BOOK OF ABSTRACTS

INTERNATIONAL CONFERENCE „CLEAN NATURE FOR HEALTH“

10 - 13 September 2023, Velingrad, Bulgaria

ORGANISING INSTITUTIONS:

INSTITUTE OF ORGANIC CHEMISTRY WITH CENTRE OF PHYTOCHEMISTRY
Bulgarian Academy of Sciences

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Project BG05M2OP001-1.002-0019:„Clean technologies for sustainable environment – water, waste, energy for circular economy“, financed by the Operational programme “Science and Education for Smart Growth” 2014-2020, co-financed by the European union through the European structural and investment funds.

CONFERENCE PROGRAM

Velingrad, Monday, 11th September, 2023

10:30 – 10:35	**WELCOME WORDS: Prof. DSc. Eng. Pavlina Dolashka**
10:35 – 11:00	**OFFICIAL GUESTS**

- **KRASIMIR VALCHEV**, Chairman of the "Commission on Education and Science" in the Parliament
- **Prof. Dr. GALIN TZOKOV,** Minister of Education and Science, Republic of Bulgaria
- **Prof. Dr. GEORGI VAISILOV**, Executive Director and Head of the Executive Agency "Programme Education" (The Governing Body of OP " Science and Education for Smart Growth")
- **Prof. Dr. KOSTADIN KOSTADINOV,** adviser of the "Commission on Education and Science" in the Parliament of Republic of Bulgaria
- **GEORGI IVANOV**, Chief Expert at the Executive Agency "Programme Education"
- **MARIA DENIZOVA,** Chief Expert at the Executive Agency "Programme Education"
- **Dr. KOSTADIN KOEV,** mayor of Velingrad municipality
- **BISER MADZIROV,** mayor of Draginovo municipality
- **Dr. ILZA POPOVA,** Director, Specialized Hospitals for Rehabilitation-Velingrad
- **ANNI PETKOVA**, Director, SS "Stoyan Zaimov", Pleven
- **KATYA TRIFONOVA**, Deputy Director, biology and health education teacher, SS "Stoyan Zaimov", Pleven

PRESENTATION OF THE SPONSORS OF THE CONFERENCE

11:00 – 11:10	**"DIACHIM" JSc.**, Dr. Tanya Rasheva "Innovative solutions in ecology"
11:10 – 11:20	**"LABIMEX" PLC**, Maria Angelova, "Sample preparation - basis of accurate analysis"
11:20 – 11:30	**"AQUACHIM" PLC**, Dr. Lydia Mihaylova, "Contemporary laboratory solutions for a clean environment and health"
11:30 – 11:40	**"ALEKS 1977" Ltd.**, Assoc. Prof. Dr. Aleksandar Dolashki, "When science meets business"
11:40 – 11:50	**„T.E.A.M." Ltd.**, Konstantin Doktorov, "Microplastic pollution: a global scientific effort"
11:50 – 12:00	**CC "Clean&Circle"**, Prof. Yana Topalova, "R&D&Inno achievements and perspectives of Clean&Circle""
12:00 – 12:10	**NNP "BioActivMed"**, Prof. Pavlina Dolashka, "„Clean nature for health"
12:10 – 12:25	**Assist. Alexander Dushkov,**"Investigating the *in vitro* antitumor and antiproliferative poten-tial of an *Amanita muscaria* (L.:Fr.) Hook-extract against lung and prostate cancer cell lines"

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SESSION 1. PLENARY LECTURES "ECOLOGY":

13:45 – 14:00	**Prof. Dr. Valentin Nenov**, "Utilization of phosphorus from wastewater and sludge"
14:00 – 14:15	**Assist. Petar Petrov**, "Mineral water and plant extracts - healing properties and potential"
14:15 – 14:30	**Assoc. Prof. Dr. Ivanka Stoycheva**, "Development of a zero-waste method of processing organic waste into useful products"
14:30 – 14:45	**Assist. Prof. Dr. Aleksandrina Kostadinova-Slaveva,** "Obtaining a soil improver through on site composting"
14:45 –1 5:00	**Assist. Dr. Savina Brankova**, "Opportunities to reduce the carbon footprint of office activities and tourism"
15:00 – 15:15	**Assist. Prof. Dr. Elena Geleva**, "Assessment of the content of uranium and polonium radionuclides in drinking water from southern Bulgaria"
15:15 – 15:30	**Assoc. Prof. Dr. Roumiana Zaharieva**, "Buildings in the sustainable development context"

SESSION 2. PLENARY LECTURES "ECOLOGY":

16:00 – 16:15	**Prof. Dr. Eng. Andrei Sarbu**, "Molecularly imprinted polymers preparation and their use for environment and health - own results"
16:15 – 16:30	**Assoc. Prof. Dr. Irina Schneider**, "Bioaugmentation during composting of garden waste"
16:30 – 16:45	**Prof. Dr. Boyko Tsyntsarski**, "Conversion of water purification sludge into carbon adsorbent and energy"
16:45 – 17:00	**Assist. Prof. Dr. Mihaela Belouhova**, "5 Years Center of Competence Clean&Circle - Fluorescence techniques based contributions to environmental biotechnologies"
17:00 – 17:15	**Assist. Prof. Dr. Angelina Kosateva**, "Applications of Raman spectroscopy for solving environmental problems"
17:15 – 17:30	**M. Sc. Selin Kyuchyuk**, "Core-Sheath and Core/Double-Sheath composite fibers prepared by single- spinneret electrospinning"

Tuesday, 12st September 2023

SESSION 4. PLENARY LECTURES "HEALTH":

09:30 – 09:45	**Prof. Dr. Reni Kalfin**, "Natural products for health: prevention and treatment of experimental Parkinson's disease with standardized *Helix aspersa* extract"
09:45 – 10:00	**Prof. Dr. Lyubka Tancheva**, "Neuroprotective mechanisms in the action of standardized extract of *Helix aspersa* in experimental model of Alzheimer's type dementia in vivo"
10:00 – 10:15	**Ventseslav Atanasov**, "Proteomic analysis of rat cortex and hippocampus in scopolamine-induced dementia of the Alzheimer type using snail extract as a neuroprotective agent"
10:15 – 10:30	**Prof. Dr. Stoyan Shishkov**, "Antiviral Activity of Extract from *Astragalus Glycyphyllos L.* against replication of human Coronavirus 229E and Simplex virus human alpha type 1 and 2 applied alone and in combination with Acyclovir"
10.30 – 10:45	**Dr. Momchil Kermedchiev**, "Natural and surgical approach in the treatment of difficult to heal and chronic wounds"
10:45 – 11:00	**Dr. Veronika Tsankova-Kermedchieva**, "Analysis of biologically active substances from natural origin used in ophthalmology"

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SESSION 5. PLENARY LECTURES "HEALTH":

11:30 – 11:45	**Assist. Prof. Dr. Ionica Deliu**, "Antimicrobial and cytotoxic activities of extracts from *Aconitum toxicum* Rchb"
11:45 – 12:00	**Corr. Member, Prof. DSc. Hristo Najdenski**, "Antimicrobial resistance as a global health threat in the new century"
12:00 – 12:15	**Assoc. Prof. Dr. Maya Zaharieva**, "Antibacterial effects of combinations of snail mucus with clinically administered antibiotics"
12:15 – 12:30	**Assoc. Prof. Dr. Ventsislava Petrova**, "Green synthesis and characterization of Cu and Zn nanoparticles with antimicrobial properties"
12:30 – 12:45	**Prof. Dr. Albena Alexandrova**, "Microplastics: review of current knowledge of sources, fates and effects on ecosystem and human health"

SESSION 6. PLENARY LECTURES "HEALTH":

14:15 – 14:30	**Assoc. Prof. Dr. Ani Georgieva**, "Assessment of the immunomodulating and anticancer activity of hemocyanins isolated from garden and marine snails in a myeloid tumor model"
14:30 – 14:45	**Assoc. Prof. Dr. Ivan Iliev**, "Safety test BALB 3T3 NRU assay for the study of natural substances"
14:45 – 15:00	**Assist. Dimitar Kaynarov**, "Environmental bioactive compounds with antitumor activity from marine and garden snail against bladder carcinoma and mechanism of action"
15:00 – 15:15	**Prof. DSc. Iva Ugrinova**, "Innovative approaches in combating drug resistance in cancer treatment: from ferrocene derivative of camphor to micellar delivery systems"
15:15 – 15:30	**Assoc. Prof. Dr. Galina Radeva**, "Soil Microbiome - a reservoir of soil health microorganisms"

SESSION 7. PLENARY LECTURES "HEALTH":

16:00 – 16:15	**Assist. Alexander Tzintzarov**, "Isolation, separation and purification of phytochemicals from *Sambucus nigra* berries for biological activity analysis"
16:15 – 16:30	**Assoc. Prof. Dr. Lyudmila Velkova**, "Bioactive compounds from the mucus of the garden snail *Helix aspersa* with antimicrobial activity"
16:30 – 16:45	**Prof. Dr. Nikolay Vassilev,** "NMR Spectroscopy in drug discovery and development from natural products"
16:45 – 17:00	**Assist. Karina Marinova,** "Water extract of chamomile as a reducing agent in the green synthesis of metal oxide nanoparticles"

SESSION 3. POSTER PRESENTATION "ECOLOGY"
Monday, 11th September, 2023

17:30 – 17:35	**P1. Dr. Teodor Sandu,** "Hybrid bead materials based on acrylic copolymer"
17:35 – 17:40	**P2. Sorin-Viorel Dolana, Daiana-Georgiana Mitrea**, "Novel polyurethanes based on recycled PET and natural feedstock polyols for wooden artifacts protective coating"
17:40 – 17:45	**P3. Assoc. Prof. Dr. Alexander Kroumov,** "An innovative system of photobioreactors for the realization of the biorefinery concept"
17:45 – 17:50	**P4. Assist. Prof. Dr. Martin Stefanov**, "Sensitivity of photosynthesis in *Zea mays* L. and *Sorghum bicolor* L. under salt stress"

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17:50 – 17:55	**P5. Assist. Prof. Dr. Hristina Lazarova**, "Photodegradation of methylene blue and crystal violet by Zr-titano silicates"
17:55 – 18:00	**P6. PhD St. Magdalena Bogdanova Angelova**, "Influence of the tourism industry on the environment based on the quantity and quality of wastewater in the region of Sunny Beach, Bulgaria"
18:00 – 18:05	**P7. Assist. Prof. Dr. Ivaylo Yotinov,** "Adaptation of key biofilm communities in a constructed wetland for mixed urban wastewater treatment"
18:05 – 18:10	**P8. Assoc. Prof. Dr. Yovana Todorova,** "Non-thermal plasma treatment to prevent biofilm formation and to control mature biofilm growth"
18:10 – 18:15	**P9. Assist. Prof. Dr. Dessislava Gerginova,** "Needles as novel bioindicators for pollution monitoring – an NMR study"
18:15 – 18:20	**P10. Assoc. Prof. Dr. Ganka Chaneva,** "Phycoremediation alleviates arsenate toxicity in maize plants"
18:20 – 18:25	**P11. Assoc. Prof. Dr. Gloria Issa,** "Nanostructured TiO_2/Mn_XO_Y mixed catalysts for enhanced hydrolysis of DNA-model phosphodiester: impact of Ti/Mn ratio"
18:25 – 18:30	**P12. Prof. Dr. Albena Alexandrova,** "Microplastics in fish and shellfish from the Bulgarian Black Sea coastal zone"
18:30 – 18:35	**P13. Georgi Georgiev,** "Carbon materials for sensors in medicine and environmental application"
18:35 – 18:40	**P14. Assist. Prof. Dr. Plamena Marinova-Dragozova**, "Surface-wave-sustained argon plasma torch for reducing leachate toxicity"
18:40 – 18:45	**P15. Prof. Dr. Dimitar Tonev,** "Investigation of the natural radioactivity of mineral waters"
18:45 – 18:50	**P16. Chemist Verzhinia Variyska,** "Study of the atmospheric radioactivity in Bulgaria for the period 2019-2022"

SESSION 8. POSTER PRESENTATION "HEALTH"
Tuesday, 12st September, 2023

17:00 – 17:05	**P1. Assist. Prof. Dr. Todor Bogdanov,** "Surface – wave-sustained plasma source for bio-medical applications"
17:05 – 17:10	**P2. Assoc. Prof. Dr. Radoslav Abrashev**, "Oxidative stress as a mechanism of antifungal activity of protein fractions from *Rapana venosa*"
17:10 – 17:15	**P3. Assist. Prof. Dr. Eng. Ivelina Tsacheva**, "Lavender oil delivery systems based on mesoporous silicate-polymer nanocomposites"
17:15 – 17:20	**P4. Assoc. Prof. Dr. Elina Tsvetanova**, "Oral treatment of mice with a combination of D-galactose and ethanol as a new aging model"
17:25 – 17:30	**P5. Assoc. Prof. Dr. Maya Zaharieva,** "Antiviral activity of extracts obtained from *Oreganum vulgare* L. and *Scenedesmus* spp."
17:35 – 17:40	**P6. Assist. Prof. Dr. Yana Ilieva,** "Preliminary in vitro toxicology tests of antimicrobial extracts and compounds from two Hypericum species"
17:40 – 17:45	**P7. Assist. Dr. Mila Kaleva,** "Antimicrobial resistance of Escherichia coli Isolated from pig farms in Bulgaria"
17:45 – 17:50	**P8. Assist. Prof. Dr. Kamelia Hristova-Panusheva**, "The combined effect of pegylated graphene oxide nanoparticles loaded with bleomycin and near-infrared irradiation treatment on hepatocellular cancer cells *in vitro*"

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17:50 – 17:55	**P9. PhD St. Trayana Kamenska,** "Hemolytic Activity of GO based nanoparticles loaded with bleomycin and NIR irradiated as a marker of their hemocompatibility"
17:55 – 18:00	**P10. Assoc. Prof Dr. Maya Zaharieva,** "Antibacterial activity of hydrogels loaded with oregano oil in combination with gentamicin or ciprofloxacin"
18:00 – 18:05	**P11. Eng. Yulia Karaivanova,** "Restorative effect of zeolite on brain acetylcholinesterase activity in rats with subchronic heavy metal intoxication"
18:05 – 18:10	**P12. Assist. Karina Marinova,** "Development of novel biological nanoparticles from the mucus of the garden snail Cornu aspersum as antimicrobial agents"
18:10 – 18:15	**P13. Assist. Stefan Genchev**, "The Bulgarian National Cyclotron Center and the benefit of public health"

SPONSORS OF CONFERENCE

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ABSTRACTS OF LECTURES

SESSION 1. PLENARY LECTURES "ECOLOGY"

R&D&INNO ACHIEVEMENTS AND PERSPECTIVES OF „CLEAN&CIRCLE"

Yana Topalova[1,2] and Team of "Clean & Circle"

[1]*Sofia University "St. Kliment Ohridski", Faculty of Biology, Bulgaria;* [2] *Center of Competence "Clean& Circle", Sofia University, Bulgaria*

The competence center is an innovation ecosystem of the year. The team won second place in the world's prestigious TripleE competition and was honored among 195 applicants from around the world. This conquest has been achieved after five years of investment in infrastructure, expertise, human resources, established technologies and innovative products. The financial part of this investment is the European infrastructure funds BGN 23,677,000 with national co-financing. But the investment is much larger, as it was complemented by numerous businesses, international projects under Horizon 2020 and national scientific programs, as well as co-financing from budget funds of Clean&Circle participants. These large financial investments led to the construction of a state-of-the-art, competitive infrastructure with equipment and updated laboratories at each partner and a common building-living lab with modern environmental features, as well as a common electronic cloud, accelerator and facilities for scientific and educational forums. In terms of content, the investment is aimed at strong research, development and implementation activity in the field of clean technologies, sustainable management of water, waste, energy efficiency, creation of circular solutions for the economy and bio-economy in the sectors-ecology, healthcare, agriculture, energy and energy efficiency.

As facts can be indicated - High scientific and technological activity, 10 developments /technologies and products/ ready for intellectual property protection, 140 scientific publications - 75% in Journal with high IF and JRS, 110 experts assigned to employment contracts in partner project organizations, 40 professors and associate professors, 35 young scientists, 26 doctoral students, 53 contracts with businesses.

Most essential to the sustainable future of Clean&Circle are the people, the STEM educational products that work sustainably for students of all levels of study and the young scientists who through "learning by doing" work and create the technologies of the future to raise the economy and bioeconomy to a high level in Bulgaria. All this is done with mind, hands and heart by the scientists building Clean&Circle.

