BOOK OF ABSTRACTS

EcoBalt 2023
Tallinn, Estonia · October 9-11

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Sustainable Conference


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EcoBalt 2023 – a sustainable and eco-friendly event in Tallinn, the **European Green Capital 2023**.

Conference venue

Nordic Hotel Forum – responsible business, with ***Green Key*** label since 2010.

- efficient water and energy use
- eco paper
- natural cleaners
- local restaurant ingredients.

Conference location

Tallinn city center – easy on foot access to Old Town, UNESCO World Heritage Site.
Fast and eco-friendly commute from:

- Tallinn Lennart Meri International Airport
- Passenger Port of Tallinn (Old City Harbor, Vanasadam)

Transportation

- Easy access to **public transportation**
- **Free public transport** in Tallinn during the conference
- Conference social events in the **walkable distance** from the conference venue

Reusable and sustainable products and meals

- Reusable tableware
- Drinking water in reusable containers
- Vegetarian and vegan meal options
- Minimized waste generation
- Electronic conference materials

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Conference Program

09 – 11 October 2023, Tallinn, Estonia, *Nordic Hotel Forum Conference Center (Viru väljak 3, Tallinn)*

Monday October 9, 2023

16:00 – 21:00	Badge pickup and registration (Foyer of the Nordic Hotel Forum Conference Center)
18:00 – 21:00	Welcome reception and special lecture by Dr. Priit Zingel, Estonian University of Life Sciences (Foyer of the Conference Center) 18:00 Welcome drinks; 18:30 – 19:15 Special lecture; 19:15 – 21:00 Welcome buffet

Tuesday October 10, 2023

8:45 – 9:00	Welcome speech by Dr. Urmas Nagel, Director of the National Institute of Chemical Physics and Biophysics (NICPB), Estonia **(Room: Sirius)**
9:00 – 9:05	Welcome and organizational information, Dr. Monika Mortimer, National Institute of Chemical Physics and Biophysics, Estonia
9:05 – 9:50	**Plenary Lecture I (Room: Sirius)** **Sampling, detection and uncertainty in environmental analysis – challenges and solutions** Dr. Steve Ellison, LGC Limited, United Kingdom (Chair: Prof. Ivo Leito, University of Tartu, Estonia)
9:50 – 10:10	*Coffee break **(Foyer of the Nordic Hotel Forum Conference Center)***

Parallel session 1: Data Quality in Environmental Analysis Chair: Prof. Ivo Leito, University of Tartu, Estonia **(Room: Sirius)**		**Parallel session 2**: The Effects of Legacy and Emerging Chemicals on Ecosystems and Humans (by the Estonian Society of Toxicology) Chair: Prof. Angela Ivask, University of Tartu, Estonia **(Room: Capella)**	
10:10 – 10:30	**Measurement quality in analysis – guidelines and software tools** Dr. Bertil Magnusson, Trollboken AB, Sweden Invited speaker	10:10 – 10:30	**The effect of environmental pollution in Baltic Sea and North Sea on marine biota and cancer development in fish** Dr. Randel Kreitsberg, University of Tartu, Estonia Invited speaker
10:30 – 10:50	Dr. Riin Rebane, University of Tartu and Estonian Environmental Research Centre, Estonia	10:30 – 10:50	**The impact of microplastics on soil invertebrates** Prof. Anita Jemec Kokalj, University of Ljubljana, Slovenia, Invited speaker
10:50 – 11:55	General discussion on data quality and environmental analysis	10:50 – 11:05	**Microplastics and associated microorganisms in the sea sediment of the Sentina Regional Natural Reserve (Central Adriatic Sea, Italy)** Prof. Cristina Miceli, University of Camerino, Italy
		11:05 – 11:20	**Novel plasticizers are emerging contaminants** Dr. Margit Heinlaan, National Institute of Chemical Physics and Biophysics, Estonia

		11:20 – 11:35	**Droplet-based technology for studying phenotypic effect of microplastic on antimicrobial resistance** Dr. Simona Bartkova, Tallinn University of Technology, Estonia
		11:35 – 11:55	**Endocrine disrupting activity of mixtures composed of pharmaceuticals and nanoplastics** Prof. Ivana Vinković Vrček, Institute for Medical Research and Occupational Health, Croatia, Invited speaker
12:00 – 13:00	*Lunch (Restaurant "Nomel" at Nordic Hotel Forum, ground floor)*		
Parallel session 3: Analytical Method Development for Environmental Pollutants (Room: Sirius) Chair: Prof. Polonca Trebse, University of Ljubljana, Slovenia		**Parallel session 4**: The Narva River, from Lake Peipsi to the Baltic Sea: Challenges and Opportunities (Room: Capella) Chair: Yaroslav Kobets, Tallinn University of Technology, Estonia	
13:00 – 13:20	**Chasing pollutants concerning public health: from food to smoke** Prof. Mojca Bavcon Kralj, University of Ljubljana, Slovenia Invited speaker	13:00 – 13:20	**Development of the harmonised methodology for the Narva River water discharge estimation** Yaroslav Kobets, Tallinn University of Technology, Estonia
13:20 – 13:40	**Identification of emerging contaminants in Estonian aquatic environment** Mailis Laht, Estonian Environmental Research Centre, Estonia	13:20 – 13:40	**Assessing biological effects of contaminants in the Gulf of Finland, north-eastern Baltic Sea, using sediment biotests with amphipods and biomarker responses in clams** Dr. Ivan Kuprijanov, Tallinn University of Technology, Estonia
13:40 – 13:55	**Detection of mycotoxins and pyrrolizidine alkaloids in a wide variety of nutritional supplements using the multianalyte HPLC-MS/MS method** Zane Berzina, University of Latvia, Latvia	13:40 – 13:55	**Estimation of the share of the total nutrient load from the territory of Estonia, coming along the Narva River to the Baltic Sea** Dr. Alvina Reihan, Tallinn University of Technology, Estonia
13:55 – 14:10	**Current approaches to the derivatization of Chemical Weapons Convention-related alcohols for on-site gas chromatographic analysis** Dr. Tomas Rozsypal, University of Defence, Czech Republic	13:55 – 14:10	**Water quality index (WQI) as a tool to assess waterbodies status. Joint WQI model development for River Narva and the rivers of the lake Peipsi basin** Kati Roosalu, Tallinn University of Technology, Estonia
14:10 – 14:25	**Cost-effective and compact measurement of Arsenic in water (ARMINE project-MITY)** Jay Pee Oña, University of Oulu, Finland	14:10 – 14:25	**Non-formal ecological education: tested innovative methods in Lake Peipsi region** Margit Säre, Peipsi Center for Transboundary Cooperation
14:25 – 14:45	**From organometallic chemistry to multifunctional nanoparticle-based devices for gas detection and degradation of air pollutants** Dr. Myrtil L. Kahn, CNRS UPR 8241, University of Toulouse, France	14:25 – 14:45	**International business, academic and RDI cooperation as drivers and accelerators of sustainable economic growth in biotechnology and the circular economy - Results of the BBC1 -project** Saija Helena Tillgren, Mikkeli Development Miksei Ltd., Finland

14:45 – 15:00	*Coffee break (Foyer of the Nordic Hotel Forum Conference Center)*
Session 5: Chemicals and Environment I (Room: Sirius); Chair: Prof. Vera Slaveykova, University of Geneva, Switzerland	
15:00 – 15:20	**Role of plant volatiles in atmospheric processes under current and future climates** Prof. Ülo Niinemets, Estonian University of Life Sciences, Estonia, Invited speaker
15:20 – 15:40	**The use of biomarkers in monitoring and assessment of chemical contamination in the Baltic Sea** Dr. Kari Lehtonen, Finnish Environment Institute, Finland, Invited speaker
15:40 – 16:00	**A Chimie Douce route to layered double hydroxides** Prof. Aivaras Kareiva, Vilnius University, Lithuania, Invited speaker
16:00 – 16:20	**The environmental safety aspects of technologically powerful materials are often overlooked** Dr. Anne Kahru, National Institute of Chemical Physics and Biophysics, Estonia, Invited speaker
16:20 – 17:30	**Poster session (Room: Vega) and Expo (Foyer of the Nordic Hotel Forum Conference Center)**
19:00 – 23:00	Conference dinner and museum visit at the Seaplane Harbour (Vesilennuki 6, Tallinn) 19:00 Museum visit; 19:30 Welcome drinks; 19:45 Buffet dinner; 21:00-21:30 Musical performance

Wednesday October 11, 2023

9:00 – 9:05	Organizational information, Dr. Monika Mortimer, National Institute of Chemical Physics and Biophysics **(Room: Sirius)**
9:05 – 9:50	**Plenary Lecture II (Room: Sirius)**
	Nanoplastic-biomolecular interactions Prof. Pu-Chun Ke, Great Bay Area National Institute for Nanotechnology Innovation, China, and Monash University, Australia Chair: Dr. Monika Mortimer, National Institute of Chemical Physics and Biophysics, Estonia
9:50 – 10:10	*Coffee break **(Foyer of the Nordic Hotel Forum Conference Center)***
Session 6: Chemicals and Environment II **(Room: Sirius)** Chair: Dr. Anne Kahru, National Institute of Chemical Physics and Biophysics, Estonia	
10:10 – 10:30	**Wood chemistry perspectives at TalTech** Dr. Tiit Lukk, Tallinn University of Technology, Estonia, Invited speaker
10:30 – 10:50	**Compounds from personal care products as emerging contaminants in swimming pool waters** Prof. Polonca Trebse, University of Ljubljana, Slovenia, Invited speaker
10:50 – 11:05	**Formation and bioaccumulation of methylmercury along vertical and spatial gradients in Baltic Sea waters** Prof. Erik Björn, Umeå University, Sweden, Invited speaker
11:05 – 11:20	**Nanocomposite metal oxide/hydroxide adsorbents for advanced wastewater treatment and toxicological risk assessment for the aquatic environment** Dr. Asya Drenkova-Tuhtan, National Institute of Chemical Physics and Biophysics, Estonia

11:20 – 11:35	**Silver-chitosan nanocomposites for biomedical application: design, synthesis and antimicrobial efficiency** Dr. Kaja Kasemets, National Institute of Chemical Physics and Biophysics, Estonia		
11:35 – 11:55	**What phytoplankton species can tell us about the implications of engineered nanoparticles in the aquatic environment** Prof. Vera Slaveykova, University of Geneva, Switzerland, Invited speaker		
12:00 – 13:00	*Lunch **(Restaurant "Nomel" at Nordic Hotel Forum, ground floor)***		
Parallel session 7: Photocatalytic Materials **(Room: Sirius)** Chair: Prof. Pu-Chun Ke, Great Bay Area National Institute for Nanotechnology Innovation, China		**Parallel session 8:** Green Materials **(Room: Capella)** Chair: Prof. Aivaras Kareiva, Vilnius University, Lithuania	
13:00 – 13:20	**Activity of nanomaterials in photocatalysis** Prof. Marina Kritsevskaja, Tallinn University of Technology, Estonia Invited speaker	13:00 – 13:20	**A brief journey on the world of heteracyclo[n]phanes, synthesis and CO_2 adsorption** Prof. Jean-Manuel Raimundo, Aix Marseille University, France, Invited speaker
13:20 – 13:40	**Innovative nanocomposites made of polymers and semiconductor photocatalysts for wastewater treatment** Dr. Giuliana Impellizzeri, National Research Council Institute for Microelectronics and Microsystems, CNR-IMM Catania, Italy	13:20 – 13:40	**Electrodeposited Cu nanofoam structures for electrochemical CO_2 reduction** Prof. Rimantas Ramanauskas, Center for Physical Science and Technology, Lithuania Invited speaker
13:40 – 13:55	**Polymeric hydrogels for the removal of emerging contaminants from water (ANTIBIO)** Dr. Sabrina Carola Carroccio, National Research Council Institute of Polymers, Composites and Biomaterials, CNR-IPCB Catania, Italy	13:40 – 13:55	**Nitrogen-doped reduced graphene oxide for electrochemical sensing applications** Dr. Justina Gaidukevič, Vilnius University, Lithuania Invited speaker
13:55 – 14:10	**Evaluation of environmental hazard of nanocomposites for wastewater treatment (ANTIBIO)** Dr. Villem Aruoja, National Institute of Chemical Physics and Biophysics, Estonia	13:55 – 14:10	**Spray-pyrolysis synthesised TiO_2 thin films for photocatalytic air treatment from VOCs** Dr. Jekaterina Sydorenko, Tallinn University of Technology, Estonia
14:10 – 14:25	**Plasma electrolytic oxidation synthesis of heterostructured TiO_2 for photoanode applications** Dr. Ramūnas Levinas, Center for Physical Sciences and Technology, Lithuania	14:10 – 14:25	**Designing of sustainable building material made of non-fired clay with various biopolymers** Yahor Trambitski, Vilnius Gediminas Technical University, Lithuania
14:25 – 14:45	**Pulsed corona discharge plasma combined with photocatalytic oxidation technology for the degradation of volatile organic compounds in air** Dr. Juri Bolobajev, Tallinn University of Technology, Estonia, Invited speaker	14:25 – 14:45	**Hemicucurbituril-porphyrin supramolecular systems for pollutants sensing and remediation** Prof. Riina Aav, Tallinn University of Technology, Estonia Invited speaker

14:45 – 15:00	*Coffee break* ***(Foyer of the Nordic Hotel Forum Conference Center)***		
Parallel session 9: Novel Approaches for Pollution Management **(Room: Sirius)** Chair: Prof. Ivana Vinković Vrček, Institute for Medical Research and Occupational Health, Croatia,		**Parallel session 10:** Sustainable Chemistry **(Room: Capella)** Chair: Prof. Marina Kritsevskaja, Tallinn University of Technology, Estonia	
15:00 – 15:20	**Anthropogenic activities and microbial populations: war, peace or adaptation?** Prof. Eglė Lastauskienė, Vilnius University, Lithuania Invited speaker	15:00 – 15:20	**Use of neoteric solvents in biomass treatment** Dr. Mihkel Koel, Tallinn University of Technology, Estonia Invited speaker
15:20 – 15:35	**Metal-phenolic network-coated nanoparticles for reducing the toxicity of metal nanomaterials** Dr. Monika Mortimer, National Institute of Chemical Physics and Biophysics, Estonia	15:20 – 15:35	**Production and characterization of polysaccharides from *Rhodotorula toruloides*** Henrique Sepulveda Del Rio Hamacek, Tallinn University of Technology, Estonia
15:35 – 15:50	**Machine learning tools can pinpoint high-risk water pollutants** Helen Sepman, Stockholm University, Sweden	15:35 – 15:50	**Elemental composition, and isotope ratio in pine needles: the impact of arginine phosphate-containing fertilizer** Maris Bertins, University of Latvia, Latvia
15:50 – 16:05	**Microplastics in influents and effluents of Estonian wastewater treatment plants** Ayankoya Yemi Ayankunle, Tallinn University of Technology, Estonia	15:50 – 16:05	**Antioxidative and anti-Borrelia effects of Plantago species** Pille-Riin Laanet, Tallinn University of Technology, Estonia
16:05 – 16:20	**The water flea as a "canary in the coal mine" – Using phenotypic and molecular endpoints to understand pollution** Prof. Konstantinos Grintzalis, Dublin City University, Republic of Ireland	16:05 – 16:20	**The study of the uptake of chromium, zinc, cadmium and lead from spiked nutrient solution in tomato plants** Dr. Katarina Marković, Jožef Stefan Institute, Slovenia
16:20 – 16:35	**Use of the midge *Chironomus riparius* larvae in plastic's eco-toxicity studies and peculiarities of their responses** Dr. Alla Khosrovyan, National Institute of Chemical Physics and Biophysics, Estonia	16:20 – 16:35	**Overlooked residue of Li-ion battery recycling waste as high-value bifunctional oxygen electrocatalyst for Zn-air batteries** Dr. Kerli Liivand, National Institute of Chemical Physics and Biophysics, Estonia
16:35 – 16:55	**Importance of viruses in carbon and nitrogen cycles in aquatic ecosystems** Dr. Sigitas Šulčius, Nature Research Centre, Lithuania Invited speaker	16:35 – 16:55	**Sustainable chemistry through catalysis and process intensification** Prof. Henrik Grénman, Åbo Akademi University, Finland Invited speaker
17:00 – 17:15	Announcement of the **Best ECR Presentation and the Best ECR Poster Awards** and closing of the conference **(Room: Sirius)**		

Plenary Speakers

Monday, October 9 - Opening lecture at Welcome Reception:

Dr. Priit Zingel, Estonian University of Life Sciences

Dr Zingel is a senior researcher at Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Centre for Limnology, Estonia. He is qualified as a hydrobiologist, and his main interests are unicellular protozoa and the functioning of food webs in lakes. Recently, he has been involved in studying the nutrition and survival of fish larvae in both lakes and seas. In collaboration with National Institute of Amazonian Research (Brazil), he has participated in expeditions in the Brazilian Amazon to investigate the structure and functioning of food chains there. He has received a scientific award of Estonian Republic for his work and two awards of popularisation of science in Estonian University of Life Sciences.

Title: The Challenge of Understanding – from Protozoa to Ecosystems

Abstract: We live in the information age, but we still do not fully understand how the surrounding nature exchanges, collects, and manages information – both at the ecosystem and cellular levels. Still, it is crucial to possess a dynamic and developing comprehension of the functioning of nature in order to understand the phenomena occurring in our ever-changing world. Sometimes we encounter an attitude that there is no need to investigate anything further, everything has already been researched. This is a very dangerous attitude. Without information we should not actually talk about the age of information. And it is worth reviewing some knowledge that is deeply ingrained in us from time to time. Occasionally, it is worth taking a step back and consider whether we ourselves may have become trapped in dogmas that may hinder understanding.

This presentation focuses on various aspects of understanding nature, starting from single-celled organisms and ending with ecosystems. The topics that will be discussed among others are:

- infochemicals (how do they actually affect the biota?)
- a remarkable process of cellular computation (does this imply that cellular materials can show a primitive intelligence?)
- the ability of plants to influence wind patterns and bring in more moisture from the ocean (what will happen to us when we excessively deforest?)

Tuesday, October 10 - Dr. Steve Ellison, LGC, the UK National Measurement Laboratory for chemical and biological measurement

Dr Ellison is a Science Fellow at LGC, the UK National Measurement Laboratory for chemical and biological measurement. Originally qualified as a chemist, his current interests are primarily in statistics, measurement uncertainty and reference material certification. He is a co-author of EURACHEM guides on measurement uncertainty, metrological traceability and qualitative analysis and contributes to a range of IUPAC, ISO, CEN, BSI and other committees involving applications of statistics for measurement. He is the present Chair of the Eurachem Measurement Uncertainty and Traceability working group and also chairs a CCQM task Group on establishing key comparison reference values for international comparisons.

Title: Sampling, detection and uncertainty in environmental analysis – challenges and solutions

Abstract: Analytical measurements are increasingly vital to inform our understanding of our changing global environment and to support regulation of human activity that affects the environment. Environmental analysis, however, experiences particular challenges for measurement reliability. Most practical environmental measurements are carried out on relatively small samples and their results taken as indicators of the much larger area sampled. Environmental monitoring can span decades, and comparability and consistency of measurement results over time and across geographical regions is important for detecting real trends. Environmentally important contaminants – often the subject of regulations – are frequently present at very low levels, often stretching the detection capability of even today’s analytical methods and instrumentation. At low levels, with significant sampling variability, and perhaps especially when environmental measurement is contentious, it is important to understand and express uncertainties clearly and accurately so that reliable policy and regulatory decisions to be made. This, with any accompanying conformity assessment decisions, can be particularly challenging in the frequent cases where sampling and even measurement distributions are far from the familiar Normal distribution. Finally, the regulatory framework controlling laboratory operations has evolved over time; for example, validation of test methods has become increasingly important as new regulatory flexibility allows wider choice of measurement methods, including ‘in-house’ methods, subject to achieving specific performance criteria. Here, these issues will be discussed in the light of experience of some of the UK’s frameworks for environmental regulation and analysis, and with attention to some important Eurachem guidance for analytical practice.

Sampling is often the first step in many environmental measurements. It is also among the most variable, simply because environmental systems themselves are variable on scales from

kilometres to centimetres. Understanding the variation due to sampling is key to developing sound sampling strategies and for planning an appropriate allocation between sampling effort and analytical work. A recently updated Eurachem guide [1] provides guidance on determination of sampling uncertainty, including relatively simple and economical methods for assessing sampling variation using a simple method based on limited duplicate sampling and analysis [2]. The latest edition includes even more economical approaches, which use a ‘staggered nested’ experimental design to reduce analytical effort [3], and also includes recent methods for summarizing uncertainty when the measurement or sampling distribution is asymmetric [4].

Once sampled, attention turns to application of an appropriate, validated, analytical method. Validation procedures for environmental analysis in the UK are specified, for example, in the MCERTS performance standard for soil analysis [5]. This sets out a specific set of validation procedures, together with criteria for acceptable performance for a wide range of analytes. A recent example of such a validation study, for an in-house modification of a standard method, [6] illustrated some of the problems of achieving reliable results across different soil matrices, even with very precise methods (**Error! Reference source not found.**); clearly, some matrices can provide individual challenges (LGC6145 for nickel in **Error! Reference source not found.**). These pose practical difficulties for achieving performance and for reporting results and uncertainty.

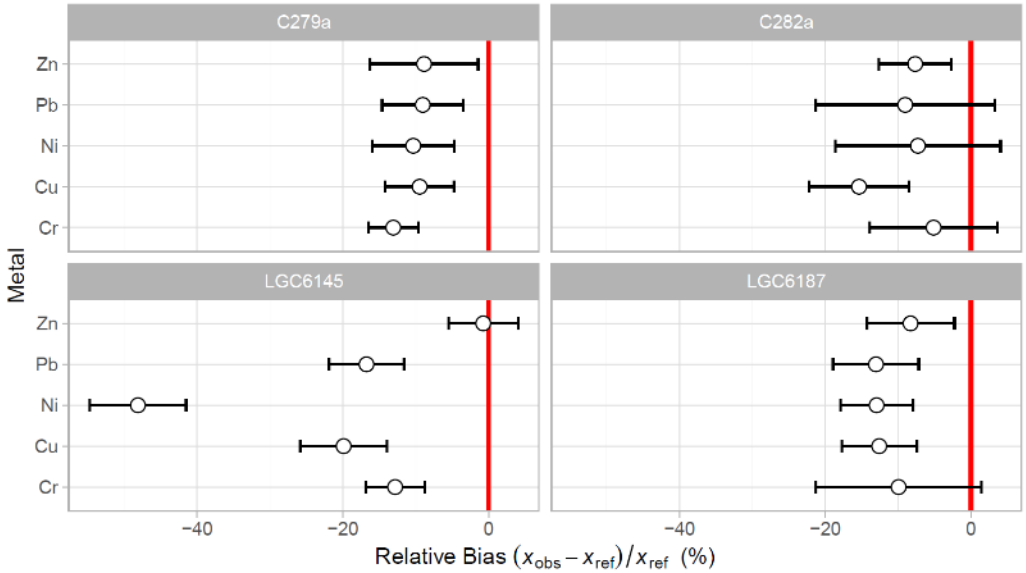

Figure 1. Relative bias for aqua regia extractable metals in four prepared soil materials. The vertical red line is at zero bias.

Detectability poses further problems, particularly for estimating averages and for summary reporting. For example, a preponderance of low levels and the wide use of ‘less than’ statements in summary emissions data for paper mills in the UK led to practical difficulties in assessing compliance with new environmental controls.

Finally, environmental analysis is not complete without a reported value, with associated measurement uncertainty and, where necessary, a statement of conformity. Measurement uncertainty evaluation is a requirement for accredited laboratories, even if it need not always be reported [8]. Originally a challenge for analytical laboratories unfamiliar with the concept, several guides exist to help with uncertainty evaluation, including a general Eurachem guide [9] and a NordTest guide specific to environmental laboratories [10]. There is also

comprehensive guidance on the use of uncertainty in conformity statements, for those laboratories required to assess conformity with a requirement [11, 12]. Recent studies show, however, that asymmetry of the kind found in environmental sampling distributions can adversely affect producer and consumer risks in conformity assessment [13], and some recent studies of the effect of asymmetry will be described.

Keywords: sampling uncertainty; measurement uncertainty; validation; detection capability

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Conflicts of Interest: The authors declare no conflict of interest

References

1. M H Ramsey, S L R Ellison and P Rostron (eds.) Eurachem/EUROLAB/ CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approaches. Second Edition, Eurachem (2019). ISBN (978-0-948926-35-8). Available from http://www.eurachem.org.
2. Ramsey M H (1998) Sampling as a source of measurement uncertainty: techniques for quantification and comparison with analytical sources. Journal of Analytical Atomic Spectrometry, 13, 97–104.
3. Rostron P and Ramsey M H (2012) Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA. Accreditation and Quality Assurance. 17, 1, 7-14. DOI:10.1007/s00769-011-0846-2.
4. Ramsey M H and Ellison S L R (2017) Combined uncertainty factor for sampling and analysis. Accreditation and Quality Assurance, 22(4), 187-189 DOI 10.1007/s00769-017-1271-y.
5. Environment Agency (2018) Performance Standard for Laboratories Undertaking Chemical Testing of Soil. version 5. Available from http://www.mcerts.net/
6. S.L.R. Ellison, M. Singh, M.G. Cox (2021) Measurement uncertainty for routine testing of metals in soil *in* Good practice in evaluating measurement uncertainty – Compendium of examples. Adriaan M.H. van der Veen and Maurice G. Cox (editors) (2021). EMUE project report. DOI: 10.5281/zenodo.5142180
7. S L R Ellison, V J Barwick, A Williams; Legislative limits below detection capability, Accred. Qual. Assur. 5 308-313 (2000)
8. ISO 17025:2017, General requirements for the competence of testing and calibration laboratories. ISO, Geneva (2017)
9. S L R Ellison and A Williams (Eds). Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (2012) ISBN 978-0-948926-30-3. Available from www.eurachem.org.
10. B. Magnusson, T. Näykki, H. Hovind, M. Krysell, E. Sahlin (2017), Handbook for calculation of measurement uncertainty in environmental laboratories, Nordtest Report TR 537 (ed. 4). Available from www.nordtest.info
11. ILAC G8:2019 – Guidelines on decision rules and statements of conformity. ILAC, Silverwater, Australia, 2019.
12. Williams and B. Magnusson (eds.) (2021) Eurachem/CITAC Guide: Use of uncertainty information in compliance assessment (2nd ed.). ISBN 978-0-948926-38-9. Available from www.eurachem.org
13. Kuselman, I., Shpitzer, S., Pennecchi, F. et al. Investigating out-of-specification test results of mass concentration of total suspended particulates in air based on metrological concepts—a case study. Air Qual Atmos Health 5, 269–276 (2012). https://doi.org/10.1007/s11869-010-0103-6

Wednesday, October 11 - Prof. Pu Chun Ke, National Institute for Nanotechnology Innovation, China

Pu Chun Ke is a Professor at the Nanomedicine Center of The Great Bay Area National Institute for Nanotechnology Innovation (Guangzhou, China) and an Adjunct Full Professor at Monash Institute of Pharmaceutical Sciences (Melbourne, Australia). Prof. Ke was a recipient of a prestigious International Senior Scientist Award (RFIS III, 2022) from the National Natural Science Foundation of China, the Inaugural Supervision Excellence Award (2019) from the ARC Center of Excellence at Monash Institute of Pharmaceutical Sciences in Australia, a Faculty Achievement in the Sciences Award (2012) from Clemson University, a CAREER Award (2008) from the National Science Foundation and an LJIS Postdoctoral Fellowship in Biophysics (2001-2003) from the University of California, San Diego in the United States. He has authored 168 peer-reviewed journal papers (senior author for 117 papers; 15 featured as journal covers) on protein corona, amyloidogenesis mitigation, nanomedicine, nanotoxicology and environmental science in journals including *Chemical Society Reviews* (3), *Nature Communications* (3), *JACS* (3), *PNAS* (3), *Advanced Materials* (1), *ACS Nano* (4), *Advanced Science* (3), *Environmental Science & Technology* (5), *Nano Letters* (4), *Nano Today* (4) and *Small* (11). His multidisciplinary research career has spanned over three continents and has been funded by NSF and EPA in the United States, by CSIRO and CBNS in Australia, and by NSFC and MOST in China. He has served on US, European and Australian federal grant panels, and as a frequent referee for 90 major journals. He is on the editorial boards of *Biophysical Chemistry* (Elsevier) and *ACS Nanoscience Au*.

