was to study the effects of drought stress on growth, photosynthesis and chlorophyll content in two wheat varieties (Katya and Zora) which differ in their sensitivity to water deficit. Thus a pot experiment has been conducted and at age of 2 weeks (during third leaf development) plants were subjected to drought for 7 days. Chlorophyll fluorescence and thermoluminescence, as well as pigment content were measured to study the performance of the photosynthetic apparatus. Then the water supply was restored to asses the ability of the plants to recover. Our results demonstrated that the two varieties respond differently to stress limitations. Katya is shown to employ more successful strategy to cope with drought, including morphological and physiological adaptations, stability in photosystem II function and the plants performed better after resumption of watering. Zora showed lower chlorophyll content, photosynthetic apparatus experienced more damages and did not fully recover. The tolerance of Katya to drought is essential for maintaining wheat production in the face of climate change and its associated challenges.

Acknowledgements: This work was supported by project № КП-06-Н81/1, financed by the Bulgarian National Scientific Fund.

P168 Fluorescence-Based Assessment of Drought Stress Tolerance in Two Wheat Cultivars

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Keywords: wheat, drought, chlorophyll fluorescence, photosynthesis

Climate change has intensified both the frequency and severity of drought events, posing a substantial threat to crop yields, particularly in staple cereals like wheat. In this research, we examined the physiological and biochemical responses of two wheat cultivars - Katya (drought-tolerant) and Zora (drought-sensitive), when exposed to controlled drought stress. To further evaluate the effects of drought on photosynthetic function, we conducted a comprehensive analysis of chlorophyll *a* fluorescence. This method refers to the emission of red to far-red light from photosynthetic tissues or organisms when they are exposed to light within the photosynthetically active radiation (PAR) range. The assessed parameters included F_o, Fm, Fv (Fm–F_o), Fv/F_o, Vj, Vi, $\varphi(P_o)$=Fv/Fm, ψ_o=1–Vj, M_o, ABS/RC, TR_o/RC, ET_o/RC, DI_o/RC, φE_o, φD_o, PI(abs), and DF(abs). All these parameters were calculated from the induction curves recorded from the prompt fluorescence (PF), based on the theory developed by Prof. Strasser. To better understand the effects of decreased water content on leaf physiology, we measure not only PF curves but also simultaneously track two other signals - delayed chlorophyll fluorescence (DF) and modulated light reflection (MR) at 820 nm, which offer additional insights into the state of the photosynthetic apparatus. Delayed chlorophyll fluorescence (DF) refers to the brief emission of light in the red to near-infrared spectrum by green plants, algae, and photosynthetic bacteria, which occurs after the light source is switched off and once prompt fluorescence has ended. On the other hand, the MR signal detected at 820 nm offers insights into electron transport processes occurring beyond plastoquinone (PQ) and toward the acceptors of photosystem I (PSI). This analysis provided detailed information on the structural and functional alterations of the photosynthetic apparatus under drought stress. Katya consistently exhibited greater photosystem II stability, higher efficiency of light energy conversion and enhanced resilience to stress compared to Zora across most measured parameters.

Acknowledgements: This work was supported by project № КП-06-Н81/1, financed by the Bulgarian National Scientific Fund.

P169 Sol-gel pure and rare earth modified ZnO (Sm_2O_3, Eu_2O_3, Gd_2O_3) powders for decomposition of analgesic utilizing friction energy

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Keywords: tribocatalysis, Paracetamol, sol-gel, rare earth

In the natural world, mechanical energy is a plentiful, sustainable, and eco-friendly energy source. Here, we successfully convert mechanical energy into ZnO and ZnO/(Sm_2O_3, Eu_2O_3, Gd_2O_3) tribocatalysts using friction. Electrons are transferred across the contact interface by the friction between the catalyst particles and the polytetrafluoroethylene (PTFE)-sealed magnetic bar under magnetic stirring. The catalyst is left with holes while the PTFE absorbs the electrons. Similar to photocatalysis, the holes in the valence band of sol-gel catalysts have strong oxidative power and can effectively oxidize organic pollutants. The tribocatalytic tests demonstrated that ZnO and ZnO/rare earth sol-gel powders could remove analgesics (Paracetamol) when magnetized in the absence of light. By varying the type of rare earth elements (Sm, Eu, Gd), stirring speed (100, 300, and 500 rpm), and magnetic rod type (glass and polytetrafluoroethylene), we further enhanced the tribocatalytic performance. In addition to developing a green tribocatalysis technique for the oxidative purification of organic pollutants, this work offers a potential route for converting environmental mechanical energy into chemical energy, which could be used for sustainable energy and environmental remediation.