Keywords: Clean&Circle, Centre of competence, innovation sustainable ecosystem, clean technologies

ACKNOWLEDGEMENTS: *This work was supported by project BG05M2OP001-1.002-0019: 'Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy', financed by Operational Program 'Science and Education for Smart Growth', co-financed by the EU through the ESIF and by the project Bulgaria– China 2019 "Investigating the microbiome during biogas production from organic fraction of municipal solid waste" – Contract №KP-06-China/11.*

CLEAN NATURE FOR HEALTH

Pavlina Dolashka, and TEAMS of CLEAN & CIRCLE and NSP "BioActivMed"

[1]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]Center of competence "Clean technologies for sustainable environment – water, waste and energy for circular economy", Bulgaria

Plants, animals and people need clean air, clean water and clean nature without solid waste. In-depth studies of pollutants in nature and developed technologies for their purification contribute to the protection of nature. The various obtained biologically active substances, analyzed by the most modern equipment, as a result of our participation in CC „Clean&Circle", and NSP "BioActivMed" gives us the opportunity to study the state of the human organism, various diseases and develop preparations to protect people's health. Based on the team's research, activated charcoal from apricot pits was created and masks used during the COVID-19 pandemic were developed. The obtained activated carbon has a high adsorption activity to both, viruses and bacterial strains. New technologies have been developed to obtained active substances and extracts from various natural raw materials, such as Black Sea crabs, marine and garden snails grown in special farms. Studies conducted on a garden snail extract with a certain composition prove the antibacterial effect against the bacterium *Helicobacter pylori*, which is the main cause of stomach ulcers, often turning into stomach cancer.

The antibacterial activity and the regenerative effect of snail mucus extract have been demonstrated during damage to the stomachs of mice with ethyl alcohol.

Moreover, a number of purified proteins from the hemolymph of rapana and garden snails suppress the development of tumor cell lines from stomach, mammary gland, bladder cancer, etc., which validates them as potential active substances for investment in pharmaceutical products to preserve human health.

Keywords: clean technologies innovative compounds

ACKNOWLEDGEMENTS: *This work was supported by project BG05M2OP001-1.002-0019: 'Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy', financed by Operational Program 'Science and Education for Smart Growth', co-financed by the EU and The BMES (Grant D01-217/30.11.2018 and agreements DO1-323/18.12.2019 and DO1-358/17.12.2020) and DO1-278/03.12.2021 under the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine" approved by DCM#658/ 14.09.2018.*

INVESTIGATING THE *IN VITRO* ANTITUMOR AND ANTIPROLIFERATIVE POTENTIAL OF AN *AMANITA MUSCARIA* (L.:Fr.) Hook. EXTRACT AGAINST LUNG AND PROSTATE CANCER CELL LINES

Alexander Dushkov[1], Zuzana Vosáhlová[2], Alexander Tzintzarov[1], Květa Kalíková[2], Tomáš Křížek[3], Maria Petrova[1], Anastas Gospodinov[1], Iva Ugrinova[1]

[1]Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague, Czech Republic; [3]Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic.

The fungus *Amanita muscaria* is universally recognizable for its iconic appearance; it is also widely regarded as poisonous, inedible, and even deadly. In spite of that, there have been documented cases of use of *A. muscaria*-containing preparations against various diseases, including cancer, to no apparent ill effect. The search for compounds that can be used to treat cancer among various plants and fungi has been intensifying in recent years. In light of this, we describe an HPLC HILIC analytical method for the evaluation of the content of the anticancer compound ergosterol (ERG) and the neuroactive alkaloids ibotenic acid (IBO) and muscimol (MUS) that contribute significantly to the unpleasant physiological syndrome associated with *A. muscaria* consumption.

A 'homemade' *A. muscaria* tincture made using 80 proof rye vodka as the solvent, an *A. muscaria* extract made with a standardized water-ethanol solution as the solvent, and fractions obtained from the second extract via liquid-liquid extraction with nonpolar solvents were analyzed.

The study also presents the results of capillary zone electrophoresis with contactless conductivity detection and UHPLC-MS/MS analyses of the IBO and MUS content of the two native *A. muscaria* extracts and an evaluation of the standardized extract's cytotoxic effect against a panel of lung cell cultures in vitro.

Our results show that the standardized extract has a significant cytotoxic effect and does not contain the compounds of interest in any significant quantity.

Keywords: cancer; fungi; alkaloids; HPLC; mass spectrometry; capillary electrophoresis; cytotoxicity; *Amanita muscaria*

ACKNOWLEDGEMENTS: *This research was funded by the National Scientific Program "BioActiveMed": grant number D01-217 and realized within the cooperation of CEEPUS project No.CIII-RO-0010-17-2223.*

UTILIZATION OF PHOSPHORUS FROM WASTEWATER AND SLUDGE

Valentin Nenov

"Professor Dr. Asen Zlatarov" University – Burgas, Bulgaria

The aim of the phosphorus recycling technologies under research is to recover the phosphorus out of the wastewater or the sludge matrix into applicable phosphate fertilizer products such as magnesium-ammonium-phosphate, the so called struvite. Struvite is formed by a basic precipitation reaction in different stages of the wastewater treatment process, from magnesium (Mg^{2+}), ammonium (NH_4^+) and ortho-phosphate (PO_4^{-3}).

This study aims to discuss: (i) the progress in extraction of P from sewage sludge and animal manure; (ii) the methods to create optimal conditions for struvite precipitation in such media; (iii) the avenues for overcoming the problems associated with choosing the right Mg source, pH adjustment and the non-acceptable level of organic matter in the initial suspension; and (iv) the implication of struvite as an alternative fertilizer for the global agriculture sector. The technology was tested by using a bench scale unit which. The results have proved the quality of the product obtained and the rate of its formation.

The agro-technical effects of struvite application were studied as well; the struvite fertilization outcome towards maize growth which was followed for 6 months of vegetation in a test field. The quantity and quality of the crops harvested was compared with control samples obtained by cultivation with conventional fertilizers. The evaluation was performed based on the yield and nutritional characteristics of the corn. The results obtained show that the struvite is efficient complement to the nitrogen fertilizers (such as ammonium nitrate and carbamate) as an alternative to the normal superphosphate and triple superphosphate in the role of P source.

Keywords: Phosphorus recovery; Struvite; Wastewater; Waste sludge; Bio- fertilizer

ACKNOWLEDGEMENTS: *The authors wish to acknowledge the support under the BG05M2OP001-1.002-0019 Clean technologies for sustainable environment – waters, waste, energy for circular economy" (Clean&Circle) Project financed by the Operational programme "Science and Education for Smart Growth" 2014-2020, co-financed by the European union through the European structural and investment funds.*

MINERAL WATER AND PLANT EXTRACTS-HEALING PROPERTIES AND POTENTIAL

Petar Petrov[1], **Karina Marinova**[1], **Valentina Lyubomirova**[2], **Lyudmila Velkova**[1], **Angelina Kosateva**[1], **Pavlina Dolashka**[1]

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Center of competence "Clean technologies for sustainable environment – water, waste and energy for circular economy", Sofia, Bulgaria;* [2]*Faculty of Chemistry and Pharmacy of Sofia University "St. Kliment Ohridski", Sofia, Bulgaria*

The main objective of this study was to determine the composition of water from several mineral springs, determine the best ones for treating different categories of illnesses use them for the preparation of traditional medicinal plant extracts and test their antimicrobial properties.

For the composition determination samples from 8 mineral springs were: subjected to an elemental analysis for the determination of 70 elements using inductively coupled plasma-mass spectrometry; dry residue samples were analyzed using Raman spectroscopy. After results processing and comparison some of the water samples were used for the preparation of medicinal plant extracts. Their antibacterial properties were tested using disk diffusion and agar well diffusion methods.

Results showed that previously obtained data for the composition of the waters is not accurate and detailed enough. Concentrations of the macro elements were high in all samples. Elements with previously reported antimicrobial properties were also found in every sample in fairly high concentrations. Raman spectroscopy revealed the presence of different crystalline structures, some of which of a sulphate nature, which presumably contribute to the waters healing properties. Mineral water plant extracts revealed a 17% and an 11% stronger antibacterial effect against *E.coli* and *B.subtilis* respectively, when compared to the dH_2O plant extracts.

The obtained information can be used for: updating and expanding data on the composition of water from mineral springs; specifying the healing effect of mineral waters; determining the possibilities for their use as additives to medicinal and cosmetic preparations.

Keywords: mineral water, elemental analysis (ICP-MS), Raman spectroscopy, plant extract, properties

ACKNOWLEDGEMENTS: *This research was carried out by the research grant Project BG05M2OP001-1.002-0019: "Clean technologies for sustainable environment – water, waste, energy for circular economy" (Clean & Circle), for development of a Centre of Competence and by the Bulgarian Ministry of Education, and the D01-217/30.11.2018 NSP "Innovative low-toxic biologically active precise medicine (BioActiveMed).*

DEVELOPMENT OF A ZERO-WASTE METHOD OF PROCESSING ORGANIC WASTE INTO USEFUL PRODUCTS

Ivanka Stoycheva, Bilyana Petrova, Boyko Tsyntsarski, Angelina Kosateva, Nartzislav Petrov, Pavlina Dolashka

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Center of competence "Clean technologies for sustainable environment – water, waste and energy for circular economy", Sofia Bulgaria

RDF (Refuse Derived Fuel) is produced from combustible components that the industry calls Municipal Solid Waste. This waste, usually taken from industrial or commercial sites, is shred, dried, baled and then finally burned to produce electricity. Refuse Derived Fuel is a renewable energy source that ensures that the waste won't be thrown into a landfill and instead it will find suitable application.

An alternative option for waste utilization is the conversion of RDF into a carbon adsorbent, and its further use for adsorption of water pollutants. Adsorption is a well established and powerful technique for treating domestic and industrial effluents.

Due to their highly developed porous structure and large specific surface area, activated carbons exhibit a considerable adsorption capacity towards various pollutants.

The aim of the research is the development of a waste-free method for processing waste (from the waste processing plant in Bogrov) to obtain useful products.

Carbon adsorbent was obtained by flash pyrolysis at 850^{o}C for 10 min. The obtained adsorbent was characterized by elemental analysis, thermogravimetric analysis, nitrogen physisorption, etc. The obtained results show that the material has a potential application for adsorption of contaminants.

Keywords: RDF fuel, waste materials, adsorbent

ACKNOWLEDGEMENTS: *This work was supported by project BG05M2OP001-1.002-0019: "Clean technologies for sustainable environment – water, waste, energy for circular economy", financed by the Operational programme"Science and Education for Smart Growth" 2014–2020, co-financed by the European Union through the European structural and investment funds.*

OBTAINING A SOIL IMPROVER THROUGH ON-SITE COMPOSTING

Aleksandrina Kostadinova-Slaveva

University of forestry, Faculty Ecology and Landscape Architecture, Bulgaria

Sustainable management of the growing amount of waste is one of the most significant challenges facing modern society. Waste management is central to the circular economy - it defines the practical implementation of the waste hierarchy. The circular economy is a key to the development of greener cities and good living conditions, as it uses a recycled resource and thus reduces carbon imprint.

A significant part of the household waste generated by households, hotels, restaurants, the catering sector and others is biodegradable waste. During the maintenance of public spaces in settlements and private green areas, organic waste is also generated in large quantities. Biodegradable waste represents about two-thirds of the total amount of waste. Improper management and disposal create conditions for the formation of gases causing global climate change. It is for this reason that it is necessary to limit their landfilling, increase their separate collection and their subsequent treatment, as biodegradable waste is processed into products to increase soil fertility and improve its structure.

This transformation of biodegradable waste into products can easily be achieved through composting processes. Due to its specificity and the possibilities it provides for the recovery of organic carbon, according to the regulatory framework, composting activities are referred to as recycling/recovery of organic substances. One of the most appropriate ways of treating biodegradable waste in smaller municipalities and suburban areas is on-site composting. On-site composting, although a relatively simple process based on natural biological processes, has its own specific requirements to ensure a quality soil improver.

The aim is to offer suitable composting recipes for biodegradable waste to ensure the correct operation of on-site composting and organic carbon recycling processes, with an emphasis on the generated biodegradable waste specific to resort settlements.

Keywords: biodegradable waste, composting, circular economy

ACKNOWLEDGEMENTS: *This work was supported by project BG05M2OP001-1.002-0019: 'Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy', financed by Operational Program 'Science and Education for Smart Growth', co-financed by the EU through the ESIF.*

OPPORTUNITIES TO REDUCE THE CARBON FOOTPRINT OF OFFICE ACTIVITIES AND TOURISM

Savina Brankova

University of Forestry, Faculty of Ecology and Landscape Architecture, Sofia, Bulgaria

In recent years, the topic related to the determination and reduction of the carbon footprint of various anthropogenic activities has become increasingly topical, due to the growing need to reduce climate impact and natural resources consumption and at the same time comprehensive environmental protection. This is also confirmed by the actions of the European Parliament, which adopted the European Climate Law that sets the EU's target of reducing net greenhouse gas emissions at least 55% by 2030.

Office activities and tourism are among the main areas, which can leave a serious carbon footprint, in case that appropriate measures for its reduction are not taken.

The current study purpose is to research the main offices and tourism activities that leave a carbon footprint, as well as the ways of decarbonization and the possibilities of achieving a "zero" carbon footprint.

Keywords: carbon footprint, office activities, tourism

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ASSESSMENT OF THE CONTENT OF URANIUM AND POLONIUM RADIONUCLIDES IN DRINKING WATER FROM SOUTHERN BULGARIA

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The results of an analysis of the content of uranium and polonium radionuclides in drinking water from southern Bulgaria are presented, as a basis for long-term scientific studies of changes in their radionuclide composition. For the purposes of the study, a complex radiochemical methodology was used to isolate the individual radionuclides from the water matrices, which includes the use of highly selective extraction-chromatographic resins.

To obtain accurate and reproducible results, nuclear-physical analysis methods such as low-level alpha spectrometry have beeb applied. The concentrations of ^{238}U, ^{234}U and ^{210}Po in drinking water from 30 settlements in the Plovdiv and Haskovo regions were precisely measured and conclusions were drawn about the levels of radioactivity in the water with the aim of their safe use by the population. The correlation between the activity concentrations of ^{238}U, ^{234}U and ^{210}Po in the water analyzed has been estimated, giving a significant correlation between ^{234}U and ^{238}U activity. There is no correlation between the uranium isotopes and ^{210}Po activities.

The expected total annual effective doses for adults have been calculated from the measured activities. The results show that the annual effective dose due to ingestion of drinking water is lower than the recommended value of 100 μSv y^{-1}, according to the limits of the World Health Organization and the Bulgarian legislation.

As a result of the conducted research, it can be concluded that the studied drinking water from Southern Bulgaria meets the requirements of the regulatory documents in terms of radiological indicators and their use in everyday life does not pose any health risks.

Keywords: Drinking water, ^{238}U, ^{234}U, ^{210}Po, total effective dose

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BUILDINGS IN THE SUSTAINABLE DEVELOPMENT CONTEXT

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The construction sector is often blamed for the huge consumption of material and energy resources, the generation of a large amount of waste (CDW) and its significant contribution to global warming. However, it is forgotten that it is the construction sector that is the backbone of the sustainable development of society, providing housing, roads, buildings to satisfy cultural and social needs, and last but not least, it is among the leading economic sectors and thus ensures prosperity.

This publication aims to present a holistic approach to the assessment of buildings in the context of sustainable development. In recent years, much has been done about new near-zero energy buildings and energy-plus buildings, capable of not only meeting the energy needs of their users, but also producing energy in excess. Intelligent technologies allow ensuring comfort and healthy conditions. At the same time, existing buildings pose a number of challenges, both in terms of energy efficiency and safety, due to the presence of building materials with hazardous properties that pose a threat to human health and the environment, especially at the end of the buildings' life cycle. Possibilities for identifying hazardous components and ways to prevent risks are discussed.

The crucial role of CDW management in the process of reversing the negative balance of the construction sector is outlined.

The publication analyzes the regulatory framework and existing good practices, as well as the challenges facing Bulgaria, which lags behind in the application of the holistic approach to the assessment of buildings in the context of sustainable development.

Keywords: construction, sustainability, environmental footprint, smart buildings, hazardous materials, CDW management

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SESSION 2. PLENARY LECTURES "ECOLOGY"

MOLECULARLY IMPRINTED POLYMERS PREPARATION AND THEIR USE FOR ENVIRONMENT AND HEALTH- OWN RESULTS

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Molecularly imprinted polymers (MIPs) are a new kind of polymer materials, containing voids with the shape, size and electronic environment complementary to a target molecule, named template. Due to these features, MIPs have great absorption and selectivity towards the template. They are obtained by the polymerization of a monomer and a crosslinker in the presence of the template. Afterwards, the template is extracted from the high croslinked polymer structure, getting voids which are also called imprinting sites [1]. Alternatively, the molecularly imprinted polymers can be prepared starting from a already prepared copolymer, by adding the template in a concentrated solution of the copolymer, followed by phase inversion in a coagulation bath and subsequent extraction of the template [2].