Title: Nanoplastic-Biomolecular Interactions

Abstract: The global-scale production of plastics has been instrumental for sustaining the modern way of life, while the accumulation of plastics in landfills, oceans, and anything in between has become a major stressor on environmental sustainability, climate, and, potentially, human health. While mechanical and chemical forces of man and nature can break down and recycle plastics, our understanding of the biological fingerprints of discharged plastics, especially of the nanoscale derivatives of plastics (i.e., nanoplastics), remains superficial.

In 2010, we first reported on algal photosynthesis impaired by nanoplastic adsorption.[1] More recently, a host of studies have been conducted to elucidate the environmental implications of micro- and nanoplastics on the molecular, cellular, or whole organism level, typically from the toxicology point of view. In this talk, I will first introduce our early representative studies focused on nano-bio/environmental interactions.[2,3] I will then report on our recent finding that anionic polystyrene and poly(methyl methacrylate) nanoparticles can elicit disruptions to vascular endothelial cadherin junctions, a new phenomenon that is biophysical/biochemical and uncorrelated with cytotoxic events such as reactive oxygen species production, autophagy, and apoptosis.[4,5] The last part of my talk will be focused on the effects of nanoplastics on the aberrant aggregation of amyloid beta and alpha synuclein, two pathogenic proteins associated

with Alzheimer’s and Parkinson’s disease. This talk aims to demonstrate the vast research potential towards elucidating the implications of plastics for environmental sustainability and human health protection.

References

1. Bhattacharya, S. Lin, J. P. Turner and P. C. Ke, *J Phys Chem* C 114, 16556 (2010).
2. Lin, J. Reppert, Q. Hu, J. S. Hudson, M. L. Reid, T. A. Ratnikova, A. M. Rao, H. Luo and P. C. Ke, *Small* 5, 1128 (2009).
3. Qiao, A. P. Roberts, A. S. Mount, S. J. Klaine and P. C. Ke, *Nano Lett*. 7, 614 (2007).
4. Lee, N. Ni, H. Tang, Y. Li, W. Wei, A. Kakinen, X. Wan, T. P. Davis, Y. Song, D. T. Leong, F. Ding and P. C. Ke, *Adv. Sci*. 8 (2021), 2102519.
5. Wei, Y. Li, M. Lee, N. Andrikopoulos, S. Lin, C. Chen, D. T. Leong, F. Ding, Y. Song and P. C. Ke, *Nat. Comm*. 13 (2022), 4757.

Invited Speakers

Analytical Chemistry

Dr. Mihkel Koel

Tallinn University of Technology, Estonia (Home Page)

Prof. Mojca Bavcon Kralj

University of Ljubljana, Slovenia (Home Page)

Dr. Bertil Magnusson

Trollboken AB, Sweden (Home page)

Ecology

Prof. Ülo Niinemets

Estonian University of Life Sciences, Estonia (Home Page)

Prof. Eglė Lastauskienė

Vilnius University, Lithuania (Home Page)

Dr. Sigitas Šulčius

Nature Research Centre, Lithuania (Home Page)

Environmental Chemistry

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Vilnius University, Lithuania (Home Page)

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Prof. Jean-Manuel Raimundo

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Implications and Applications of Nanomaterials

Prof. Vera Slaveykova

University of Geneva, Switzerland (Home Page)

Dr. Marina Krichevskaya

Tallinn University of Technology, Estonia (Home Page)

Dr. Juri Bolobajev

Tallinn University of Technology, Estonia (Home Page)

Prof. Justina Gaidukevič

Vilnius University, Lithuania (Home Page)

List of Oral Presentations

Scientific and legal responsibility for the abstract belongs to the authors. Spelling and punctuation are kept without changes.

Abstracts of Oral Presentations

Hemicucurbituril-Porphyrin Supramolecular Systems for Sensing and Remediation

Riina Aav *, Victor Borovkov, Dzmitry Kananovich, Nele Konrad, Tatsiana Jarg, Mari-Liis Ludvig, Marko Šakarašvili

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Abstract: Hemicucurbiturils are members of the single-bridged cucurbituril family, formed through templated synthesis in a single step. This class of compounds is known for its ability to form inclusion complexes with electron-rich species. We have developed sustainable synthesis methods for chiral hemicucurbit[n]urils (where n = 6 or 8) and their derivatives [1-4]. Additionally, we have demonstrated that cyclohexanohemicucurbiturils can form external complexes with metalloporphyrins [5]. Porphyrins are well-known for their optical and photochemical properties, which are extensively utilized in sensing and catalysis applications. Chirality sensing adds another viewpoint to sensing systems and we have shown that toxic organocatalyst can be sensed via complex formation with Zn-porphyrins. [6] Merging chiral hemicucurbiturils and metalloporphyrins by non-covalent interactions into solid thin material, one can build an enantioselective electronic nose and have a very selective discrimination of different analytes and their handedness. [7] In this conference, we will present our findings on how hemicucurbiturils and porphyrins, as supramolecular receptors, can be employed for optical and gravimetric sensing, as well as for remediation of chemical pollutants.

Keywords: chiral receptor; pollutant; sensing; absorption; supramolecular systems

Author Contributions: Conceptualization, R.A..; methodology, R.A, V.B., D.K; investigation, N.K., T.J., M.-L. B. and M.Š.; writing—original draft preparation, R.A..; writing—review and editing, R.A..; project administration, R.A; funding acquisition, R.A. All authors have read and agreed to the published version of the abstract.

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References

1. Aav, R.; Shmatova, E.; Reile, I.; Borissova, M.; Topic, F.; Rissanen, K. *Organic Letters*, **2013**, 15 (14), 3786
2. Prigorchenko, E.; Öeren, M.; Kaabel, S.; Fomitšenko, M.; Reile, I.; Järving, I.; Tamm, T.; Topic, F.; Rissanen, K.; Aav, R. *Chem. Commun.*, **2015**, 51, 10921
3. Kaabel S., Stein R. S., Fomitsenko M., Jarving I., Friscic T., Aav R., *Angew. Chem. Int. Ed*., **2019**, 58, 6230 –6234
4. Dalidovich, T.; Mishra, K. A.; Shalima, T.; Kudrjašova, M.; Kananovich, D. G.; Aav, R. *ACS Sustainable Chem. Eng.* **2020**, 8, 41, 15703
5. Ustrnul, L.; Kaabel, S.; Burankova, T.; Martõnova, J.; Adamson, J.; Konrad, N.; Burk, P.; Borovkov, V.; Aav, R. *Chem. Commun.* **2019**, *55*, 14434
6. Konrad, N.; Horetski, M.;Sihtmäe, M. Truong, K.N.,Osadchuk, I.;Burankova,T.; Kielmann,M.; Adamson, J. Kahru, A.; Rissanen, K.;Senge, M.O.; Borovkov, V.; Aav, R.; Kananovich, D. *Chemosensors* **2021**, 9 (10), 278.
7. Magna, G.; Šakarašvili, M.; Stefanelli, M.;Giancane, G.; Bettini, S.; Valli, L.; Ustrnul, L.; Borovkov, V.; Aav, R.; Monti, D.;, Di Natale, C.; Paolesse, R. ACS Applied Materials & Interfaces 2023, 15, 25, 30674.

Evaluation of Environmental Hazard of Nanocomposites for Wastewater Treatment

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Abstract: Antibiotics are not completely metabolized nor degraded in the traditional wastewater treatment leaving a large proportion of them in the wastewater. Consequently, the water bodies become reservoirs of antibiotics, where antibiotic resistance can develop. Therefore, a new strategy for efficient, selective, and cost-effective removal of antibiotics from wastewater is being developed within the ANTIBIO project [1], using imprinted magnetic nanomaterials. However, in order to apply the new materials in large scale their potential harm to the environment has to be evaluated. We used aquatic ecotoxicology tests according to OECD/ISO to evaluate the environmental hazard of the novel nanocomposites. We used bacteria *Vibrio fischeri*, water fleas *Daphnia magna* and algae *Raphidocelis subcapitata* to study the toxicity of the nanocomposites as well as their component materials, including organic polymers, TiO_2, ZnO and magnetite (Fe_3O_4). The studied concentration range was 0.01-100 mg/l. Out of the tested samples only ZnO was toxic to all organisms with EC_{50} values (mg/l) 3.9 for *V. fischeri*, 3.3 for *D. magna* and 0.1 for *R. subcapitata*. When the inhibition was calculated based on mg Zn per liter, ZnO and $ZnSO_4$ were equally toxic, indicating shedding of Zn ions as a possible mechanism of ZnO toxicity. Magnetite was toxic to algae, EC_{50} 2.2 mg/l, however, the composite containing magnetite core was not harmful to algae. In contrast to our previous studies [2-4] TiO_2 did not inhibit algal growth at 100 mg/l and entrapment of algal cells within TiO_2 aggregates was not witnessed. According to our results the organic polymers as well as the TiO_2 produced within this study are environmentally not harmful while the toxicity of ZnO has to be considered when designing nanocomposites.

Keywords: nanomaterials; environmental toxicology; antibiotic; wastewater treatment

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Author Contributions: Conceptualization, A.K.; methodology, V.A., M.S., I.B..; writing—original draft preparation, V.A.; writing—review and editing, V.A., M.S.; visualization V.A, M.S.; project administration, funding acquisition, A.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. *ANTIBIO*. Available from: https://www.antibio.it/.
2. Aruoja, V., et al., *Toxicity of 12 metal-based nanoparticles to algae, bacteria and protozoa.* Environmental Science-Nano, 2015. **2**(6): p. 630-644. DOI: 10.1039/C5EN00057B
3. Bondarenko, O.M., et al., *Multilaboratory evaluation of 15 bioassays for (eco)toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID.* Nanotoxicology, 2016. **10**(9): p. 1229-42. DOI:10.1080/17435390.2016.1196251
4. Aruoja, V., et al., *Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae Pseudokirchneriella subcapitata.* Science of the Total Environment, 2009. **407**(4): p. 1461-1468. DOI: 10.1016/j.scitotenv.2008.10.053

Microplastics in Influents and Effluents of Estonian Wastewater Treatment Plants

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Abstract: This study is the first systematic investigation of microplastics in influents and effluents of Estonian wastewater treatment plants (WWTPs) using continuous sampling filtration method and FTIR-spectroscopy. The aim of the study was to evaluate the efficiency of WWTPs in removing microplastics from the treated water. In total, six WWTPs from all over Estonia were included in the study. For sampling, 14, 25 and 1450 L volume of wastewater was collected from the influents before and after the screen and effluents, respectively and filtrated over 24 h on three layers (5, 0.4, 0.33 mm) metal sieves. For microplastics analysis, organics of the sample was oxidized, and the sample transferred to 10 µm polycarbonate filter for microscope-aided characterization. Selected particles were identified by µFTIR. Contamination controls from sample collection, preparation and characterization steps were analyzed in parallel. Results showed that microplastics were present in all the samples, with the highest concentrations observed in the influent, sampled before the screen. The most common polymer types identified were polyester, polyethylene, and polypropylene. The study found that the treatment process was effective in removing larger microplastics size fractions but less effective in removing smaller ones. The obtained data are also important for estimating microplastics load that reaches the environment in WWTP sludge, used in agriculture and landscaping. According to Koelmans et al., (2019) [1], the results of the study can be considered of high quality and hence important for implementing microplastics mitigation strategies and control. The study established baseline levels of microplastics in influent and effluent of Estonian WWTPs**.**

Keywords: polymers; µFTIR; plastic pollution

Author Contributions: Conceptualization, A.Y.A. and M.H.; methodology, A.Y.A., M.H. and N.B.; software, A.Y.A., E.L., A.L. and M.H.; validation, A.Y.A., M. H., A.L. and N. B.; formal analysis, A.Y.A. and M.H.; investigation, A.Y.A., M.H., A.L. and N.B.; resources, A.Y.A., M.H., E. L. and K. P.; data curation, A.Y.A., E.L., N. B. and M.H.; writing—original draft preparation, A.Y.A. and M.H..; writing—review and editing, A.Y.A., E.L. and M. H.; visualization, A.Y. A., E.L. and M.H.; supervision, M.H., E. L. and K. P.; project administration, M.H., E. L. and K. P.; funding acquisition, M.H. and K. P. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Koelmans, A. A., Mohamed Nor, N. H., Hermsen, E., Kooi, M., Mintenig, S. M., & De France, J. (2019). Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Research, 155, 410–422. https://doi.org/10.1016/J.WATRES.2019.02.054

Droplet-Based Technology for Studying Phenotypic Effect of Microplastic on Antimicrobial Resistance

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Abstract: Plastic pollution is a global emergency [1][2]. One key problem is that microplastics (MPs) (1µm - 5mm) [3] and nanoplastics (NPs) (≤ 1µm) [4] enhance the already severe threat of antimicrobial resistance (AMR), by providing a micro-environment termed "plastisphere" for bacteria to form biofilm [5][6]. However, exact knowledge of the severity of plastisphere and its impact on AMR is currently still scarce [7]. We show how droplet-based technology can be used to study potential phenotypic effect of MPs on AMR. For this we used (i) polydisperse water-in-oil droplets generated via vortexing, (ii) GFB-labelled *Escherichia coli* JEK 1036 as our study object, (iii) Cefotaxime as the test antibiotic, and (iv) 10 µm carboxylated polystyrene microspheres (PS). In parallel, we encapsulated single cells of *E. coli* into droplets with different concentrations of Cefotaxime and with or without PS. After overnight incubation at 37°C, we imaged droplets as a monolayer via confocal microscopy and analyzed droplets via Software Ilastik [8], CellProfiler™ [9] and EasyFlow [10].

Our results show that *E. coli* minimal inhibitory concentration (MIC) shifts slightly towards a higher Cefotaxime concentration when PS is present in droplets. Image analysis of *E. coli* growth patterns in individual droplets illustrates that *E. coli* tends to clump together in droplets with PS, versus exhibiting an evenly distributed growth pattern in droplets without PS.

In conclusion, we see that PS in droplets enhances MIC of *E. coli* resistance against Cefotaxime. This possible enhanced resistance may be related to the observed tendency of clumping pattern (indication of biofilm formation) of *E. coli* when PS is present. Droplet-based technology is thus a suitable tool for studying phenotypic effect of MPs on AMR. Further experiments with different antibiotics and MPs types and sizes will shed more light on the interesting and worrying tendency of MP potentially enhancing AMR that was found in this study.

Keywords: Antimicrobial resistance; Microplastic pollution; Droplet microfluidics, Droplet-based technology, Biofilm formation, Plastisphere, Phenotypic analysis, Single cell incubation, Fluorescence microscopy

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Author Contributions: Conceptualization, S.B. and O.S.; methodology, S.B., F.S; and P.P.; software, S.B. and I.S.; validation, S.B. and F.S.; formal analysis, S.B., F.S. and O.S.; investigation, S.B. and O.S.; resources, O.S.; data curation, S.B. and I.S.; writing—original draft preparation, S.B.; writing—review and editing, S.B. and O.S.; visualization, S.B., F.S. and I.S.; supervision, S.B. and O.S.; project administration, O.S.; funding acquisition, S.B. and O.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. E. M. Veidis, A. D. LaBeaud, A. A. Phillips, and M. Barry, "Tackling the Ubiquity of Plastic Waste for Human and Planetary Health," *Am. J. Trop. Med. Hyg.*, vol. 106, no. 1, p. 12, Nov. 2022, doi:

10.4269/AJTMH.21-0968.
2. J. Brahney *et al.*, “Constraining the atmospheric limb of the plastic cycle,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 118, no. 16, Apr. 2021, doi: 10.1073/pnas.2020719118.
3. J. P. G. L. Frias and R. Nash, “Microplastics: Finding a consensus on the definition,” *Mar. Pollut. Bull.*, vol. 138, pp. 145–147, Jan. 2019, doi: 10.1016/j.marpolbul.2018.11.022.
4. J. Gigault *et al.*, “Current opinion: What is a nanoplastic?,” *Environ. Pollut.*, vol. 235, pp. 1030–1034, Apr. 2018, doi: 10.1016/j.envpol.2018.01.024.
5. Y. Zhang, J. Lu, J. Wu, J. Wang, and Y. Luo, “Potential risks of microplastics combined with superbugs: Enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system,” *Ecotoxicol. Environ. Saf.*, vol. 187, p. 109852, 2020, doi: 10.1016/j.ecoenv.2019.109852.
6. M. Imran, K. R. Das, and M. M. Naik, “Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat,” *Chemosphere*, vol. 215, pp. 846–857, Jan. 2019, doi: 10.1016/J.CHEMOSPHERE.2018.10.114.
7. S. Bartkova, A. Kahru, M. Heinlaan, and O. Scheler, “Techniques Used for Analyzing Microplastics, Antimicrobial Resistance and Microbial Community Composition: A Mini-Review,” *Front. Microbiol.*, vol. 12, p. 413, Mar. 2021, doi: 10.3389/FMICB.2021.603967/BIBTEX.
8. S. Berg *et al.*, “ilastik: interactive machine learning for (bio)image analysis,” *Nat. Methods*, vol. 16, pp. 1226–1232, 2019, doi: 10.1038/s41592-019-0582-9.
9. A. E. Carpenter *et al.*, “CellProfiler: image analysis software for identifying and quantifying cell phenotypes,” *Genome Biol.*, vol. 7, no. 10, p. R100, Oct. 2006, doi: 10.1186/gb-2006-7-10-r100.
10. I. Sanka *et al.*, “User-friendly analysis of droplet array images,” *Anal. Chim. Acta*, vol. 1272, pp. 341–397, Sep. 2023, doi: 10.1016/J.ACA.2023.341397.

Elemental Composition, and Isotope Ratio in Pine Needles: The Impact of Arginine Phosphate-Containing Fertilizer Application in Pine Planting Sites

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Abstract: Forests in Latvia are crucial, covering over half of the country's territory and expanding continuously through afforestation and natural growth. However, like forests globally, they face challenges requiring attention. Climate and biodiversity changes call for sustainable forest management practices different from those in the past to ensure the long-term health, resilience, and ecological value of Latvia's forests. This study focuses on investigating the impact of arginine phosphate containing fertilizer on nitrogen uptake, carbon content, and elemental concentrations in pine needles across different forest types in Latvia. By examining the effects of this fertilizer in the context of Latvia's specific forest-related issues, the research aims to contribute valuable insights into nutrient dynamics and concurrence in first years after planting. The study encompasses three distinct forest types: Vacciniosa, Aegopodiosa, and Myrtillosa. Soil treatment was implemented during planting of the seedlings, followed by the analysis of pine needle samples. Isotope ratio mass spectrometry and inductively coupled plasma mass spectrometry were employed to determine nitrogen and carbon mass fraction, nitrogen isotope ratio, and elemental concentrations. Chemometric analysis facilitated data evaluation. The findings reveal diverse patterns in nitrogen uptake and isotope ratio changes among the forest types. Aegopodiosa and Myrtillosa forests exhibited increased nitrogen mass fraction and decreased $\delta^{15}N$ values in pine needles, indicating arginine phosphate as the primary nitrogen source. Conversely, Vacciniosa forests displayed elevated $\delta^{15}N$ values in control samples, suggesting alternative nitrogen uptake due to low soil nitrogen content. All samples exhibited a significant increase in carbon content and a decrease in $\delta^{13}C$ values, associated with transplantation and environmental shifts. Aegopodiosa forests demonstrated the least variation in $\delta^{13}C$ values, indicating a more consistent response during transplantation. Chemometric analysis highlighted correlations between elemental concentrations, seedling age, and forest types [1]. This study highlights the importance of considering forest type and environmental conditions when assessing fertilizer efficacy. It provides insights into the varying effects on nitrogen uptake and carbon content in pine needles across different forest types in Latvia, contributing to our understanding of nutrient dynamics in forest ecosystems and guiding sustainable forest management practices.

Keywords: pine needles; arginine phosphate; isotope ratio; chemometric analysis

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Author Contributions: Conceptualization, M.B. and D.L.; methodology, A.V.,D.L., M.K.; software, M.B. and L.A.B.; validation, D.L., M.K. and A.V.; formal analysis, M.B., J.S., L.B., K.D., A.Z, V.V., T.A.S., and S.Z.; data curation M.B., J.S., L.B., K.D., A.Z, V.V., T.A.S., and S.Z.; resources, A.V., D.L. M.K.; writing—original draft preparation, M.B., J.S., L.A.B.; writing—review and editing, M.B., L.A.B., D.L., M.K., A.V.; visualization, M.B., J.S. and L.A.B.; supervision, D.L., M.K. and A.V. All authors have read and agreed to the published version of the manuscript.

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elements in cooperation with Latvian State Forest initiated research “Working methods and technologies for restoration, planting, care and protection of forest stands” No. 5-5.9.1_007O_101_21_77)

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bērtiņš, M., Buša, L., Lazdina, D., Dumins, K., Zake, S., Kļaviņš, M., Vīksna, A. Impact of Arginine Containing Fertilizer on Nitrogen Isotope Ratio and Elemental Content in Young Conifer Stands. *Key Engineering Materials* **2022**, Vol. 933, pp. 185–192. Trans Tech Publications, Ltd. https://doi.org/10.4028/p-558697

Detection of Mycotoxins and Pyrrolizidine Alkaloids in a Wide Variety of Nutritional Supplements Using the Multianalyte HPLC-MS/MS Method

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Abstract: Plant-based nutritional supplements are considered a natural means of enriching everyday diets, but they are also a source of various food contaminants such as mycotoxins [1] and pyrrolizidine alkaloids [2]. Both groups of food contaminants include chemical compounds that can significantly affect consumer health. In this context, the present study aimed to perform a multi-mycotoxin and pyrrolizidine alkaloid (PA) analysis (79 compounds in total) of 47 herbal dietary supplements containing at least one herbal ingredient. The extraction was performed by the QuEChERS method supplemented with the extract freezing-out procedure. Alkaloids were separated by a Luna Omega C18 column and quantified by TSQ Quantiva. The method's LOQs ranged from 0.25 to 500 µg kg^{-1} and recoveries ranged from 86% to 119%. The majority of samples contained detectable mycotoxins and PA. Total concentrations ranged up to 5 mg kg^{-1}. High concentrations of alternariol monomethyl ether (AME) and tentoxin were found with levels reaching up to 2479 µg kg^{-1} and 307 µg kg^{-1} respectively. As reported before, many emerging mycotoxins were detected, such as enniatin group mycotoxins and beauvericin as well as regulated mycotoxins – deoxynivalenol, T-2 and HT-2 toxins. Regarding PA, echinatine was determined at the highest concentrations reaching up to 790 µg kg^{-1} (on average 191 µg kg^{-1}), but the total PA concentration in positive samples was in the range of 0.62 – 1097 µg kg^{-1}. Two samples exceeded the maximum level of 400 µg kg^{-1} for such food supplements. Obtained estimated daily intakes for AME ranged from 2.4 to 2479 ng day^{-1} and contributed 1.4 – 1417 % of toxicological concern (TTC) value respectively. 4 of 19 positive samples exceeded the TTC value, and five samples represented 60% and higher of TTC. The daily intake of mycotoxins and pyrrolizidine alkaloids might significantly increase with the regular use of such nutritional supplements.

Keywords: Dietary supplements, mycotoxins, pyrrolizidine alkaloids

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Author Contributions: **Zane Bērziņa**: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, visualization; **Vadims Bartkevics**: supervision, project administration, funding acquisition, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Veprikova, Z., Zachariasova, M., Dzuman, Z., Zachariasova, A., Fenclova, M., Slavikova, P., Hajslova, J. Mycotoxins in Plant-Based Dietary Supplements: Hidden Health Risk for Consumers. *Journal of Agricultural and Food Chem.* **2015**, *63(29)*, 6633–6643.
2. Jansons, M., Fedorenko, D., Pavlenko, R., Berzina, Z., Bartkevics, V., Nanoflow liquid chromatography mass spectrometry method for quantitative analysis and target ion screening of pyrrolizidine alkaloids in honey, tea, herbal tinctures, and milk, *Journal of Chrom. A*, **2022**, 1676.

Formation and Bioaccumulation of Methylmercury Along Vertical and Spatial Gradients in Baltic Sea Waters

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Abstract: Methylmercury (MeHg) is a neurotoxic compound that bioaccumulates in the aquatic food web and causes threats to ecosystem viability and human health [1]. The processes for formation [2] and bioaccumulation [3] of MeHg are governed by complex interactions between chemical and biological factors. While these processes have been extensively studied, critical knowledge gaps remain and hamper quantitative predictions of environmental concentrations of MeHg, in particular for different environmental change scenarios [4]. The Baltic Sea is characterized by substantial spatial and vertical gradients in several of the key factors identified to control MeHg formation and bioaccumulation, most importantly redox conditions [5, 6], organic matter composition [7] and salinity [8]. Using such gradients as study objectives can advance the understanding of how future changes in critical parameters will impact MeHg cycling. In this presentation will be discussed (i) formation, (ii) incorporation at the base of the food web and (ii) magnification through the food web of MeHg across spatial and vertical gradients in the Baltic Sea. Controlling processes and factors and implications of environmental change scenarios will be highlighted. Focus will be on how redox conditions affect MeHg formation and how organic matter composition, directly and indirectly, affects incorporation and magnification of MeHg in the food web.

Keywords: Methylmercury; mercury methylation; bioaccumulation; redox stratification; organic matter composition.

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References

1. C.T. Driscoll, R.P. Mason, H.M. Chan, D.J. Jacob, N. Pirrone, Mercury as a Global Pollutant: Sources, Pathways, and Effects, Environ. Sci. Technol. 47(10) (2013) 4967-4983.
2. A.G. Bravo, C. Cosio, Biotic formation of methylmercury: A bio-physico-chemical conundrum, Limnol. Oceanogr. 65(5) (2020) 1010-1027.
3. K.C. Kidd, M.; Jardine, T., Bioaccumulation and biomagnification of mercury through food webs, in: G.C. Liu, Y.; O'Driscoll,N. (Ed.), Environmental chemistry and toxicology of mercury2012.
4. J.E. Sonke, H. Angot, Y.X. Zhang, A. Poulain, E. Bjorn, A. Schartup, Global change effects on biogeochemical mercury cycling, Ambio (2023).

5. A.L. Soerensen, A.T. Schartup, A. Skrobonja, S. Bouchet, D. Amouroux, V. Liem-Nguyen, E. Bjorn, Deciphering the Role of Water Column Redoxclines on Methylmercury Cycling Using Speciation Modeling and Observations From the Baltic Sea, Global Biogeochemical Cycles 32(10) (2018) 1498-1513.
6. E. Capo, C.Y. Feng, A.G. Bravo, S. Bertilsson, A.L. Soerensen, J. Pinhassi, M. Buck, C. Karlsson, J. Hawkes, E. Bjorn, Expression Levels of hgcAB Genes and Mercury Availability Jointly Explain Methylmercury Formation in Stratified Brackish Waters, Environ. Sci. Technol. 56(18) (2022) 13119-13130.
7. A.G. Bravo, S. Bouchet, J. Tolu, E. Bjorn, A. Mateos-Rivera, S. Bertilsson, Molecular composition of organic matter controls methylmercury formation in boreal lakes, Nat. Commun. 8 (2017) 14255.
8. B.L. Reinhart, K.A. Kidd, R.A. Curry, N.J. O'Driscoll, S.A. Pavey, Mercury bioaccumulation in aquatic biota along a salinity gradient in the Saint John River estuary, Journal of Environmental Sciences 68 (2018) 41-54.