Acknowledgment: The authors are grateful to be financially supported by the Bulgarian NSF project KP-06-N89/07 (КП-06-Н89/07).

P170 *Elymus repens* (L.) Gould as a potential phytotherapeutic agent for arthritis: pharmacognostic characterisation and in silico pharmacodynamic evaluation

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Keywords: *Elymus repens* (L.) Gould, arthritis, phytotherapy, flavonoids, *in silico*

Elymus repens (L.) Gould has been traditionally used in European ethnomedicine for the treatment of rheumatism, gout, and urinary tract disorders. It exhibits a broad spectrum of pharmacological activities, including anti-inflammatory, antioxidant, diuretic, spasmolytic, and antibacterial effects. The aim of this study is to investigate the potential of *Elymus repens* (L.) Gould as a phytotherapeutic agent for the treatment of arthritis. The phytochemical composition and therapeutic potential of *Elymus repens* (L.) Gould were evaluated through an integrated pharmacognostic and *in silico* pharmacodynamic approach.

In silico analyses were performed on four flavonoids identified in the rhizome of *Elymus repens* (L.) Gould (luteolin, apigenin, quercetin, and tricin) using SwissADME and CB-Dock2. Molecular docking was conducted against COX-2, ALOX5, MMP-13, and IKKβ, with binding poses assessed based on AutoDock Vina scores and predicted amino acid interactions.

Among the compounds analysed, luteolin demonstrated the highest binding affinity, with Vina scores of –10.3 kcal/mol (MMP-13), –9.7 kcal/mol (COX-2), and –8.9 kcal/mol (ALOX5), forming multiple hydrogen bonds and hydrophobic interactions with catalytically relevant residues. Quercetin also exhibited strong interactions with COX-2 and ALOX5 (–9.4 and –8.6 kcal/mol, respectively), consistent with its documented anti-inflammatory properties. Apigenin and tricin showed moderate binding affinities, particularly towards MMP-13, COX-2, and ALOX5.

The findings provide compelling preliminary evidence supporting the anti-inflammatory and chondroprotective potential of the active constituents of *Elymus repens* (L.) Gould rhizome. Luteolin and quercetin demonstrated the strongest binding affinities, while apigenin and tricin exhibited complementary interactions suggestive of a plausible multi-target mechanism of action. These results support the traditional use of *Elymus repens* (L.) Gouldin joint disorders and highlight its potential as an alternative therapeutic agent in the management of arthritis.

P171 Impact of hypothyroidism on BDNF levels in early cognitive decline: evidence from a rat model

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Keywords: BDNF, hypothyroidism, cognitive impairment, neurotrophins, rat model

Thyroid hormones are essential for brain growth and function, and there is mounting evidence that hypothyroidism is associated with cognitive decline and depressive-like behaviour. One possible mechanism linking the hypothyroid state and cognitive impairment is brain-derived neurotrophic factor (BDNF), a crucial modulator of synaptic plasticity and memory.

Assessing BDNF levels in brain tissue during the early phases of cognitive decline brought on by hypothyroidism in rats was the goal of the current study. Experimental groups of male Wistar albino rats were given 0.01% propylthiouracil (PTU) in drinking water for five weeks in order to induce experimental hypothyroidism. BDNF levels were assessed by ELISA in hippocampal and cerebellar homogenates, as well as in blood serum of both hypothyroid and control animals.