The conference presents the original results obtained, concerning the preparation of MIPs by various polymerization methods, such as block polymerization microemulsion polymerization, dispersion polymerization , electropolymerization and sol- gel, as well as the preparation of MIPs by phase inversion, using acrylonitrile copolymers.

The obtained MIPs proved useful for environment application, such as biomimetic sensors for detection of pollutants or for extraction of bioactive substances from complex matrices such as phytoextracts.

References: [1]. Vitalys Mba Ekomo, et. al, Biosensors and Bioelectronics, 112, 156-161, 2018; [2]. Ana-Mihaela Florea, et. al, Talanta (2016), 148, pp. 37-45.

Keywords: molecularly imprinted polymers, pollutants, sensors, bioactive substances, phytoextracts.

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BIOAUGMENTATION DURING COMPOSTING OF GARDEN WASTE

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The increasing waste generation requires the introduction of circular solutions in waste management. An example of such during utilization of biodegradable waste is composting. This biotechnology can be applied to the treatment of excess sludge after aerobic or anaerobic treatment, for garden waste, incl. fruits and vegetables and others. Starting the composting process can be done by applying bioaugmentation using stabilized compost or microbiological and enzymological preparations.

The purpose of the study is to evaluate the effect of bioaugmentation on composting in a pilot plant at the Faculty of Biology to the Sofia University "St. Kliment Ohridski". The first part of the research presents information on the microbiological composition and biodegradation activity of the bioaugmentation products, and the second part analyzes the composting process depending on the initially used inoculation material. Both the main factors on which the process depends (humidity, temperature, C:N:P) and the microbial biodegradation activity through the indicator aerobic dehydrogenase activity were investigated. The elemental composition during composting was analyzed at Institute of Organic Chemistry with Center of Phytochemistry to Bulgarian Academy of Science and the Faculty of Chemistry and Pharmacy to Sofia University "St. Kliment Ohridski".

Keywords: circular solution, waste management, bioaugmentation, microbial activity, composting

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CONVERSION OF WATER PURIFICATION SLUDGE INTO CARBON ADSORBENT AND ENERGY

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Sewage sludge is the residual, semi-solid material which is by-product from sewage treatment of industrial or municipal waste waters. When wastewater enters a primary settling tank, approximately 50% of the suspended solid matter will settle out in 90 min. This matter called n of solids is called "raw sludge". US States municipal wastewater treatment plants in 1997 produced about 7.7 million dry tons of sewage sludge, and about 6.8 million dry tons in 1998 according to EPA estimates. As of 2004, about 60% of all sewage sludge was applied to land as a soil amendment and fertilizer for growing crops. In 2012 it was reported, that 10 million tones sludge per year were produced in 27 European countries. Thus the sludge utilization becomes a significant environmental issue, which needs to be solved quickly. Original thermal treatment procedure, Fast pyrolysis /heat shock/, of water treatment sludge material is performed in the temperature range between 500 and 850°C. The sample is placed directly into an oven heated to the appropriate temperature. This is made in order to achieve maximum rate of thermal destruction of the organic mass of the material. As a result, the amount of volatile substances /which could be applied as energy source/ is increased and a solid product with a high surface area is obtained, whereas large number of pores formed during the release of volatile substances. BET results show moderately high specific surface area. The obtained carbon material is characterized by high ash content (20%). The material balance is as follows: 30% solid product, 20% volatoles and 50% gaseous products. The gas mixture contains 2% hydrogen, 18% methane, 30% CO, and can be used as energy source.
Thermal treatment of water treatment sludge material is performed at temperature 500-850°C. Fast pyrolysis /heat shock/ was used, as the sample is put directly into an oven heated preliminary to the selected temperature. The obtained samples were characterised by nitrogen low-temperature sorption, technical and elemental analysis, etc. A solid product with a moderately high surface area is obtained, whereas large number of pores formed during the release of volatile substances. Fast pyrolysis is suitable method to obtain volatiles with possible energy application, as well as a solid product with a moderately high surface area. Our study demonstrated a new promising way for sludge utilization.

Keywords: water purification, carbon adsorbents, sedimentary keg, sludge

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5 YEARS CENTER OF COMPETENCE CLEAN&CIRCLE – FLUORESCENT TECHNIQUES BASED CONTRIBUTIONS TO ENVIRONMENTAL BIOTECHNOLOGIES

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Within the Center of competence "Clean technologies for sustainable environment - water, waste and energy for circular economy (Clean&Circle), the Faculty of Biology of Sofia University made a significant investment in fluorescent technologies (about BGN 1.5 M). The equipment of the highest class allowed to develop and introduce various methods - innovative and internationally recognized, to carry out precise research on various topics and to obtain results in the form of both scientific and applied products. The use of fluorescence techniques combines advantages such as speed, accuracy, precision of information, sensitivity regarding biological processes, combining quantitative and visual, 3D information and last but not least – a high degree of applicability in challenging environmental matrices.

The approach that was used included the following algorithm – selection of a fluorescence technique with a special design for environmental samples; development of methods aimed at biodegradation, detoxification and intoxication; digital processing of the received visual information to obtain numerical, objective data; placing the information in the context of biological processes in order to build effective control systems; verification based on different biotechnological situations; automation to create a product. The fluorescence methods used are based on a combination of analyzes with CTC (5-Cyano-2,3-ditolyl tetrazolium chloride), DAPI (4',6-Diamidino-2-Phenylindole) and fluorescence in-situ hybridization. Image segmentation and analysis software products (author's software and DAIME) were used for digital analysis. In this way a product (control system) for evaluation of methanogenesis in anaerobic digesters was developed. It was based on study of the complex biogas-producing communities including both taxonomic data, spatial structure and autofluorescence. Another method, based on the quantification of metabolic activity and detection of morphological changes by CTC/DAPI analysis, was also developed to determine toxicity. It is also applicable in studies on the antibacterial effect of various substances and on the biodegradation activity of pollutants. The method has been verified in various matrices - activated sludge, pure cultures (*Escherichia coli*, *Pseudomonas* sp., *Brevibacillus laterosporus*), river sediments, biogas-producing communities, soil, microbiological contamination of water bodies. Through the innovative approach, various processes were characterized - biodegradation of xenobiotics; intoxication in environmental biotechnologies (leachate from solid waste landfills, PFAS, phenol); antibacterial action of peptides and proteins from molluscs; activity of the biodegradation of pollutants in treatment plants for household wastewater (WWTP of Sofia city) and industrial wastewater (business enterprises); biogas production activity in full-scale anaerobic digesters; activity of soil communities during toxic load and after

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application of bioaugmentation; abiotic detoxification based on plasma technologies; control of industrial purification processes in critical conditions (treatment plants to waste landfills and wood processing plant). A large part of the described results have also been verified in a real business environment, which is a prerequisite for bringing them out of the laboratories at the service of the practitioners in the field of environmental biotechnology.

Keywords: fluorescence, control of biological processes, toxicity, microbial communities in environmental biotechnology

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APPLICATIONS OF RAMAN SPECTROSCOPY FOR SOLVING ENVIRONMENTAL PROBLEMS

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Raman spectroscopy was used to analyze different type of carbon materials, microorganisms in water samples and biological objects. All the analyses were performed on Bruker Senttera II Raman microscope, equipped with laser with 532 nm wavelength (green) and uCuv-(N) cuvette holder for liquid sample measurement Adaptor for microscope (M25-0.75 threaded). Studied carbon materials are: activated carbon (AC), carbonized and green carbon foam (CF), and carbon adsorbents, produced from refuse derived organic fuel (RDF). Raman spectroscopy was able to detect structural changes during the activation of the carbon materials.

This allows to be studied the changes in the structure and the formation of the porous texture as a result of the physical activation of the carbon materials which significantly determine their applicability in the purification industry. Activated carbon materials are effective in removal of organic and inorganic pollutants from water, due to their high surface area and micro-meso porosity, as well as simple design and easy operation of activated carbon adsorption process.

Applying Gram staining technique we can observe Gram positive and Gram negative bacteria on the Raman Microscope. The technique allows seeing how contaminated with microorganism a given sample is, such as mineral water sample.

The uCuv-(N) cuvette holder for liquid sample measurement which is adaptor for the Raman microscope was usedto analyze different liquid samples of mineral water of Velingrad, Bulgaria. Raman spectroscopy was used to study carbon materials, which have different industrial applications.

Keywords: Raman spectroscopy, carbon materials, activated carbons, ecology, bacteria, proteins

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CORE-SHEATH AND CORE/DOUBLE-SHEATH COMPOSITE FIBERS PREPARED BY SINGLE-SPINNERET ELECTROSPINNING

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Core-sheath composite fibers prepared by electrospinning have gained increasing interest due to the possibility of combining the properties of two polymers or a polymer and a low-molecular-weight substance. In addition, this type of architecture is attractive from a biomedical viewpoint due to the ability for targeted loading of bioactive agents in the fiber core or sheath. The conventional approach for preparation of core-sheath fibers is coaxial electrospinning; however, the use of auxiliary coaxial device is required. Making use of the self-organization occurring during the flight of the spinning jet we have prepared core-sheath composite fibers by single-spinneret electrospinning of homogeneous blend solutions of poly(ethylene oxide) (PEO) and beeswax (BW) [1].

The core-sheath structure of the fibers was evidences by transmission electron microscopy (TEM), water contact angle study, X-ray photoelectron spectroscopy and selective extraction of PEO or BW. Furthermore, applying the same approach we have prepared core-double sheath composite fibers by single-spinneret electrospinning of homogeneous blend solutions of PEO, poly(L-lactide) (PLA) and BW [2].

The core-double sheath architecture was evidenced by TEM and selective extraction of PLA and BW. In order to assess the possibility, the prepared fibers to serve as drug carriers 8-hydroxyquinoline derivatives (8QD) were used as model bioactive substances [1,2]. 8QD-loaded core-sheath and core-double sheath fibers demonstrated antibacterial and antifungal activities against human pathogenic microorganisms [1,2] as well as against phytopathogenic microorganisms [3]. Therefore, the new materials are promising for wound healing application as well as for plant protection.

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Keywords: core-sheath fibers, core/double-sheath fibers, electrospinning, poly(ethylene oxide), beeswax, poly(L-lactide), 8-hydroxyquinoline derivatives, antibacterial and antifungal activities

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SESSION 4. PLENARY LECTURES "HEALTH"

NATURAL PRODUCTS FOR HEALTH: PREVENTION AND TREATMENT OF EXPERIMENTAL PARKINSON'S DISEASE WITH STANDARDIZED *HELIX ASPERSA* EXTRACT

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Good health is the first step to overall wellness. Especially with age it is necessary to pay more attention on it. Neurodegenerative disorders such as Alzheimer's, Parkinson's and dementia continue to be a clinical concern in most older people. Unfortunately, there are no strategies that can stop the brain cell injury in Parkinson's disease (PD).

Since snail extract (SE) possess antioxidant, anti-inflammatory and antiapoptotic properties, we studied its effects in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice model of PD. The mucus was collected from *Helix aspersa* snails, grown in Bulgarian eco-farms using patented technology without the suffering of snails. Male mice (C57BL/6, male, 8 weeks old) were used for mouse Parkinson's disease model via MPTP treatment. MPTP+SE (0.1 ml/10 g b.w) group received fresh snail extract through a special food tube for 12 consecutive days. All animals were submitted to motor coordination and memory tests on the 16th and 17th day. At the end of experiment two brain structures related to memory (prefrontal cortex and hippocampus) were separated for the biochemical and histological analyses. After 12 days with SE treatment (7 days before and 5 days simultaneously with MPTP) an improvement in motor and memory performance in Parkinsonian animals was observed. SE protected dopaminergic neurons as proved via histological studies as well as with biochemical evaluation. Twelve days after first MPTP treatment the reduction in brain DA content was by 73 % as compared to control.

Multiple SE administration increased DA brain level (by 176 %) and decreased those of NA (by 42 %,) as compared to Parkinsonian group. We also observed significant anti-inflammatory effect of SE. In conclusion, our results provide insight into the protective effects of *Helix aspersa* extract in an experimental model of Parkinson's disease *in vivo*.

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NEUROPROTECTIVE MECHANISMS IN THE ACTION OF STANDARDIZED EXTRACT OF *HELIX ASPERSA* IN EXPERIMENTAL MODEL OF ALZHEIMER'S TYPE DEMENTIA IN VIVO

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Complex neurodegenerative mechanisms of Alzheimer's disease require new multi-target strategies of prevention and treatment. They are based on the concept of using natural products with different complementary mechanisms, attacking several targets at the same time. On the other hand, mucus from snail *Helix aspersa* is a mixture of bioactive molecules with antimicrobial, anti-inflammatory, antioxidant and anti-apoptotic effects. Hence, we investigated mechanisms of action underlying beneficial effects of snail extract (SE) from *Helix aspersa* on learning and memory in Alzheimer's type dementia (ATD) induced by scopolamine (Sco) in male Wistar rats. SE (0.5 mL/100 g) was applied orally through a food tube for 16 consecutive days: 5 days before and 11 days simultaneously with Sco (2 mg/kg, i.p). At the end of Sco treatment, using behavioural methods, we evaluated memory performance. Additionally, in cortex and hippocampus the acetylcholinesterase (AChE) activity, acetylcholine and monoamines (dopamine, noradrenaline and serotonin) content, levels of main oxidative stress markers, expression of brain-derived neurotrophic factor (BDNF), and cAMP response element-binding protein (CREB) were determined. SE significantly improved the cognitive deficits induced by Sco. Furthermore, SE possessed AChE inhibitory activity, moderate antioxidant properties and the ability to modulate monoamines content in brain structures related to memory. Moreover, multiple SE applications not only restored the depressed by Sco expression of CREB and BDNF, but significantly upregulated it. Summarizing results we conclude that complex mechanisms underlie beneficial effect of SE on impaired memory in ATD.

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PROTEOMIC ANALYSIS OF RAT CORTEX AND HIPPOCAMPUS IN SCOPOLAMINE-INDUCED DEMENTIA OF THE ALZHEIMER TYPE USING SNAIL EXTRACT AS A NEUROPROTECTIVE AGENT

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Alzheimer's disease is the most common cause of dementia - a gradual deterioration of memory, thinking, behavior and social skills that gradually progresses over time and ends in death. Scopolamine is a commonly used agent for the induction of dementia of the Alzheimer type in experimental animals. A scopolamine model was applied to evaluate the potential neuroprotective effect of *Helix aspersa* garden snail extract on neurodegenerative processes *in vivo*, in male sexually mature experimental Wistar rats. Three groups were studied: (a) control group of healthy rats, (b) scopolamine group (treated with scopolamine) and (c) experimental group treated simultaneously with scopolamine and snail extract. Changes in the expression of proteins in brain homogenates from the two structures related to learning and memory - cortex and hippocampus in rats in the scopolamine group and in the experimental group treated simultaneously with scopolamine and an extract from the mucus of snails *H. aspersa* were investigated by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) and mass spectrometry (MALDI-TOF-MS and MALDI-TOF-MS/MS analyses). Proteins with altered expression from the cerebral cortex and hippocampus were identified by MASCOT Peptide Mass Fingerprint® and NCBI BLAST online databases, IQTL® software and Melanie™ Coverage 9. We found that *H. aspersa* snail mucus extract has a protective role on the cortex and hippocampus of rats in an experimental model of scopolamine-induced dementia of the Alzheimer type. The mechanism of this protective role remains to be elucidated.

Keywords: Alzheimer type dementia, cerebral cortex, hippocampus, scopolamine, snail extract, proteins

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ANTIVIRAL ACTIVITY OF EXTRACT FROM *ASTRAGALUS GLYCYPHYLLOS* L. AGAINST REPLICATION OF HUMAN CORONAVIRUS 229E AND SIMPLEXVIRUS HUMANALPHA TYPE 1 AND 2 APPLIED ALONE AND IN COMBINATION WITH ACYCLOVIR

Stoyan Shishkov[1], Ilina Krasteva[2] Anton Hinkov[1], Aleksandar Shkondrov[2], Kalina Shishkova[1], Venelin Cvetkov[1], Daniel Todorov[1], Krasimira Yocheva[2]

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More than 200 types of viruses can infect humans, and the number of emerging viruses, albeit slowly, is increasing every year. Mortality and morbidity caused by viral infectious diseases have a negative impact on every aspect of life. Drugs used in the therapy of Simplexvirus humanalpha (SVHA) (eg, nucleoside analogues such as acyclovir (ACV) and its derivatives) manage the symptoms of primary and recurrent infections, but their use often leads to the development of resistant viral mutants. The pandemic caused by SARS‑CoV‑2 has been a challenge for scientists around the world in the search for effective preparations to combat the etiological agent that caused the pandemic. Despite the availability of effective vaccines, there are few known drugs that inhibit SARS-CoV-2 to some extent. In this regard, plants are of increasing interest as a source of substances with biological activity and an alternative to overcome the often developing resistance to antiviral drugs used in medical practice.