Pulsed Corona Discharge Plasma Combined with Photocatalytic Oxidation Technology for the Degradation of Volatile Organic Compounds in Air

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Abstract: The anthropogenic impact on the environment has long been known to negatively affect the quality of air. Volatile organic compounds (VOCs) are widely used in domestic and industrial applications, generally as solvents. They are mobile in both gaseous and aqueous phases, and thus their spread in environment could have massive effect with dramatically negative consequences.

Pulsed corona discharge (PCD) and photocatalytic oxidation (PCO) are considered as efficient and eco-friendly methods for the energy-efficient abatement of gaseous hazardous pollutants. One of the main problems of PCD application in air treatment, however, is residual ozone, a side product of air ionization considered as secondary air pollution. Photocatalytic processes are known to degrade ozone extending simultaneously the photocatalyst lifetime. Thus, combining PCD and PCO in a two-step treatment system could solve the problem of the presence of residual ozone and complement each other's strengths. In this study, experiments were conducted in separate systems, i.e. photocatalysis and plasma, making a prerequisite for the progress in the combined PCD/PCO applications. A prototype PCO reactor was built and tested with ozone and 2-methoxyethanol (2ME) in combinations. 2ME was chosen as a hazardous model VOC used in industry in solvents and paints. For the PCD experiments xylene was tested. Being refractory air pollutant, extensively studied for its removal, xylene provides a basis for the comparison of its abatement methods. The PCD treatment showed unequalled energy efficiencies in gaseous xylene oxidation. With respect to PCO experiments, the degradation of 2ME and ozone was 40% and 95%, respectively. High ozone degradation performed by PCO confirms the expediency of proposed air cleaning combination.

Keywords: volatile organic pollutant; heterogeneous photocatalysis; pulsed corona discharge

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Polymeric Hydrogels for the Removal of Emerging Contaminants from Water

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Abstract: A proof of concept was herein reported, concerning the formulation of methacrylic and styrene-based hydrogels with adsorption properties versus emerging pollutants (Dyes, pesticides, pharmaceuticals). The synthesis of the hydrogels was achieved by functionalizing the HEMA monomer with specifically chelating groups such as amino acids (lysine and histidine) [1], cyclodextrins [2], and meglumine [3]. The as-prepared monomers were polymerized in water at different temperatures to obtain macroporous samples. All samples were characterized by chemical-physical and morphological analyses confirming the success of the reactions. The resulting systems were successfully tested to adsorb 2, 4 D, methylene blue, and lomefloxacin [1]. In addition, regeneration tests up to five cycles were performed, confirming the aptitude of samples to be used several times without losing efficiency and maintaining their mechanical properties.

Keywords: wastewater; purification; hydrogel; Photocatalysis; emerging contaminants

Author Contributions: "Conceptualization, S.C.C., and C.Z; methodology, G.C. and C.Z., validation, G.C. and C.Z., investigation, G.C. and C.Z.; data curation, S.C.C.; writing—original draft preparation, S.C.C.; writing—review and editing S.C.C.; funding acquisition, S.C.C.; All authors have read and agreed to the published version of the manuscript."

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References

1. Zagni, C.; Dattilo, S.; Mecca, T.; Gugliuzzo, C.; Scamporrino, A. A.; Privitera, V.; Puglisi, R.; Carroccio, S.C.; Single and dual polymeric sponges for emerging pollutants removal, *Eur. Pol. J.* **2022**, *179*, 111556 https://doi.org/10.1016/j.eurpolymj.2022.111556.
2. Zagni, C.; Coco, A.; Mecca, T.; Curcuruto, G.; Patamia, V.; Mangano, K.; Rescifina, A.; Carroccio S.C., Sponge-like macroporous cyclodextrin-based cryogels for controlled drug delivery, *Mater. Chem. Front.* **2023**, 7, 2693 https://doi.org/10.1039/D3QM00139C.
3. Mecca, T.; Ussia, M.; Caretti, D.; Cunsolo, F.; Dattilo, S.; Scurti, S.; Privitera, V.; Carroccio, S.C.; N-Methyl-D-Glucamine Based Cryogels as Reusable Sponges to Enhance Heavy Metals Removal from Water, *Chem. Eng. J.* **2020**, *399*, 125753, https://doi.org/10.1016/j.cej.2020.125753.

Nanocomposite Metal Oxide/Hydroxide Adsorbents for Advanced Wastewater Treatment and Toxicological Risk Assessment for the Aquatic Environment

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Abstract: Phosphorus (P) is a key nutrient for agriculture [1], but also an environmental pollutant causing eutrophication, commonly removed from wastewater [2]. Engineered nanostructured materials, predominantly metal oxides/hydroxides, are frequently reported as excellent adsorbents for phosphate [3], able to selectively remove P from wastewater to ultra-low concentrations, and facilitate P-recovery through reversible sorption [4], but their environmental safety is rarely addressed. This study assesses the ecotoxicological hazard of 10 highly-efficient metal oxide/hydroxide nanocomposite P-adsorbents using toxicity tests involving two different food-web level test organisms: the naturally bioluminescent marine bacterium *Vibrio fischeri* and the crustacean *Daphnia magna*. Nanocomposites were synthesized based on published procedures [5,6] via co-precipitation of 2-, 3- and 4-valent metal precursors (Zn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Zr^{4+}) at different molar ratios, and characterized with laser diffraction, ICP-OES, XRD and SEM. Among these, the pilot-scale tested ZnFeZr-6:1:1-oxyhydroxide [7] was modified by reducing the zinc-fraction to minimize leaching of toxic Zn^{2+} ions. Composites stability was investigated in deionized water and 2% NaCl (*V. fischeri* test medium) addressing agglomeration, settling and solubilization (release of metal ions and/or potentially hazardous nanoparticles). All composites, their filtered supernatants and precursor metal salts were evaluated for their toxic potency (half-effective concentration, EC_{50} and minimum bactericidal concentration, MBC) using 3 different tests: *Vibrio fischeri* 30-min bioluminescence inhibition assay (ISO-21338:2010), *V. fischeri* 24-h viability assay ('Spot test') and *Daphnia magna* 48-h acute immobilization test (OECD-202). Only the Zn-containing composites showed inhibitory effects to both organisms. Those with the highest zinc-fraction (ZnFeZr-18:5:1; ZnFeZr-10:1:1) were classified "harmful" to *V. fischeri* ($10<EC_{50}\leq100$ mg/L) and toxic to *D. magna* ($1<EC_{50}\leq10$ mg/L), therefore, environmentally unsafe for engineering applications. The ZnFeZr-6:1:1 (*V. fischeri* EC_{50}=118 mg/L; *D. magna* EC_{50}=7.7 mg/L) proved assumingly safe for both aquatic organisms once deposited on magnetic particles ZnFeZr-6:1:1@MPs (EC_{50} >>100 mg/L, MBC >1000 mg/L). All other composites without Zn were non-toxic, neither to *V. fischeri*, nor to the more sensitive *D. magna*.

Keywords: Aquatic toxicity; Magnetite; Metal-based adsorbents; Phosphorus recovery; Phosphorus removal; Safe-by-design; Nanomaterials; Wastewater effluent polishing; Zinc

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References

1. Withers, P.J.A.; Forber, K.G.; Lyon, C.; Rothwell, S.; Doody, D.G.; Jarvie, H.P.; Martin-Ortega, J.; Jacobs, B.; Cordell, D.; Patton, M.; Camargo-Valero, M.A.; Cassidy, R.. Towards resolving the phosphorus chaos created by food systems. *Ambio* **2020**, *49 (5)*, 1076–1089. DOI: https://doi.org/10.1007/s13280-019-01255-1
2. Dodds, W.K.; Bouska, W.W.; Eitzmann, J.L.; Pilger, T.J.; Pitts, K.L.; Riley, A.J.; Schloesser, J.T.; Thornbrugh, D.J. Eutrophication of U.S. freshwaters: analysis of potential economic damages. *Env. Sci. & Technol.* **2009**, *43 (1)*, 12–19. DOI: https://doi.org/10.1021/es801217q
3. Ooi, K.; Sonoda, A.; Makita, Y.; Torimura, M. Comparative study on phosphate adsorption by inorganic and organic adsorbents from a diluted solution. *J. Env. Chem. Eng.* **2017**, *5 (4)*, 3181–3189. DOI: https://doi.org/10.1016/j.jece.2017.06.015
4. Drenkova-Tuhtan, A.; Schneider, M.; Mandel, K.; Meyer, C.; Gellermann, C.; Sextl, G.; Steinmetz, H. Influence of cation building blocks of metal hydroxide precipitates on their adsorption and desorption capacity for phosphate in wastewater—A screening study. *Colloids Surf. A: Physicochem. Eng. Asp.* **2016**, *488*, 145–153. DOI: https://doi.org/10.1016/j.colsurfa.2015.10.017
5. Mandel, K.; Drenkova-Tuhtan, A.; Hutter, F.; Gellermann, C.; Steinmetz, H.; Sextl, G. Layered double hydroxide ion exchangers on superparamagnetic microparticles for recovery of phosphate from waste water. *J. Mater. Chem. A* **2013**, *1 (5)*, 1840–1848. DOI: https://doi.org/10.1039/C2TA00571A
6. Schneider, M.; Drenkova-Tuhtan, A.; Szczerba, W.; Gellermann, C.; Meyer, C.; Steinmetz, H.; Mandel, K.; Sextl, G. Nanostructured ZnFeZr oxyhydroxide precipitate as efficient phosphate adsorber in waste water: understanding the role of different material-building-blocks. *Environ. Sci.: Nano* **2017**, *4 (1)*, 180–190. DOI: https://doi.org/10.1039/C6EN00507A
7. Drenkova-Tuhtan, A.; Schneider, M.; Franzreb, M.; Meyer, C.; Gellermann, C.; Sextl, G.; Mandel, K.; Steinmetz, H. Pilot-scale removal and recovery of dissolved phosphate from secondary wastewater effluents with reusable ZnFeZr adsorbent @ Fe_3O_4/SiO_2 particles with magnetic harvesting. *Water Res.* **2017**, 109, 77–87. DOI: https://doi.org/10.1016/j.watres.2016.11.039

Nitrogen-Doped Reduced Graphene Oxide for Electrochemical Sensing Applications

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Abstract: Graphene-based derivatives, such as graphene oxide (GO) and reduced graphene oxide (rGO), have gained significant attention in the field of electrochemical sensors [1]. These materials offer several advantages, including a time-efficient and cost-effective synthesis procedure and unique chemical, physical, and electronic properties [2]. rGO-based materials, in particular, possess a high surface area, chemical stability, and electrical conductivity [3]. However, despite these favorable characteristics, the development of rGO-based sensors with high sensitivity and rapid response times remains a challenge. One of the promising strategies to meet this challenge. is the doping of rGO with heteroatoms. This approach involves introducing some atoms (e.g. N, B, P, or S) into the graphene lattice, which can modify the structural and electrochemical rGO properties [4]. Therefore, this study focuses on the synthesis and structural characterization of N-doped rGO-based materials and their application in electrochemical sensing of dopamine and H_2O_2. The rGO modification with nitrogen species was achieved using two different synthesis approaches. To functionalize the rGO surface with a cationic Bismarck Brown dye, a hydrothermal synthesis method was employed. Also, the rGO surface was modified with gaseous ammonia at temperatures of 950°C or 850°C for 8 or 4 h, respectively. The obtained materials were characterized by different methods (XPS, BET, SEM, and Raman spectroscopy). Electrochemical measurements, such as cyclic voltammetry and chronoamperometry were used to evaluate the obtained samples toward dopamine or H_2O_2 detection. The results demonstrated that various nitrogen species, including pyridinic-N, pyrrolic-N and quaternary-N, were detected in the N-doped rGO. Moreover, it was observed, that the amount and type of N-species introduced to the rGO surface contribute to the improved performance of the sensing platform, enabling the sensitive and selective detection of analytes.

Keywords: nitrogen doping; graphene oxide; dopamine; electrochemical sensor

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References

1. Suvarnaphaet P, Pechprasarn S. Graphene-Based Materials for Biosensors: A Review. *Sensors* **2017** 17(10):2161. https://doi.org/10.3390/s17102161.
2. Jiříčková A, Jankovský O, Sofer Z, Sedmidubský D. Synthesis and Applications of Graphene Oxide. *Materials (Basel)* **2022** 25;15(3):920. doi: 10.3390/ma15030920.
3. Ray S. C., Chapter 2 - Application and Uses of Graphene Oxide and Reduced Graphene Oxide. In *Applications of Graphene and Graphene-Oxide Based Nanomaterials*, Ray, S. C., Ed. William Andrew Publishing: Oxford, 2015; pp 39-55.
4. Arvas M. B., Gürsu H., Gencten M., Sahin, Y. Preparation of different heteroatom doped graphene oxide based electrodes by electrochemical method and their supercapacitor applications. *Journal of Energy Storage* **2021,** *35*, 102328. https://doi.org/10.1016/j.est.2021.102328

Sustainable Chemistry Through Catalysis and Process Intensification

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Abstract: The shift away from fossil resources is revolutionizing our industrial carbon sources and the developments in legislation demand increased overall efficiency in processes and emissions abatement. Catalysis plays a key role in enabling the green transition in the chemicals process industry and environmental protection. They also act as a bridge between chemical reactions and reaction mechanisms and moving from the molecular to the process scale. Besides enhancing reaction rates, increasing the selectivity is in key role and both of these factors are tightly linked also to the process design and optimization for which modern process intensification provides good tools. The current presentation displays three examples of combining heterogeneous catalysis with process intensification for wastewater treatment and direct conversion of CO_2 recently studied at our research group. The wastewater treatment includes removing of hemicelluloses from dilute biorefinery effluents with the help of catalytic aqueous phase reforming in a continuous reactor [1,2]. The second case focuses on removal of pharmaceuticals from communal wastewaters by combining ozonation with heterogeneous catalysis in a semi-batch reactor operating at ambient pressure [3-5]. In the case focusing on gas phase processing, CO_2 is converted to renewable natural gas utilizing a bi-functional catalytic material in a periodically operating continuous reactor concept [6-10]. Chromatography was used as the main analysis method in all of the experiments. High yields and good selectivity were obtained in all of the cases and the next steps are related to process optimization, stability testing and preparative studies for scale up studies. The obtained results display significant potential for green process technology and process efficiency by combining catalyst development with process design for being able to efficiently utilize effluent streams and minimize the effects on environment.

Keywords: heterogeneous catalysis; process intensification; effluents treatment; liquid and gas phase processing

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References

1. Aho, A.; Alvear, M.; Ahola, J.; Kangas, J.; Tanskanen, J.; Simakova, I.; Santos, J.; Eränen, K.; Salmi, T.; Murzin, D.Yu.; Grénman, H., Aqueous phase reforming of birch and pine hemicellulose hydrolysates, *Bioresource Technology* **2022**, *348*, 126809. https://doi.org/10.1016/j.biortech.2022.126809
2. Aho, A.; Õna, J.P; Rosales, C.; Eränen K.; Salmi, T.; Murzin, D. Yu.; Grénman, H. Biohydrogen from dilute side streams - influence of reaction conditions on the conversion and selectivity in aqueous phase reforming of xylitol. *Biomass and Bioenergy* **2020**, *138*, 105590. https://doi.org/10.1016/j.biombioe.2020.105590
3. Saeid, S.; Tolvanen, P.; Kumar, N.; Eränen, K.; Peltonen, J.; Peurla, M.; Mikkola, J.P.; Franz, A.; Salmi, T. Advanced oxidation process for the removal of ibuprofen from aqueous solution: A non-catalytic and catalytic ozonation study in a semi-batch reactor, *Appl. Catal. B Environ.* **2018**, *230*, 77–90. doi: 10.1016/j.apcatb.2018.02.021.
4. Saeid, S.; Kråkström, M.; Tolvanen, P.; Kumar, N. ; Eränen, K.; Mikkola, J.P.; Kronberg, L.; Eklund, P.; Peurla, M.; Aho, A.; Salmi, T. Advanced oxidation process for degradation of carbamazepine from aqueous solution: Influence of metal modified microporous, mesoporous catalysts on the ozonation process, *Catalysts* **2020**, *10(1)*, 90. doi.org/10.3390/catal10010090
5. Kråkström, M.; Saeid, S.; Tolvanen, P.; Salmi, T.; Kronberg, L.; Eklund, P. Catalytic ozonation of the antibiotic sulfadiazine: Reaction kinetics and transformation mechanisms, *Chemosphere.* **2020**, *247*, 1-12. doi.org/10.1016/j.chemosphere.2020.125853

6. Wei, L. Haije, W.; Grénman, H.; de Jong, W. Sorption enhanced catalysis for CO2 hydrogenation towards fuels and chemicals with focus on methanation *Heterogeneous Catalysis*, Elsevier, **2022**, 95-119.
7. Wei, L.; Azad, H.; Hamza; Haije, W.; Grénman, H.; de Jong, W. Pure methane from CO2 hydrogenation using a sorption enhanced process with Catalyst/Zeolite bifunctional materials, *Applied Catalysis B: Environmental* **2021**,120399. https://doi.org/10.1016/j.apcatb.2021.120399
8. Wei, L.; Grénman, H.; Haije, W.; Kumar, N.; Aho, A.; Eränen, K.; Wei, L.; de Jong, W. Sub-nanometer ceria-promoted Ni 13X zeolite catalyst for CO2 methanation *Applied Catalysis A: General* **2021**, *612*, 118012. https://doi.org/10.1016/j.apcata.2021.118012
9. Wei, L.; Haije, W,; Kumar, N.; Peltonen, J.; Peurla, M.; de Jong, W; Grénman, H., The influence of nickel precursors on the properties and performance of Ni impregnated zeolite 5A and 13X supported catalysts in CO2 methanation. *Catalysis Today* **2021**, *362*, 35-46. https://doi.org/10.1016/j.cattod.2020.05.025
10. Wei, L.; Haije, W.; Kumar, N.; de Jong, W; Grénman, H. Can bi-functional nickel modified 13X and 5A zeolite catalysts for CO2 methanation be improved by introducing ruthenium?, *Molecular Catalysis A* **2020**, *494*, 111115. https://doi.org/10.1016/j.mcat.2020.111115

The Water Flea as a "Canary in the Coal Mine" – Using Phenotypic and Molecular Endpoints to Understand Pollution

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Abstract: Assessment of pollution is a serious issue and a major consequence of the overgrowing human population and its activities. Focusing on the aquatic ecosystem, traditional approaches of water chemistry mainly provide a minimal monitoring with the detection of pollutants, while they fail to produce mechanistic or predictive insight. As such, effect-based methods have gained significant attention for the better mechanistic understanding of aquatic pollution. Among the key species used, daphnids have acquired a central position in aquatic toxicology and ecology. In this study, a novel feeding assay was developed and applied in a battery of exposures to different pollutants. Furthermore, in combination with biochemical markers and sensitive metabolomic analyses, the responses of daphnids following exposures were uncovered in molecular detail. Specific categories of metabolites were identified as significant indicators to predict pollution.

Keywords: molecular ecotoxicology; *Daphnia*; metabolomics; feeding; pharmaceuticals; pollutants

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Novel Plasticizers Are Emerging Contaminants

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Abstract: In recent years plastic use and pollution has gained a lot of attention. Plastic particles have been identified from the Mariana Trench [1] to high mountains [2] however the impact of plastic goes beyond that of particles [3]. Plastics are chemically very diverse. In addition to the principal polymeric component, plastics contain over 10 000 different additives [4] that may be non-intentional (e.g. production impurities) or intentional (functional) for obtaining certain properties. Flexibility is an important property for which plasticizers - a group of functional additives, are used. Until recently, phthalates have been the most used plasticizers but advanced knowledge on the hazardous properties of several commonly used phthalate plasticizers has led to their strict regulation in the European Economic Area since 2020. This has created the need for alternative plasticizers, especially in sensitive human applications. Plastic industry has started producing and using novel non-phthalate plasticizers and high molecular weight phthalate plasticizers to the extent they have become emerging contaminants [5, 6]. Due to hydrophobicity, plasticizers sorb to particulate matter and tend to accumulate in e.g. wastewater treatment sludge. In the current research, sludge from wastewater treatment plants was sampled from all over Estonia (20 samples) to analyse occurrence of selected novel plasticizers in the Estonian environment. Samples were analysed by gas chromatography–mass spectrometry (GC-MS). The first results showed that the most used DEHT (Bis(2-ethylhexylterephthalate), DPHP (Di(2-propylheptyl)phthalate), DINCH (1,2-cyclohexanedicarboxylic acid diisonyl ester) were above the GC-MS quantification limits in the majority of the analysed samples and their levels were comparable to those of the regulated phthalate plasticizer DEHP (Bis(2-ethylhexyl) phthalate).

Keywords: phthalates, wastewater sludge, DEHP, DPHP, DEHT, DINCH, plastic

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References

1. Jamieson, A. J., Brooks, L. S. R., Reid, W. D. K., Piertney, S. B., Narayanaswamy, B. E. and Linley, T. D. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *Roy Soc Open sci.* **2019**. 6(2). https://doi.org/10.1098/rsos.180667
2. Tsering, T., Sillanpää, M., Viitala, M., Reinikainen, S-P. Variation of microplastics in the shore sediment of high-altitude lakes of the Indian Himalaya using different pretreatment methods. *Sci Total Environ*. **2022**. 849, 157870. https://doi.org/10.1016/j.scitotenv.2022.157870
3. Zimmermann, L., Dierkes, G., Ternes, T.A., Völker, C., Wagner, M. Benchmarking the *in Vitro* Toxicity and Chemical Composition of Plastic Consumer Products. *Envir Sci Tech.* **2019** 53 (19), 11467-11477. DOI: 10.1021/acs.est.9b02293
4. Wiesinger, H., Wang, Z., Hellweg, S. Deep Dive into Plastic Monomers, Additives, and Processing Aids. *Envir Sci Tech*. **2021.** 55 (13), 9339-9351. DOI: 10.1021/acs.est.1c00976
5. Lemke, N., Murawski, A., Lange, R., Weber, T., Apel, P., Dębiak, M., Koch, H.M., Kolossa-Gehring, M. Substitutes mimic the exposure behaviour of REACH regulated phthalates – A review of the German HBM system on the example of plasticizers. *Int J Hyg Envir Heal*. **2021**. 236,

113780. https://doi.org/10.1016/j.ijheh.2021.113780
6. Nagorka, R., Birmili, W., Schulze, J., Koschorreck, J. Diverging trends of plasticizers (phthalates and non-phthalates) in indoor and freshwater environments—why? *Environ Sci Eur*. **2022**. 34, 46. https://doi.org/10.1186/s12302-022-00620-4

Innovative Nanocomposites Made of Polymers and Semiconductor Photcatalysts for Wastewater Treatment

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Abstract: An improvement of water supply and sanitation and a better management of water resources, especially in terms of water reuse, is one of the priority of the European Green Deal. In this context, it is crucial to find new strategies to recycle wastewater efficiently, in a low-cost and eco-friendly manner. The immobilization of inorganic nanomaterials on polymeric matrices has been drawing a lot of attention in the recent years due to the extraordinary properties characterizing the as-obtained nanocomposites. The hybrid materials, indeed, combine the properties of the polymers such as flexibility, low-cost, mechanical stability, high durability, ease of availability, with the properties of the inorganic counterpart. In particular, if the inorganic fillers are nanostructured photocatalysts, the materials will be able to utilize the energy delivered by light to catalyze chemical reactions for an efficient wastewater treatment [1-4]. Additionally, since the anchoring of the nanomaterials to the polymers, the dispersion of the materials in the environment is prevented, thus overcoming one of the main limits that impedes the application of nanostructured photocatalysts on a large scale. In this talk, I will present nanocomposites, made of polymers (polymethyl methacrylate - PMMA), photocatalytic semiconductors (ZnO or TiO_2 in the forms of nanoparticle). MoS_2 nanoparticles were also added as co-catalyst, to improve the photocatalytic performance of the ZnO or TiO_2. The hybrid materials were prepared by the sonication and solution casting method. The nanocomposites were deeply characterized and their remarkable photocatalytic abilities were evaluated by the degradation of several organic water pollutants (such as, dyes and drugs). The acting mechanisms were investigated. The relevance of the obtained results will be discussed, opening the route for the application of these materials in photocatalysis, and especially for novel wastewater remediation.

Keywords: nanomaterials; nanocomposites; photocatalysis; environment; water.

Author Contributions: Conceptualization, M.C. and G.I.; methodology, G.I.; investigation, M.C., V.S., M.Z. and V. S.; data curation, M.C., V.S., M.Z. and V. S.; writing—original draft preparation, G.I.; writing—review and editing, M.C., V.S., M.Z. and V. S.; supervision, G.I.; funding acquisition, G.I. All authors have read and agreed to the published version of the manuscript.

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References

1. Cantarella, M.; Impellizzeri, G.; Di Mauro, A.; Privitera, V.; Carroccio, S. C. Innovative Polymeric Hybrid Nanocomposites for Application in Photocatalysi. *Polymers* **2021**, *13*, 1184 1-22. https://doi.org/10.3390/polym13081184
2. Di Mauro, A.; Farrugia, C.; Abela, S.; Refalo, P.; Grech, M.; Falqui, L.; Nicotra, G.; Sfuncia, G.; Mio, A.; Buccheri, M. A.; Rappazzo, G.; Brundo, M. V.; Scalisi, E. M.; Pecoraro, R.; Iaria, C.; Privitera, V.; Impellizzeri, G. Ag/ZnO/PMMA Nanocomposites for Efficient Water Reuse. *ACS Appl. Bio. Mater.* **2020**, *3*, 4417-4426. https://dx.doi.org/10.1021/acsabm.0c00409
3. Ussia, M.; Di Mauro, A.; Mecca, T.; Cunsolo, F.; Nicotra, G.; Spinella, C.; Cerruti, P.; Impellizzeri, G.; Privitera, V.; Carroccio, S. C. ZnO-pHEMA Nanocomposites: An Ecofriendly and Reusable Material for Water Remediation. *ACS Appl. Mater. Interfaces* **2018**, *10*, 40100-40110. http://dx.doi.org/10.1021/acsami.8b13029
4. Di Mauro, A.; Cantarella, M.; Nicotra, G.; Pellegrino, G.; Gulino, A.; Brundo, M. V.; Privitera, V.; Impellizzeri, G. Novel synthesis of ZnO/PMMA nanocomposites for photocatalytic applications. *Sci. Rep.* **2017**, 7:40895 1-12. http://dx.doi.org/10.1038/srep40895

From Organometallic Chemistry to Multifunctional Nanoparticle-Based Devices for Gas Detection and Degradation of Air Pollutants

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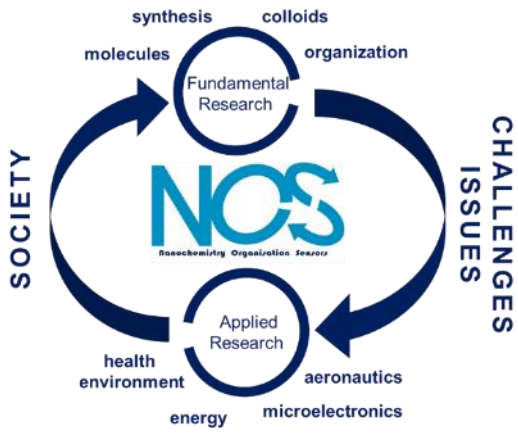
Abstract: Considering the climate, societal and health related current and emerging issues facing the world, our group as part of the (nano-)material science community will play a part in providing materials and technology, that can tackle these issues. Our strategy focuses on the design and development of complex hybrid nano-objects and nano-materials with unprecedented properties, with the aim of developing functional and innovative solutions to societal challenges. To achieve this, we are applying organometallic approach for the synthesis of well-defined nanoparticles (NPs) and nanomaterials [1]. This bottom up approach allows to control the NPs synthesis (size, shape, colloidal stability) on a molecular level with the help of cleverly designed starting molecular precursor(s), under mild reaction conditions and in safe-by-design approaches [2]. The presentation will focus on our team’s research related to the synthesis and properties of NPs and nanomaterials, their implementation into devices for either gas detection (i.e. sensors based on Cu [3], Zn [4,5] and Sn oxide NPs [6,7]) or degradation of air pollutants [8], and the interconnection between different fields (chemistry, physical chemistry, physics and biology).