Results revealed a tendency of increase in BDNF levels in both hippocampal and cerebellar tissues of hypothyroid rats compared to euthyroid controls. The elevation was particularly notable in the cerebellum, where inter-individual variability was also higher among hypothyroid animals. In contrast, control animals exhibited more consistent and lower BDNF expression in both regions. Additionally, serum BDNF levels in hypothyroid rats demonstrated substantial variation across individuals, with a subset showing markedly elevated concentrations. This suggests a possible dysregulation of peripheral BDNF expression in response to thyroid hormone deficiency, which may reflect or influence central neurotrophic alterations. These region-specific and systemic changes support the hypothesis that hypothyroidism disrupts neurotrophin balance early in the course of cognitive decline.

These findings highlight the vulnerability of the brain to thyroid hormone deficiency and suggest that BDNF may serve as both a biomarker and potential therapeutic target in the prevention of hypothyroidism-induced cognitive impairment and depression. Early detection and intervention strategies modulating neurotrophic pathways could prove beneficial in preserving cognitive function in thyroid-related neuropsychiatric disorders.

P172 Comparative evaluation of *in vivo* and *ex vivo* ozone adjuncts to University of Wisconsin solution in rat kidney cold preservation models

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Keywords: Cold ischemia, Organ model, Ozone treatment, Wistar rat

Cold ischemia–reperfusion injury remains the Achilles' heel of kidney transplantation, as standard University of Wisconsin (UW) solution alone cannot fully prevent the cascade of oxidative stress and inflammation that compromises graft viability. To explore whether ozone, a low-cost, readily implementable adjuvant, can bolster UW's protective effects, we developed four rat kidney preservation models combining ozone administration with conventional cold storage. Twenty kidneys from ten male Wistar rats were randomized into four groups (n = 5 each). In the UW-Only control group, kidneys were submerged in UW solution and stored at 4 °C for six hours. In the Ex Vivo Ozone model, excised kidneys received a five-minute pretreatment with an ozone–oxygen mixture (20 µg/mL) immediately before identical UW storage. The In Vivo Ozone Preconditioning model involved a single intraperitoneal ozone injection (1 mL of 20 µg/mL) administered 30 minutes before nephrectomy, after which kidneys underwent six hours of UW preservation. Finally, the combined Ozone model applied both *in vivo* preconditioning and a five-minute ex vivo ozone exposure prior to UW storage. These four models, we aim to identify the optimal strategy—*ex vivo*, *in vivo*, or combined ozone treatment—that maximizes graft protection during cold ischemia. Establishing these models lays the groundwork for mechanistic investigations and paves the way toward improved protocols for organ preservation and enhanced transplant outcomes.

Acknowledgements: This work was supported by Çanakkale Onsekiz Mart University the Scientific Research Coordination Unit, Project number: TDK-2025-5114

P173 Application of argumentation maps in 10th grade Biology education

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Keywords: argumentation maps, argumentation skills, biology education

The aim of this study is to determine the effect of using argumentation maps on the development of students' argumentation skills in the context of 10th grade "Biology and Health Education" classes. The methodological framework is based on S. Toulmin's theory of argument structure and the pedagogical model of Argument-Driven Inquiry (ADI) developed by V. Sampson, adapted to the curriculum content in genetics, ecology, and evolution for 10th grade.

The model was implemented with a sample of 90 students over a period of 12 school weeks. A key component of the instructional process was the metacognitive strategy of constructing and analyzing argumentation maps through collaborative (group) work. Students' argumentation skills were assessed using two diagnostic tests – Test 1 (pre-test) administered before the experiment and Test 2 (post-test) conducted afterward. The data from both tests were statistically analyzed using IBM SPSS Statistics, Version 19.

The results of the statistical analysis demonstrate a significant improvement in students' ability to construct arguments when investigating scientific problems in a simulated classroom environment. The findings indicate that argumentation maps substantially enhance students' skills in generating arguments and counterarguments, support the coordination of evidence with claims, and contribute to the evaluation of the validity of arguments and rebuttals of alternative positions.

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ISBN 978-619-281-077-1

UNIVERSITY OF PLOVDIV “PAISII HILENDARSKI”