The lack of reports on the antiviral effects of Astragalus glycyphyllos L., its traditional use in folk medicine and the existing knowledge of its phytochemical constituents prompted us to investigate the effect of defatted aerial part extract (DEAG) in vitro against SVHA type 1 replication. (strain F) and type 2 (strain DD) and human coronavirus 229E (HCoV-229E). HCoV-229E is part of the coronavirus family and this makes it a suitable laboratory model for testing the effect of biologically active substances on its replication in vitro. The most active of them could be used in the fight against SARS-CoV-2. The experiments were carried out at different times and with two batches of the extract prepared from plant material collected in different years. Against replication of SVHA type 1 and 2 first batch of DEAG was used. Maximum non-toxic concentration (MNC) (0.6 mg/ml) and cytotoxic concentration (CC_{50}) (2.5225 mg/ml) were determined by colorimetric method (MTT assay) at 72 hours. The MNC of DEAG resulted in 65.065% and 61.48% cell protection (effective concentration 50 (EC_{50}) was 0.363 mg/mL and 0.385 mg/mL) when added simultaneously with the virus suspension and 74.49% and 68.86% cell protection (EC_{50} was 0.4 mg/ml and 0.268 mg/mL) when added 1 h after virus adsorption against strain F and strain DD respectively.

To determine the combined antiviral effect of DEAG and ACV on SVHA type 1 replication, we used the three-dimensional model of Prichard and Shipman. The effect of simultaneous application is antagonistic. DEAG is not suitable to be used as a partner in combination with ACV.

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Against replication of HCoV-229E second batch of DEAG was used. We also tested saponine mixture (SM) and three isolated saponins (S1, S3 and S9). In all experiments MDBK (Madine and Darby bovine kidney) cells were used. MNC (1 mg/ml, 0.025mg/ml, 0.00625mg/ml, 0.025 mg/ml and 0.00625mg/ml respectively for DEAG, SM, S1, S3 and S9) and CC_{50} (1.27 mg/ml, 0.075 mg/ml, 0.117 mg/ml, 0.163 mg/ml and 0.104 mg/ml respectively for DEAG, SM, S1, S3 and S9) were determined by colorimetric method (MTT assay) at 72 hours. By MTT-based colorimetric assay for detection of HCoV-229E replication inhibition, DEAG and the SM administered at the maximal non-toxic concentration showed 100% of protection of cells inoculated with the virus and inhibitory concentration 50 equal to 0.091 mg/ml (when DEAG was applied simultaneously with inoculation) and 0.114 mg/ml (when DEAG was applied 1.5 hour after inoculation) and 0.0045 mg/ml (when SM was applied simultaneously with inoculation) and 0.0048 mg/ml (when SM was applied 1.5 hour after inoculation). The three isolated saponins did not show any antiviral activity. Selectivity index for DEAG and SM was ≥ 10 making them suitable for *in vivo* tests. The antiviral activity for SM and the absence of such activity for the three isolated saponins shows that the effect is due to the cumulative action of all saponins included in the SM.

Keywords: SVHA, SARS-CoV-2, *Astragalus glycyphyllos* L., DEAG, combined antiviral effect

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NATURAL AND SURGICAL APPROACH IN THE TREATMENT OF DIFFICULT TO HEAL AND CHRONIC WOUNDS

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Some types of wounds arise that defy the standard teaching of tissue healing and present a challenge. They can be considered hard-to-heal and chronic wounds. Many chronic systemic diseases lead to the formation of such wounds, and physicians and scientists must consider all factors related to the wound. In addition to the surgical treatment of the wound as the "Gold Standard", appropriate and modern measures should be taken to correct the underlying causes of the difficult healing of the wound. In view of the new results and scientific findings, a serious revision in the methods of treatment of such patients is necessary. Knowledge of the therapeutic effect of snail slime and various types of herbs is known from written history. Pliny the Elder (AD 23) wrote that snails were "a sovereign remedy for pain connected with burns, abscesses, and other wounds". Patients with difficult-to-heal and chronic wounds of different ages and genesis (diabetic gangrene, chronic venous insufficiency, chronic arterial insufficiency, operative wounds and decubitus) as well as with various concomitant diseases of different gender and age were prospectively selected.
A prospective new protocol, a new approach to wound antiseptics, a new rate and type of dressings, a treatment plan, education of the patient and relatives on self-help and dressings were applied. During the treatment, the results of a number of parameters were reported: local wound status, pH-metry, local inflammatory process, bacterial flora, pain level, healing speed and comorbidity analysis.
Intermediate results: Presentation of intermediate results of the treatment of difficult-to-heal and chronic wounds of different genesis. A new approach used in the treatment of hard-to-heal wounds with snail mucus from Helix Aspersa and herbal essential oils shows promising results. A significant reduction in wound healing time, rapid and effective reduction of the local inflammatory process and chronic pain is observed. Improving the general and psycho-emotional condition of the patient, significantly improve the quality of life. The results obtained suggest that the healing of such wounds is promoted not only by limiting bacterial infection, but also by stimulating tissue growth and providing them with an adequate local alkaline-acid balance, humidity and nutrient environment. Early and regular application of our proposed new treatment approach will lead to the prevention of leg amputation and serve as the main therapy for the treatment of chronic wounds.

Keywords: Difficult Wounds, Chronic Wounds, Treatment, Snail - Helix Aspersa, Essential Oils.

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ANALYSIS OF BIOLOGICALLY ACTIVE SUBSTANCES FROM NATURAL ORIGIN USED IN OPHTHALMOLOGY

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This presentation offers a retrospective analysis of the biologically active substances from natural origin used in ophthalmology. Nature has always provided an unlimited source of biologically-active compounds. Since the beginning of mankind, humans have used resources from the fauna and flora to treat eye diseases. The use of herbal extracts to treat ophthalmic conditions dates back to ancient times. The topical use of the macerated fruit of *Atropa belladonna* by the Egyptians is the first known use of a nature-derived agent to treat an ophthalmic disease.

Two major challenges faced by ocular pharmacology are the following: developing drugs that are able to reduce blindness due to glaucoma; and controlling the pain associated with eye surgery. The search for a drug that effectively lowers intraocular pressure and controls the progression of glaucoma has led to the development of various ocular hypotensive agents, such as physostigmine from the Physostigma venenosum plant.

The anesthetic properties of cocaine, extracted from *Erythroxylon coca*, finally enabled surgical procedures in the eye. Due to the side effects of these agents in the eye they have been replaced by safer, more effective drugs with fewer side effects like the Pilocarpine, isolated from the leaves of *Pilocarpus jaborandi*. Several new natural compounds have been investigated in an attempt to identify substances with the potential to provide additional benefits to eye tissue and vision. The pharmaceutical industry still seeks new active compounds from natural sources as well as from revisiting already-established naturally derived compounds. This presentation focuses on the bioactive substances from natural origin with scientifically-proven benefits for the ocular tissues

Keywords: ophthalmology, eye diseases, plants, pharmacology.

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SESSION 5. PLENARY LECTURES "HEALTH"

ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF EXTRACTS FROM *ACONITUM TOXICUM* RCHB

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The wealth of primary and secondary metabolites from plants and their potential applications have always attracted considerable interest. Most of active substances are carbohydrates, pectin, mucilage, fatty oils, volatile oils, organic acids, glycosides, saponins, vitamins, alkaloids, plant dyes, antibiotics, phytoncides. The genus *Aconitum* has almost 400 species, ornamental and medicinal plants, widely spread in Eurasia and North America, some of them used in various therapeutic purposes because of their content in diterpene alkaloids (for instance aconitine, an alkaloid toxine). A lot of diterpene alkaloids express pharmacological properties like antimicrobial, anticancer, anti-inflammatory, antiarrhythmic, hypotensive, local anesthetic and analgesic, neurotropic and muscle relaxer. The aim of this paper was to determine the bioactivity of some extracts of *Aconitum toxicum* Rchb. The plants were processed to obtain hydroalcoholic extracts by modern methods: microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE). The plant extracts were supplemented with silver nanoparticles (AgNPs). Sixteen experimental variants were established, according to type of extraction method, type of solvent, extract concentration, presence or absence of AgNPs. The antimicrobial activity was performed by disk diffusion method using four reference bacterial strains: *Staphylococcus aureus ATCC 25923, Bacillus subtilis ISM 68/53, Klebsiella pneumoniae ATCC 10031* and *Escherichia coli ATCC 25922* cultivated on Mueller-Hinton agar and 10µL per disk of each extract. The positive control was Gentamycine, the negative controls were ethanol and methanol. The cytotoxic activities were performed by *Allium* test. In this test, the negative control was distilled water and the positive control was methyl-methanesulfonate. The extracts obtained by microwave assisted extraction with AgNPs, showed antimicrobial effect against tested bacteria, especially Gram+ (*S. aureus* and *B. subtilis)*, but also against Gram- *E. coli*. The hydroethanolic samples determined larger growth inhibition zones than hydromethanolic samples in the most cases, but all zones had smaller diameters than those determined by positive control.
The mitotic index showed a different variation depending on the type of extract. The largest number of cells arrested at *prophase, respectively in metaphase* was determined by hydroethanolic extracts obtained by UAE, and respectively by those obtained by MAE. Among chromosomal aberrations and nuclear anomalies, the binuclear cells (more frequent, in case of MAE extracts), C-mitosis, sticky chromosomes, vagrant chromosomes, fragments, bridges were observed.

Keywords: antimicrobial, cytotoxic, plant extracts, nanoparticles

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ANTIMICROBIAL RESISTANCE AS A GLOBAL HEALTH THREAT IN THE NEW CENTURY

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Antimicrobial resistance (AMR) is one of the Global Health challenges of the 21st century. Available knowledge about AMR has mostly come from the high-income countries and is scattered in studies across various fields, focused on infectious diseases, transmission pathways, pathogen reservoirs, the extent of the problem at a population level (public health), etc. That is why there is a huge need to reframe AMR in the current public health and the global health in the context of One Health concept.

The differences and similarities between individual stakeholder goals and the gaps and challenges in combating AMR at different levels require a comprehensive analysis of host and microbial heterogeneities, the role of surrounding ecosystems and the challenges they pose to surveillance, antimicrobial stewardship and infection control which are the traditional cornerstones of controlling AMR in human and animal health.

Rapidly growing worldwide AMR to clinically administered antibiotics and chemotherapeutics necessitates the search for alternative sources of antimicrobial agents and approaches for the prevention and treatment of infectious diseases in human and veterinary medicine, for the preservation of food products in the food industry, disinfection of surfaces, etc. In this aspect, intensive research is conducted in several directions, namely: search for new substances of natural origin with low-toxic antimicrobial action, testing of new drug delivery systems, incl. nanoparticles etc. complex systems, research of various combinations of biologically active substances and antibiotics in order to increase their activity and reduce their application dose.

These modern approaches are the subject of the studies presented here.

Keywords: antimicrobial resistance, infectious diseases, antibiotics, One Health concept, new antimicrobial agents.

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ANTIBACTERIAL EFFECTS OF COMBINATIONS OF SNAIL MUCUS WITH CLINICALLY ADMINISTERED ANTIBIOTICS

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Chronic wounds often occur in patients with diabetes mellitus due to the impairment of wound healing. Considering the growing prevalence of diabetes, this problem will become a significant medical, social, and economic burden in the near future. In addition, the increasing incidence of multi-resistant germs poses difficult challenges to therapy and turns antimicrobial resistance (AMR) into one of the most serious global public health threats in this century. Natural products have long played a major role in medicine and science. The garden snail *Cornu aspersum* can be regarded as a rich source of biologically active compounds with antimicrobial activity and such accelerating wound epithelialization.

Aim of our study was to isolate pathogenic bacterial strains from diabetic wounds and to investigate the combination effects of snail mucus (fraction >30 kDa) and the fluoroquinolone ciprofloxacin or the aminoglycoside gentamicin. Four isolates belonging to three bacterial species (*Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*) were isolated from the diabetic wounds. The biochemical identification was conducted using the BD Phoenix device. The isolates were tested for antimicrobial resistance, as well. The Gram-positive isolates were sensitive to fluoroquinolones and had intermediate susceptibility to the aminoglycoside gentamicin. Both *P. aeruginosa* isolates showed resistance to the fluoroquinolone norfloxacin and intermediate sensitivity to gentamicin. One *P. aeruginosa* isolate has intermediate susceptibility to ciprofloxacin. After combining different concentrations of ciprofloxacin or gentamicin (in accordance with the clinical breakpoints of EUCAST) with snail mucus (4 mg/mL) we observed additive to synergistic effect in all four bacterial isolates, wherein the effective concentrations of the antibiotic drugs were diminished two-fold to four-fold.

In conclusion, the snail mucus is an active natural product with potential to enhance the antibacterial activity of clinically used antibiotics and chemotherapeutics in the treatment of diabetic wounds which might play an important role in combating antimicrobial resistance.

Keywords: snail mucus, antimicrobial resistance, diabetic wounds, fluoroquinolones, aminoglycosides

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GREEN SYNTHESIS AND CHARACTERIZATION OF Cu AND Zn NANOPARTICLES WITH ANTIMICROBIAL PROPERTIES

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In recent decades, nanoparticles have gained the interest of biologists because of their widespread therapeutic and theranostic applications. Among metal nanoparticles, zinc oxide (ZnO-NPs) and copper oxide (Cu_2O-NPs) nanoparticles have emerged as semiconductor nanomaterials with widespread applications in electronic and optoelectronic devices, photocatalysis, cosmetic products, and most importantly, in biomedicine due to their biocompatible nature.

Recently, a promising "Green" method has appeared using various plant extracts for producing such nanoparticles. In this regard, the main objective of the present study was to develop an environmentally friendly process for obtaining organometallic zinc and copper nanoparticles. For this purpose, various biologically active compounds were used, such as ascorbic acid, whey, and extracts from *Cotinus coggygria* and *Arnica montana*. NPs were characterised by their absorption spectrum, and their synthesis was optimised with respect to the incubation time, pH and T^0C. The resulting preparations showed strong antimicrobial activity against Gram (+) and Gram (–) bacteria, completely inhibiting their growth within 3 to 12 hours.

Fungicidal effects for some of the obtained nanopreparations were also found. It was shown that the antimicrobial activity of the obtained NPs is due to the disruption of redox homeostasis in microorganisms, with a 3- to 8-fold increase in the levels of ROS and oxidised lipids and a 40-60% higher concentration of carbonylated proteins.

In view of the obtained results, it can be assumed that the preparation of Zn-NPs and Cu-NPs through "Green" synthesis may prove to be an efficient strategy for the treatment of various microbial infections.

Keywords: Zn nanoparticles (NPs), Cu nanoparticles (NPs), "Green" synthesis, antimicrobial properties

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MICROPLASTICS: REVIEW OF CURRENT KNOWLEDGE OF SOURCES, FATES AND EFFECTS ON ECOSYSTEM AND HUMAN HEALTH

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Plastic particles, less than 5 mm in size, are known as microplastics (MPs) and are found all over the world, even in the most remote areas as Mount Everest and Antarctica. They can penetrate into organisms from the environment and accumulate in their tissues and organs. MPs may have direct toxic effects or indirect impacts, due to their high absorption potential, which can transfer various chemical pollutants and pathological microorganisms.

More and more evidence shows the negative effects of MPs on growth, development and reproduction of living organisms, especially of those inhabiting aquatic ecosystems. Moreover, the negative changes in individual organisms can also further affect higher hierarchical levels.

Our research group carried out for the first time a screening of MPs in key bivalve and fish species from the Bulgarian Black Sea coast and established a high degree of their bioaccumulation. Through food consumption, drinking water and air inhalation MPs can also reach humans and affect human health. Laboratory tests have demonstrated that MPs cause damage to human cells. However, until now, there are no epidemiological studies clearly documenting the link between MPs exposure and human health.

The present work reviews known sources of MPs, their categorization according to different characteristics, bioaccumulation and translocation pathways, biomagnification ability and health effects. In conclusion, considering the widespread distribution, diversity and persistence of MPs, it is obvious that more research is urgently needed to elucidate the cellular and molecular mechanisms of their toxicity and associated pathology, as well as potential means to mitigate these effects.