Keywords: organometallic approach; nanoparticles; mild conditions; size control; pollutant degradation

References

1. Amiens, C.; Chaudret, B.; Ciuculescu-Pradines, D.; Collière, V.; Fajerwerg, K.; Fau, P.; Kahn, M.; Maisonnat, A.; Soulantica, K.; Philippot, K. Organometallic approach for the synthesis of nanostructures. *New J. Chem.* 2013, *37*, 3374-3401.
2. Carnide, C.; Champouret, Y.; Valappil, D.; Vahlas, C.; Mingotaud, A.-F.; Clergereaux, R.; Kahn, M.L. Secured Nanosynthesis–Deposition Aerosol Process for Composite Thin Films Incorporating Highly Dispersed Nanoparticles. *Adv. Sci.*, 2022, 2204929
3. Jońca, J.; Ryzhikov, A.; Palussière, S.; Esvam J.; Fajerwerg, K.; Menini, P.; Kahn, M.L.; Fau, P. Organometallic Synthesis of CuO Nanoparticles: Application in Low-Temperature CO Detection. *ChemPhysChem* 2017, *18*, 2658-2665.
4. Jońca, J.; Ryzhikov, A.; Kahn, M.L.; Fajerwerg, K.; Chaudret, B.; Chappelle, A.; Menini, P.; Fau, P. Shape-controlled ZnO Nanistructures for Gas Sensing Applications. *Procedia Eng.* 2014, *87*, 907-910.
5. Ryzhikov, A.; Jońca, J.; Kahn, M.; Fajerwerg, K.; Chaudret, B.; Chappelle, A.; Menini, P.; Shim, C.H.; Gaudon, A.; Fau. P. Organometallic synthesis of ZnO nanoparticles for gas sensing: towards selectivity through nanoparticles morphology. *J. Nanopart. Res.* 2015, *17*, 280.
6. Jońca, J.; Ryzhikov, A.; Fajerwerg, K.; Kahn, M.L.; Chaudret, B.; Chappelle, A.; Menini, P.; Fau, P. A Novel SnO_2 Sensor and its Selectivity Improvement with Catalytic Filter. *Procedia Eng.* 2014, *87*, 923-926.
7. Jońca, J.; Ryzhikov, A.; Kahn, M.L.; Fajerwerg, K.; Chappelle, A.; Menini, P.; Fau, P. SnO_2 “Russian Doll” Octahedra Prepared by Metalorganic Synthesis: A New Structure for Sub-ppm CO Detection. *Chem. Eur. J.* 2016, *22*, 10127-10135.
8. Castelló Lux, K.; Hot ; J; Fau, P.; Bertron, A.; Kahn, M.L.; Ringot, E.; Fajerwerg, K. Nano-gold decorated ZnO: An alternative photocatalyst promising for NO_x degradation. *Chem. Eng. Sci.* 2023, *267*, 118377.

The Environmental Safety Aspects of Technologically Powerful Materials Are Often Overlooked

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Abstract: Novel materials and their combinations are the basis of the societal progress: stone - Stone Age, bronze - Bronze Age, iron - Iron Age. For the current stage of the development of mankind, there is not yet a commonly agreed strategic key material (silicon, polymers, graphene, nano*) but it is generally agreed that novel materials and their combinations are creating the basis of the technological progress.

Not all technologically powerful materials are intrinsically safe and may harm humans and our surrounding ecosystems already at relatively low concentrations (copper, silver, zinc, cadmium, lead, mercury, nickel, chromium, platinum, lithium, cobalt). That does not mean that intrinsically harmful materials cannot be harnessed to offer mankind new developments (incl. for the generation of green energy and destruction of environmental pollutants). Indeed, some of the intrinsically harmful materials are highly conducting electricity (copper), have magnetic properties (cobalt, nickel, neodymium), or possess multiple technologically beneficial properties (graphene). However, progress cannot be built threatening the health of people and the environment. To find a balance between venture and precaution, the environmental fate and safety aspects of technologically powerful materials can no longer be overlooked to be in line with the UN Sustainable Development Goals, The Green Deal, and One Health programs. In addition, environmental toxicity data are imperatively needed for all materials sold or marketed in Europe in large quantities as regulated by REACH legislation. Moreover, the data on the toxicity of almost all elements in the periodic table as well as on plenty of organic compounds to conduct the initial risk assessment are available in various databases and scientific resources. Unfortunately, the communities of material scientists and engineers who create novel materials and devices and environmental scientists who have the knowledge on harmful effects of materials are educated separately and do not share the same information space also in their professional life. Due to that, there is a big risk that the novel technologies will be introduced on large scale before their environmental aspects (but also human health aspects) have been deeply evaluated. To avoid that, the holistic approach covering also safety aspects [1] is needed while novel technologies are planned and designed, analogous to that applied in nanomaterials safety research about 20 years ago when physicists, biologists, chemists, material scientists, environmental scientists and medical doctors joined forces in analysis of potential harmful effects of nanomaterials - cornerstones for nanotechnologies [2,3].

Keywords: environmental hazard, heavy metals, rare earth elements, green technologies, Green Deal, Sustainable-by-Design, One Health

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References

1. Bondarenko, O., Mortimer, M., Kahru, A., Feliu, N., Javed, I., Kakinen, A., Lin, S., Xia, T., Song, Y.,

Davis, T.P., Lynch, I., Parak, W.J., Leong, D.T., Ke, P.C., Chen, C., Zhao, Y. Nanotoxicology and nanomedicine: The Yin and Yang of nano-bio interactions for the new decade. *Nano Today* **2021**, *39*, 101184. https://doi.org/10.1016/j.nantod.2021.101184

2. Kahru, A., Dubourguier, H.-C. From ecotoxicology to nanoecotoxicology, *Toxicology* **2010**, *269*, 105-119. https://doi.org/10.1016/j.tox.2009.08.016
3. Kahru, A., Ivask, A. Mapping the dawn of nanoecotoxicological research, *ACC Chem Res* **2013**, *46*, 823-833. https://doi.org/10.1021/ar3000212

A Chimie Douce Route to Layered Double Hydroxides

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Abstract: Recently layered double hydroxides (LDHs) have attracted substantial attention due to a wide range of important application areas, e.g. catalysis, photochemistry, biomedical science and environmental applications [1, 2]. LDHs can be fabricated by different synthesis methods. The most common preparation techniques are co-precipitation [3] and anion-exchange [4] methods. The aim of this study is to show the advantages of Chimie Douce route to LDHs. The indirect sol-gel synthesis route for the preparation of LDHs recently was developed and suggested [5]. The synthesized precursor gels were converted to the mixed metal oxides (MMO) by heating the gels at 650 °C. The LDHs were fabricated by reconstruction of MMO in water at 80 °C. In this study, the co-precipitation and novel indirect sol-gel synthesis techniques for the preparation of Mg-Al LDHs were compared and luminescent properties have been investigated. The peculiarities of intercalation of organic anions to the LDHs structures were also studied. In conclusion, the proposed sol-gel synthesis route for LDHs shows some benefits over the co-precipitation method such as simplicity, high homogeneity and good crystallinity of the end synthesis products, effectiveness, cost efficiency and suitability for different systems. It was also demonstrated, that the luminescence of lanthanide element in the $Mg_3Al_{1-x}RE_x$ could be induced by intercalation of organic reagents to the LDHs structure. The Mg_3Al LDH coatings have been also successfully fabricated using the same sol-gel processing route.

Keywords: layered double hydroxides 1; sol-gel synthesis 2; optical properties

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Author Contributions: Conceptualization, A.K.; methodology, A.K. and D.S.; formal analysis, D.S.; investigation, D.S.; writing—original draft preparation, A.K.; writing—review and editing, A.K. All authors have read and agreed to the published version of the manuscript.

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References

1. Mohanty, U.A.; Sahoo, D.P.; Paramanik, L.; Parida, K. A critical review on layered double hydroxide (LDH)-derived functional nanomaterials as potential and sustainable photocatalysts. *Sustain. Energy Fuels*. **2023**, *7*, 1145-1186.
2. Constantino, V.R.L.; Figueiredo, M.P.; Magri, V.R.; Eulalio, D.; Cunha, V.R.R.; Alcantara, A.C.S.; Perotti, G.F. Biomaterials Based on Organic Polymers and Layered Double Hydroxides Nanocomposites: Drug Delivery and Tissue Engineering. *Pharmaceutics.* **2023**, *15*, 413.
3. Theiss, F.L.; Ayoko, G.A.; Frost, R.L. Synthesis of layered double hydroxides containing Mg^{2+}, Zn^{2+}, Ca^{2+} and Al^{3+} layer cations by co-precipitation methods-A review. *Appl. Surf. Sci.* **2016**, *383*, 200-213.
4. Kameda, T.; Takaizumi, C.; Kumagai, S.; Saito, Y.; Yoshioka, T. Preparation of Zn-Al layered double hydroxide intercalated with carboxymethyl-beta-cyclodextrin by anion exchange method and its Ni^{2+} adsorption property. *Soft Mater.* **2020**, *19*, 139-147.
5. Sokol, D.; Salak, A.N.; Ferreira, M.G.S.; Beganskiene, A.; Kareiva, A. Bi-substituted Mg_3Al-CO_3 layered double hydroxides. *J. Sol-Gel Sci. Technol.* **2018**, *85*, 221-230.

Silver-Chitosan Nanocomposites for Biomedical Application: Design, Synthesis and Antimicrobial Efficiency

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Abstract: Hospital-acquired infections are serious medical problems worldwide. Therefore, novel antimicrobials for the treatment of infections, especially those caused by antibiotic-resistant microbes are urgently needed. We have previously shown that Ag, CuO and ZnO nanoparticles are toxic also against pathogenic microbes and relatively safe to animal cells [1] thus being promising for medical use, e.g. in wound treatment. Moreover, combining these NPs with biologically active polymers may enhance their efficacy and specificity. Chitosan (CS) is a biocompatible, antimicrobial and immuno-modulating polymer and is already used for wound treatments. Therefore, crosslinking of chitosan with antimicrobial nanoparticles can yield novel antimicrobials with both, biocidal and immune-modulating effects.

The study aimed (i) to design and synthesize silver-chitosan nanocomposites (nAgCSs) with different silver-chitosan (Ag/CS) weight ratios (1:0.3, 1:1 and 1:3), (ii) to evaluate their efficacy against bacteria and fungi which can cause wound infections and (iii) to elucidate the mode of antimicrobial action of nAgCSs.

nAgCSs were synthesized by reduction of $AgNO_3$ with trisodium citrate and stabilized/coated with the low molecular weight chitosan. The antimicrobial activity of nAgCSs against bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* and fungi *Candida albicans* and *C. glabrata* was studied using a Spot test [2]. In this test, microbes are exposed to toxicants in deionized water for 1, 4 and 24 h and then plated on an agar medium for the quantification of the minimum biocidal concentration (MBC).

The synthesized nAgCSs' primary and hydrodynamic sizes were ~50 and ~100 nm, respectively, and the surface charge was ~+25 mV. The shedding of Ag ions was in the range of 2–4%.

The synthesized nAgCSs were efficient antimicrobials acting already at sub-mg per litre concentrations. In general, the nAgCSs were more toxic towards bacteria than fungi (24-h MBC 0.07–0.56 and 9.3–44 mg Ag/L, respectively) and nAgCSs with an Ag/CS mass ratio of 1:3 were the most efficient. The high antimicrobial efficiency was most likely due to the absorption of nAgCSs onto the surface of the microbes as shown by confocal laser scanning microscopy and flow cytometry. Interestingly, the shed Ag ions (the most reported toxicity mechanism of AgNPs) did not explain the biocidal effect of nAgCSs indicating a synergy between chitosan and silver.

Keywords: wound infections, antimicrobial resistance, bacteria, fungi, novel antimicrobials, silver, chitosan, nanocomposites, synergy, particle-cell interactions.

Author Contributions: Conceptualization, K.K. and A.K.; methodology, formal analysis and investigation, K.K.; J.L, M.S., H.V., I.K.; M.O.; writing, project administration and funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

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References

1. Suppi, S.; Kasemets, K.; Ivask, A.; Künnis-Beres, K.; Sihtmäe, M.; Kurvet, I.; Aruoja, V.; Kahru, A. A novel method for comparison of biocidal properties of nanomaterials to bacteria, yeasts and algae. Journal of Hazardous Materials 2015, 286, 75−84. DOI: 10.1016/j.jhazmat.2014.12.027.
2. Bondarenko, O.; Juganson, K.; Ivask, A.; Kasemets, K.; Mortimer, M.; Kahru, A. Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. Archives of Toxicology 2013, 84, 1181−1200. DOI: 10.1007/s00204-013-1079-4.

Use of the Midge *Chironomus riparius* Larvae in Plastic's Ecotoxicity Studies and Peculiarities of Their Responses

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Abstract: *Chironomus riparius*, a standard text organism [1], has been widely used for the assessment of the ecotoxicity of metal and organic contaminants in aquatic systems. Due to both aquatic and sediment-based developmental stages, the midge larvae can be exposed to both waterborne and sediment-bound contaminants and hence represents a versatile model for toxicity studies. Recently, *C. riparius* has also been widely used in the environmental risk assessment of microplastic contamination in water bodies. The responses of chironomid larvae which were experimentally exposed to nano or microplastic particles were shown to range from molecular to whole organism level [e.g., 2,3, 4]. However, these responses have not been strongly related to the microplastics concentrations used for exposure or load within the organism (the number of ingested particles). Instead, although the presence of microplastic particles in the gut of *C. riparius* was demonstrated [5,6], the populations of *C. riparius* which were continuously exposed to microplastic during three generations showed recovery after the first generation [7]. Moreover, despite the greater number of ingested particles at higher microplastic concentration (1 g kg^{-1}), compared to the 10 times lower concentration (0.1 g kg^{-1}), the emergence of adult midges in both cases was not significantly different from each other [6]. In this work, a review of the responses of *C. riparius* to plastic exposure based on published literature and own data is provided and peculiarities of the observed responses are discussed.

Keywords: multi-generation; UV-weathering.

Author Contributions: "Conceptualization, A.K. (Alla Khosrovyan); methodology, A.K. (Alla Khosrovyan and Anne Kahru); formal analysis, A.K. (Alla Khosrovyan), investigation, A.K. (Alla Khosrovyan); resources, A.K. (Anne Kahru).; writing—original draft preparation, A.K. (Alla Khosrovyan); writing—review and editing, A.K (Anne Kahru); project administration, A.K. (Alla Khosrovyan and Anne Kahru); funding acquisition, A.K. (Alla Khosrovyan and Anne Kahru). All authors have read and agreed to the published version of the manuscript."

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References

1. Organization for Economic CO-operation and Development (OECD). 2010. Sediment-water chironomid life-cycle toxicity test using spiked water or spiked sediment. Test N 233.
2. Silva, C.J, M., Silva, A.L.P.., Gravato, C., Pestana, J.L.T., 2019, Ingestion of small-sized and irregularly shaped polyethylene microplastics affect Chironomus riparius life-history traits. Sci Total Environ. 672:862-868. https://doi.org/10.1016/j.scitotenv.2019.04.017
3. Khosrovyan, A., Doria, H.B., Kahru, A., Pfenninger, M., 2022, Polyamide microplastic exposure elicits rapid, strong and genome-wide evolutionary response in the freshwater non-biting midge Chironomus riparius, Chemosphere, 299, 134452, https://doi.org/10.1016/j.chemosphere.2022.134452
4. Almroth, B.C., Carle, A., Blanchard, M., Molinari, F., Bour, A., 2023, Single-use take-away cups of paper are as toxic to aquatic midge larvae as plastic cups, Environ. Pollut. 830, 121836. https://doi.org/10.1016/j.envpol.2023.121836.
5. Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M. 2017, Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. Sci. Rep. 7:17006. https://doi.org/10.1038/s41598-017 17191-7
6. Khosrovyan, A., Gabrielyan, B., Kahru, A. 2020, Ingestion and effects of virgin polyamide microplastics on Chironomus riparius adult larvae and adult zebrafish Danio rerio. Chemosphere 259, 127456, https://doi.org/10.1016/j.chemosphere.2020.127456

7. Khosrovyan, A.; Kahru, A. 2021, Evaluation of the potential toxicity of UV-weathered virgin polyamide microplastics to non-biting midge Chironomus riparius. Environ. Pollut. 2021, 287, 117334.

Development of the Harmonised Methodology for the Narva River Water Discharge Estimation

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Abstract: The Narva River takes its source in Lake Peipsi, flowing into the Gulf of Finland. It is divided between Estonia and Russia along the river's fairway. Estonia is a member of the European Union while Russia is not. In addition to that, the countries use completely different techniques for discharge estimation. That all creates difficulties in the river runoff calculations, affecting calculations of pollution load, providing data inconsistencies when the countries report to Helsinki Commission (HELCOM) as they are required to. Besides that, the river has a unique feature called "backwater effect", which affects the stage-discharge relationships thus the usage of simple stage-discharge rating curve provides a lot of error.

The differences in the numbers from both parties can go up to 80% for certain months.

The NARVAWATMAN project, which was implemented under the European Neighbourhood Instrument and co-financed by the European Union, strived to analyse the existing discharge estimation techniques, conduct additional field measurements, come up with a new harmonised solution, and proved recommendations for future analysis in the Narva River runoff estimation [1].

Keywords: river discharge estimation; transboundary rivers; Narva River; rating curves.

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References

1. NarvaWatMan project, Final Report, Tallinn, 2022. Location: Taltech, Water Environmental Engineering Research Group. By special request: Alvina.reihan@taltech.ee

Use of Neoteric Solvents in Biomass Treatment

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Abstract: The research on the use of neoteric solvents is motivated by the increase of pollution-control legislation and more regulations of common solvents, especially related to the treatment of biomass. The most widely used neoteric solvent in biomass treatment is supercritical carbon dioxide, especially for the extraction of essential oils and other bioactive compounds from plants. A great reason to use CO_2 in its supercritical condition is to expand the spectrum of solvent solubility, polarity and volatility [1].

Ionic liquids (IL) and the deep eutectic solvents (DES) are very attractive for extraction and separation sciences as replacements for volatile organic solvents. Unfortunately, traditional ILs despite of their high chemical flexibility and non-volatility are not green solvents.

DESs are representing other type of tailor-made solvents, where for both hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) compounds available from renewable resources can be used resulting in natural deep eutectic solvents (NADES) which have preferable intrinsic characteristics, low cost, easy preparation, and low toxicity. The HBD and HBA of a DES dictate the solvent properties, that have a direct impact on the extraction efficiency. The role of water in DES' composition is to alter the pH, viscosity and polarity, which results in a significant increase in extraction efficiency.

There are studies that have shown excellent performances of DESs in the extraction of biomolecules (such as polyphenols, iridoids, alkaloids), providing comparable or even higher efficiency than conventional solvents [2,3].

A variety of analytical methods could be applied for characterization of obtained extracts, including HPLC-DAD-MS, which is well suited for analysis of most complicated samples.

Keywords: supercritical fluids; ionic liquids; deep eutectic solvents; extraction, chromatography; essential oils; bioactive compounds

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References

1. Gretšušnikova, T., Järvan, K., Orav, A., Koel, M. Comparative analysis of the composition of the essential oil from the shoots, leaves and stems the wild Ledum palustre L. from Estonia. *Procedia Chemistry*, **2010,** 2(S1),168 – 173; doi: 10.1016/j.proche.2009.12.024
2. Koel, M., Kuhtinskaja, M., Vaher, M., Extraction of bioactive compounds from *Catharanthus roseus* and *Vinca minor*, *Sep. Pur:Tech.*, 252 (**2020**) 117438; doi.org/10.1016/j.seppur.2020.117438
3. Saar-Reismaa, P., Koel, M., Tarto, R., Vaher, M., Extraction of bioactive compounds from *Dipsacus fullonum* leaves using deep eutectic solvents, *J.Chromatogr. A*, 1677**(2022)** 463330; doi.org/10.1016/j.chroma.2022.463330

The Impact of Microplastics on Soil Invertebrates

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Abstract: As a result of plastic pollution and intentional use of plastics in agriculture, small plastic particles called microplastics (<1mm), are commonly found in soils. This has raised many concerns on the possible effects of microplastics on soil physico-chemical properties, soil biota and the ecosystem services they provide [1,2]. Hazard assessment of microplastics on soil biota relies on using model terrestrial invertebrates, which are exposed to microplastics either by ingesting the soils or through body surface contact. In recent years we have gathered considerable information regarding the effects of microplastics on key ecotoxicity test invertebrates: woodlice *Porcellio scaber*, mealworms *Tenebrio molitor*, springtails *Folsomia candida*, enchytraeids *Enchytraeus cripticus* and earthworms *Eisenia andrei* [3-6]. The organisms were exposed through soil or food spiked with environmentally relevant microplastic concentrations (0.005- 5%) for typically 3-4 weeks. Microplastics were generated from various plastic materials generating: tire wear particles, textile fibers, polypropylene microplastics from disposable medical masks, low density polyethylene fragments from packaging, low-density-polyethylene fragments milled from mulching films and starch blend polybutylene adipate-co-terephthalate mulching films fragments. A number of endpoints was followed: survival, reproduction, moult, growth, energy related biomarkers and immune-response. In general, our results show that tested microplastics are not lethal to tested invertebrates, but microplastic exposure can induce sublethal effects, such as alternation in reproduction, reduced growth, changes in metabolic activity and induction of immune response. We will present an overview of the various effects observed on different organisms and discuss how the responses differ between types of microplastics and whether plastics-associated chemicals might contribute to observed effects.

Keywords: microplastics, agricultural plastics, plastics-associated chemicals, terrestrial invertebrates, crustaceans, insects, soil, reproduction, growth, immune response

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References

1. de Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC. Microplastics as an emerging threat to terrestrial ecosystems. *Glob Change Biol*, **2017,** *24:1405-1416.*
2. Nizzetto L, Futter M, Langaas S. Are agricultural soils dumps for microplastics of urban origin? *Environ Sci Technol*, **2016**, *50:10777-10779.*
3. Selonen S, Andraz D, Jemec Kokalj A, Skalar T, Parramon Dolcet L, Hurley R, van Gestel CAM. Exploring the impacts of plastics in soil – The effects of polyester textile fibers on soil invertebrates. *Sci Tot. Environ*, **2020**, *700:134451.*
4. Selonen, S., Dolar, A., Jemec Kokalj, A. J., Sackey, L. N., Skalar, T., Fernandes, V. C., ... & van Gestel, C. A. Exploring the impacts of microplastics and associated chemicals in the terrestrial environment–Exposure of soil invertebrates to tire particles. *Environmental Research*, **2021**, *201, 111495.*
5. Jemec Kokalj, A.., Dolar, A., Drobne, D., Škrlep, L., Škapin, A. S., Marolt, G., ... & van Gestel, C. A. Effects of microplastics from disposable medical masks on terrestrial invertebrates. Hazmat, **2022**, *438, 129440.*
6. Jemec Kokalj, A., Dolar, A., Titova, J., Visnapuu, M., Škrlep, L., Drobne, D., ... & Heinlaan, M. Long term exposure to virgin and recycled LDPE microplastics induced minor effects in the freshwater and terrestrial Crustaceans *Daphnia magna* and *Porcellio scaber*. *Polymers*, **2021**, *13(5), 771.*

Chasing Pollutants Concerning Public Health: From Food to Smoke

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Abstract: Sample preparation is the key step in determining low concentrations of pollutants from food, biological, plant, industrial and environmental matrices. Solid phase microextraction (SPME) is a solvent-free, cost-effective, robust, and high-throughput sample preparation technique [1] usually coupled with gas chromatography (GC). It is especially versatile for sampling of volatile organic compounds (VOCs) not only present in the analysis of wine bouquet or cheese aromas [2], but also in cigarettes' smoke [3] or in identification of microplastics (MPs) [4]. The Sorption of VOCs on the SPME fibre in the headspace (HS) of the sample depends on numerous parameters: the type of fiber, the extraction time, and the temperature. The mixed polarity phase SPME fibres (DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) were used in all analyses from Nanos cheese, MPs identification to cigarettes' smoke. The HS-SPME method enabled the VOCs' profiles study of Nanos cheese. The evolved cheese aroma profiles were affected by cheesemaking parameters: the amount of starter culture, ripening temperature and media, and were independent of the geographical origin of raw milk as well as the location of ripening [2]. Further, by employing the HS-SPME-GC–MS, identification of five most common polymer types (PVC, PS, PET, PP, PE) of MPs was possible. The well-controlled melting process, which generates characteristic compounds of each polymer enabled the classification of MPs from real mixtures. Studying other VOCs concerning public health included also HS-SPME of flavours in tobacco products (classic cigarettes – CCs, electronic cigarettes (ECs), and heat-no-burn products – HNB). Flavours are the most common reason for promoting smoking initiation, duration, and they make smoking cessation more difficult among adolescents. However, the lack of simple smoke/aerosol/vapour (S/A/V) analyses for comparison of CCs, ECs and HNB makes legislation or prohibition of such products impossible. It would be of general interest if a simple standardised method would exist.

Keywords: solid phase microextraction, food aromas, microplastics identification, smoking comparison, volatile organic compounds

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References

1. Jalili, V.; Barkhordari, A.; & Ghiasvand, A. A comprehensive look at solid-phase microextraction technique: A review of reviews. *Microchem. J.* **2020**, 152, 104319.
2. Boltar, I.; Majhenič, A. Č.; Jarni, K.; Jug, T.; & Kralj, M. B. Research of volatile compounds in cheese affected by different technological parameters. *J. Food Nutr. Res.* **2019**, 58(1).
3. Poljšak, B., Gošnak Dahmane, R.; Podobnik, L.; Bavcon Kralj, M. Comparing smoke and aerosols from classic and electronic cigarettes, and from »heat-not-burn« devices. V: Bavcon Kralj, Mojca (ed.), et al. 22nd European Meeting on Environmental Chemistry: book of abstracts: 5-8 December, 2022, Ljubljana, Slovenia. 1st e ed. Ljubljana: University of Ljubljana Press, 2022. Str. 85, ilustr. ISBN 978-961-297-035-2.
4. Šunta, U.; Trebše, P.; & Kralj, M. B. Simply applicable method for microplastics determination in environmental samples. Molecules **2021**, 26(7), 1840.

The Effect of Environmental Pollution in the Baltic Sea and North Sea on Marine Biota, Focusing on Cancer Development in Fish

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Abstract: Current knowledge of natural cancer defense mechanisms is limited, often using model organisms living in controlled environments. In natural habitats, anthropogenic contamination has resulted in increases of oncogenic substances. Studies have shown that wild populations can adapt to highly contaminated environments. We suggest that this process can be used to study the evolution of cancer defenses, and polluted seas provide the perfect 'wild laboratories' for this. Moreover, gene databases allow us to gain novel information about the evolution and function of these genes in different species, but also to understand cancer as a driving force in biological systems and species life histories. As fish are evolutionarily old and a genetically diverse group, comparative studies with cancer-related genes in different species could yet be a largely unexplored treasure trove for understanding the evolution and ecology of cancer. Here, we provide an overview of two studies on adaptations to cancer defenses: First, intraspecific research on different populations of the flounder (Platichthys spp.) and dab (Limanda limanda) in the North and Baltic Seas. Flatfish populations inhabit the whole gradient of contamination. We have found different prevalence of liver tumours and cancer related gene expressions along that gradient [1]. Second, we present a comparative study of cancer-related gene copy number variation (CNV) in different fish species. Our study demonstrates a relationship with cancer-related CNV and maximum lifespan in fish species, suggesting that higher tumour suppressor gene CNV lengthens and oncogene CNV shortens lifespan [2]. In addition, other potential defense mechanisms, including antioxidant defenses and biotransformation, are discussed.

Keywords: cancer defense mechanisms, wildlife cancer genetics, pollution-induced cancer, fish cancer, liver cancer

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Author Contributions: RK: conceptualization, investigation, writing – original draft, writing - review & editing. CB: investigation, methodology, writing – original draft, writing - review & editing. RM: conceptualization, data curation, formal analysis, methodology, software, visualization, writing - review & editing. PN: investigation, methodology, writing - review & editing. TS: conceptualization, funding acquisition, project administration, supervision, writing – original draft, writing - review & editing. All authors have read and agreed to the published version of the manuscript.

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References

1. Baines, C.; Meitern, R.; Kreitsberg, R.; Fort, J.; Scharsack, J. P.; Nogueira, P.; Giraudeau, M.; Sepp, T. 2023. Correlations between oxidative DNA damage and formation of hepatic neoplasms in two flatfish species from contaminated environments. *Biol. Lett*. **2023**, *19*, 20220583. DOI: 10.1098/rsbl.2022.0583
2. Baines, C.; Meitern, R.; Kreitsberg, R.; Sepp, T. Comparative study of the evolution of cancer gene duplications across fish. *Evol. Appl*. **2022**, *15*, 1834-1845. DOI: 10.1111/eva.1348

Activity of Nanomaterials in Photocatalysis

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Abstract: Several environmental issues need to be addressed, including air pollution and human exposure to the low concentrations of volatile organic compounds that have been detected indoors. Replacing fossil fuels with, for example, green and renewable hydrogen to reduce carbon dioxide emissions and air pollution is another recent challenge. Photocatalysis is an attractive technology with the potential to do both: abate air pollutants and synthesize hydrogen. In order to increase the potential of the environmental applications of photocatalysis, the synthesis of catalytic materials with significantly higher activity is a priority. The semiconductor oxide TiO_2 in the form of an immobilized nano-powder and in the form of thin films is the most studied and most promising photocatalyst. The photocatalytic activity of TiO_2 is associated with its defects, where both types of charge carriers - electrons and holes, can reach the surface in nanosized materials to form effective interactions [1]. Favorable defect distribution can be determined by the presence of specific facets, although obtaining high purity anatase crystals with controlled crystallographic facet purity remains a challenge [2]. It has also been found that oxygen vacancies are critical for the adsorption of oxygen on the particle surface and the capture of photogenerated electrons. However, the engineering of oxygen vacancies has not yet been developed for practical applications of photocatalytic air purification or hydrogen generation [3,4]. At present, there is still a gap in the efficient use of photocatalytic nanomaterials between laboratory scale research and practical applications. The development of materials science in the field of nanomaterials, along with developments in the field of photocatalytic systems engineering, should provide solutions for increasing the efficiency of photocatalysis.

Keywords: photocatalysis; titanium dioxide; nanoparticles; air purification; hydrogen generation

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References

1. Beni, A.A.; Jabbari, H. Nanomaterials for environmental applications. *Results Eng.* **2022**, *15*, doi.org/10.1016/j.rineng.2022.100467.
2. Chen, F.; Cao, F.; Li, H.; Bian, Z. Exploring the important role of nanocrystals orientation in TiO_2 superstructure on photocatalytic performances. *Langmuir* **2015**, *31*, 3494–3499 doi.org/10.1021/la5048744.
3. Moiseev, A.; Krichevskaya, M.; Qi, F.; Weber, A.P.; Deubener, J. Analysis of photocatalytic performance of nanostructured pyrogenic titanium dioxide powders in view of their polydispersity and phase transition: Critical anatase particle size as a factor for suppression of charge recombination. *Chem. Eng. J.* **2013**, 228, 614-621 dx.doi.org/10.1016/j.cej.2013.05.038.
4. Lu, Y.; Chen, M; Jiang, L.; Cao, J.; Li, H.; Lee, S.C.; Huang, Y. Oxygen vacancy engineering of photocatalytic nanomaterials for enrichment, activation, and efficient removal of nitrogen oxides with high selectivity: a review. *Environ. Chem. Lett* **2022**, *20*, 3905-3925 doi.org/10.1016/j.rineng.2022.100467.

Assessing Biological Effects of Contaminants in the Gulf of Finland, North-Eastern Baltic Sea, Using Sediment Biotests with Amphipods (*Monoporeia affinis*) and Biomarker Responses in Clams (*Macoma balthica*)

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Abstract: The Gulf of Finland, in the north-eastern Baltic Sea, is experiencing ongoing adverse effects due to human activities, leading to a decline in the quality of the marine environment [1]. The current emphasis in environmental monitoring and assessment lies in chemical and ecological measurements, with little attention given to the connection between these measurements and their biological effects. The neglect of examining biological effects hampers our understanding of the overall influence that various contaminants have on marine organisms, which results from complex combinations of multiple effects. We have collected sediments from moderately to highly contaminated offshore and coastal areas with subsequent analyses of selected chemicals. Where available, clams (*M. balthica*) were collected for biological effects measurements. From seven sites the whole-sediment bioassays with amphipods (*M. affinis*) was conducted, to determine the effect of contaminants with the registration of mortality rate and activity of three biochemical biomarkers. In sediment biotest mortality rate was mostly uniformly low - around 8%. The comparison of the amphipod and clam biomarker data revealed that the amphipod, which was exposed to sediments from Narva Bay, did not exhibit significant changes in biomarker activities, except for catalase (CAT) which indicates oxidative stress. In clams, peaks and falls in enzymatic activities primarily reflect *in situ* exposure to harmful compounds and conditions. The lowest glutathione S-transferase (GST) activity in clams might be related to the impact of contaminants, as high levels of mercury registered simultaneously in the sediments near the Narva river mouth, while near Kunda harbour, the normalized content of PAH anthracene exceeded more than five times the HELCOM threshold. The highest GST in Narva bay clams might be related to the mixed impact of toxic biocide TBT, which exceeded almost ten times the GES threshold, and moderate contamination by PAHs and non-dioxin-like PCBs found in the sediments there. According to the calculated integrated biomarker response index, the highest value at the Sillamäe harbour reflects the most stressful conditions within the studied area. In addition, the elevated level of oxidative stress hints at the unfavourable hydro-physic and chemical conditions in this location.

Keywords: Gulf of Finland; biological effects; chemical contamination; sediment biotests; biomarkers; *Monoporeia affinis*; *Macoma balthica*.

Author Contributions: Conceptualization, I.K.; methodology, I.K., N.K., M.L. and K.K.L.; formal analysis, I.K.; investigation, I.K, N.K., M.L.; writing—original draft preparation, I.K.; writing—review and editing, I.K. and K.K.L.; supervision, I.K. and K.K.L.; project administration, I.K.; funding acquisition, I.K., N.K. and K.K.L.; All authors have read and agreed to the published version of the manuscript.

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References

1. Kuprijanov, I.; Väli, G.; Sharov, A.; Berezina, N.; Liblik, T.; Lips, U.; Kolesova, N.; Maanio, J.; Junttila, V.; Lips, I. Hazardous substances in the sediments and their pathways from potential sources in the eastern Gulf of Finland. *Mar. Pollut. Bull.* **2021**, *170*, 112642.

Antioxidative and Anti-*Borrelia* Effects of *Plantago* Species

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Abstract: *Borrelia burgdorferi sensu lato* bacteria are the causative agent of Lyme disease, the most common vector-borne disease in Europe. In Estonia, the spread of ticks carrying the pathogenic bacteria along with the case numbers of the illness are rapidly raising [1]. The infection can affect multiple organ systems and withstand several rounds of antibiotic treatment [2]. Therefore, novel treatment options are needed to combat the persister forms of the bacteria responsible for the chronic illness [3]. The screening of natural resources has shown promise in helping discover lead compounds with distinct anti-*Borrelia* activity for future therapeutic approaches. The antioxidative and antibacterial properties of several plants found in Estonia have been demonstrated by our group. In this presentation, the chemical characterisation and anti-*Borrelia* activity determination of *Plantago major* and *Plantago lanceolata*, are discussed. The main groups of bioactive compounds in the plants were quantified by colorimetric tests, total flavonoids by the $AlCl_3$, total iridoids by the Trim-Hill, and total polyphenols by the Folin-Ciocalteu method. The results show that dried aerial parts of *Plantago mojor* and *Plantago lanceolata* contain up to 31.3 and 48.4 mg/g gallic acid equivalents of phenolic compounds and up to 17.9 and 27.4 mg/g asperuloside equivalents of iridoids, respectively. The extracts were chemically characterised using HPLC-DAD-MS/MS. The antioxidative activity of all extracts was evaluated using the $ORAC_{FL}$ method. The anti-Borrelia activity of the plant extracts was tested on the latent bacterial forms using the SYBR Green I and Propidium Iodide assay. The residual viability of *B. burgdorferi* bacteria after incubation with the plant extracts was as low as 15.1% for both *Plantago* species tested. Therefore, as our results demonstrate that both *Plantago major* and *Plantago lanceolata* contain considerable amounts of phytochemicals with antioxidant properties and show significant anti-*Borrelia* effects on the latent forms of *Borrelia burgdorferi*, these plants should be considered for further therapeutic research.

Keywords: Lyme disease; *Borrelia burgdorferi*; phytochemicals; antioxidants

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Author Contributions: Conceptualization, M.V.; methodology, P.S.-R., M.V.; formal analysis, R.D.; P.-R.L., P.S.-R., O.B.; investigation, P.-R.L., P.S.-R., M.V.; data curation, P.-R.L., P.S.-R.; writing – original draft preparation, P.-R.L.; writing – review and editing, P.-R.L., M.V.; visualization, P.-R.L.; supervision, P.S.-R., M.V.; project administration, M.V.; funding acquisition, M.V. All authors have read and agreed to the published version of the manuscript.

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References

1. J. Geller et al., National Institute for Health Development, 2022.
2. S. K. Singh, H. J. Girschick, Clinical Microbiology and Infection 2004, 10(7), 598-614.
3. B. Sharma et al., Antimicrobial Agents and Chemotherapy 2015, 59(8), 4616-4624.

Identification of Emerging Contaminants in Estonian Aquatic Environment

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Abstract: Internationally agreed long-term monitoring indicators do not consider the risks of new synthetic substances. Screening a wide range of chemical substances gives early identification data about substances that exceed ecotoxicological effect values in the aquatic environment.

The work summarized the pollutants found in the Estonian water environment, which can cause both short-term and long-term effects on aquatic life. Data has been collected within the period 2016-2023 in the framework of various projects, including Estonian national environmental monitoring[1]. Information on substances is collected both with substance-group-based multi-methods (HPLC/MS; GC/MS ca 300 substances), with wide-scope target screening (2,500 substances by LC-ESI-HRMS and GC-APCI-HRMS) and non-target suspect screening (more than 65,000 compounds in each of the samples including their semi-quantification using LC-ESI-HRMS) [2].

In the screening results, both completely new substances previously not associated with environmental risk, as well as substances that have already been regulated and considered an important risk factor are found. In Estonian waters, 10 substances were found in all sea fish and shellfish samples examined. Five of these substances also exceeded the PNEC value on all samples (5'-Methylthioadenosine; 1-Eicosanol, phosphate, compd. with 2,2'-iminobis[ethanol]; Misoprostol; Butyl acrylate; 1-Propanone; 1-(4-dodecyl phenyl)-2-hydroxy-2-methyl-). PAHs and PFASs still pose an environmental risk. The screening identifies regional peculiarities. Not all substances are spread all over the Baltic Sea. Some have significant effects only in Estonian waters.

Screening studies of man-made substances found in the environment with the latest analytical methods and knowledge taking into account the latest scientific developments will be necessary in the future in order to prevent long-term environmental problems. The presence of substances in the environment depends on the properties of the substance, and therefore it is important to monitor different matrices (water, sediment, biota).

Keywords: environmental monitoring; target screening; non-target screening.

Author Contributions: Conceptualization, M.L.; K.M.; and L.P.; methodology, K.M. and L.P.; validation, M.L., K.M. and L.P.; formal analysis, K.M; L.P.; investigation, M.L.; resources, M.L.; data curation, M.L.; writing—original draft preparation, M.L.; writing—review and editing, K.M. and L.P; project administration, M.L.; funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript.

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References

1. Riiklik keskkonnaseire programm https://kese.envir.ee/kese/welcome.action
2. HELCOM PreEMP Final report (will be published by HELCOM secretariat https://helcom.fi/helcom-at-work/projects/pre-empting-pollution-by-screening-for-possible-risks-preempt/)

Anthropogenic Activities and Microbial Populations: War, Peace or Adaptation?

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Abstract: Aquaculture is one of the fastest-growing food sectors in Central, North, and Eastern Europe. Freshwater farming is changing the biodiversity of the fishing ponds to fulfill industrial needs, and these changes can impact the adjusting water bodies. Precautions should be taken to protect the ecosystems and keep them sustainable. The main objective of this research was to evaluate the influence of intensive fish farming on the condition of fishery ponds themselves and the surrounding water ecosystems as well as to evaluate the possibility of transferring the pollutants and antibiotic resistance genes to both environment and the human hosts. Sediment samples and fish gut microbiome samples were collected during September 2019 and the Summer of 2020 in 3 locations in Lithuania: Fishery ponds, Simnas Lake upstream from the fishery ponds, and Dusia Lake – downstream from the fishery ponds. The heavy metals and antibiotic residues were measured in the samples. Genomic DNA was isolated from the samples by using ZymoBIOMICSTM DNA Miniprep Kit according to the manufacturer's recommendations. The composition of the bacterial community was determined by next-generation sequencing (NGS) by scanning the amplicons of the bacterial 16S rRNA gene. The V3–V4 16S rRNA regions were chosen for sequencing because they are capable to detect both bacterial and archaea taxons with high resolution [1], [2]. NGS was performed by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) on Illumina paired-end platform to generate 250 base pairs (bp) length paired-end raw reads. All the tested sediment samples did not show significantly elevated heavy metal concentrations and no substantial veterinary antibiotic pollution. From the antibiotic resistance genes tested, the presence of aminoglycoside and b-lactam resistance determinants as well as the presence of integrons could be of concern for the possibility of transfer to humans. The microbiome beta-diversity analysis results clearly indicated the differences between the microbiota composition of all pond sediments and entrance point, treated as a clean area.

Keywords: keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the abstract yet reasonably common within the subject discipline.)

Author Contributions: For abstracts with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, E.L. and J.A.; methodology, E.L. and J.A..; resources, E.L. and J.A.; data curation, E.L. and J.A.; writing—original draft preparation, E.L.; writing—review and editing, E.L.; visualization, E.L. and J.A.; supervision, E.L.; project administration, E.L.; funding acquisition, E.L. and J.A. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. E. K. Wear, E. G. Wilbanks, C. E. Nelson, and C. A. Carlson, 'Primer selection impacts specific population abundances but not community dynamics in a monthly time-series 16S rRNA gene amplicon analysis of coastal marine bacterioplankton', *Environ Microbiol*, 2018, doi: 10.1111/1462-2920.14091.
2. Y. S. Bukin, Y. P. Galachyants, I. V. Morozov, S. V. Bukin, A. S. Zakharenko, and T. I. Zemskaya, 'The effect of 16s rRNA region choice on bacterial community metabarcoding results', *Sci Data*, 2019, doi: 10.1038/sdata.2019.7.

The Use of Biomarkers in Monitoring and Assessment of Chemical Contamination in the Baltic Sea

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Abstract: Chemical pollution affects the health of marine biota, key functions of the ecosystem, and endangers its biodiversity. The current environmental monitoring and assessment approach is based on chemical and ecological measurements, while the linking between them, biological effects at the individual level, has been largely neglected. It is currently well acknowledged that the monitoring of chemical concentrations alone is not sufficient to protect populations since it considers only a tiny number of substances while a myriad of others is left unnoticed. In addition, the hazards related to contaminant mixtures remain undetected. Biological effects of contaminants can be detected and measured at different levels of biological organization, i.e., from the molecular/biochemical level up to changes in populations and communities. In Baltic Sea monitoring programmes, the few observations on the effects of contaminants have for decades mostly been made only at the higher biological organization levels, applying reproduction success parameters in top predators such as raptor birds and seals as indicators. However, recording early warning signals of contaminants exposure and effects on the health of individuals at the lower biological levels makes it possible to anticipate and prevent damage at the higher levels. Moreover, it is also crucial to monitor effects at the lower levels of the marine food web. The so-called biomarkers focus on changes in various biological functions and include parameters related to detoxification of xenobiotics, oxidative stress, neurotoxicity, geno- and cytotoxicity and reproductive disorders, among others. Research on biomarkers aiming at improving their use in monitoring and assessing the contamination status of marine areas has been intensive during the recent decades albeit the implementation of the methods to monitoring programmes has been slow. Here, the current state of biological effects monitoring using biomarkers as well as selected case studies in the Baltic Sea are presented, followed by recommendations on their application in this sea area.

Keywords: Baltic Sea; biological effects; biomarkers; monitoring; chemical contaminants.

Plasma Electrolytic Oxidation Synthesis of Heterostructured TiO_2 for Photoanode Applications

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Abstract: In the renewable energy field, conversion of solar light into electrical or chemical energy is considered essential in order to move towards a truly green energy economy. Solar energy can be harnessed not just through generating electricity with photovoltaic cells, but also by driving photoelectrochemical (PEC) reactions such as water splitting or pollutant oxidation. In this study TiO_2 films were synthesized electrochemically by a procedure called plasma electrolytic oxidation (PEO). Under specific conditions, as the Ti substrate dissolves and the oxide film grows, electron discharges occur across the film and this ionizes both the oxide and some amount of electrolyte that had been in contact. The mixture then cools, leaving a macroporous TiO_2 structure. What is particularly interesting for PEC applications is that the films can be crystalline and doped after synthesis. XRD analysis revealed that a TiO_2 film that had been obtained at a voltage of 200 V has an anatase crystal structure. In addition, during ionisation and cooling ions from the solution can be incorporated into the film. By adding 0.1 M of Cu_2SO_4 into the synthesis electrolyte we were able to inorporate Cu into the films, as proven by EDX and XPS. The TiO_2 and heterostructured films show good PEC water splitting activity and stability in alkaline media when illuminated with 365 nm LED light. It was found that the obtained photocurrent depends on the synthesis voltage, and that the heterostructured films would generate ~ 2 times larger photocurrents. In addition, further surface functionalization (e.g., with Au) was investigated. The electron-hole recombination was evaluated by an advanced non-stationary photoelectrochemical technique – intensity modulated photocurrent spectroscopy (IMPS). Generally the films have very little recombination and only at lower overpotentials up to ~ 1 V). Overall, synthesis of oxide films by PEO may provide an efficient alternative to obtaining crystalline films by annealing, and various heterostructures can be created simply by modifying synthesis conditions.

Keywords: titanium oxide, plasma electrolytic oxidation, heterostructures, photoelectrochemistry, photoanode, water splitting

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Author Contributions: Conceptualization, R.L. and E.N.; methodology, R.L. E.N.; formal analysis, R.L.; investigation, R. L., V. P., A. S., T.M., R. V., A. J., I.S.; resources, E.N.; writing—original draft preparation, R.L.; writing—review and editing, R.L., E. N.; visualization, R.L.; supervision, E.N.; All authors have read the published version of the manuscript."

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Overlooked Residue of Li-Ion Battery Recycling Waste as High-Value Bifunctional Oxygen Electrocatalyst for Zn-Air Batteries

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Abstract: The rapidly growing demand for Li-ion batteries (LIBs) is raising concerns regarding the end-of-life handling, recycling, and recovery of secondary materials. To turn the battery value chain more sustainable the reuse of all battery materials needs to be assured, to prevent the losses of critical resources. Current industrial recycling solutions are still inefficient and various battery materials are not recovered. Used graphite is currently lost during the industrial state-of-the-art hydrometallurgical recycling of black mass, regardless of its criticality as a raw material. Consequently, there is a need for strategies that can recover and valorize the spent graphite present in the battery waste. This research outlines a novel approach utilizing industrially produced and hydrometallurgically leached black mass as a raw material source in bi-functional oxygen electrocatalyst production. Spent graphite in the post-metallurgical waste residue was turned into valuable graphene-like material, which was spontaneously doped by cobalt due to the traces of cathode metals present remaining in the battery leaching waste after the hydrometallurgical recycling treatment. The Bat-N-rGO electrocatalyst, prepared from this industrial waste, not only exhibited a very high ORR activity, comparable with a 19.8% Pt/C commercial benchmark catalyst, but also an advantageous OER activity, similar to RuO_2 catalyst. Moreover, the Bat-N-rGO bifunctional oxygen electrocatalyst was used in a rechargeable Zn-air battery as an air cathode catalyst and achieved a high-power density of 113 $mWcm^{-2}$ and specific capacity 719 $mAhg^{-1}$, whilst maintaining a good cycling stability [1]. This research for the first time, clearly demonstrates the significant potential of spent Li-ion battery black mass residue as a resource for the more sustainable production of high performance and high value catalyst materials for the next-generation electrochemical energy storage and conversion devices required to mitigate climate change.

Keywords: Li-ion battery recycling, spent graphite, bifunctional oxygen electrocatalyst, oxygen reduction reaction, metal-air battery

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Author Contributions: K. L. – Conceptualization, Funding acquisition, Investigation, Project administration, Writing – original & reviewed draft.
J. S. – Investigation (XPS), Writing – original & reviewed draft.
B. P. W. - Writing – original & reviewed draft.
I. K. – Resources, Supervision, Writing – original & reviewed draft.
M. L. – Resources, Supervision, Writing – original & reviewed draft.

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References

1. K. Liivand, J. Sainio, B.P. Wilson, I. Kruusenberg, M. Lundström, Overlooked Residue of Li-Ion Battery Recycling Waste as High-Value Bifunctional Oxygen Electrocatalyst for Zn-Air Batteries, Appl. Catal. B Environ. 332 (2023). https://doi.org/10.2139/ssrn.4349474.

Wood Chemistry Perspectives at TalTech

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Abstract: Wood is the most abundant natural renewable resource in Estonia. Albeit abundant, for Estonia wood is also one of the locally least valorized natural resources. To unlock its full potential, for high level of valorization, wood needs to be processed to its core components either chemically, thermochemically or in combination with enzymes to fractionate it to fibres, wood sugars and its polyphenolic component, lignin. The multiple research groups working on wood valorization at TalTech work on developing the full value chain that involve food additives, thermoplastics, coating materials and fine chemicals. For decades the lignin component from the wood fractionation process was burned just for just thermal energy. As a paradigm shift, the researchers from TalTech are now converting lignin into novel thermoplastics, catalytic materials or feeding it to environmental microbial isolates to produce fine chemicals via fermentation processes.

Keywords: wood chemistry; lignin; functional materials; fermentation; environmental microbiology; TalTech;

Measurement Quality in Analysis – Guidelines and Software Tools

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Abstract: Measurement quality is about fulfilling analytical requirements, which should be based on the intended use of the results. Within the European Union environmental requirements can be set in a directive based on maximum allowable concentration of a substance in air, soil or water, e.g an Environmental Quality Standard EQS or an Emission Limit Value EVS. The requirements on measurement quality, e.g. limit of quantification, LOQ, within-laboratory reproducibility s_{Rw} or measurement uncertainty MU. This presentation is about public available guidelines that can help the analytical chemist working in the laboratory with a specific method to 1) set up the internal quality control over the whole concentration range based on the requirements – use of target control limits 2) perform on-going internal quality control based on only two rules 3) plan the method validation and 4) estimate the MU based on quality control and validation data. Normally for instrumental methods the MU is estimated as a relative uncertainty at higher concentrations and as an absolute uncertainty at lower concentrations close to the LOQ. For quality control and MU estimation, free open-source softwares will be presented.

Keywords: measurement quality; quality control; validation; uncertainty; target control limits

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The Study of the Uptake of Chromium, Zinc, Cadmium and Lead from Spiked Nutrient Solution in Tomato Plants

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Abstract: Reuse of treated wastewater for irrigation can preserve freshwater resources. However, treated wastewater carries health risks due to residual contaminants. For safe food production, it is essential to understand the uptake of potential contaminants and their distribution in edible parts. Chromium (Cr), zinc (Zn), cadmium (Cd) and lead (Pb) are commonly found in municipal wastewaters. In the present study, the uptake of Cr, Zn, Cd and Pb was studied. Tomatoes were cultivated in Hoagland nutrient solution (pH 7), prepared using potable water and nutrients [1]. Treated tomatoes were grown in the Hoagland solution spiked with naturally abundant elements ^{52}Cr (100 ng/mL), ^{66}Zn (100 ng/mL), ^{111}Cd (50 ng/mL) and ^{208}Pb (100 ng/mL) and their enriched stable isotopes ^{53}Cr, ^{70}Zn, ^{106}Cd and ^{204}Pb at same concentration levels. The use of enriched isotopes enabled distinguishing between the concentration of an individual element, which is naturally present in Hoagland's solution and is uptaken by plant during growth, and the concentration of an individual element uptaken by plant, as a result of its addition in the nutrient solution. Tomatoes were raised 5 weeks in 40 L pots. Nutrient solution, or spiked nutrient solution were weekly replenished to volume. Plants were harvested 5-week after exposure and divided into roots, stems, leaves and fruits. Samples were decomposed using microwave digestion and element concentrations were determined by ICP-MS. The results showed that the roots exhibited the highest accumulation of Cr, Zn, Cd and Pb, while low concentrations of Cr and Pb were determined in fruits. In the fruits, the highest accumulation was observed for the

essential element Zn. In compliance with Commission Regulation, the concentration of Cd in fruits exceeded four-times the permissible levels set for fruiting-like vegetables. This study contributes to the understanding of pathways of accumulation of essential and toxic elements in plants and to food safety.

Keywords: wastewater, irrigation, Cr, Zn, Cd, Pb, food safety, ICP-MS

Author Contributions: Conceptualization, J.Š. R.M. E.H. N.K.M. and M.P.; methodology, R.M. J.Š. E.H. N.K.M. and M.P.; formal analysis, K.M.; investigation, R.M., J.Š. and K.M.; resources, E.H.; data curation, R.M., J.Š. and K.M.; writing—original draft preparation, K.M. R.M. and J.Š.; writing—review and editing: J.Š., R.M. and E.H. and visualization, K.M.; supervision, J.Š. and R.M. and.; project administration, E.H.; All authors have read and agreed to the published version of the manuscript."

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References

1. D. A. E.-A. Ahmed, D. F. Slima, H. M. Al-Yasi, L. M. Hassan, and T. M. Galal, "Risk assessment of trace metals in Solanum lycopersicum L. (tomato) grown under wastewater irrigation conditions," *Environ Sci Pollut Res*, vol. 30, no. 14, pp. 42255–42266, Jan. 2023, doi: 10.1007/s11356-023-25157-8.

Microplastics and Associated Microorganisms in the Sea Sediment of the Sentina Regional Natural Reserve (Central Adriatic Sea, Italy)

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Abstract: (1) Background: Large dispersion of microplastics (MPs) in the marine environment has effects on living organisms health [1-3]. The aim of this study was to identify MPs and their associated microorganisms in Adriatic Sea sediments and to evaluate the antibiotic susceptibility patterns of the microbial communities.

(2) Methods: A beach transect, parallel to the shoreline where the waves break, has been identified for the samplings. A protocol to perform MPs isolation from sandy sediments at different seasons, suitable for biological samples upkeeping, and based on plastic floating in high salinity water, has been optimized. From floating MPs, aerobic and anaerobic cultivable microorganisms were isolated and total DNA extraction was performed for the shotgun metagenomic analysis. Susceptibility to a panel of 14 antibiotics, belonging to 12 different categories, was assessed [4,5]. Chemical characteristics of the isolated MPs were analysed using a Thermo Nicolet 6700 FT-IR Spectrometer with "Smart Orbit" diamond micro-ATR accessory and Thermo Nicolet iN10 MX FT-IR microscope.