Keywords: Bulgarian Black Sea, ecosystem health, human health, microplastics

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SESSION 6. PLENARY LECTURES "HEALTH"

ASSESSMENT OF THE IMMUNOMODULATING AND ANTICANCER ACTIVITY OF HEMOCYANINS ISOLATED FROM GARDEN AND MARINE SNAILS IN A MYELOID TUMOR MODEL

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Hemocyanins are oxygen-transporting glycoproteins in the hemolymph of some invertebrate species that attracted the scientific interest as potential anticancer agents. The present study aims to assess the immunomodulating and anticancer activity of hemocyanins isolated from the garden snails *Helix aspersa*, *Helix lucorum* and the marine snail *Rapana venosa* in Graffi myeloid tumor model. Nine samples, including native hemocyanins, their subunits and *H. Aspersa* mucus, were isolated and purified by dialysis, ultrafiltration, ultracentrifugation, gel filtration and anion exchange chromatography. The molecular masses and purity of the hemocyanins and their subunits were confirmed by a native PAGE. The cytotoxicity of the tested hemocyanins was evaluated on a cell line derived from the Graffi tumor and a non-tumorigenic fibroblast cell line BALB/3T3. The *in vitro* antitumor activity of the tested hemocyanin samples was determined by a standard colorimetric cell viability assay and cytomorphological analysis of fluorescent and transmission electron microscopic images of hemocyanin-treated cancer cells. The immunomodulating and antineoplastic effects of the hemocyanins were examined in experimental hamsters transplanted with Graffi cancer cells after immunization with hemocyanin-containing vaccinal preparations. The serum titres of antibodies against the tested hemocyanins and against the tumor antigen were determined by ELISA. Histopathological analysis of the tumor tissues and visceral organ samples from the treated experimental animals was performed to assess the morphological features related to the antitumor effect, immune system response and toxicity. The results indicated that the tested hemocyanins suppress the proliferation of the tumor cells and induce apoptotic cell death. The applied experimental immunotherapy resulted in significantly increased titres of antibodies cross-reacting with the Graffi tumor antigen, reduced transplantability, suppression of tumor growth and metastasis and prolonged survival time without any signs of toxic side effects. The present study indicated that the antitumor activity of the studied hemocyanins is due to both immune stimulation and direct effects on the cancer cells and shows their potential as therapeutic agents against hematological malignancies.

Keywords: hemocyanins, myeloid tumor, immunomodulating activity, antitumor activity

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SAFETY TEST BALB 3T3 NEUTRAL RED UPTAKE ASSAY FOR RESEARCH OF NATURAL SUBSTANCES

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Phototoxicity is a common phenomenon caused by certain substances of synthetic or natural origin that absorb sunlight. The phototoxic effect is due to benzene nuclei and heterocyclic rings in the chemical structure of the substance. For this reason, it is important that substances used in cosmetics and pharmaceuticals are tested for photosafety. T

he safety test *in vitro BALB/c 3T3 Neutral Red Uptake assay* is a basic biological test for the determination of cytotoxicity and phototoxicity of new natural products and substances. Through this test, the average values of CC_{50}, PC_{50} and Photo-Iritation Factor can be determined in a short time.

The high sensitivity of the mouse embryonic fibroblast cell line used to toxic effects allows reproducible results to be obtained. The safety test is mandatory for substances that will be used in the pharmaceutical, cosmetic, food industry, etc.

The aim of this research is to perform an initial biological screening to determine the cytotoxicity and possible phototoxicity of new natural products and substances isolated from different snail species. In this study, nine hemocyanins and their derivatives isolated from the hemolymph of *Helix lucorum*, *Helix aspersa* and *Rapana venosa* were investigated. The results showed that the investigated hemocyanins are use in the pharmaceutical and cosmetic industries.

Keywords: Safety test, Phototoxicity, Hemocyanin

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BIOACTIVE COMPOUNDS WITH ANTITUMOR ACTIVITY FROM MARINE AND GARDEN SNAILS AGAINST BLADDER CARCINOMA AND MECHANISM OF ACTION

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Cancer is a complex multifactorial disease that results from alterations in many physiological and biochemical functions. Over the last few decades, it has become clear that cancer cells can acquire multidrug resistance to conventional anticancer drugs, resulting in tumor relapse.Therefore, the search for new, more effective and selective anticancer drugs is urgent. Hemocyanins are extracellular copper-containing respiratory proteins, dissolved in the hemolymph of many arthropods and molluscs as a key componet comprising about 90–95% of the total protein. Some molluscan hemocyanins (Hcs) show significant immunological and antitumor potential, which allows their application in oncology.The antitumor activity of Hcs from marine snails *Rapana venosa* (RvH), giant snail *Megathura crenulata* (KLH) and garden snail *Helix lucorum* (HlH) as well as their various derivatives were investigated in vitro on a permanent bladder cancer cell line T24 and normal urothelial cell line HL 10/29 compared to doxorubicin.

The antiproliferative activity of the tested Hcs was determined by WST-1 and BrdU ELISA assays. Morphological changes in both urothelial cell lines were confirmed by fluorescence microscopy. Proteomic analysis of bladder cancer cell line T24 before and after treatment with functional unit (FU) βc-HlH-h using two-dimensional gel electrophoresis (2D-PAGE) and mass spectrometry revealed differences in the expression of some proteins.

Selective inhibitions of T24 cell growth was observed after incubation with structural subunits βc-HlH, RvHI and RvHII and FUs (βc-HlH-h and RvHII-e). The functional unit βc-HlH-h demonstrated the highest antiproliferative effect (doxorubicin-like), in which mainly apoptotic and less late apoptotic or necrotic changes in tumor cells were observed by fluorescence electron microscopy. A significant change in protein expression of T24 tumor cell line under the action of βc-HlH-h was shown for the first time, and a number of proteins, associated with the apoptosis pathway have been identified by proteomic analysis.

Keywords: hemocyanis *Rapana venosa* (RvH), *Megathura crenulata* (KLH) and *Helix lucorum* (HlH), isoforms, functional unit βc-HlH-h, anti-tumour effect, proteomic analysis, bladder carcinoma T24.

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INNOVATIVE APPROACHES IN COMBATING DRUG RESISTANCE IN CANCER TREATMENT: FROM FERROCENE DERIVATIVE OF CAMPHOR TO MICELLAR DELIVERY SYSTEMS

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Discovering new anticancer drugs with enhanced specificity and reduced side effects compared to conventional chemotherapeutic agents presents a significant challenge in current medical research and development.

Our recent study demonstrated the promising antiproliferative activity of a newly synthesized organometallic compound, specifically a ferrocene-containing camphor sulfonamide (DK164), against breast and lung cancer cells. However, one limitation faced by DK164 is its poor solubility in biological fluids. To address this issue, we incorporated DK164 into biodegradable micelles based on a triblock copolymer (PEO113-b-P(CyCL3 -co-CL46)-b-PEO113) of poly(ethylene oxide) and poly(α-cinnamyl-ε-caprolactone-co-ε-caprolactone). We comprehensively studied the physicochemical properties (size, size distribution, zeta potential, encapsulation efficiency) and biological activity of the resulting micellar system. Cytotoxicity assays and flow cytometry were employed to determine the type of cell death induced. At the same time, immunocytochemistry allowed us to evaluate the impact of the encapsulated drug on crucial cellular proteins (p53 and NFkB) and the process of autophagy.

Our results indicate that the micellar formulation of the organometallic ferrocene derivative (DK164-NP) offered several advantages compared to the free compound. These advantages include improved metabolic stability, enhanced cellular uptake, increased bioavailability, and prolonged activity while maintaining the drug's potent anticancer properties.

Keywords: cancer, ferocen, camphor, chemotherapeutic, triblock copolymer

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SOIL MICROBIOME - A RESERVOIR OF SOIL HEALTH MICROORGANISMS

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Soil microbiomes drive key functions in agroecosystems, determine soil health, crop productivity and stress tolerance. The microbiome is intricately linked with soil structure and its physicochemical properties.

To assess changes in microbial community structure due to long-term heavy metal contamination, 16S rRNA gene retrieval, 16S rRNA gene target sequencing and qPCR calculations of microbial abundance were applied in two regions in Bulgaria: Srednogorie area and non-ferrous metal plant KCM -2000, near Plovdiv town. Ecological insight into soil microbiome structure and function will be discussed in relation to main abiotic factors.

The potential of the identified native bacterial species will be presented in the context of their use in microbial remediation.

Keywords: microbial communities, soil, heavy metals

SESSION 7. PLENARY LECTURES "HEALTH"

ISOLATION, SEPARATION AND PURIFICATION OF PHYTOCHEMICALS FROM *SAMBUCUS NIGRA* BERRIES FOR BIOLOGICAL ACTIVITY ANALYSIS

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Reactive oxygen species generated through various extracellular and intracellular processes have attracted the attention of scientists as novel signaling molecules involved in biological processes such as growth differentiation and cell death. ROS arise both as a result of natural metabolic processes at the cellular level and in conditions of cellular stress induced by a variety of exogenous factors. Excessive formation of ROS leads to a condition known as oxidative stress. Antioxidants are compounds that regulate ROS levels in cells and prevent a part of their chemical interactions. Many antioxidants are exogenous to human cells and are present in various medicinal plants. Such plant is the black elder – *S. nigra*. Elderberry is rich in nutrients, such as carbohydrates, proteins, fats, fatty acids, organic acids, minerals, vitamins and essential oils. Elderberry also contains polyphenols and flavonoids, known for antioxidant activity [1].

The aim of the present study is to evaluate the bioactivity of *S. nigra* ethanolic extracts and to understand if this activity is related to a specific phytochemical profile of the extracts. For this purpose, we prepared tinctures from elderberries with different concentrations of ethanol. For the phytochemical screening of the extracts we performed a qualitative colorimetric analysis and UHPLC/DAD analysis [2]. Our results show presence of polyphenols from the flavonoid subclass of compounds. To test the bioactivity of the extracts we performed a MTT test for cytotoxicity and DCF-DA assay for in vitro antioxidant activity. Elderberry extracts are not cytotoxic and demonstrate good antioxidant capacity in living cells.

Referencies: [1]Młynarczyk, K., Walkowiak-Tomczak, D., & Łysiak, G. P. (2018). *Journal of functional foods*, 40, 377–390. [2]Egbuna, Chukwuebuka & Ifemeje, et. al, 2019. Phytochemistry, Volume 1; ISBN: 978-1-77188-759-5.

Keywords: *S. nigra*; phytochemicals; HPLC/DAD

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BIOACTIVE COMPOUNDS FROM THE MUCUS OF THE GARDEN SNAIL *HELIX ASPERSA* WITH ANTIMICROBIAL ACTIVITY

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In recent years, the growth of microbial resistance is among the main threats to public health and poses new challenges for scientists, related to the discovery of new more effective antimicrobial agents with different mechanisms of action and low levels of resistance. The slime of the garden snails is a complex multicomponent mixture, including substances with various bioactive properties, such as antimicrobial, antioxidant and regenerative.

Different fractions, isolated from the mucus of garden snail *Helix aspersa* obtained by ultrafiltration were in vitro tested against a number of $Gram^+$ and $Gram^-$ bacteria. The results show that the peptide fraction below 10 kDa exhibits a broad spectrum of antimicrobial activity. Other fractions with antimicrobial activity are the fractions with Mw<20 kDa, 10–30 kDa, as well as the protein fraction with Mw>20 kDa. By combining chromatographic and electrophoretic analyzes with mass spectrometry and bioinformatics we determined the compounds in the composition of the active fractions. It has been established that the fraction below 10 kDa includes low molecular weight metabolites, peptides and some glycopeptides. Using tandem mass spectrometry (MALDI-TOF-MS/MS and Q-Trap LC/MS/MS), we identified the amino acid sequences of more than 50 new peptides and some glycopeptides, and we determined their physicochemical parameters. Most of them belong to a new class of Gly/Leu-rich antimicrobial peptides. Analysis of proteins in the fraction above 20 kDa revealed high homology to antimicron proteins with molecular weights between 34-90 kDa found in other land snails.

To elucidate the mechanism of antibacterial action, we performed proteomic analysis by 2D-gel electrophoresis, before and after treatment of *Escherichia coli* with an active peptide fraction <10 kDa. The results showed changes in the expression of key proteins related to the replication and transcription of DNA and RNA, carbohydrate metabolism, as well as the passive diffusion of nutrients, toxins and drug molecules. The observed antibacterial effect is due to a disturbance in the vital functions and cellular metabolism of *E. coli* caused by the synergistic action of low molecular weight metabolites, peptides and glycopeptides in the active fraction. The results presented are a basis for further research on antimicrobial peptides from natural sources and their potential biomedical application.

Keywords: *Helix aspersa* mucus, antibacterial activity, peptides, proteins, tandem mass spectrometry

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NMR SPECTROSCOPY IN DRUG DISCOVERY AND DEVELOPMENT FROM NATURAL PRODUCTS

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NMR spectroscopy is the most powerful method for structure elucidation of unknown substances in solution. Among the variety of applications of NMR spectroscopy in drug discovery, NMR metabolic profiling of various natural extracts plays a key role.

Metabolic profiling based on ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy was applied with the aim to investigate the functional role of the metabolites in lyophilized mucus from garden snail *Helix aspersa*. Twenty metabolites were unambiguous identified by ^{1}H, 1D TOCSY, 2D J-resolved, 2D COSY and 2D HSQC NMR spectra with water suppression. The developed protocol for the determination and assignment of metabolites in mucus from *H. aspersa* by NMR spectroscopy was adopted to study the low molecular weight fractions of hemolymph from *H. lucorum* (<1kD and <3kD) and fourteen metabolites were unambiguously identified. The same protocal was applied to the low molecular weight fractions of hemolymph from *R. venosa* (<3kD) and eleven metabolites were unambiguously identified.
In the all studies metabolites with known antioxidant, antibacterial and antimicrobial activity have been detected. Some of them were confirmed by mass spectrometric analysis. The primary structure of several peptides was identified in low molecular weight fractions (Mw<1 kDa) by tandem mass spectrometry.

Keywords: NMR, mucus, hemolymph, mass spectrometry, peptides, metabolites, *Helix aspersa, Helix lucorum, Rapana venosa*

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WATER EXTRACT OF CHAMOMILE AS A REDUCING AGENT IN THE GREEN SYNTHESIS OF METAL OXIDE NANOPARTICLES

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Chamomile (*Matricaria chamomilla* L.) is a well-known medicinal plant species from the Asteraceae family often referred to as the "star among medicinal species." Nowadays it is a highly favored and much used medicinal plant in folk and traditional medicine. Its multitherapeutic, cosmetic, and nutritional values have been established through years of traditional and scientific use and research.

HPLC analysis was obtained to determine the extract contains a large group of therapeutically interesting and active compound classes. Sesquiterpenes, flavonoids, coumarins, and polyacetylenes are considered the most important constituents of the chamomile drug.

The aim of this work was to develop a method of green synthesis of CuO, ZnO and Ag nanoparticles using water chamomile extract as a reducing agent. The plant-based metal nanoparticles were obtained by two methods and the parameters of the synthesis were optimized.

The nanoparticles were characterized by UV-Vis spectrometry, XRD, FTIR, SEM and photocatalytic degradation of organic dye.

The antimicrobial properties of the nanoparticles were tested against Gram (+) and Gram (-) strains. The MIC concentration of the nanoparticles was determined.

Keywords: green synthesis, sustainable, nanoparticles, antibacterial

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ABSTRACTS OF POSTERS

POSTER PRESENTATIONS "ECOLOGY"

P1. HYBRID BEAD MATERIALS BASED ON ACRYLIC COPOLYMER

Teodor Sandu[1], Raul Augustin Mitran[2], Anamaria Zaharia[1], Marinela Victoria Dumitru[1], Sorin Viorel Dolana[1], Tanța Verona Iordache[1], Andrei Sârbu[1], Georgi Georgiev[3], Boyko Tsyntsarski[3]

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This work aims to identify Red Mud (RM) recycling methods. Hence, hybrid beads with applications for water treatments were developed using acrylonitrile-acrylic acid copolymers and RM and characterized by FTIR spectra and TGA.

The production of aluminum by the Bayer process generates significant amounts of RM as a by-product. Therefore, using RM for catalysis (based on its high content of iron oxides) has emerged as a recycling option. However, RM cannot be used for this purpose as it is an alkaline powder. Therefore, in this study, RM was first neutralized, and then integrated into acrylic polymer beads (prepared using five acrylonitrile-acrylic acid copolymers). FTIR spectra displayed the characteristic peaks of monomers, but also those of metal oxides from RM. The properties of the materials can be well controlled, the incorporation of RM and the thermal stability depending on the copolymer. As a future work, the materials will be characterized morphologically, but also by water studies.

References: [1] A. H. Bhat, H. P. S. A. Khalil, I. H. Bhat, A. K. Banthia, J. Polym Environ 20, 2012, 395-403; [2] C. Brunori, C. Cremisini, P. Massanisso, V. Pinto, L., J.Haz.Mat.B117, 2005, 55- 63

Keywords: recycling, red mud, polymer composites

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P2. NOVEL POLYURETHANES BASED ON RECYCLED PET AND NATURAL FEEDSTOCK POLYOLS FOR WOODEN ARTIFACTS PROTECTIVE COATING

Sorin-Viorel Dolana[1], Daiana-Georgiana Mitrea[2], Andrei Sarbu[1], Tanta-Verona Iordache[1], Ana-Maria Zaharia[1], Toma Fistos[1], Boyko Tsyntsarski[3], Sandu Teodor[1], Georgi Georgiev[3], Ivanka Stoycheva[3]

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Polyurethane is one of the most used polymers in the world, owing its versatility and it is used in foam production (67% of all production), CASE applications (Coatings, Adhesives, Sealants and Elastomers), athletic footwear, fibers, vibration damping devices etc. [1]. It is ranked as the 6th most produced plastics in the world by mass, with an estimated production volume of 21.5 Mt in 2022 [2]. The most common process for polyurethane manufacturing involves the polyaddition reaction between a monomeric or polymeric isocyanate and a polyol. Commonly, the components are derived from petroleum, but petroleum being a finite and resource will push the polymer industry to find renewable resources as raw materials. As renewable alternatives, polyols for polyurethane production can be readily synthesized from vegetable feedstock such as vegetable oils, starch, lignin, cellulose, proteins etc.[3]. Also, polyol can be made from chemical recycling of some polymers such as polyamides, polyurethanes and polyesters. One of the most used polyesters is poly(ethylene terephthalate) (PET). It is widely used in the production of beverage bottles, food packaging and in fiber production. The more and more stringent environmental policies that are adopted throughout the world will require the use of polymers in a circular economy. One of the strategies involved is the recycling of polymers that are at the end of their life to produce new materials/articles. PET can be recycled mechanically, by cutting in pieces, re-melting and then reforming, or chemically by cleavage of the covalent bonds and subsequently by depolymerization, to produce shorter chain polymers, oligomers or even monomers. PET is suitable to undergo depolymerization in the presence of short chain diols or polyols and metallic catalysts and to produce polyols that can be used in further polymerization reactions (e.g. to produce polyurethanes, polyesters, polyamides). This reaction is known as glycolysis. We used chemical depolymerization of PET in the presence of PEG_{600} and various homogenous metallic-based catalysts to produce polyols further used in the production of polyurethane to be used as a binder (resin) in film-forming formulations intended for preserving and protecting wooden heritage objects. The polyolic part of the mixture was also composed of glycerol as a bio-based polyolic cross-linker (f=3) and castor oil as a natural polyol and plasticizer. The isocyanate used was isophorone diisocyanate and the

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ratio between NCO and OH that we used in formulations was 1.1. The polyurethanes synthesized were characterized by various tehniques such as FTIR, DMA, TGA or DSC.

References: [1] J.O. Akindoyo, et. al., RSC Adv. 6 (2016) 114453–114482; [2] https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/; [3] H. Sardon, et. al., ACS Sustainable Chem. Eng. 9 (2021) 10664–10677. https://doi.org/10.1021/acssuschemeng.1c02361.

Keywords: PET recycling; Polyols; Polyurethane; Coatings

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P3. AN INNOVATIVE SYSTEM OF PHOTOBIOREACTORS FOR THE REALIZATION OF THE BIOREFINERY CONCEPT

Alexander Kroumov[1], Maya M. Zaharieva[1], Snejana Russinova[1], Yana Ilieva[1], Anna Brachkova[1], Tanya Kim[1], Mila Kaleva[1], Reneta Gevrenova[2], Dimitrina Jeleva-Dimitrova[2], Vessela Balabanova-Bozushka[2], Hristo Najdenski[1]

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A complex photobioreactor system with the purpose of CO_2 sequestration from flue gases (waste industrial gases) was created. The photobioreactors (PBRs) in the scheme are designed by using the achievements in the field of fluid dynamics and modern knowledge in the physiology of microalgae linked with the PBR geometry. Controlling the state parameters of the cultivation process-light irradiation, temperature, pH and components of the nutrient medium it was possible to obtain highest absorption of CO_2 from the gas phase. Further, during the microalgae growth, synthesis of high and low value products was optimized. The results have shown that in the developed innovative PBR system, products such as biofuels, pigments and metabolites used in infectious microbiology, virology, pharmacy and medicine, as well as the overall utilization of the resulting biomass can be realized fulfilling the ideas of integral biorefinary strategy.

Keywords: innovative photobiorector system, integral biorefinery concept, flue gas, CO_2 sequestration, microalgae

ACKNOWLEDGEMENTS: *This work was supported by project BG05M2OP001-1.002-0019: 'Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy', financed by Operational Program 'Science and Education for Smart Growth'.*

P4. SENSITIVITY OF THE PHOTOSYNTHESIS IN *ZEA MAYS* L. AND *SORGHUM BICOLOR* L. UNDER SALT STRESS

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Climate change is one of the main causes of soil salinization, which in recent years has become a growing problem in agriculture and leads to a decrease in the cultivation area, harming plant growth, crop yield, and quality. In this study, the impact of salinity on photosynthesis in maize (*Zea mays* L. Kerala) and sorghum (*Sorghum bicolor* L. Shamal) were investigated.

The plants had grown in Hoagland solution with different NaCl concentrations (0-250 mM) for different periods of time (5 days and 15 days). Chlorophyll fluorescence (PAM and JIP test), P700 photo-oxidation, pigment analysis and antioxidant markers were used for characterization of the protective role of NO under salt stress.

The salinity treatment leads to: (i) an influence on the effective PSII antenna size (ABS/RC); (ii) a decrease of the photochemical quenching (qp), the electron transport from Q_A to the PSI end electron acceptors (REo/RC) and the possibility to their reductions; (iii) an increase of the regulated and non-regulated energy losses.

All these salt-induced changes influence the PSI photochemical activity, the electron transport rate as well as the rate the photosynthesis, depending on the NaCl concentration and the time of the treatment. The alteration in functions of the photosynthetic apparatus under salinity corresponds with a reduction of the pigment content, an increase in the amount of stress markers and a decrease in membrane stability. The parameters that can be used to estimate the sensitivity of plants to salinity were indicated.

Keywords: maize, sorghum, chlorophyll fluorescence, salinity

ACKNOWLEDGEMENTS: *This work was supported by the Bulgarian Science Fund, КП-06-Н36/9, 2019.*

P5. PHOTODEGRADATION OF METHYLENE BLUE AND CRYSTAL VIOLET WITH Zr TITANO SILICATE

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Our society is confronted with a critical challenge related to the increasing water consumption and inadequate reserves. Currently, water pollution from the textile, metallurgical, pharmaceutical and other industries is one of the main problems affecting the whole world. Wastewater from textile industry containing dyes is a significant environmental pollutant, which also impacts on human health. Therefore, it is necessary to develop new techniques for purifying water and removing unwanted and harmful pollutants. The present work focuses on the pollution of water by dyes and in particular crystal violet (CV) and methylene blue (MB). In view of this and for obtaining highly efficient dye removal catalysts Zr modified catalysts based on porous titanium silicates - Engelhard Titanium Silicate (ETS) - were developed and synthesized. The catalysts newly synthesized catalysts were characterized by XRD powder diffraction, XRF, N_2 physisorption, DTA-TG and FTIR. The photocatalytic properties of Zr-ETS catalysts were tested for removal of crystal violet (CV) and methylene blue (MB) from contaminated water. To better understand the major rate-determining steps, such as mass-transfer processes, pseudo first order, pseudo second order etc. models have been employed.

The determined equilibrium sorption capacity (qe) values obtained from the pseudo-second order model replicate the experimental data with correlation coefficient values close to unity. As a result, the process can be effectively described by the pseudo-second order kinetic model for both dyes.

Keywords: photocatalysis, Zr, Engelhardt titan silicate, methylene blue, crystal violet

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P6. INFLUENCE OF THE TOURISM INDUSTRY ON THE ENVIRONMENT BASED ON THE QUALITY AND QUANTITY OF WASTEWATER IN THE REGION OF SUNNY BEACH, BULGARIA

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Sunny Beach is a resort of national importance for Bulgaria, which occupies the largest share in the tourism sector of the country. With a total of 196 accommodation places and nearly 5 million overnight stays on an annual basis, the resort has the most significant impact on the environment. Considering the seasonality of tourism in the area, we are talking about mass tourism concentrated in the months of June, July, and August. The treatment plant - Ravda was put into operation in the current state of technology in 2015 and has modern facilities for the joint elimination of carbon, nitrogen and phosphorus and possibilities for expansion and contraction depending on the seasonality and load of wastewater. These are the most modern management technological mechanisms in water purification technologies. WWTP-Ravda has anaerobic facilities for treating sludge with the production of biogas, which covers 30% of the energy needs of the municipality in the most developed version of the water treatment process.

This is also observed from the data on the quantity and quality of the wastewater of the Ravda WWTP. The aim of this study is to analyze the correlation between tourism and ecology, as well as their mutual impact, through statistical data and comparative data on COD, BOD5, COD:BOD ratio, wastewater quantity, and number of tourists for the period 2018-2022. Sustainable water management practices are proposed that will help to move from a linear to a circular economy that will reduce the negative impact of mass tourism. This includes the reduction of water consumption and wastewater generation, improving wastewater collection and its reuse, as well as promoting the implementation of different total quality management (TQM) standards such as ISO, ESG, and Travelife.

Keywords: wastewater, water treatment, sustainable tourism, circular economy

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P7. ADAPTATION OF KEY BIOFILM COMMUNITIES IN A CONSTRUCTED WETLAND FOR MIXED URBAN WASTEWATER TREATMENT

Ivaylo Yotinov[1,2], Mihaela Belouhova[1,2], Yovana Todorova[1,2], Irina Schneider[1,2], Jaklina Tsvetkova[1], Dean Stoyanov[3], Irina Kostova[2,3], Yana Topalova[1,2]

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In recent decades, humanity has faced various challenges. The cases of wastewater and drinking water treatment, storage and reuse for various purposes come to the forefront. Among the EU members, there is an increasing awareness of working towards a circular economy, which involves reusing water resources, using them economically and eliminating the possibility of their pollution. Constructed wetlands provide precisely this opportunity for purifying highly polluted waters in as simple a technological way as possible. At the basis of the ongoing biodegradation processes, there is a forming biofilm with a high purification capacity.

The working hypothesis of this study aims to track the formation and adaptation of a bacterial consortium. In this way, a biofilm is formed that is adapted to the pollutants in wastewater. The aim is to trace the adaptation of the key microbiological groups that are relevant to the biodegradation of pollutants in three main stages - initial, middle and final. The study of the key groups of bacteria is carried out in a controlled bioreactor of the "wet zone" type. Through these controlled conditions, information can be gained on the time of formation, adaptation and activity of microbial communities. The key bacteria with biodegradation potential studied in this experiment are the genus *Acinetobacter* and *Pseudomonas*, as well as denitrifying bacteria. Also aerobic and anaerobic heterotrophic bacteria. Therefore, it is extremely important to trace the process covering the three main stages of the experiment - beginning, middle and end.

In managing the biodegradation processes of xenobiotic pollutants, the model bioreactor can be scaled up and applied to larger projects such as small towns or seasonal recreation sites where they would find application with smaller volumes of wastewater.

Keywords: biofilm, wetlands, biodegradation, bacteria, wastewater

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8. NON-THERMAL PLASMA TREATMENT TO PREVENT BIOFILM FORMATION AND TO CONTROL MATURE BIOFILM GROWTH

Yovana Todorova[1,2], **Plamena Marinova-Dragozova**[2,3], **Ivaylo Yotinov**[1,3], **Todor Bogdanov**[3,4], **Evgenia Benova**[2], **Yana Topalova**[1,2]

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Because of the rapid achievement of good bactericidal efficiency, ease of operation, and lack of residual toxicity, non-thermal plasma is the focus of intensive scientific investigation in the last years as an innovative sterilization approach for different purposes in medicine, biology, agriculture, etc. The non-thermal plasma generated at atmospheric pressure contains a wide variety of radicals, charged particles, reactive oxygen and nitrogen species, UV radiation, and electrical fields with a high synergic effect for the mitigation of bacterial growth.

The purpose of this study is to evaluate the inactivation of biofilm-forming bacteria (*E. coli* ATCC 700728) at the initial and mature phase of biofilm development by a plasma torch produced in argon at atmospheric pressure by a surfatron-type electromagnetic wave launcher. The plasma demonstrated a definite inactivation effect on the bacterial biofilms.

The well-presented zones without cells were obtained and the sizes varied with exposure time and initial bacterial density. Mature biofilms were less sensitive to plasma treatment, but bacterial growth was nevertheless reduced by more than 50% at high initial densities. Plasma-based technologies have significant potential as a new sterilization technology that can be used for effective microbial control, especially to prevent biofilm formation in different devices in the water supply, medicine, and food processing.

Keywords: bacterial biofilms, non-thermal plasma, *E. coli*, sterilization

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P9. NEEDLES AS NOVEL BIOINDICATORS FOR POLLUTION MONITORING – AN NMR STUDY

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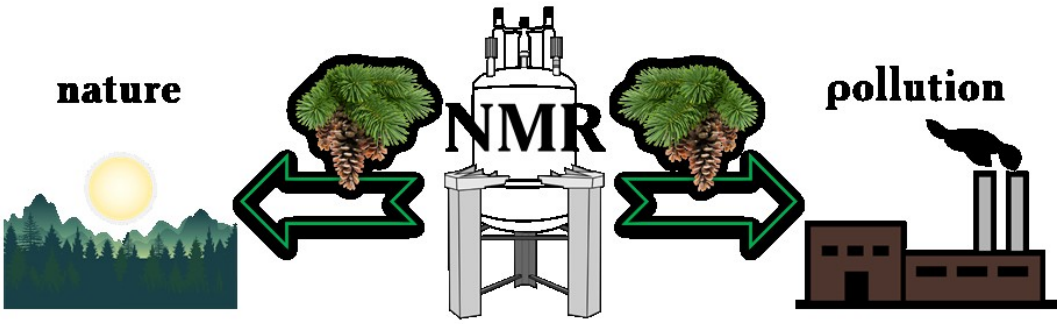
Rapid industrialization and urbanization have undoubtedly improved human life, but they have also posed environmental challenges, especially in terms of polluting the planet. Pollution poses a significant risk to ecosystems and human health. Researchers are actively seeking innovative and cost-effective approaches to address the complexity of pollution monitoring, as traditional methods have limitations in spatial coverage, cost, and accessibility. To overcome these challenges, our research focuses on exploring the potential of conifer needles for air pollution monitoring, offering a practical, economical and reliable solution.



The present study aims to investigate the relationships between metabolites present in spruce and fir trees, commonly found in different geographical areas, and the pollution levels of their growing areas. To achieve this, we used ^{1}H NMR profiling and statistical analysis. Additionally, we aimed to explore potential differences in the chemical composition of spruce and fir needles and the altitudes at which they were grown.

Keywords: NMR metabolomics, pollution monitoring, spruce and fir needles, chemometrics

ACKNOWLEDGEMENTS: *This research was funded by the Bilateral project between Romanian and Bulgarian Academy of Sciences. Equipment purchased by Project No BG05M2OP001-1.002-0012, funded by the OP "Science and Education for Smart Growth" 2014-2020, co-financed by the EU through the European Regional Development Fund, was used. Financial support of the Ministry of Education and Science-Bulgaria (INFRAMAT project) is gratefully acknowledged for maintaining the NMR infrastructure.*

P10. PHYCOREMEDIATION ALLEVIATES ARSENATE TOXICITY IN MAIZE PLANTS

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The present study discussed the possibility of reducing arsenate toxicity in maize (*Zea maize* L.) by the method of phycoremediation. The investigations were carried out in two experimental systems – ES 1, in which plants were treated with 0.1 mM, 0.2 mM, 0.5 mM and 1 mM $NaAsHO_4.7H_2O$ added to the Knop's nutrition medium, and ES 2 - in which the roots of plants were grown in algal suspension. The microalgal strain used for co-cultivation, *Arthronema africanum* (Cyanoprokaryota), was selected due to its well-pronounced heavy metal uptake properties. It showed a particularly high tolerance to the presence of arsenate in the medium, up to 10 mM As. Growth and physiological parameters of algal culture and vascular plant were determined on the 3rd, 5th and 7th day of the treatment, by monitoring the changes of dry weight, pigments and total protein content.

The positive effect of the co-cultivation was most evident on the 5th and 7th day of the experiment, especially on the length and weight of the maize roots. It was found that the pigment content of maize leaves was favorably influenced by the presence of algal suspension. Chlorophyll *a* appeared to show the highest sensitivity to As toxicity among the photosynthetic pigments. It was severely reduced at 0.5 mM and 1 mM As in ES 1 (38% - 44% below the control), but co-cultivation with *A. africanum* mitigated the negative impact of arsenate. The changes of chlorophyll *a* were not consistent with those in chlorophyll *b* content. In ES 2, the concentration of chlorophyll *b* was still enhanced at 0.1 and 0.2 mM As in the medium. Further, the most significant changes were noticed in the carotenoid content - it was elevated in all ES 2 variants, especially at 0.1 and 0.2 mM As, where it was increased by 70% - 90% over control. That result could be considered to confirm their protective role towards heavy metal stress in plant tissues. In addition, total protein content of maize leaves was not significantly affected in ES 2, in contrast to ES 1 where it was highly reduced at 1 mM As.