(3) Results: By chemical analysis, polypropylene microplastics were estimated in the highest percentage, followed by polyethylene, poly-methyl acrylate and poly-vinyl chloride. Metagenomics data revealed differences in bacterial abundances during seasons and in floated MPs with respect to total sand. The differential gene analysis showed specific metabolic pathways in MPs associated microorganisms, including antibiotic resistance. By microbial cultivation and MALDI-TOF MS identification, bacteria promising for plastic degradation, such as *Lysinobacillus fusiformis*, *Exiguobacterium sp.*, *Pseudomonas oleovorans* were also found, as well as potentially pathogens, like *Clostridium septicum, Clostridium novyi*, *Shewanella putrefaciens*. Only 17.2% resulted susceptible to all the tested antibiotics. High percentages of resistance were observed for penicillins (85.7%), monobactams (80.9%), and tetracyclines (64.3%).

(4) Conclusions: MPs work as a vehicle of potential pathogens and antibiotic resistant microorganisms in the Central Adriatic Sea

Keywords: Microplastics; antibiotic resistance, metagenomics, bioremediation, microplastics isolation, sediment analysis.

Author Contributions: "Conceptualization, C.M.; FM.P.; M.M. FM.P.; methodology, A.P.; A.A.; M.M.S.V.; formal analysis and data curation, A.P.; A.A.; P.C.; writing—review and editing, A.P; M.M; C.M.; All authors have read and agreed to the published version of the manuscript."

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References

1. Ogonowski et al. 2018. Evidence for selective bacterial community structuring on microplastics. *Environmental Microbiology*, 20(8): 2796–2808.
2. Lu et al. 2019. Interaction between microplastics and microorganism as well as gut microbiota: A consideration on environmental animal and human health. *Science of the Total Environment*, 667: 94–100.

3. Silva et al. 2018. Microplastics in the environment: Challenges in analytical chemistry - A review. *Analytica Chimica Acta*, 1017: 1e19
4. Zhang et al. 2022. Potential risks of microplastics combined with superbugs: Enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system. *Ecotoxicology and Environmental Safety*, 187: 109852.
5. Su et al. 2021. Microplastics act as vectors for antibiotic resistance genes in landfill leachate: The enhanced roles of the long-term aging process. *Environmental Pollution*, 270: 116278.

Metal-Phenolic Network-Coated Nanoparticles for Reducing the Toxicity of Metal Nanomaterials

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Abstract: Growing use of metal nanomaterials (including Ag-, Cu- and Zn-based nanoparticles, NPs) in medical applications but also in various green technologies is expected to result in an elevated environmental burden of toxic metals and NPs [1]. Since soluble metal-based NPs cause toxicity via released toxic metal ions [2,3], safe use of NPs requires strategies for mitigating their toxicity via removal of these metal ions. Here, a novel class of nano-sized specific adsorbent materials, metal-phenolic networks, are proposed as suitable materials for this purpose due to their biocompatibility, high specific surface area and presence of functional groups specific for metal ion binding. Iron-tannic acid network-coated Au nanoparticles (Fe-TA@Au NPs) were synthesized and characterized for their physicochemical properties and metal adsorption profile using Cu ions as model toxicants. The morphology, size, composition, and stability in water of synthesized adsorbent materials were characterized using electron microscopy, Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering, ultraviolet-visible spectrophotometry (UV-Vis) and TXRF spectrometry (S2 PICOFOX, Bruker). Metal ion adsorption capacity, kinetics and specificity of synthesized Fe-TA@Au NPs were determined in aqueous solutions containing Cu^{2+} ions. Facile two-step synthesis in aqueous medium at room temperature yielded TA-stabilized Au NPs with primary size of 25±7 nm that were coated with Fe-TA amorphous layer (thickness 7.6±3 nm). The hydrodynamic diameter of the Fe-TA@Au NPs was ~60 nm and surface charge was highly negative both in MilliQ water (pH 6.0) and in HEPES buffer (pH 7.4; Zeta potential -45 and -60 mV, respectively). Aqueous suspensions of Fe-TA@Au NPs were stable over several days. FTIR analysis indicated presence of metal coordination bonds between TA and Fe atoms in the metal-phenolic network essential for the formation of network structure. Fe-TA@Au NPs effectively adsorbed Cu^{2+} in aqueous media as determined by TXRF spectrometry. When unicellular freshwater protozoa *Tetrahymena thermophila* [4, 5, 6] were co-incubated with Fe-TA@Au NPs and $CuSO_4$, Fe-TA@Au NPs completely rescued protozoa from the toxicity of $CuSO_4$, suggesting efficient adsorption of Cu ions by synthesized metal-phenolic networks. The results indicate that NPs coated with metal-phenolic networks have promising applications in environmental remediation.

Keywords: iron; tannic acid; gold nanoparticles; biocompatibility; toxicity; metals; adsorbent; aquatic

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References

1. Mortimer, M.; Kahru, A. Nanomaterials and Microorganisms: From Green Synthesis to Antibacterial Applications in Medicine and Agriculture. *Nanomaterials* **2022**, *12*, 4265, doi:10.3390/nano12234265.
2. Ivask, A.; Juganson, K.; Bondarenko, O.; Mortimer, M.; Aruoja, V.; Kasemets, K.; Blinova, I.; Heinlaan, M.; Slaveykova, V.; Kahru, A. Mechanisms of toxic action of Ag, ZnO and CuO nanoparticles to selected ecotoxicological test organisms and mammalian

cells in vitro: a comparative review. *Nanotoxicology* **2014**, *8 Suppl 1*, 57-71, doi:10.3109/17435390.2013.855831.
3. *Advances in Toxicology and Risk Assessment of Nanomaterials and Emerging Contaminants*, Guo, L.-H., Mortimer, M., Eds.; Springer Nature, Singapore, 2022; pp. 1–371., 10.1007/978-981-16-9116-4.
4. Mortimer, M.; Kasemets, K.; Kahru, A. Toxicity of ZnO and CuO nanoparticles to ciliated protozoa *Tetrahymena thermophila*. *Toxicology* **2010**, *269*, 182-189, doi: 10.1016/j.tox.2009.07.007.
5. Mortimer, M.; Kasemets, K.; Vodovnik, M.; Marinsek-Logar, R.; Kahru, A. Exposure to CuO nanoparticles changes the fatty acid composition of protozoa *Tetrahymena thermophila*. *Environ. Sci. Technol.* **2011**, *45*, 6617-6624, doi:10.1021/es201524q.
6. Juganson, K.; Mortimer, M.; Ivask, A.; Pucciarelli, S.; Miceli, C.; Orupold, K.; Kahru, A. Mechanisms of toxic action of silver nanoparticles in the protozoan *Tetrahymena thermophila*: From gene expression to phenotypic events. *Environ. Pollut.* **2017**, *225*, 481-489, doi: 10.1016/j.envpol.2017.03.013.

Role of Plant Volatiles in Atmospheric Processes Under Current and Future Climates

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Abstract: Several plant species emit different volatile organic compounds constitutively under typical physiological conditions, and all plant species can be elicited to emit volatiles under stress conditions when physiological activity is curbed. Plant emissions constitute the biggest global source of reactive volatile organic compounds in the atmosphere. These volatiles play major biological roles in plant-plant interactions and in plant interactions with other organisms. In addition, plant-emitted volatiles often the dominate the volatile-driven large-scale biosphere-atmosphere processes. In particular, plant volatiles participate in ozone- and secondary organic aerosol (SOA)-forming reactions and in cloud formation. Both enhanced SOA and cloud formation contribute to the cooling of Earth surface, implying that plants can alter their own life environment. At the global scale, amelioration of vegetation growing conditions via volatile emissions can reduce the rate of global environmental change, but there are currently major uncertainties in understanding of how plant emissions change in future climates and the quantitative impact of plant emissions on future climate change. Climate change is a complex phenomenon that entails alterations in a series of environmental factors. For plants, climate change is expected to enhance the severity and duration of stress periods when plant physiological activity is strongly reduced. This includes both enhancement of abiotic stresses such as rising temperatures and more severe drought episodes in many parts of the globe, and more devastating biotic stresses such as frequent outbreaks of herbivore and pathogen attacks. These changes in the frequency and duration of stress episodes can strongly impact volatile emissions. Stress typically reduces the emissions of constitutively released plant volatiles, and elicits emissions of specific volatiles in stress severity-dependent manner. Thus, the plant-dependent feedbacks on global change are expected to become stronger in more stress-prone climates.

Keywords: air chemistry; biotic stress; cloud formation; environmental stress; global change; plant stress; plant volatiles; secondary organic aerosols; stress-induced volatiles; volatile organic compounds

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Cost-Effective and Compact Measurement of Arsenic in Water (ARMINE Project-MITY)

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Abstract: Contamination of groundwater by arsenic is a serious concern due to the acute and long-term effects of arsenic toxicity to human health. Arsenic contamination is observed in at least 70 countries where the concentration of arsenic in drinking water exceeds the WHO provisional limit of 10 μg/L [1]. In Finland can also be found areas with high concentrations of arsenic in groundwater [2] that affects the quality of well water used for the gardening. Through watering the vegetables and fruits with contaminated well water arsenic can enter the food chain. Although the contamination in Finland mostly originates from the natural arsenic, the growing mining activities represent the risk of additional pollution from artificial sources. Therefore, proper monitoring of water supply is necessary to ensure safe levels of arsenic in water for human consumption. Currently, reliable methods for arsenic content determination (e.g. AAS, ICP-MS) are time consuming and must be carried out under laboratory conditions [3-4]. The aim of project was to develop a compact and inexpensive method for measuring arsenic concentration in water samples using an electrochemical sensor. The method technique involves voltametric stripping [5], which allows for rapid measurement of arsenic at very low concentrations (ppb levels). The hand-held potentiostat with the measurement and evaluation software and mobile phone application were also made. This technology is designed for on-field monitoring of arsenic in industrial and residential areas. The project started with a laboratory-scale development of the electrochemical method for arsenic analysis, which was later implemented on a pilot scale in southern Finland. This research was performed under the ARMINE project of the Measurement Technology (MITY-Kajaani) unit of the University of Oulu (Finland), and one of the application areas of research for health and clean technology.

Keywords: Arsenic; electrochemistry; water analysis; stripping voltammetry

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References

1. *Guidelines for drinking-water quality*, 4th ed.; World Health Organization, **2022**; pp. 340-343.
2. Ruskeeniemi, T., Backman, B., Loukola-Ruskeeniemi, K., Sorvari, J., Lehtinen, H., Schultz, E., Mäkelä-Kurtto, R., Rossi, E., Vaajasaari, K. & Bilaletdin, Ä. Arsenic in the Pirkanmaa region, southern Finland: From identification through to risk assessment to risk management. *Geological Survey of Finland, Special Paper 49*, **2011**, 213–227.
3. Feng, Y. L.; Chen, H. Y.; Tian, L. C.; Narasaki, H. Off-line separation and determination of inorganic arsenic species in natural water by high resolution inductively coupled plasma mass spectrometry with hydride generation combined with reaction of arsenic(V) and L-cysteine. *Anal. Chim. Acta*, *375*, **1998**, 167-175. https://doi.org/10.1016/S0003-2670(98)00285-2.

4. Anezaki, K., Nukatsuka, I. & Ohzeki, K. Determination of Arsenic(III) and Total Arsenic(III,V) in Water Samples by Resin Suspension Graphite Furnace Atomic Absorption Spectrometry. *Anal. Sci.*, *15*, 1999, 829–834. https://doi.org/10.2116/analsci.15.829.

5. Mays D.E, Hussam A., Voltammetric methods for determination and speciation of inorganic arsenic in the environment—A review, *Anal. Chim. Acta*, *646 (1-2)*, **2009**, 6-16. https://doi.org/10.1016/j.aca.2009.05.006.

A Brief Journey on the World of Heteracyclo[n]phanes: Synthesis and CO_2 Adsorption

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Abstract: A Optoelectronic properties of π-conjugated chromophores have been widely used since the discovery in the late 70’s of the conducting properties of synthetic organic polymers. Indeed, due to their chemical structures organic conducting polymers exhibit electrical properties that provide useful materials such as batteries, light-emitting diodes, antistatic packaging and coating, microelectronic devices, photovoltaic cells, corrosion inhibitors, sensors etc. Among them, polyaniline and polysulfide have been extensively studied for this purpose due to their environmental stability, oxidation or protonation-adjustable electrical properties and low cost production.
Nevertheless, these structures are generally depicted as linear structures and there is an interest in achieving hemicyclic or cyclic analogues structures to extend their potential of use as metal-gas sequestering agents and/or sensing materials as well as new organic cyclic semiconductors exhibiting different properties from their linear counterparts. In this context, we have recently developed a simple strategy based on a nucleophilic aromatic substitution reaction to achieve such cyclic derivatives, that are parent compounds of calixarenes with an heteroatoms on the bridge, affording the desired heteracalixarenes and mixed heteracalixarenes or the hemicyclic scaffold in high yields. Some examples and properties will be presented herein as well as the on-going research toward nanoarchitectures that can be used into devices such as sensors.

Keywords: Pi-conjugated systems, macrocycles, CO_2 capture, advanced organic materials

Electrodeposited Cu Nanofoam Structures for Electrochemical CO_2 Reduction

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Abstract: An exclusive Cu surface feature is a capability to convert CO_2 into hydrocarbons with significant Faradaic efficiency [1]. The catalytic activity of metal is highly sensitive to electrolysis conditions including surface structure, morphology and a real surface area (S_R). Simple polycrystalline Cu electrode possess rather small surface area therefore, its efficiency is low. A three-dimensional nanoramified Cu electrodes or foams, can be produced by metal electrodeposition accompanied by intensive hydrogen evolution [2]. An accurate estimation of porous electrode S_R is of high importance, as the precise knowledge of this parameter is a crucial for comparison of the behaviour of various catalytic systems.

The aim of this study was to evaluate the suitability of the known Cu real surface area determination methods [3] for their application for Cu 3D nanostructures. To reach the goal the following design was created. The initial Cu electrode, with the known S_R value was employed as a basis for Cu 3D structure electrodeposition from acidic sulphate solution. Electrochemical methods employing underpotential deposition of Tl and Pb, as well the double layer capacitance measurements, applying cyclic voltammetry and electrochemical impedance spectroscopy were applied for Cu 3D structures S_R evaluation.

The obtained results imply that non-porous Cu electrodes are not sensitive to the applied S_R determination method, while this parameter for Cu 3D structures depends significantly on the evaluation mode. The most reliable data for Cu foam characterization have been obtained by double-layer capacity measurements, while all other applied methods yielded inaccurate results. The electrodeposited Cu 3D layer structure and hence S_R depends on the plating solution composition [4]. An attempt has been done to investigate the influence of HCl additives to the deposited Cu foam S_R values. The obtained results indicate that addition of HCl threefold increases Cu S_R.

Keywords: 3D copper; electrochemically active real surface area; copper foam

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Author Contributions: Conceptualization, R.R.; data curation, B.S.; L.G.; R.R.; formal analysis, B.S., L.G., R.R.; investigation, B.S., L.G.; methodology, B.S., L.G., R.R.; software, B.S., L.G. supervision, R.R.; writing—original draft, R.R.; writing—review and editing, R.R. All authors have read and agreed to the published version of the manuscript.

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References

1. Xie, H.; Wang, T.; Liang, J.; Li, Q.; Sun, S. Cu-Based Nanocatalysts for Electrochemical Reduction of CO2. *Nano Today* **2018**, *21*, 41–54, doi:10.1016/j.nantod.2018.05.001.
2. Materials, W.C.S.P.; Xu, M.; Chen, Y.; Liang, J.; Mo, D.; Lyu, S. Electrodeposition Patterned Copper Foam with Micro / Nanostructures for Reducing Supercooling In. *Appl. Sci.* **2020**.
3. Trasatti, S.; Petrii, O.A. Real Surface Area Measurements in Electrochemistry. *Pure Appl. Chem.* **1991**, *63*, 711–734, doi:10.1351/pac199163050711.
4. Shin, H.C.; Liu, M. Copper Foam Structures with Highly Porous Nanostructured Walls. *Chem. Mater.* **2004**, *16*, 5460–5464, doi:10.1021/cm048887b.

Quality of Analytical Data – From Validation to Interpretation of Results

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Abstract: Carrying out analytical measurements has been a main task for routine laboratories to have a better understanding what is happening in the environment. However, with new emerging contaminants and constant improvement of instrumentation in recent decade has made the task for analytical chemists more challenging. There is a constant need to validate new analytical methods and interpret obtained data so that it would help with to deciding on further actions needed. To carry out method validations in a way that these would fit the purpose, there is a limited availability of suitable matrix matched standards in necessary concentration range, supporting proficiency testing schemes, standard methods etc. To validate such methods is a challenge even for experienced analytical chemists. Furthermore, depending on why the analytical measurements are carried out, aspects such as sampling, amount of samples etc. will have an influence on the interpretation. Therefore, there is a lot to account for in order to obtain analytical data with necessary quality.

In conclusion, the challenges to obtain analytical data with high quality will remain but understanding of the purpose of the data, experienced personnel in the laboratory and unified guidelines for data interpretation are steps that will help along the way.

Keywords: validation; analytical data; environmental analysis

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Estimation of the Share of the Total Nutrient Load from the Territory of Estonia, Coming Along the Narva River to the Baltic Sea

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Abstract: The annual inputs of nitrogen and phosphorus by the Narva River into the Baltic Sea were distributed between Estonia and Russia according to the catchment area shares of 33% and 67% respectively [1]. A comparison of the total nutrient load shows that the proportion between countries may vary depending on what input data and estimation methods are used. The aim of the study was to improve the comparability of estimates in order to obtain more accurate information on the distribution of nutrients from Estonia. The results contain testing of the Estonian and Russian methodologies [2] for the period 2006-2018, as well as the distribution of sources calculated for the direct catchment area of the transboundary Narva river. Detailed calculations for each sub-basin were carried out using mainly national coefficients. Comparison of the calculated total input with monitoring data showed that both countries' estimates of nitrogen and phosphorus loads correlate quite well with monitoring data. The main sources of Ntot in the immediate catchment were point sources and agriculture. The main difference was in Ptot input from natural and urban areas and agriculture. Therefore, point sources, agriculture and runoff from urban areas can be considered as priority sources. According to the Estonian methodology, the contribution of Ntot was 23% and Ptot 29% [3].

Keywords: nitrogen and phosphorus**;** nutrient load estimation; transboundary rivers; Narva river; Baltic Sea.

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References

1. HELCOM, 2018. The Sixth Pollution Load Compilation (PLC-6). https://helcom.fi/wp-content/uploads/2019/08/PLC-6-Executive-Summary.pdf
2. Applied methodology for the PLC-6 assessment Edited by Lars M. Svendsen, DCE Aarhus University, PLC-6 project manager, 60 p. https://helcom.fi/wp-content/uploads/2020/01/PLC-6-methodology.pdf
3. NarvaWatMan project, Final Report, Tallinn, 2022. Location: Taltech, Water Environmental Engineering Research Group. By special request: Alvina.reihan@taltech.ee

Water Quality Index (WQI) as a Tool to Assess Waterbodies Status. Joint WQI Model Development for River Narva and the Rivers of the Lake Peipsi Basin (Based on Results of NarvaWatMan Project)

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Abstract: One of the aims of The European Water Framework Directive (WFD) is that the Member States monitor and assess the ecological and chemical status of all surface water bodies (rivers, lakes, transitional and coastal waters) and to ensure that they achieve good status by 2027, with an interim deadline of 2021. The question is, what is meant by good status and how to assess it? A good status of a water body can depend on several factors such as its physical, chemical, and biological characteristics, which can mean a lot of data. Water quality index (WQI) is a tool to provide a numerical value that indicates the overall health of the water in a particular area. A summary of how the WQI has developed and how it can be used was provided. When managing joint water bodies, it is especially important that decisions are made on a uniform basis and the status of the water body is assessed in a similar way. During the project NarvaWatMan the comparison was made how the status of the river Narva is assessed both in Estonia and Russia. Based on the collected data a joint water quality index was developed, and the received results were presented [1].

Keywords: WQI; WFD; pollution load; transboundary rivers; Narva River; Baltic Sea.

Author Contributions: “Conceptualization and methodology, Kati Roosalu; formal analysis, Kati Roosalu; data curation, Kati Roosalu; writing—original draft preparation, Kati Roosalu.

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References

1. NarvaWatMan project, Final Report, Tallinn, 2022. Location: TalTech, Water Environmental Engineering Research Group. By special request: Alvina.reihan@taltech.ee

Current Approaches to the Derivatization of Chemical Weapons Convention-Related Alcohols for On-Site Gas Chromatographic Analysis

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Abstract: The task of deployable military laboratories is to perform fast identification of chemical warfare agents (CWAs) and related chemicals in various types of samples in field conditions. Identification is limited by the time and equipment of mobile laboratories. Polar degradation products are commonly derivatized by a procedure using *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) [1,2], which is time-consuming and the resulting chromatograms often contain a number of artifacts that hinder the identification [3]. This work describes the development and optimization of an alternative trimethylsilylation (TMS) procedure for on-site identification. The goal was to develop a fast and robust method that would be efficient without heating and produce clean chromatograms. The analytes were precursors and degradation products of blistering (thiodiglycol, ethyldiethanolamine, methyldiethanolamine, triethanolamine), nerve (*N*,*N*-diisopropylaminoethanol) and psychoactive (3-quinuclidinol) CWAs. Ten TMS-reagents were compared in terms of derivatization efficiency. The solvent effect, catalyst addition effect, time and temperature of derivatization were studied and optimized. The time stability of the derivatives was observed and chromatogram artifacts were monitored. The original recommended and widely used method of derivatization of alcohols for 30 min at 60 °C in acetonitrile using BSTFA was overcome by 3 optimized procedures using different TMS-reagents, which achieved high and stable yields in an acetone environment already at room temperature and a reaction lasting 5 min. Due to the same chemical structure of the resulting compounds (TMS-derivatives), it is possible to use established mass spectral databases. Optimized procedures were applied to environmental (water, sand) and urban (acrylic paint, asphalt-aluminum paint, concrete) samples contaminated with the studied alcohols. The results obtained on a benchtop gas chromatograph were afterwards validated on a field device that is being used by the deployable chemical laboratory of the Czech Army following their standard operating procedures for sample preparation. The developed methods are useful for military teams and also for stationary analytical laboratories whose task is the unambiguous identification of CWAs and related compounds in various samples.

Keywords: military identification; chemical warfare agents; silylation; field analysis; environmental sample; urban sample.

Author Contributions: One author.

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References

1. Black, R.; Read, R; Riches, J. Derivatisation for gas chromatography. In *Recommended Operating Procedures for Analysis in the Verification of Chemical Disarmament*, 2nd ed.; Vanninen, P., Ed.; University of Helsinki: Helsinki, Finland, 2017; pp. 131–152.
2. Witkiewicz, Z.; Sliwka, E; Neffe, S. Chromatographic analysis of chemical compounds related to the Chemical Weapons Convention. *Trends Anal. Chem.* **2016**, *85*, 21–33. DOI: 10.1016/j.trac.2016.05.006

3. Rozsypal, T. Development of a method for the derivatization of ethanolamines and its application to sand samples. *J Serb. Chem. Soc.* **2022**, 87, 233–245. DOI: 10.2298/JSC210312047R

Non-Formal Ecological Education: Tested Innovative Methods in Lake Peipsi Communities

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Abstract: Our continent faces serious problems in areas such as biodiversity loss, limited natural resources, climate change impacts etc. Thus, during the last decade environmental education has gained importance globally as the “alarm clock” in ringing now really loud and educators as well as politicians understand the urgent need to better educate the young people on complexity and interconnection of ecological, economical, political, cultural, etc. issues and develop creative problem solving skills. This requires new types of educational methods as the recent research also suggests that non-formal and interactive approaches in ecological education are more effective than purely fact-based teaching. The article describes innovative, interactive methods tested by Estonian NGO Peipsi Center for Transboundary Cooperation (www.ctc.ee) in regional schools, but also during public events. Deliverables such as the textbook/ worksheets on Peipsi ecosystems, LoqQuiz orientation game; crossword and educational videos with quizzes on Peipsi ecology, Educational Live-Action Role Play (EduLARP), were of most interest among our target group [1], Our experience suggests that interactive and narrative approaches in environmental education such as problems-solving exercises, role-plays can be more effective in a long run and engaging for the pupils than fact-based (classroom) teaching. Non-formal learning is characterized by learning by doing, as learners learn foremost from the specific situations they experience, while their attitudes and values would shape their future behavior [2]. Also, the ecological education should focus more of the importance of individual responsibility as well as the role of collective action and t is very important to constantly work with educators, especially in more peripheral areas.

Keywords: non-formal ecological education, innovative environmental education methods, Lake Peipsi region

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References

1. Formal, non-formal and informal learning, Council on Europe. Available online: https://www.coe.int/en/web/lang-migrants/formal-non-formal-and-informal-learningL (accessed 20.06.2023)
2. Publications, Peipsi Center for Transboundary Cooperation. Available online https://ctc.ee/publications

Machine Learning Tools Can Pinpoint High-Risk Water Pollutants

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Abstract: Liquid chromatography – high-resolution mass spectrometry (LC/HRMS) is a powerful tool for detecting chemicals that are present in low concentrations. While this technique has revealed thousands of ionizable pollutants in environmental samples [1,2], the expanding list of emerging contaminants highlights the urgency to speed up their risk assessment [3,4].
Generally, risk assessment workflow starts with structural identification, followed by obtaining the analytical standard for confirmation, toxicity assessment, and quantification with a calibration curve. To speed up the process, machine learning has found use in predicting toxicity and ionization efficiency; however, most of the models in use still require chemical structure as an input. Therefore, detected but unidentified chemicals are frequently discarded from further analysis and the bioactivity of samples often remains partially unexplained [5]. Still, the fragmentation spectrum provides information about the structure which can be related to the properties of the chemical. We developed a workflow for estimating the risk of chemicals detected in non-target screening based on their MS^2 data. Two prediction models, MS2Quant [6] for ionization efficiency and MS2Tox [7] for acute fish toxicity were trained based on structural fingerprints. While structural fingerprints can be calculated from structure, a recently developed SIRIUS+CSI:FingerID software [8] offers the possibility to predict these fingerprints based on the MS^2 spectrum, and therefore predict chemical properties without structural assignment. Based on the validation set, the root mean square error of MS2Quant and MS2Tox were 5.9× (39 chemicals) and 7.8× (219 chemicals), respectively. These models were applied in a non-target screening workflow on wastewater analysis. The preliminary results show that MS2Quant and MS2Tox help to pinpoint chemicals that pose a higher risk compared to a top 5 approach. Therefore, this approach provides a possibility to evaluate the risk of unidentified LC/HRMS features and prioritize the high-risk chemicals in identification.

Keywords: high-resolution mass spectrometry; toxicity; quantification; machine learning; non-targeted screening; suspect screening; risk assessment.

Author Contributions: H.S., P.P., and A.K. designed the research study. H.S. and P.P. developed the models and wrote the code. L.J., H.S., and L.M. performed the measurements. A.K., M.P. and M.M.2 performed supervision. A.K., J.M., M.M.1, and M.B. acquired funding for the project. All authors have read and agreed to the published version of the abstract.

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References

1. Papazian, S.; D'Agostino, L. A.; Sadiktsis, I.; Froment, J.; Bonnefille, B.; Sdougkou, K.; Xie, H.; Athanassiadis, I.; Budhavant, K.; Dasari, S.; Andersson, A.; Gustafsson, Ö.; Martin, J. W. Nontarget Mass Spectrometry and in Silico Molecular Characterization of Air Pollution from the Indian Subcontinent. Commun. Earth Environ. 2022, 3 (1), 35. https://doi.org/10.1038/s43247-022-00365-1.
2. Schymanski, E. L.; Singer, H. P.; Slobodnik, J.; Ipolyi, I. M.; Oswald, P.; Krauss, M.; Schulze, T.; Haglund, P.; Letzel, T.; Grosse, S.; Thomaidis, N. S.; Bletsou, A.; Zwiener, C.; Ibáñez, M.; Portolés, T.; de Boer, R.; Reid, M. J.; Onghena, M.; Kunkel, U.; Schulz, W.; Guillon, A.; Noyon, N.; Leroy, G.; Bados, P.; Bogialli, S.; Stipaničev, D.; Rostkowski,

P.; Hollender, J. Non-Target Screening with High-Resolution Mass Spectrometry: Critical Review Using a Collaborative Trial on Water Analysis. Anal. Bioanal. Chem. 2015, 407 (21), 6237–6255. https://doi.org/10.1007/s00216-015-8681-7.