In conclusion, that study showed the higher potential of the cyanoprokaryote *Arthronema africanum* for modulation of arsenate toxicity in maize plants. It could contribute to expanding knowledge about the mechanisms and future successful application of the phycoremediation method.

Keywords: As, *Arthronema africanum*, microalga, phycoremediation, growth, pigments

ACKNOWLEDGEMENTS: *That study was supported by grant № 80-10-68/25.04.2023 Sofia University'.*

P11. NANOSTRUCTURED TiO_2/Mn_XO_Y MIXED CATALYSTS FOR ENHANCED HYDROLYSIS OF DNA-MODEL PHOSPHODIESTER: IMPACT OF Ti/Mn RATIO

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Many basic biological processes, as well as cellular structures, are based on the reactivity of phosphorus compounds, phosphoesters, and phosphodiesters. The most typical example can be the structures of DNA and RNA that contain extremely stable phosphodiesters binding together individual nucleoside units. The functions of phosphor(di)esters in biological systems reveal a remarkable paradox from the extreme stability of phosphoesters in nucleic acids (the half-life of spontaneous DNA hydrolysis in water is approx. 30 million years) compared to very fast cellular processes regulation. This places very high demands on enzymatic catalysis, and it is therefore not surprising that evolution has developed natural enzymes capable of catalyzing the cleavage of the phosphoester bond (phosphomonoesterases including acid and alkaline phosphatases, phosphodiesterase, and phosphotriesterase), which are among the most efficient enzymes in terms of their activity.

In the last few years, TiO_2 has also attracted strong attention as a photocatalyst. The achievement of high photocatalytic activity of TiO_2 is determined by various factors: crystal structure, specific surface area, porosity, particle size and size distribution, the surface density of hydroxyl groups, etc. On the other hand, manganese-containing catalysts are of great interest because of their importance in the chemical industry, particularly in selective oxidation reactions and those oxidative dehydrogenations. The particularly high catalytic activity of manganese-containing materials is related mainly to the simultaneous presence of Mn^{2+}/Mn^{4+} or Mn^{3+}/Mn^{4+} oxidation-reduction pairs and the possibility of active participation of the oxygen atom from the lattice in the catalytic process.

Titanium-manganese oxide materials showed themselves as effective catalysts with enzyme-mimetic properties for the degradation of DNA-model phosphodiester and the production of alternative energy sources *via* methanol decomposition. The obtained materials were characterized by low-temperature nitrogen physisorption, XRD, SEM, TEM, XPS, and thermo-programmed reduction with hydrogen. The found differences in the binary materials, which could be tuned by the variation in the Ti/Mn ratio, provoked significant changes in their textural, surface, and redox properties, which is in close relation with the registered catalytic activity and selectivity in the studied catalytic processes. The most effective catalyst in both reactions was 2Ti8Mn material due to the predominant presence of manganese oxide species with a low degree of crystallinity, either deposited around the titania surface or as relatively finely dispersed manganese oxide particles, that provide a high amount of metal ions in low oxidation state as well as the formation of reactive oxygen species.

ACKNOWLEDGEMENTS: *The authors thank to the project KII-06-H27/9 for the financial support and bilateral project BAS-AS CR for the help with the physicochemical characterization of the samples*

P12. MICROPLASTICS IN FISH AND SHELLFISH FROM THE BULGARIAN BLACK SEA COASTAL ZONE

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Environmental pollution with microplastics (MPs) is constantly increasing and they accumulate in marine organisms and can be further biomagnified along food chains. MPs in marine species are found in different numbers with different size and form, and can have negative consequences on all hierarchical levels of living matter. The purpose of the present study was to screen for the presence, frequency and quantity of MPs in several target species from the Bulgaria.n Black Sea coastal area including fish (*Mullus barbatus* Linnaeus, 1758) and bivalves (*Mytilus galloprovincialis* Lamarck, 1819 and *Donax trunculus* Linnaeus, 1758). In the laboratory, the gastrointestinal tract of *M. barbatus* and the soft tissues of *M. galloprovincialis* and *D. trunculus* were digested with 10% potassium hydroxide at 40°C for 48–72 h and filtered with glass microfiber filters with 1.2 µm pore size.

The identified MPs were characterized by type, color and size using a stereomicroscope and chemical composition by FTIR spectroscopy. In *M. barbatus* from the Bulgarian coast MPs were found in 31.65% of all examined individuals and they were mostly in the form of filaments. The MPs found in this fish species were more than twice as numerous (0.92 ps/ind.) as those found in the same species along the Turkish Black Sea coast. Examination of the bivalves revealed the presence of significant amounts of MPs in the soft tissues of both species studied. As a whole, MPs were found in most of the examined bivalve specimens. In terms of shape, they were filaments, pellets and irregularly shaped, with filaments predominating in *M. galloprovincialis* and pellets predominating in *D. trunculus*. According to size, in both bivalve species MPs with size ≤ 25 µm prevailed. Most of the isolated MPs particles were of polyethylene and polyethylene terephthalate. Differences were found in the number of accumulated MPs in the studied species sampled from different regions.

The highest number of MPs was present in the bivalves from the region of Varna Bay and Ahtopol. For the first time in this study seasonal differences in the bioaccumulation of MPs in *D. trunculus* were found and, in general, the presence of MPs particles was higher in the autumn-winter period. The data obtained from the present research clearly showed that MPs of various types, shapes and colors were massively accumulated in the studied target species of fish and bivalves inhabiting the Bulgarian Black Sea coastal zone. Given that fish and bivalves are essential components of the marine ecosystem, their health condition and well-being are critical for maintaining ecosystem resilience and its ability to provide ecosystem services to humans. On the other hand, fish and shellfish represent an important food resource with recently

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increasing consumption also in Bulgaria. Consumption of contaminated food can result in increased number of MPs in the human body and consequently cause health damages. It is necessary to regularly monitor MPs pollution of the marine environment and take measures to reduce their spread. On the other hand, more research is urgently needed to elucidate the specific effects of MPs on human health.

Keywords: bivalves, Black Sea, Bulgaria, fish, microplastics

ACKNOWLEDGEMENTS: *This work was supported by grants № КП-06-Н41/6 and № КП-06-Н61/10 of the National Science Fund, Bulgaria.*

P13. CARBON MATERIALS FOR SENSORS IN MEDICINE AND ENVIRONMENTAL APPLICATION

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There are numerous reports on magnetism in carbon. Magnetic carbon structures usually contain ferromagnetic phases, due to the presence of π-delocalized spins caused by the presence of hetero-element impurities in the carbon matrix. Organic molecules can produce a ferromagnetic, carbon-based material after heating in a vacuum. Magnetic properties of carbon materials could be often due to the existence of small amounts of ferromagnetic metals – iron, cobalt, nickel, manganese, chromium. The mechanism of ferromagnetism in carbon structures that do not contain metal atoms is not well studied. N-containing carbon ferromagnetic materials have amorphous structure. Carbon structures containing B or P can also have some degree of magnetization.

The main objectives of this study are preparation and physico-chemical characterization of novel carbon materials with magnetic properties, suitable for applications as sensors in medicine and ecology. Carbon materials are synthesized from polymer precursors by thermo-chemical treatment with HNO_3 at 200-300^{o}C, subsequent carbonizations at 700^{o}C, and hydropyrolysis at 900^{o}C. The obtained samples are characterized by XRD, SEM, BET.

The obtained carbon materials are characterized by moderately high surface area and high degree of graphitization. The samples demonstrated very good magnetic properties, and they have possible application as sensors in gas and water purification, electronics, medicine.

Keywords: carbon materials, sensors, magnetic properties, heteroatoms.

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P14. SURFACE-WAVE-SUSTAINED ARGON PLASMA TORCH FOR REDUCING LEACHATE TOXICITY

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The leachate from solid waste landfills contains high concentrations of various organic and inorganic recalcitrant compounds, most of which are highly toxic. The treatment of this complex mixture of contaminants of environmental concern, including per- and polyfluoroalkyl substances (known as PFAS) is a challenge for waste/wastewater management and conventional technologies usually have low efficiency. Plasma methods have the potential to successfully contribute to solving existing treatment problems and to be the basis of effective hybrid technologies for the complete removal of hazardous pollutants.

The effect of non-thermal plasma treatment of leachate and sludge on their toxicity is presented. The plasma is produced by a microwave plasma torch sustained by an electromagnetic surface wave at 2.45 GHz in Argon at atmospheric pressure. The toxicity of treated and untreated samples was studied by fluorescence staining with CTC (5-cyano-2,3-ditolyl tetrazolium chloride) and DAPI (4',6-diamidino-2-phenylindole) on a test bacterial culture E. coli NBIMCC 8785 at an incubation time of 1 hour. The plasma-based technologies have the potential to improve the efficiency of the removal of recalcitrant compounds.

ACKNOWLEDGEMENTS: *This work is supported by Clean&Circle Center of Competence, Grant № BG05M2OP001-1.002-0019: "Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy", financed by the Science and Education for Smart Growth Operational Program (2014-2020) and co-financed by the EU through the ESIF.*

P15. INVESTIGATION OF THE NATURAL RADIOACTIVITY OF MINERAL WATERS

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The activity of naturally occurring radionuclides in mineral water from certain most frequently used sources in Bulgaria has been measured with high precision by means of nuclear and radiochemical methods. The survey is pointed at natural uranium (nat. U), radium-226 (^{226}Ra) and lead-210 (^{210}Pb) in mineral water, which are most important radiation sources from the point of view of public health in Bulgaria, because of their high toxicity and radiotoxicity.

The concentration of nat. U in the mineral water was in the range ≤ 3 to 860 µg/L. The activity concentrations of ^{226}Ra and ^{210}Pb varied from 13 to211 mBq/L and from ≤ 1.8 to 104 mBq/L respectively.

The annual effective doses were calculated for all investigated waters for adult inhabitants assuming yearly consumption of 730 litres. The results have shown that all values of the annual effective dose of the investigated mineral waters were below the individual dose criterion of 100 µSv/y reported by World Health Organization.

The obtained new results are used to assess the radiation status of the investigated waters. They will support timely and adequate measures to reduce the harmful impact of ionizing radiation on the population in cases of increased radioactivity.

Key words: ^{226}Ra, ^{210}Pb, nat. U, mineral water.

ACKNOWLEDGEMENTS: *This research has been supported by the Bulgarian Science Fund under Contract No KP-06-N44/1, 27.11.2020.*

P16. STUDY OF THE ATMOSPHERIC RADIOACTIVITY IN BULGARIA FOR THE PERIOD 2019 – 2022

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Institute for Nuclear Research and Nuclear Energy – Bulgarian Academy of Sciences

Systematic studies on the content of natural and technogenic radionuclides have been carried out in aerosol filters collected on the peak Moussala. Measurements of the gamma-ray background on the peak Moussala and at 7 different points on the territory of the Institute for Nuclear Research and Nuclear Energy at the Bulgarian Academy of Sciences in Sofia have been were also carried out.

Analysis of data obtained for the period 2019-2022, including the start of the war in the areas of the Chernobyl and Zaporozhia nuclear power plants, showed that no technogenic radionuclides were recorded in aerosol filters. The results for the permanently present in the air cosmogenic radionuclide ^{7}Be are well below the permissible annual average values, according to the Radiation Protection Ordinance. They do not represent any health risk and a danger to the population and the environment. There is no increase in gamma-ray background at the selected control points.

There are no adverse trends in the radiation situation on the territory of Bulgaria after the war in Ukraine that began in February 2022.

Keywords: radioactivity, aerosol filters, gamma-background.

ACKNOWLEDGEMENTS: *This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science of Bulgaria.*

POSTER PRESENTATIONS "HEALTH"

P1. SURFACE-WAVE-SUSTAINED PLASMA SOURCE FOR BIO-MEDICAL APPLICATIONS

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Electromagnetic wave travelling along a dielectric tube can produce plasma inside the tube which is the typical cylindrical plasma column of surface-wave-sustained discharges. Such configuration could be used for biomedical applications.

This plasma source allows varying of the: geometrical parameters (length, diameter, cross-section of the discharge tube), main plasma parameters (wave power, electron, and gas temperatures, concentration of charged particles and reactive species, UV and microwave radiation), gas and gas mixture parameters (flow velocity, gas mixture ratio). The fact that we are able to vary these parameters allows us to sustain low-temperature plasma torch (gas temperature up to 30–37 ^{o}C).

The investigation is focused on the effect of a microwave plasma torch sustained by traveling electromagnetic wave with the usage of different gasses and gas mixtures on in vivo treated biological systems. Till now this type of plasma source has not been used for bio-medical purposes so often.

This low-temperature plasma torch contains active agents, microwave radiation, and UV radiation which can be successfully used for sterilizations, bacteria tretament, and activation of the healing agents in the wound, for example.

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P2. OXIDATIVE STRESS AS A MECHANISM OF ANTIFUNGAL ACTIVITY OF PROTEIN FRACTIONS FROM *RAPANA VENOSA*

Ekaterina Krumova[1], **Pavlina Dolashka**[2], **Radoslav Abrashev**[1], **Aleksandar Dolashki**[2], **Lyudmila Velkova**[2], **Dr. Jeny Miteva-Staleva**[1], **Vladislava Dishlijska**[1], **Maria Angelova**[1]

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The marine mollusks are a huge source to discover bioactive natural products but they demonstrated in most cases only antibacterial activity. The compounds exhibiting antifungal effects are very rare found. Even less is known about the mechanism of action of these compounds on the fungal cell. In our previous studies, we investigated a broad range of peptide fractions isolated from marine mollusks for their antifungal properties. The fraction from *R. venosa* with molecular mass 50-100 kDa (Rv/50-100) inhibited the growth of *Penicillium griseofulvum* and *Aspergillus niger* in a concentration-dependent manner, equal to that of nystatin. The present study was designed to evaluate the participation of oxidative stress in the mechanism of antifungal activity of Rv/50-100 on the fungal cells from *P. griseofulvum* and *A. niger*.

The protein fraction Rv/50-100 used in the experiments, was purified from *R. venosa* hemolymph. The fungal strains were cultivated in 300 ml Erlenmaer flasks on liquid PDB with or without Rv/50-100 for 72 h. The changes in the level of oxidative stress biomarkers and antioxidant enzyme activity were measured.

The addition of Rv/50-100 to the culture medium resulted in a significant decrease in biomass and total protein content in both strains. Simultaneously, an increase in oxidatively damaged protein was detected. The value of lipid peroxidation found in *A. niger* is also in confirmation of this trend in contrast to the data for *P. griseofulvum*. Concentrations of 0.05 and 0.1% sharply increase the level of the first antioxidant enzyme, superoxide dismutase (SOD). The catalase was significantly higher than that of the control. In *P. griseofulvum*, a certain decrease was observed at 0.3%, which is probably related to the extremely weak development of the strain under these conditions.

In conclusion, the results suggested the involvement of oxidative stress in the mechanism of antifungal action of Rv/50-100. Despite the increased level of antioxidant defense in fungal cells, it is not enough adequate for the level of oxidative stress, which leads to growth inhibition.

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P3. LAVENDER OIL DELIVERY SYSTEMS BASED ON MESOPOROUS SILICATE-POLYMER NANOCOMPOSITES

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Lavender oil has proven industrial applications in the manufacturing of cosmetics and perfumes, as therapeutic agents in aromatherapy, and nutraceutical applications. Following the new paradigm of a more natural and sustainable lifestyle, essential oils are rather appealing due to their chemical, physical, and physiological actions in human beings. The encapsulation of lavender oil in microporous structures as natural zeolites is an attractive solution since these host materials are cheap and non-toxic to biological environments [1].

Here we present our preliminary study on two newly synthesized composites from natural zeolite, KH_2PO_4 and Cellulose phosphate as candidates for lavender oil delivery systems.

The new lavender oil delivery systems were characterized by infrared spectrograms using a Thermo Scientific™ Nicolet™ iS50 FTIR Spectrophotometer.

References: [1]. Ferreira, A.P.; et. al,: An Overview, Molecules 2022, 27(23), 8525; https://doi.org/10.3390/ molecules27238525.

Keywords: lavender oil, zeolite, nanocomposites

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P4. ORAL TREATMENT OF MICE WITH A COMBINATION OF D-GALACTOSE AND ETHANOL AS A NEW AGEING MODEL

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Ageing is a process characterized by the progressive decline and loss of tissue and organ function. These losses, as well as a number of age-related pathologies (cardiovascular disease, liver damage, inflammation, neurodegenerative diseases, cancer etc. are associated with the hypothesis of accumulation of damages caused by oxidative stress.