3. Richardson, S. D.; Ternes, T. A. Water Analysis: Emerging Contaminants and Current Issues. Anal. Chem. 2022, 94 (1), 382–416. https://doi.org/10.1021/acs.analchem.1c04640.
4. McCord, J. P.; Groff, L. C.; Sobus, J. R. Quantitative Non-Targeted Analysis: Bridging the Gap between Contaminant Discovery and Risk Characterization. Environ. Int. 2022, 158, 107011. https://doi.org/10.1016/j.envint.2021.107011.
5. Houtman, C. J.; ten Broek, R.; van Oorschot, Y.; Kloes, D.; van der Oost, R.; Rosielle, M.; Lamoree, M. H. High Resolution Effect-Directed Analysis of Steroid Hormone (Ant)Agonists in Surface and Wastewater Quality Monitoring. Environ. Toxicol. Pharmacol. 2020, 80, 103460. https://doi.org/10.1016/j.etap.2020.103460.
6. Sepman, H.; Malm, L.; Peets, P.; MacLeod, M.; Martin, J.; Breitholtz, M.; Kruve, A. Bypassing the Identification: MS2Quant for Concentration Estimations of Chemicals Detected with Nontarget LC-HRMS from MS 2 Data. Anal. Chem. 2023, 95 (33), 12329–12338. https://doi.org/10.1021/acs.analchem.3c01744.
7. Peets, P.; Wang, W.-C.; MacLeod, M.; Breitholtz, M.; Martin, J. W.; Kruve, A. MS2Tox Machine Learning Tool for Predicting the Ecotoxicity of Unidentified Chemicals in Water by Nontarget LC-HRMS. Environ. Sci. Technol. 2022, 56 (22), 15508–15517. https://doi.org/10.1021/acs.est.2c02536.
8. Dührkop, K.; Fleischauer, M.; Ludwig, M.; Aksenov, A. A.; Melnik, A. V.; Meusel, M.; Dorrestein, P. C.; Rousu, J.; Böcker, S. SIRIUS 4: A Rapid Tool for Turning Tandem Mass Spectra into Metabolite Structure Information. Nat. Methods 2019, 16 (4), 299–302. https://doi.org/10.1038/s41592-019-0344-8.

Production and Characterization of Polysaccharides from *Rhodotorula toruloides*

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Abstract: The increasing demand for sustainable material production has fueled extensive research on alternative polysaccharide sources. Microbial polysaccharides, particularly exopolysaccharides (EPS) in yeast, have garnered significant attention in the industrial sector for their unique properties and production methods [1]. *Rhodotorula toruloides*, an unconventional yeast known for intracellular lipids and carotenoid production, also possesses the capacity to produce EPS [2]. This EPS is primarily composed of mannose and glucose, alongside non-carbohydrate components such as proteins and glycoproteins [3]. Therefore, this study aimed to investigate, characterize, and evaluate the chemical properties of EPS derived from *R. toruloides*. The yeast was cultivated in shaker flasks under varied growth conditions, including pH levels, salt concentrations, agitation speeds, and C:N ratios, allowing identification of the optimal environment for EPS production. Previous research has shown that a high carbon-to-limiting nutrient ratio in the growth media increases EPS production [4]. Additionally, an increase in the ionic strength of the medium hinders *R. toruloides* flocculation [5]. EPS purification was achieved through absolute ethanol precipitation, followed by characterization via HPLC for monosaccharide composition, GC-MS for glycosidic linkages after methylation using iodomethane/sodium hydroxide, high-performance gel permeation chromatography for homogeneity and molecular weight, and FT-IR spectrometric analysis to identify functional groups [6-8]. Determining the chemical properties of EPS is crucial for potential applications in industries such as food, pharmaceuticals, and bioplastics. This study contributes to the growing knowledge of sustainable polysaccharide production using yeast, particularly through the utilization of *R. toruloides* as a microbial source. In conclusion, this study underscores the potential of *R. toruloides* as a promising candidate for microbial polysaccharide production. The optimization of cultivation conditions and EPS characterization pave the way for exploring yeast-based polysaccharides in various industrial applications.

Keywords: yeast; polysaccharides; sustainable material production; Rhodotorula toruloides

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References

1. Saadat, Y.R.; Khosroushahi, A.Y. Yeast exopolysaccharides and their physiological functions. *Folia Microbiol* **2021**, *66*, 171-182. DOI: 10.1007/s12223-021-00856-2.
2. Buck, J.W.; Andrews, J.H. Attachment of the yeast Rhodosporidium toruloides is mediated by adhesives localized at sites of bud cell development. *Appl Environ Microbiol* **1999**, 65, 465-471. DOI: 10.1128/AEM.65.2.465-471.1999.
3. Breierová, E.; Kocková-Kratochvílová, A. Cryoprotective effects of yeast extracellular polysaccharides and glycoproteins. *Cryobiology*

1992, 29, 385–390. DOI: 10.1016/0011-2240(92)90039-5.
4. Pavlova, K.; Panchev, I., Krachanova, M.; Gocheva, M. Production of an exopolysaccharide by Antarctic yeast. *Folia Microbiol* **2009**, 54, 343–348. DOI: 10.1007/s12223-009-0049-y.
5. Illarionov, A.; Lahtvee, P.-J.; Kumar, R. Potassium and Sodium Salt Stress Characterization in the Yeasts *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Rhodotorula toruloides*. Appl Environ Microbiol **2021**, *87*, e03100-20. DOI: 10.1128/AEM.03100-20.
6. Van Dyk, J.S.; Kee, N.L. A.; Pletschke, B.I. Extracellular polysaccharide production in Bacillus licheniformis SVD1 and its immunomodulatory effect. *BioResources* **2012**, *7*, 4976-4993.
7. Li, H.; Huang, L.; Zhang, Y.; Yan, Y. Production, Characterization and Immunomodulatory Activity of an Extracellular Polysaccharide from Rhodotorula mucilaginosa YL-1 Isolated from Sea Salt Field. *Mar. Drugs* **2020**, *18*, 595. DOI: 10.3390/md18120595.
8. Hamidi, M.; Gholipour, A.R.; Delattre, C.; Sesdighi, F.; Seveiri, R.M.; Pasdaram, A.; Kheirandish, S.; Pierre, G.; Kozani, P.S.; Kozani, P.S.; Karimitabar, F. Production, characterization and biological activities of exopolysaccharides from a new cold-adapted yeast: Rhodotorula mucilaginosa sp. GUMS16. International Journal of Biological Macromolecules. *Int. J. Biol. Macromol.* **2020**, 151, 268-277. DOI: 10.1016/j.ijbiomac.2020.02.206.

What Phytoplankton Species Can Tell Us About the Implications of Engineered Nanoparticles in the Aquatic Environment

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Abstract: Nanotechnology is considered as the "sixth truly revolutionary technology" introduced in the modern world. The central question how to benefit by this powerful technology, while maximizing and avoiding possible risks represents a challenge for regulatory agencies and an important area of scientific research. With examples from our own research we will illustrate what happens when aquatic microorganisms are inadvertently exposed to engineered nanoparticles (ENPs) that are increasingly released into the environment. The specific focus will be on nanoAg and nanoTiO_2 as representatives of mostly widely used nanomaterials. We compared the ENPs-induced responses in two representative phytoplankton species: presumably "particle-proof" green alga *Chlamydomonas reinhardtii* and "particle-ingesting" microalgal predator flagellate *Poterioochromonas malhamensis*. Generation of the highly reactive oxygen species (ROS), disturbing the cellular pro- and antioxidant equilibrium was followed. The results revealed significant increase of the cellular ROS upon exposure to ENPs, but the intensity of the effects was dependent on the nature, size and concentration of the ENPs, the exposure duration and the feeding pattern of the species. Liquid chromatography - based targeted metabolomics revealed that in all cases the abundance of metabolites involved in various pathways corresponding to amino acid, nucleotides, fatty acids, tricarboxylic acid cycle, and antioxidant metabolism was altered in various treatments. The metabolomics results correlated well with the physiological results and confirmed that (i) oxidative stress is a major toxicity mechanism for nanoTiO_2 exposure [1]; (ii) dissolved Ag released by nanoAg seems to be a major toxicity driver, even though nanoAg is internalized in the food vacuoles of *P. malhamansis* [2]. However, nanoAg plays an important role in the perturbation of amino acid metabolism, TCA cycle and oxidative stress. The implications of the obtained results for the assessment of the ENPs toxicity and tolerance responses in phytoplankton and for enabling a discovery of sensitive markers for early warning will be highlighted.

Keywords: nanomaterials, phytoplankton, metabolomics

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References

1. Liu, W.; Li, M.; Li, W.; Keller, A.A. and Slaveykova, V.I. Metabolic alterations in alga *Chlamydomonas reinhardtii* exposed to nTiO_2 materials. *Environ. Sci. Nano*, **2022**, *9*, 2922-2938.
2. Liu, W.; Majumdar, S.; Li, W.; Keller, A.A. and Slaveykova, V.I. Metabolomics for early detection of stress in freshwater alga *Poterioochromonas malhamensis* exposed to silver nanoparticles. *Sci. Rep*. **2020**, *10*, 20563.

Viral Effect on Carbon and Nitrogen Cycling in Bloom-Forming Cyanobacteria

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Abstract: Viruses can significantly influence the biogeochemical cycling of major nutrients through the infection and lysis of cyanobacteria, a globally important primary producers [1]. However, surprisingly little attention has been given to understand how viruses alter the metabolism of carbon (C) and nitrogen (N) in bloom forming cyanobacteria, distributed worldwide in fresh and brackish water ecosystems. Moreover, there is a lack of information about how co-occurring microbial community respond to the lysis of these primary producers. Therefore, we employed an ecologically relevant filamentous diazotrophic cyanobacteria *Aphanizomenon flos-aquae* [2] and *Nodularia spumigena* [3] and their lytic cyanophages [4,5] as a host-virus model systems in combination with a series of incubation experiments, to investigate the effect of viral infection and lysis on photosynthetic activity, nitrogen assimilation and enrichment rates, expression level of genes involved in photosynthesis, carbon and nitrogen metabolism as well as on the concentration of some central and secondary cellular metabolites. In addition, we analyzed the variation in the composition of associated bacterial assemblages in response to viral additions and in relation to uninfected cyanobacterial cultures throughout their cultivation periods. We found that the effect of cyanophages on carbon and nitrogen cycling and cellular metabolism was significant yet varied widely depending on the stage of the infection process (e.g. cyanophage adsorption vs. DNA replication vs. release), and the state of the host culture (culture undergoing infection/lysis vs. recovering culture). Our observations suggest that cyanobacteria underwent a physiological state-shift toward lower efficiency of carbon and energy cycling as well as to the reduced nitrogen transport from heterocytes (N fixing cells) to vegetative cells [6,7]. Lysis of cyanobacterial cells was associated with a release of ammonium and other compounds that promoted changes in co-occurring microbes. The shift in the associated bacterial community was related to the infection rate and increased with higher initial cyanophage density. On the contrary, the initial infection rate, although affected the timing, had no effect on the magnitude of net population loss or changes in population structure. Our observations indicate that cyanophage infection and lysis have implications across multiple levels of ecological organization, from cell to population and the entire community [5,6].

Keywords: *Aphanizomenon flos-aquae*, Cyanophage vB_AphaS-CL131, Harmful cyanobacterial blooms, Metabolic reprograming

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References

1. Jover, L. F.; Effler, T. C.; Buchan, A.; Wilhelm, S. W.; Weitz, J. S. The elemental composition of virus particles: Implications for marine biogeochemical cycles. Nature Rev Microbiol 2014, 12, 519–528. https://doi.org/10.1038/nrmicro3289
2. Cirés, S.; Ballot, A. A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon* spp. and related species within the *Nostocales* (cyanobacteria). Harmful Algae 2016, 54, 21–43. https://doi.org/10.1016/j.hal.2015.09.007
3. Bertos-Fortis, M.; Farnelid, H. M.; Lindh, M. V.; Casini, M.; Andersson, A.; Pinhassi, J.; Legrand, C. Unscrambling cyanobacteria community dynamics related to environmental factors. Front Microbiol 2016, 7, 625. https://doi.org/10.3389/fmicb.2016.00625
4. Šulčius, S.; Šimoliūnas, E.; Alzbutas, G.; Gasiūnas, G.; Jauniškis, V.; Kuznecova, J.; Miettinen, S.; Nilsson, E.; Meškys, R.; Roine, E.; Paškauskas, R.; Holmfeldt, K. Genomic characterization of cyanophage vB_AphaS-CL131 infecting filamentous diazotrophic cyanobacterium *Aphanizomenon flos-aquae* reveals novel insights into virus-bacterium interactions. Appl Environ Microbiol 2019, 85, e01311-18. https://doi.org/10.1128/AEM.01311-18
5. Šulčius, S.; Mazur-Marzec, H.; Vitonytė, I.; Kvederavičiūtė, K.; Kuznecova, J.; Šimoliūnas, E.; Holmfeldt, K. Insights into cyanophage-mediated dynamics of nodularin and other non-ribosomal peptides in *Nodularia spumigena*. Harmful Algae 2018, 78, 69–74. https://doi.org/10.1016/j.hal.2018.07.004
6. Kuznecova, J.; Šulčius, S.; Vogts, A.; Voss, M.; Jürgens, K.; Šimoliūnas, E. Nitrogen flow in diazotrophic cyanobacterium *Aphanizomenon flos-aquae* is altered by cyanophage infection. Front Microbiol 2020, 11, 1–14. https://doi.org/10.3389/fmicb.2020.02010
7. Antosiak, A.; Šulčius, S.; Malec, P.; Tokodi, N.; Łobodzińska, A.; Dziga, D. Cyanophage infections reduce photosynthetic activity and expression of CO2 fixation genes in the freshwater bloom-forming cyanobacterium *Aphanizomenon flos-aquae*. Harmful Algae 2022, 116, 102215. https://doi.org/10.1016/j.hal.2022.102215

Spray-Pyrolysis Synthesised TiO_2 Thin Films for Photocatalytic Air Treatment from VOCs

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Abstract: A wide range of mixtures of volatile organic compounds (VOCs), which are present at indoor air in low concentrations, can strongly affect human health. Therefore, the scientific interest to photocatalytic oxidation as a cost-effective and efficient technology for removal of VOCs from indoor air is growing. The aim of the study was to deposit TiO_2 thin films by ultrasonic spray pyrolysis with different titanium isopropoxide (TTIP): acetylacetone (AcacH) molar ratios in the spray solution. Other objectives were to determine the optimal TTIP:AcacH ratio and to study the ability of the film to purify air from VOCs under different experimental conditions. TiO_2 films were deposited onto the borosilicate glass at 350°C and heat-treated at 500°C for 1 h. TTIP:AcacH molar ratio in spray solution varied from 1:1 to 1:20. At first, the photocatalytic activity of all obtained films was estimated by degradation of 8.8 mM stearic acid (SA) deposited on top of the film [1]. In the second step of studies, TiO_2 films were tested for oxidation of VOCs (acetone, acetaldehyde, heptane and toluene) as separate pollutants in the concentration ranges 5-40 ppm [2] and as 9-ppm VOCs mixtures [3]. The oxidation of VOCs was studied in the gas-phase multi-section reactor under ultraviolet and visible light. An increase in the amount of AcacH in the spray solution enhanced the photocatalytic performance of the films due to the carbon incorporation and changes in electronic structure. The reaction rate constant of SA oxidation on the film with the TTIP:AcacH molar ratio 1:8 was ten times higher than that of the 1:3 film. TiO_2 film with molar ratio 1:8 showed promising ability in VOCs degradation, oxidizing up to 9 ppm VOCs mixtures at 1.5 min at catalyst surface areas of 600 cm^2 under ultraviolet and up to 90% of the mixture under visible light.

Keywords: spray pyrolysis, TiO_2, thin films, photocatalysis, indoor air, purification, volatile organic compounds.

Author Contributions: Conceptualization, J.S., I.O.A. M. Krichevskaya; methodology, I.O.A., M. Krichevskaya; software, A.M, M. Krichevskaya; validation, J.S.; formal analysis, J.S., I.O.A., M. Krichevskaya; investigation, I.O.A., M. Krichevskaya, M. Krunks, A.M.; resources, I.O.A.; data curation, J.S.; writing—original draft preparation, J.S.; writing—review and editing, I.O.A., M. Krichevskaya, M. Krunks, A.M.; visualization, J.S.; supervision, I.O.A, M. Krichevskaya; project administration, I.O.A.; funding acquisition, I.O.A. All authors have read and agreed to the published version of the manuscript.

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References

1. J. Spiridonova, A. Katerski, M. Danilson, M. Krichevskaya, M. Krunks, I. Oja Acik. Effect of the Titanium Isopropoxide: Acetylacetone Molar Ratio on the Photocatalytic Activity of TiO_2 Thin Films. Molecules **2019**, 24, 4326, http://doi.org/10.3390/molecules24234326.
2. J. Spiridonova, A. Mere, M. Krunks, M. Rosenberg, A. Kahru, M. Danilson, M. Krichevskaya, I. Oja Acik. Enhanced Visible and Ultraviolet Light-Induced Gas-Phase Photocatalytic Activity of TiO_2 Thin Films Modified by Increased Amount of Acetylacetone in Precursor Solution for Spray Pyrolysis. Catalysis **2020**, 10, 1011, http://doi.org/10.3390/catal10091011.
3. J. Sydorenko, A. Mere, M. Krunks, M. Krichevskaya, I. Oja Acik. Transparent TiO_2 thin films with high photocatalytic activity for indoor air purification. RCS Advances 12, **2022**, 35531-35542, https://doi.org/10.1039/D2RA06488J.

International Business, Academic and RDI Cooperation as Drivers and Accelerators of Sustainable Economic Growth in Biotechnology and the Circular Economy - Results of the BBC1 - Project

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Abstract: A common argument is that Europe is the world's leading producer of science, but is falling behind in its application [1]. The emergence of new commercial solutions of environmental technology on the market requires long-term technological product development, a suitable development environment and successful technology transfer from invention to innovation. Technology transfer requires business development, legal knowledge, negotiation, networking, market knowledge and marketing.

In the BBC1 project promoted the emergence and commercialisation of biotechnology and circular economy solutions to achieve sustainable economic growth in the cross-border area between Finland and Russia, in which the Finnish side requires a special regional economic support measures. The project staff worked in cooperation with companies, students and experts from universities and business development organisations to develop and test new collaborative models for commercialising innovations and increasing business.

The project organised networking and expert events for businesses in different sectors of the circular economy to grow their international business. Courses, competitions, lectures and programmes were organised for students to develop their business skills while stimulating new solutions and innovation in the circular economy. The Business symbiosis programmes brought businesses and students together and used the expertise of researchers as sparring partners in the process. In the project developed a digital platform "ecosairila.fi", which was used in the implementation of the project and serves as an international information portal and knowledge exchange platform for businesses, students, experts and citizens. The EcoSairila framework provided an excellent environment to implement the project and to visualise the concrete and optimal implementation of the water circularity in particular.

Systematic cooperation between students, businesses and experts can significantly boost market access for new environmental technology solutions. In the BBC1 project developed models and methods for cooperation. However, cooperation is not yet well established and models, methods and platforms for cooperation need to be further developed and expanded. Cooperation also needs to be driven and continuous, because there is and will continue to be a huge global demand for new technological solutions to improve the state of the environment. It would be valuable and desirable to develop cooperation: different actors could learn from and support each other and exchange best practices [1]. It would also be useful for the sector to make efforts to raise the profile of ecosystem managers, whose skills and professionalism play a key role in promoting innovation [1].

Keywords: environmental technology; commercialization; innovation; business development; biotechnology; circular economy; economic growth; internationalization; bisness symbiosis; ecosystem manager

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References

1. Mikko Huuskonen. Kansainvälisiä oppeja keksintöjen kaupallistamisesta (International lessons on the commercialisation of inventions). Yrityskatsaus (Business review) 2019, Työ- ja elinkeinoministeriö (Ministry of Economic Affairs and Employment of Finland), Suomi Finland. Available online: https://yrityskatsaus.fi/kansainvalisia-oppeja-keksintojen-kaupallistamisesta/

Designing of Sustainable Building Material Made of Non-Fired Clay with Various Biopolymers

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Abstract: This study is dedicated to the development of an environmentally friendly building material made from non-fired clay, stabilized with various biopolymers (starch, alginate, and chitosan). The application of a natural polymers have already proven their efficacy in geotechnical industry for soil stabilization [1-3]. Authors believe, that the use of natural polymers will have a positive impact on the physical-mechanical and operational properties of the newly-obtained building material made from non-fired clay. With addition of different concentrations (2.5%, 5%, 7.5%, and 10%) of biopolymer solutions, the density of the clay composites was reduced by up to 9% (from 1990 to 1810 kg/m^3). The inclusion of starch and alginate contributed to an increase in the strength of the clay composite, up to 42% (from 6.6 to 9.4 MPa). However, the application of 10% chitosan resulted in a 20% decrease in strength (from 6.6 to 5.3 MPa). The conducted research confirms that the use of biopolymers is a promising stabilizer for non-fired clay composites. Nevertheless, to create an effective building material, it is essential to consider the type of clay and biopolymer, as well as their interaction potential and compatibility.

Keywords: clay; biopolymers; stabilization; modification; building materials; sustainability

Author Contributions: Conceptualization, Y.T. and O.K.; methodology, O.K. and V.K.; validation, Y.T., O.K. and V.K.; data curation, O.K.; writing—original draft preparation, Y.T.; writing—revie and editing, O.K. and V.K.; visualization, Y.T.; supervision, O.K.; All authors have read and agreed to the published version of the manuscript.

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References

1. Chang, I.; Im, J.; Prasidhi, A.K.; Cho, G.C. Effects of Xanthan gum biopolymer on soil strengthening. *Constr. Build. Mater.* 2015, 74, 65–72. https://doi.org/10.1016/j.conbuildmat.2014.10.026.
2. Losini, A.E.; Grillet, A.C.; Bellotto, M.; Woloszyn, M.; Dotelli, G. Natural additives and biopolymers for raw earth construction stabilization – a review. *Constr. Build. Mater.* 2021, 304, 124507. https://doi.org/10.1016/j.conbuildmat.2021.124507.
3. Soldo, A.; Miletić, M.; Auad, M.L. Biopolymers as a sustainable solution for the enhancement of soil mechanical properties. *Sci. Rep.* 2020, 10(1), 1–13. https://doi.org/10.1038/s41598-019-57135-x.

Compounds from Personal Care Products as Emerging Contaminants in Swimming Pool Waters

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Abstract: (1) Background: Ultraviolet (UV) light, coming from the sun, is particularly harmful in biological systems, where it causes the damage to skin cells, resulting in accelerated aging of the skin and the emergence of various diseases, from inflammatory processes to cancer. To protect against UV light, various substances, named UV filters, are used that either reflect or absorb UV light. Increasingly, they are used in personal care products (e.g. sunscreen, lipsticks, shampoos and hair sprays) as a result of the growing awareness of the harmful exposure to the sun. Sunscreen products are used primarily in settings, such as swimming pools and sea, in the snow and in the mountains, where thorough protection is needed. However, several studies showed that UV filters may degrade by action of oxidant or react by light [1-3]; (2) Methods: In our study we focused on transformation of selected UV filters as well as some antioxidants in sunscreens under disinfection conditions. Various experimental condition (media, light source, addition of ions, disinfectants and their combinations were used in order to simulate conditions under which different products may be formed. They were identified by HPLC-MS, and GC-MS. Some compounds were even synthesized independently to prove the presence of specific products. (3) Results: Over 60 disinfection by-products were identified as transformation products of avobenzone in different disinfection reactions of chlorination and bromination in fresh and seawater. The formation of halogenated byproducts in chlorinated waters is inevitable, since compounds possess double bonds, phenolic, keto, or amino moieties. From the environmental point of view, we need to mention formation of chloroanhydrides and chlorophenols (chlorination experiments) as well brominated phenols and substituted acetophenones; (bromination experiments) [4-8]; (4) Conclusions: The mechanism of aquatic chlorination or bromination is a very complex process and rather difficult to predict. The assortment and level of by-products depend on the concentration and type of disinfecting agent, ratio substrate/active chlorine, pH, temperature, reaction time and water composition. All these influence on the toxicity of reaction mixture.

Keywords: UV filters; sunscreens; water disinfection; disinfection by-products

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References

1. Shaath, N.A. Ultraviolet filters. Photochem. Photobiol. Sci. **2010**, 9 (4), 464–469. DOI: 10.1039/b9pp00174c
2. Lebedev, A. Mass spectrometry in the study of mechanisms of aquatic chlorination of organic substrates. Eur. J. Mass Spectrometry **2007**, 13, 51–56. https://doi.org/10.1255/ejms.852
3. Ul'yanovskii, N.V.; Kosyakov, D.S.; Varsegov, I.S.; Popov, M.S.; Lebedev, A.T. Identification of Novel

Disinfection Byproducts in Pool Water: Chlorination of the Algaecide Benzalkonium Chloride. Chemosphere **2020**, 239, 124801. https://doi.org/10.1016/J.CHEMOSPHERE.2019.124801.

4. Trebše, P., Polyakova, O.V., Baranova, M., Dolenc, D., Sarakha, M., Kutin, A., Lebedev,A.T. Transformation of avobenzone in conditions of aquatic chlorination and UV-irradiation. **2016**, Water Res. 101, 95–102. http://dx.doi.org/10.1016/j.watres.2016.05.067
5. Chugunova, A.A., Bavcon Kralj, M., Polyakova, O.V., Artaev, V., Trebse, P., Pokryshkin, S.A., Lebedev, A.T., Study of the chlorination of avobenzone in sea water by gas chromatography – high resolution mass spectrometry. **2017**, J. Anal. Chem. 72 (14), 1369–1374 Original Russian version in Mass-spektrometrya (Rus) 2017. Vol. 14. N 2.73-78. DOI: 10.1134/S1061934817140039
6. Detenchuk, E.A., Chen, J., Polyakova, O.V., Trebse, P., Pokryshkin, S.A., Lebedev, A.T., Study of aquatic chlorination of UV filter avobenzone in the presence of inorganic salts by gas chromatography combined with high resolution mass spectrometry. J. Anal. Chem. **2019**, 74, 1271–1276. https://doi.org/10.1134/S1061934819130069. Original Russian version in Mass-spectrometrya (Rus), 2018. Vol. 15 N 4. doi:10.25703/MS.2018.15.42.
7. Lebedev, A.T.; Bavcon Kralj, M.; Polyakova, O.V.; Detenchuk, E.A.; Pokryshkin, S.A.; Trebše, P. Identification of Avobenzone By-Products Formed by Various Disinfectants in Different Types of Swimming Pool Waters. Environ. Int. **2020**, 137, 105495. https://doi.org/10.1016/j.envint.2020.105495.
8. Detenchuk, E.A.; Trebše, P.; Marjanović, A.; Kosyakov, D.S.; Ul’yanovskii, N.V.; Kralj, M.B.; Lebedev, A.T. Transformation of Resveratrol under Disinfection Conditions. Chemosphere **2020**, 260, 127557. https://doi.org/10.1016/j.chemosphere.2020.127557.

Endocrine Disrupting Activity of Mixtures Composed of Pharmaceuticals and Nanoplastics

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Abstract: Endocrine disrupting chemicals (EDCs) are defined as "an exogenous chemical or mixture of chemicals that can interfere with any aspect of the hormone action" [1,2]. Thus, they have been considered among strong risk factors in the development of obesity, metabolic disorders, infertility, endocrinopathies, diabetes, and hormone-dependent cancers globally. Combined exposure to EDCs may have more pronounced adverse effects on human health and may trigger stronger (or occasionally weaker) toxicological effects than exposure to individual chemical, even at concentrations regarded as non-adverse (i.e. where no effects are expected) [3]. This study is focused on endocrine disruptive (ED) effects of the mixture of pharmaceuticals with nanoplastics.