The intensive study of ageing processes would be facilitated by using accelerated aging models. One of the most recently used models is the subcutaneous treatment of mice and rats with D-galactose (D-gal). The model is standardized and widely used but labor intensive and stressful for the animals. The aim of our study was to obtain a new accelerated ageing model using a combination of oral treatment with D-galactose and a low dose of ethanol. Materials and Methods: Male ICR mice were divided into four groups: 1) Control, 2) D-galactose, 3) Ethanol (Eth) and 4) D-gal+Eth. D-galactose (3 mg/ml) was added to the drinking water of the D-gal and D-gal+Eth groups and 1% ethanol to the Eth and D-gal+Eth groups. Animals were treated for 6 weeks and at the end of the experiment the brains and the livers were removed and frozen untill the analysis. After defrosting the following oxidative stress (OS) markers: lipid peroxidation (LPO), total glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) were measured spectrophotometrically in both organs. The obtained results in brain showed significantly increased levels of LPO in D-gal and D-gal+Eth groups compared to control, accompanied by raised SOD activity in D-gal and Eth groups. GPx and AChE in D-gal+Eth group were also increased, but not statistically reliable. In liver were observed elevated LPO levels in D-gala and Eth groups, significantly increased activity of CAT in D-gal group and not significant in Eth and D-gal+Eth (as well as lower content of GSH) and insignificantly decreased GPx in all tested groups vs control group.

Conclusions: The changes in the investigated oxidative stress markers showed that our proposed new less stressful for the experimental animals model provides opportunities for studying natural substances affecting the ageing processes and reliably mimics aging processes and related diseases.

Keywords: ageing, new model, oxidative stress

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P5. ANTIVIRAL ACTIVITY OF EXTRACTS OBTAINED FROM *OREGANUM VULGARE* L. AND *SCENEDESMUS* SPP.

Maya Zaharieva[1], Pelagia Foka[2], Dimitrina Zheleva-Dimitrova[3], Tanya Chan Kim[1], Eirini Karamichali[2], Yana Ilieva[1], Mila Kaleva[1], Vessela Balabanova-Bozushka[3], Niko Benbassat[3], Reneta Gevrenova[3], Alexander Kroumov[1], Krassimira Yoncheva[3], Urania Georgopoulou[2], Hristo Najdenski[1]

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Coronaviruses are worldwide distributed RNA-viruses affecting several species, causing a broad spectrum of diseases with a zoonotic potential and the ability to jump from one host species to a different one, including humans. The pandemic outbreak coronavirus disease 2019 (COVID-19) has become a global health problem and serious economic burden in more than 200 countries across the six continents. The causative agent SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) belongs to the family Coronaviridae, genus Betacoronavisures and represents an enveloped, positive-strand RNA virus. Currently, the therapeutic strategies to combat CoV infections could be classified into three categories: the first one refers to preventive measures aiming at reducing the transmission in the community; the second one modulates the immune system, while the third should inhibit the virus itself. Many natural and synthetic agents are under investigation and development, including specific antiviral drugs that are already approved for clinical use for other viral infections or completely new are the oregano essential oil and the microalgae extracts. Aim of this study was to investigate the antiviral activity of encapsulated oregano oil and extracts obtained from the microalgal species *Scenedesmus acutus*. As a suitable BSL II in vitro model a strain of bovine coronavirus which belongs to the genus of betacoronaviruses was used and propagated in the MDBK (Madin-Darby bovine kidney) cell line.

The effects of the natural products on the coronaviral strain were evaluated using the following three approaches: pre-treatment, treatment and direct inactivation. The virus titre was determined using digital droplet PCR (ddPCR, TaqMan methodology) targeting the gene for the nucleocapsid, the plaque and $TCID_{50}$ assays. The cytotoxicity of the extracts on MDBK cells and the antiviral activity of at least 5 non-toxic concentrations of the extracts were determined according to ISO 10993-5, Annex C. The results obtained showed a concentration dependant antiviral activity, most strongly expressed after direct inactivation. The cytopathic effect diminished with increasing the applied non-toxic concentrations of the encapsulated oregano oil or the microalgal extracts. The inhibition of the viral replication was proven by ddPCR.

In conclusion, the encapsulated oregano oil and both studied microalgal extracts have the potential to be developed in natural products with antiviral activity.

Keywords: betacoronaviruses, encapsulated oregano oil, *Scenedesmus acutus* extracts, antiviral potential

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P6. PRELIMINARY IN VITRO TOXICOLOGY TESTS OF ANTIMICROBIAL EXTRACTS AND COMPOUNDS FROM TWO *HYPERICUM* SPECIES

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The active ingredients from *Hypericum* spp. (St. John's wort) have a long-proven antimicrobial and antineoplastic effect, but their influence on normal cells of the human body is poorly studied. The aim of the present study was to determine the in vitro toxicological potential of proven antibacterial agents from *Hypericum* on non-tumorigenic eukaryotic cell lines. The MTT-assay and four immortalized cell lines (three human and one mouse) were used: HaCaT (keratinocytes), HEK-293 (kidney epithelial cells) HGF (gingival fibroblasts) and CCL-1 (mouse fibroblasts, a line recommended by the International Standard Organization). Extracts and phloroglucinol derivatives of *H. rochelii* and *H. hirsutum* were applied. The results show that the agents exert in vitro cytotoxic activity on healthy cells at low micromolar concentrations (below 5 mcg/mL). Since some of the clinically used anticancer drugs, e.g. cisplatin also have a toxic effect on healthy cells approximately as much as on tumor cells (low selectivity), these results do not exclude the possibility of developing the agents tested here as drugs. In addition, often in vitro results are very different from those in vivo, because the cells in a multicellular organism possess defense systems. The obtained results indicate that *in vivo* studies are needed to more precisely establish the selectivity of the active extracts of *Hypericum* against tumor and normal cells and tissues and their prospect to be developed as drugs.

Keywords: *Hypericum*, toxicology, cell lines

ACKNOWLEDGEMENTS: *This study was funded by Bulgarian National Science Fund, Contract KП-06-M51/4.*

P7. ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM PIG FARMS IN BULGARIA

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The aim of this study was to evaluate the presence, virulence, antibiotic resistance and biofilm formation of *E. coli* in three swine farms in Bulgaria. The samples were taken from feces, wastewater in a separator, lagoons, soils and transport vehicles. The isolation of single bacterial colonies was performed by standard procedures with some modifications and *E. coli* isolates were identified by MALDI-TOF-MS and polymerase chain reaction (PCR). The antimicrobial resistance was assessed by disk diffusion method. PCR was used to detect genes for antibiotic resistance (GAR) (*qnr*, *aac*(3), *amp*C, *bla*SHV/*bla*TEM and *erm*) and virulence genes (*stx*, *stx*2all, LT, STa, F4 and *eae*). The possibility of biofilm formation of each isolate was investigated the protocol of Stepanovic.A total of 84 isolates from different samples were identified as *E. coli*. Almost all strains demonstrated antimicrobial resistance and most of them- resistance to multiple antibiotics from different classes. No GAR from those tested for quinolones, aminoglycosides and macrolides were found. A total of 56 isolates that were resistant to a penicillin class antibiotic had β-lactamase-producing plasmid genes-all of them had *amp*C, and 34 of them had *bla*TEM. A total of 14 isolates formed strongly adherent biofilms. There were not any virulence genes detected coding the Shiga toxin, enterotoxins or enteropathogenicity. These results in a country where the use of antibiotics for growth promotion and prophylaxis in farms is highly restricted corroborate that the global implemented policy on antibiotics in human and veterinary medicine needs revision.

Keywords: *Escherichia coli*; pigs; antibiotic resistance; resistance genes; β-lactamase; ESBL; MALDI-TOF-MS; biofilm.

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P8. THE COMBINED EFFECT OF PEGYLATED GRAPHENE OXIDE NANOPARTICLES LOADED WITH BLEOMYCIN AND NEAR-INFRARED IRRADIATION TREATMENT ON HEPATOCELLULAR CANCER CELLS *IN VITRO*

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Combining photothermal therapy (PTT) and chemotherapy has been considered a promising strategy to improve therapeutic efficiency and minimize side effects in cancer therapies. To implement the maximal synergistic effect, nanoparticles can be used simultaneously as drug delivery systems and photothermal agents. That approach increases the permeability and sensitivity of cancer cells to the drug and accomplishes more selective and accurate drug delivery in tumor cells.

In this work, we have developed a near-infrared (NIR)-responsive thermosensitive GO-PEG system combined with bleomycin (GO-PEG-Bleo), and the efficacy and mechanisms of this combined treatment approach were evaluated. Cell morphology observations indicated that the GO-PEG-Bleo nanosystem increases cell swelling upon irradiation. Lactate Dehydrogenase (LDH) assay shows an increase in the permeability of the cell membrane without NIR irradiation and vice versa - after NIR, cell permeability decreases significantly. Measurement of reactive oxygen species (ROS), nuclear and mitochondria staining showed that NIR irradiation reduced the generation of ROS by the GO-PEG-Bleo system, disturbed nucleus morphology, and led to shrinkage of the cell's nucleus as well it affected mitochondrial structure. These data demonstrated that GO-PEG-Bleo can be used as a NIR-responsive system in a combined thermo-chemotherapy to treat hepatocellular cancer.

Keywords: HepG2 cells, photothermal therapy, drug delivery systems, nanoparticles

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P9. HEMOLYTIC ACTIVITY OF GO BASED NANOPARTICLES LOADED WITH BLEOMYCIN AND NIR IRRADIATED AS A MARKER OF THEIR HEMOCOMPATIBILITY

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With the expansion of nanoparticle (NPs) application in medicine, the need to assess the toxicity of the new nanomaterials also grows. In the human body NPs enter the bloodstream and interact with various blood cells, including red blood cells (RBCs), the most abundant cellular component in circulation. The exposure of RBCs to NPs leads to various biochemical/biophysical and morphological changes that can significantly affect their functionality. At present, the tests for NPs' hemolytic activity are the most widely adopted to assess NPs' hemotoxicity. Therefore, our study assessed hemolysis and overall morphology of RBCs after treatment with GO and GO-PEG NPs, loaded with bleomycin, and irradiated with near-infrared (NIR) light. Additionally, we have studied the haemocoagulation of RBCs after exposure to GO-Bleomycin and GO-PEG-Bleomycin and NIR irradiation by measurement of the activated partial thromboplastin (APTT), prothrombin time (PT), and fibrinogen concentration.

Upon NIR irradiation, the hemolysis of GO and GO-bleomycin NPs increased significantly, while the hemolytic activity of RBCs treated with GO-PEG was very weak. The overall morphology of RBCs also alters after different treatments depending on the exposure times and type of treatments. Our results show that RBCs are sensitive models for evaluating the hematotoxicity of different nanomaterials.

Keywords: PEGylated graphene oxide; activated partial thromboplastin time (APTT); prothrombin time (PT); erythrocytes, red blood cells, haemocoagulation

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P10. ANTIBACTERIAL ACTIVITY OF HYDROGELS LOADED WITH OREGANO OIL IN COMBINATION WITH GENTAMICIN OR CIRPOFLOXACIN

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Methicillin-resistant *Staphylococcus aureus* (MRSA) constitutes one of the major threats among antibiotic-resistant agents today. As this pathogen is very often isolated from wounds, the World Health Organization has listed it among the high priority multidrug-resistant organisms. Therefore, aim of the present study was to evaluate the antimicrobial activity of combinations between encapsulated oregano oil and two of the most commonly used antibiotics (ciprofloxacin or gentamicin) by patients with skin infections against methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* strains.

Chitosan-alginate nanoparticles loaded with oregano oil and the selected antibiotics were included in methylcellulose hydrogels. Consistency, spreadability, pH of the hydrogel and in vitro release rate of the oil were determined accordingly and considered appropriate for topical application.

The combinations between encapsulated oil and gentamicin or ciprofloxacin in the hydrogel system led to synergism or additive effect, respectively. The effective concentration of gentamicin was reduced fourfold and the bacterial metabolic activity was inhibited up to 98%. The effective concentration of ciprofloxacin was diminished two-fold and 96% reduction in the bacterial metabolic activity was observed. Both combinations inhibited the formation of MRSA biofilm by more than 90%. The in vivo test for evaluation of skin irritation in rabbits proved the safety profile of the synergistic combination between encapsulated oregano oil and gentamicin.

In conclusion, the combinations represent an attractive option for further pharmacological studies aiming at the development of pharmaceutical formulations for the treatment of skin infections caused by methicillin-resistant staphylococci.

Keywords: Methicillin-resistant *Staphylococcus aureus*, wound infections, encapsulated oregano oil, gentamicin, ciprofloxacin

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P11. RESTORATIVE EFFECT OF ZEOLITE ON BRAIN ACETYLCHOLINESTERASE ACTIVITY IN RATS WITH SUBCHRONIC HEAVY METAL INTOXICATION

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Environmental pollution is inextricably linked to various diseases, and this interconnection is expected to increase in the future. In searching for alternative detoxification methods that affect acetylcholinesterase (AChE), natural zeolite clinoplinolite (Zeolite) was used in rats intoxicated with heavy metals (HM). The use of Zeolite (hydrated natural or synthetic microporous crystal containing AlO_4 and SiO_4 tetrahedra) in medicine is a relatively recent subject of interest. Our previous studies have demonstrated damaging effects of heavy metals on some cognitive functions in rats. Zeolite significantly decreases bioaccumulation of Pb, Zn and their combination (Pb+Zn). But how it affects cholinergic function, changed by HM exposure, remains unclear.

The aim of this study was to assess the effects of Zeolite on AChE activity in rats, subchronically intoxicated with HM, namely Pb, Zn and Pb+Zn.

Male Wistar rats were treated with HM salts: $ZnCl_2$, $Pb(CH_3COO)_2$ or their combination. The HM salts were administered via drinking water in doses 100 mg/kg drinking water for 30 days. The Control group drank clean water. Half of the rats received 2% Zeolite for 30 days with their regular food. On the 24th hour after the last intake, three brain structrures related to memory: cortex, hippocampus and striatum were isolated and AChE activity was measured according to Ellman's method.

HM intoxication decreased AChE activity in the brain indicating cholinergic synapses deficiency. This effect was most pronounced in the hippocampus and cortex. Zeolite administration normalized cortex AChE activity that was changed by Zn and Pb+Zn. In the striatum only the combination of Pb+Zn and Zeolite significantly increased AChE activity.

The restorative effect of Zeolite on AChE activity in some brain structures is part of its detoxifying mechanisms against HM intoxication and deserves further studies.

Keywords: Heavy metals, Zeolite, Acetylcholinesterase

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P12. DEVELOPMENT OF NOVEL BIOLOGICAL NANOPARTICLES FROM THE MUCUS OF THE GARDEN SNAIL *CORNU ASPERSUM* AS ANTIMICROBIAL AGENTS

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The development of green, low cost and sustainable synthetic routes to produce metal nanoparticles (NPs) is of outmost importance, as these materials fulfill large scale applications in a number of different areas. Herein, snail slime extracted from *Cornu aspersum* snails was successfully employed both as bio-reducing agent of metal salts and as bio-stabilizer of the obtained nanoparticles.

The influence of different preparation conditions, such as metal salt concentration, volume of the reagents and process temperature, were investigated to find the best biogenic pathway to produce metal nanoparticles. UV–Visible Spectroscopy, SEM, XRD, FTIR were used for a detailed characterization of the nanoparticles. The role of proteins and glycoproteins in the biogenic production of NPs was elucidated. Infrared spectra clearly showed the presence of proteins all around the metal core.

The macromolecular shell is also responsible of the effectiveness of the synthesized NPs to inhibit Gram-positive and Gram-negative bacterial growth. The known antibacterial properties of nanoparticles are strongly enhanced by the presence of slime macromolecules wrapping the nanoparticles.

Keywords: *Cornu aspersum*, antibacterial, nanoparticles, protein.

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P13. THE BULGARIAN NATIONAL CYCLOTRON CENTER AND THE BENEFIT OF PUBLIC HEALTH

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The Institute for Nuclear Research and Nuclear Energy at the Bulgarian Academy of Sciences is establishing a National Cyclotron Center. The main tasks that will be solved with its commissioning are to perform research in the fields of radiopharmacy, as well as the production of radiopharmaceuticals for the diagnosis and treatment of tumor diseases.

The center will also be the only accelerator complex in Bulgaria for training in the field of radiopharmacy, radiobiology, radiochemistry, nuclear physics and nuclear energy.

The work of the National Cyclotron Center will make a significant contribution to the improvement of Bulgarian healthcare.

Keywords: Cyclotron TR24, radiopharmacy, PET and SPECT studies.

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