Paracetamol, ibuprofen and fluoxetine were selected as scientific cases of pharmaceuticals, while commercially available 25 nm sized polystyrene nanoparticles (PNP) were used as nanoplastics. ED effects of each pharmaceutical and PNP as well as of their mixtures were evaluated using in vitro estrogen receptor activity assay based on T47D-KBluc cell line [4]. This cell line is stably transfected with a triplet ERE (estrogen-responsive elements)-promoter-luciferase reporter gene construct and therefore can be used to screen chemicals for estrogenic and anti-estrogenic effects.

Obtained results showed estrogenic effects of PNP and all tested pharmaceuticals. The mixture of pharmaceuticals with PNP demonstrated higher agonistic affinity towards estrogen receptors (ER) compared to individual components of the mixture.

This study unambiguously shows that health hazard potential of environmental contaminants should not be investigated exclusively as individual pollutants, but as complex mixtures components.

Keywords: estrogen receptors; polystyrene nanoparticles; paracetamol; ibuprofene; fluoxetine; mixture effects

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Author Contributions: Conceptualization, IVV; methodology, LB, NP and NK.; validation, LB; formal analysis, LB, NP and NK; investigation, LB; resources, IVV; data curation, LB, NP and NK; writing—original draft preparation, LB, NP and NK; writing—review and editing, IVV; visualization, LB, NP and NK; supervision, IVV; project administration, IVV; funding acquisition, IVV. All authors have read and agreed to the published version of the manuscript.

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References

1. Schug, TT; Johnson, AF; Birnbaum, LS; Colborn, T; Guillette, LJ; Crews, DP; et al. Minireview: Endocrine Disruptors: Past Lessons and Future Directions. *Mol Endocrinol* **2016,** 30(8), 833–847.
2. Lauretta, R; Sansone, A; Sansone, M; Romanelli, F; Appetecchia, M. Endocrine Disrupting Chemicals: Effects on Endocrine Glands. *Front Endocrinol (Lausanne)* **2019**, 10, 178.
3. https://ec.europa.eu/environment/pdf/chemicals/2020/10/SWD_mixtures.pdf.
4. USEPA, Office of Prevention P and TS: Endocrine Disruptor Screening Program Test Guidelines - OPPTS 8901300: Estrogen Receptor Transcriptional Activation. **200**

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Scientific and legal responsibility for the abstract belongs to the authors. Spelling and punctuation are kept without changes.

Abstracts of Poster Presentations

Burning of Fountain Candles Indoor – A Moment of Joy Versus Indoor Air Quality Concerns

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Abstract: Burning candles, magic candles, incense, and pyrotechnics such as fountain candles creates a sense of joy and celebration in various life and social events. Unfortunately, most people never even thought about the smoke, particulate matter produced and the pollution that occurs and remains in indoor air after such activities. A lot of studies have raised the issue whether the use of such products indoors can worsen indoor air quality [1,2]. Moreover, contrary to that the exposure to fine ambient particulate matter has been associated to cardiovascular and respiratory diseases, relevance with particulate matter from different candle burning remain unexplored [3].

The objective of the current study was to characterize number concentration and mass concentration of particulate matter originated indoors after burning fountain candles, which are commercially available in Latvia. Therefore, simulating studies of burning fountain candles were performed in a close laboratory for quantitative analysis of obtained particulate matter. For the mass concentration of PM_{10}, $PM_{2.5}$, PM_1 and number concentration of ultra fine and fine particulate matter with diameters from 0.265 up to 2.750 µm measuring the spectrometer GRIMM EDM – 365 (Grim Aerosol Technik) was used. In order to determine possible exposure of emitted particulate matter, detecting device was placed 1 m from fountain candle. In another experiment detecting device was placed 4 m from fountain candle to characterize particulate matter distribution dynamics (see Fig.1).

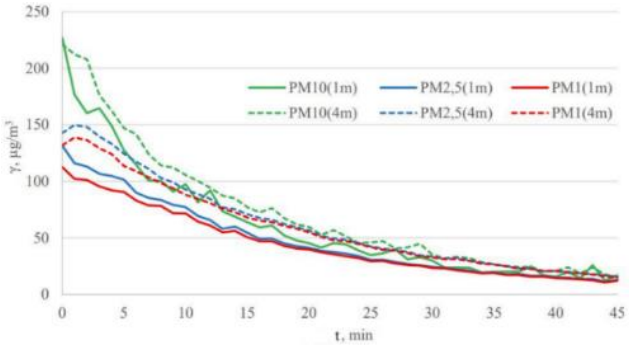

Figure 1. Mass concentrations of PM_{10}, $PM_{2.5}$ and PM_1 emitted from fountain candle burning depending on the distance of the detecting devise.

Model experiments showed that most of the particulate matter released indoors after fountain candles burning are in size ≤ 0.265 µm, which are the most harmful to human health. Tha mass and number concentration of particles depend on the distance at which the emission source is located, e.g., ultra fine particles move further in the room and their concentrations are higher at 4 m compared to 1 m.

Keywords: particulate matter; number concentration**;** fountain candles; indoor air; Grimm spectrometer.

Author Contributions: Conceptualization, A.A. and M.R..; methodology, A.A., M.R.; software, L.P.; validation, M.B., L.P.; formal analysis, L.P.; investigation, A.A.; resources, A.V.; data curation, L.P.; writing—original draft preparation, A.A.; writing—review and editing, M.R.; visualization, L.P.; supervision, A.A.; project administration, A.V. All authors have read and agreed to the published version of the manuscript.

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References

1. Derudi, M.; Gelosa, S.; Sliepcevich, A.; Cattaneo, A.; Cavallo, D.; Rota, D.; Nano, G. Emission of air pollutants from burning candles with different composition in indoor environments. *Environ Sci Pollut Res* **2014**, *21*, 4320–4330, DOI 10.1007/s11356-013-2394-2.
2. Niu, X.; Jones, T.; BéruBé, K.; Chuang, H.; Sune, J.; Ho, H.F. The oxidative capacity of indoor source combustion derived particulate matter and resulting respiratory toxicity. *Sci Tot Environ* **2021**, *767*, https://doi.org/10.1016/j.scitotenv.2020.144391.
3. Loft, S.; Andersen, Z.A.; Jørgensen, J.T.; Kristiansen, A.D.; Dam, J.K.; Cramer, J.; Rudi G. J.; Westendorp, R.G.J.; Lund, R.; Lim, J.H. Use of candles and risk of cardiovascular and respiratory events in a Danish cohort study. *Indoor Air* **2022**, *32*, 1-10, https://doi.org/10.1111/ina.13086.

The Effects of Pesticides on the Bioenergetics of Intestinal Cells

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Abstract: Colorectal cancer is considered the second most common type of cancer [1]. It is assumed for this type of cancer that environmental factors are more important than hereditary factors [2]. Pesticides present in our food can alter the metabolism of our intestinal cells and this might be related to potential impact on overall cellular metabolism.

The aim of this project was to investigate whether pesticides Glyphosate, Glyphosate-based Roundup, Boscalid, and NeemAzal, alter the energy metabolism of human intestinal cells.

The study analyses if prolonged exposure and different growth environments increase the sensitivity of Caco-2 cells to pesticides. The rate of oxygen consumption based on electron flow through individual respiratory chain complexes and the overall oxygen consumption rate of respiratory chain were analyzed using the method of high resolution respirometry.

The results demonstrated that lower concentrations of pesticides, which do not affected cells in the short term, significantly decreased cell viability with prolonged use. The experiments also showed that in a plasma-like medium, similar to physiological conditions, the toxic effect of pesticides is higher or equivalent to that observed in cells grown in a regular medium. Analysis of mitochondrial oxidative phosphorylation revealed a significant decrease in oxygen consumption rate through the electron transport chain at concentrations reducing cell viability by 20% for all pesticides. At lower pesticide concentrations reducing viability by up to 10%, the effect was detectable only for Boscalid and Roundup. The results of the study confirm that commercially available pesticide Roundup, along with their accompanying additives, exhibit stronger toxic effects than the declared active ingredient Glyphosate alone.

The results of the study indicate that low pesticide concentrations, which have no immediate impact, may exert toxic effects over a longer period and this influence should be studied in a plasma-like medium.

Keywords: Mitochondria; Pesticides; High resolution respirometry; *Oxidative phosphorylation*.

Author Contributions: Conceptualization, K.T. and T.K.; methodology, K.T.; formal analysis, K.T.; investigation, K.T.; G.L.A. and K.K.; writing—original draft preparation, G.L.A. and K.T.; writing—review and editing, T.K.; visualization, G.L.A. and K.T.; supervision, K.T. and T.K.; funding acquisition, K.T. All authors have read and agreed to the published version of the manuscript.

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References

1. R.L. Siegel, N.S. Wagle, A. Cercek, R.A. Smith, A. Jemal, Colorectal cancer statistics, 2023, CA Cancer J Clin, (2023) https://doi.org/10.3322/caac.21772.
2. J. Tian, K.O. Afebu, A. Bickerdike, Y. Liu, S. Prasad, B.J. Nelson, Fundamentals of Bowel Cancer for Biomedical Engineers, Annals of biomedical engineering, 51 (2023) 679-701 https://doi.org/10.1007/s10439-023-03155-8.

Structural and Morphological Investigations of N- and B-Modified Reduced Graphene Oxide

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Abstract: The successful application of reduced graphene oxide (rGO) in supercapacitors, (bio)sensors, fuel or solar cells strongly depends on its physicochemical properties. Present studies have demonstrated that doping with heteroatoms (B, N, P, and S) could be a key strategy to alter the electrochemical, electronic, and structural characteristics of rGO [1,2]. It is well known that nitrogen-rich sites in rGO can effectively improve electrochemical activity, especially, in the presence of pyrrolic-N and pyridinic-N bonding configurations. N-doping also increases the electrical conductivity of graphene-based materials due to the formation of N-graphitic atoms in the lattice and enhances the specific surface area by larger defect sizes and a more porous structure [2]. In the case of B-doping, the incorporation of B atoms can lead to extra sites grafted onto the carbon surface, enabling both enhanced hydrophilicity and durability for the carbon materials [3]. Therefore, the boron- and nitrogen-codoped rGO (BN-rGO) samples synthesized with improved structural and electrochemical properties may be promising candidates as metal-free and non-expensive electrode materials for the development of supercapacitors or (bio)sensors. This study focusses on the synthesis procedure as well as the structural and morphological characterisation of BN-rGO nanostructures. BN-rGO samples are prepared by a two-stage synthesis method. In the first step, a homogeneous suspension of GO mixed with different amounts of NH_4BF_4 is hydrothermally treated in a Teflon-lined stainless-steel autoclave at a temperature of 180 °C for 20 hours. In the second step, the resulting materials are thermally annealed in a tube furnace at 850 °C temperature for 30 min under Ar atmosphere. The impact of B- and N-codoping on the morphology and structure of rGO is analyzed by scanning electron microscopy (SEM), energy dispersive X-ray (EDX), and Raman spectroscopies. The BN-rGO materials are also studied by measuring nitrogen adsorption-desorption isotherms at 77 K.

Keywords: reduced graphene oxide; doping with N and B; structural and morphological properties

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Author Contributions: Conceptualization, R.A.; methodology, R.A.; investigation, R.A.; data curation, R.A.; writing—original draft preparation, R.A; writing—review and editing, J.G. and J.B.; visualization, R.A.; supervision, J.G. All authors have read and agreed to the published version of the manuscript.

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References

1. Kaushal, S.; Kaur, M.; Kaur, N.; Kumari, V.; Singh, P. P. Heteroatom-doped graphene as sensing materials: a mini review. RSC Adv 2020, 10, 28608–28629.
2. Li, D.; Duan, X.; Sun, H.; Kang, J.; Zhang, H.; Tade, M.O.; Wang, S. Facile synthesis of nitrogen-doped graphene via low-temperature pyrolysis: The effects of precursors and annealing ambience on metal-free catalytic oxidation. *Carbon* **2017**, 115, 649–658.
3. Gul, I.; Yar, M.; Ahmed, A.; Hashmi, M.A.; Ayub, K. Permeability of boron- and nitrogen-doped graphene nanoflakes for protium/deuterium ion. *RSC Adv* **2022**, 12(7), 3883–3891.

Variation of Carbon and Nitrogen Stable Isotope Ratios in Conventionally and Organically Fertilized Cereals at Different Growth Stages

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Abstract: Over the past decades, the cereal grain market has experienced notable changes. Due to the demand for healthier and more sustainable food options, significant growth in the production of organically grown cereals has been observed in the EU and Latvia [1]. Climate change and its impact on agricultural productivity have created challenges to cereal grain production leading to a focus on resilient crop varieties and sustainable farming practices. Research on stable carbon and nitrogen isotope ratio changes of barley and triticale at different growth stages gives valuable insights into the metabolic processes and nutrient uptake patterns of these crops. This research contributes to the improvement of sustainable agricultural practices by allowing the optimization of fertilization strategies and the development of more efficient crop management techniques. For this study barley and triticale samples at the stages of tillering, jointing, booting and maturity stages from conventionally and organically fertilized sample plots were collected at Institute of Agricultural Resources and Economics Priekuli Research Centre. Roots, leaves and grains at maturity stage of the collected crop samples were analyzed using stable isotope ratio mass spectrometer (Nu Horizon, Nu Instruments, UK). $\delta^{13}C$ and $\delta^{15}N$ values, and total carbon and nitrogen content were determined. The results showed decrease in $\delta^{15}N$ values and total nitrogen content in both barley and triticale roots and leaves during the growth of analyzed crop samples. No significant changes in $\delta^{13}C$ values and total carbon content were observed. Differences in total nitrogen content and nitrogen stable isotope ratios between conventionally and organically fertilized crops were not definite both for barley and triticale samples. These findings highlight the dynamic nature of nitrogen uptake and utilization in barley and triticale crops during growth stages and suggest that other factors beyond fertilizer type may influence nitrogen content and isotope ratios in these crops.

Keywords: stable isotope ratios; barley; triticale; organic farming; conventional farming; cereal grains; cereal growth stages

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Author Contributions: Conceptualization, L.B. and A.V.; methodology, A.V., L.B., L.L. and I.L.; software, L.B.; validation, L.B., A.V. and G.C.; formal analysis, L.B. and G.C.; investigation, L.B. and G.C.; resources, A.V., L.L. and I.L.; data curation, L.B. and G.C.; writing—original draft preparation, L.B. and G.C.; writing—review and editing, L.B., G.C. and A.V.; visualization, L.B.; supervision, A.V., L.L. and I.L. All authors have read and agreed to the published version of the manuscript.

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References

1. Busacca, E.; Gernert, M.; Moeskops, B.; Steu, A.; Willer, H. Organic in Europe: Recent Developments. In *The World Of Organic Agriculture. Statistics and Emerging Trends 2023*, Willer, H.; Schlatter, B.; Travnicek, J., Eds.; Research Institute of Organic Agriculture FiBL, Frick, and IFOAM – Organics International: Bonn, Germany, 2023, pp. 225-233. doi:10.5281/ZENODO.7572890.

Study of Pesticides in Bolivian Quinoa (*Chenopodium quinoa Willd*) by Gas Chromatography with Mass Detector GC-MS

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Abstract: Quinoa has been recognized for centuries as an important food crop in the Andes of South America, its grains are highly nutritious with a significant amount of protein and bioactive compounds, surpassing traditional cereals in biological value, it is also considered one of the more important in cultivation and export in Bolivia. Bolivian quinoa exports reach at least 60 countries in the world, among the main ones are the United States, Canada, China, France, Germany, Belgium, Australia, Spain. The forms of sale are classified into organic quinoa and conventional quinoa.

US MRLs in cereals for chlorpyrifos are 0.5 mg/Kg, chlorpyrifos methyl 6 mg Kg, 0.05 mg/Kg L cyhalothrin; The MRL for cypermethrin according to the European Union 0.3 mg/Kg These mentioned values apply to conventional type crops. To be called organic quinoa, the values must be below 0.01 mg/Kg, so sensitive quantification techniques such as GC MS and extraction methods are used that manage to determine concentrations of the order of 0.01 mg/Kg.

The method used for the determination of pesticides is based on the UNE EN 15662 standard by means of GCMS, extraction with acetonitrile and the dispersive purification method QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) finding the presence of Cypermethrin, L cyhalothrin , chlorpyrifos chlorpyrifos methyl, which have a minimum value of <0.01mg/Kg and 0.01mg/Kg and the maximum values vary depending on the pesticide 0.02; 0.014; 0.145; 0.036 mg/Kg in chlorpyrifos, methylchlorpyrifos, cypermethrin and L-cyhalothrin respectively. These data were found by analyzing 200 samples from the departments of Potosí and Oruro, which are the main producing departments in Bolivia. The analyzes were carried out from 2016 to 2021, from which it is concluded that 69% of the quinoa analyzed would be called organic. and the rest would be conventional, positioning Bolivia as a producer of organic quinoa.

Analysis and Usage Perspective of Solid Digestate

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Abstract: Extremely intensive and chemicalized agriculture severely damages the naturally formed eco-system [1]. This issue encourages concern about soil conservation and improvement of its quality, human health, and environmental protection, therefore it is necessary to look for alternative methods of agriculture. One of these methods is organic farming, where mineral fertilizers are not used, and nutrients needed for plants are provided using biological substances of organic origin. On the other hand, the accumulation of such biodegradable organic waste and its disposal in landfills causes various environmental, economic and social problems. One such waste is digestate [2]. Most of the digestate (about 120 million tons) is agricultural digestate (a mixture of manure and plants). The rest is obtained by mechanical biological treatment from municipal solid waste, separated biological waste, sewage sludge, agriculture or food industry. In various EU countries, renewable energy policies and subsidies for the production of electricity, gas and heat from bio-mass have improved the economic conditions for the anaerobic digestion of biological waste or food waste but in this process digestate is also produced. However, it was also noted that a lot of detailed scientific research should be done on risk factors, environmental effects, fertilizer treatment methods and enrichment during long-term use [3-5]. Because the digestate is rich in organic matter, during the study when three different digestates where used, firstly the organic carbon content was determined. The tests were carried out on dried digestates, which were of two types: whole and chopped. It was found that the content of organic carbon is higher in whole samples and ranges from 39.0 to 50.0 %, and in chopped 34.8 – 45.2 %. In order to obtain the maximum concentration of organic carbon from the used digestates, extracts were produced on the basis of water and potassium alkali, and by applying different production conditions. The samples were kept for 3, 6 and 9 days at room temperature and for 3, 6 and 9 hours at different temperatures, mixed with a mechanical stirrer and in an ultrasonic bath. Also, the samples were heated at temperatures of 50 ℃, 70 ℃ and 90 ℃. A higher concentration of organic carbon was found in potassium-based extracts, and the higher temperature had a positive effect on the concentration of organic carbon in the samples.

Keywords: digestate; organic farming; organic carbon.

References

1. Parikh, S. J. and B. R. James. Soil: The Foundation of Agriculture. Nature Education Knowledge. 2012, 3(10):2. https://www.nature.com/scitable/knowledge/library/soil-the-foundation-of-agriculture-84224268/
2. Paull, John. Attending the First Organic Agriculture Course: Rudolf Steiner’s Agriculture Course at Koberwitz, 1924. European Journal of Social Sciences. 2011, 21 (1). https://orgprints.org/id/eprint/18809/1/Paull2011KoberwitzEJSS.pdf
3. D. Kovacic, Z. Loncaric, Applied sciences, 12 (2022) 9216.
4. N. Cheffins, D. Stainton, Final project report for OMK006-06. WRAP, Banbury UK, 2015.
5. Baştabak, B. ir Koçar, G., (2020). A review of the biogas digestate in agricultural framework. Journal of Material Cycles and Waste Management, 22, p. 1318–1327.

Exploring Gut Microbiota Metabolism – New Chemical Biology Tools for Metabolomics Analysis

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Abstract: The impact of the gut microbiota on human physiology through their vast metabolic activities has surfaced as a remarkable scientific discovery over the past decade. It has provided new avenues for biomarker discovery, especially via the analysis of known and unknown metabolites using mass spectrometry-based metabolomics. In the face of limited tools in chemical biology for metabolomics, we've designed advanced methodologies utilizing ultra-performance liquid chromatography-coupled with tandem mass spectrometry (UPLC-MS/MS) [1-4], with an intent to uncover unknown metabolites from human samples, specifically from pancreatic cancer patients.

Understanding the bioactive metabolites produced by the gut microbiome, especially thiol-containing metabolites, is key to the discovery of potential novel drug scaffolds and dietary or disease biomarkers. To overcome the challenge posed by the lack of specific tools for analyzing thiol-containing metabolites, we've synthesized a unique chemoselective probe, coupled to magnetic beads. This facilitates easy extraction of metabolites and enhances the mass spectrometric sensitivity significantly. Application of this technique on fecal samples has unveiled previously unknown metabolites and boosted the detection limit for most metabolites [1-4].

In a novel approach, we've incorporated bicyclobutane into our methodology for the chemoselective and irreversible capturing of thiol metabolites. Applying this tool to human plasma, fecal samples, and bacterial cultures, we've identified the core bacterial thiol metabolome of 394 features and specific bacterial metabolites for each bacterium, including first-time detections in human plasma [1].

This novel chemical biology method surmounts analytical limitations and simplifies the investigation of bioactive thiol-containing metabolites [1]. It uncovers a host of previously unidentified metabolites from dietary, bacterial, and human origins and paves the way for comprehensive mass spectrometric investigations. This holds significant potential for the discovery of disease biomarkers and therapeutic interventions.

Keywords: Metabolomics, Chemoselective probe, Gut microbiota, LC-MS/MS, Mass Spectrometry

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References

1. Kaur, A., Lin, W., Dovhalyuk, V., Driutti, L., Di Martino, M. L., Vujasinovic, M., Löhr, J.-M., Sellin, M. E., & Globisch, D. Chemoselective bicyclobutane-based mass spectrometric detection of biological thiols uncovers human and bacterial metabolites. In Chemical Science (2023). (Vol. 14, Issue 20, pp. 5291–5301). Royal Society of Chemistry (RSC). https://doi.org/10.1039/d3sc00224a
2. Lin, W., Conway, L. P., Vujasinovic, M., Löhr, J. - Matthias, & Globisch, D. Chemoselective and Highly Sensitive Quantification of Gut Microbiome and Human Metabolites. In Angewandte Chemie International Edition (2021). (Vol. 60, Issue 43, pp.

23232–23240). Wiley. https://doi.org/10.1002/anie.202107101

3. Garg, N., Conway, L. P., Ballet, C., Correia, M. S. P., Olsson, F. K. S., Vujasinovic, M., Löhr, J. - Matthias, & Globisch, D. Chemoselective Probe Containing a Unique Bioorthogonal Cleavage Site for Investigation of Gut Microbiota Metabolism. In Angewandte Chemie International Edition (2018). (Vol. 57, Issue 42, pp. 13805–13809). Wiley. https://doi.org/10.1002/anie.201804828
4. Conway, L. P., Garg, N., Lin, W., Vujasinovic, M., Löhr, J.-M., & Globisch, D. Chemoselective probe for detailed analysis of ketones and aldehydes produced by gut microbiota in human samples. In Chemical Communications (2019). (Vol. 55, Issue 62, pp. 9080–9083). Royal Society of Chemistry (RSC). https://doi.org/10.1039/c9cc04605d

Extraction and Determination of Total Phenolic, Flavonoid Contents, and Volatile Compounds in *Epilobium angustifolium* and *Cannabis sativa* Varieties

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Abstract: Plants are a natural source of phytochemicals, many of which have favorable bioactive properties for human health and are therefore used in ethnomedicine for preventing and treating a variety of diseases [1]. *Epilobium angustifolium* (fireweed) is a popular medical plant that is known and used worldwide. Due to the abundance of secondary metabolites such as polyphenols, tannins, and terpenes, fireweed has antioxidant, anti-inflammatory, and anti-aging properties [2]. *Cannabis sativa* (industrial hemp) is mainly cultivated as a source of industrial fiber or seed oil. In addition to cannabinoids, the chemical composition of industrial hemp includes many other active compound groups, mostly tannins and polyphenols [3]. Biological activity of the herb is dependent on its geographical origin and on sample preparation procedure [4].

The aim of the present study was to quantitatively determine the total phenolic, flavonoid contents and characterize the volatile compositions of three different plants growing in Estonia: fireweed, and industrial hemp varieties Finola and Estica. The volatile compounds were extracted from dried samples using headspace SPME (solid-phase microextraction) and analyzed by GC-MS. Polyphenols and flavonoids were investigated in plant ethanol extracts by colorimetric tests. As a result of SPME-GC-MS, over 15 volatiles were quantified (≥1%) in each sample. Volatile compound compositions were similar in all the samples and the volatiles with the highest contents detected were β-caryophyllene, humulene. Colorimetric tests showed high concentrations of polyphenols (up to 157.6 ± 8.1 mg GAE/g) and flavonoids (up to 11.7 ± 0.9 mg QE/g), whereas fireweed extracts indicated higher levels. The obtained results show that the investigated plants, fireweed, Finola, and Estica, are valuable sources of phenolic and volatile phytochemicals.

Keywords: *Epilobium angustifolium*; phenolic compounds; flavonoids; colorimetry; plant extracts; volatile compounds; *Cannabis sativa;* solid-phase microextraction;

Author Contributions: Conceptualization, P.J. and M.V.; methodology, software, validation, formal analysis, investigation P.J., and K.D.; resources, M.V.; data curation, writing—original draft preparation, writing—review and editing, and visualization, P.J. and K.D.; supervision, P.J.; project administration and funding acquisition, M.V. All authors have read and agreed to the published version of the manuscript.

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References

1. E. Salmerón-Manzano, J. A. Garrido-Cardenas, ja F. Manzano-Agugliaro, „Worldwide Research Trends on Medicinal Plants", *Int. J. Environ. Res. Public. Health*, kd 17, nr 10, lk 3376, 2020, doi: 10.3390/ijerph17103376.

2. A. Nowak *et al.*, „Epilobium angustifolium L. Extracts as Valuable Ingredients in Cosmetic and Dermatological Products", *Molecules*, kd 26, nr 11, lk 3456, 2021, doi: 10.3390/molecules26113456.

3. C. M. Andre, J.-F. Hausman, ja G. Guerriero, „Cannabis sativa: The Plant of the Thousand and One Molecules", *Front. Plant Sci.*, kd 7, lk 19, 2016, doi: 10.3389/fpls.2016.00019.

4. V. Kaškonienė *et al.*, „Evaluation of phytochemical composition of fresh and dried raw material of introduced Chamerion angustifolium L. using chromatographic, spectrophotometric and chemometric techniques“, *Phytochemistry*, kd 115, lk 184–193, 2015, doi: 10.1016/j.phytochem.2015.02.005.

Comparison of the Accumulation of Free Radicals in Two Moss Species Growing in Latvia

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Abstract: Moss have a high radionuclide sorption capability (Cs-137, U-238, Th-228, K- 40). Therefore, when moss is exposed to ionizing radiation even in quantities of a few mGy and due to other environmental factors free radicals are formed in the moss. In the study, two moss species – Abietinella abietina (A) and Pleirozium schreberi (B) were investigated using electron spin resonance (ESR) spectroscopy method before and after irradiation with accelerated electrons, absorbed dose 10 – 1000 mGy. Samples of both of the species were divided into two parts: one was irradiated while it was still wet and only dried before the ESR spectra was registered, while the other was dried before irradiation. ESR spectra were recorded separately for the alive part of the moss (leaves and stalks) and the rhizoids at room temperature (EMX plus, BRUKER). The ESR signal of the organic radicals is asymmetric and differs for both moss species. Its form, line width, g-factor and radical concentration changes depending on the radiation absorbed dose and storage conditions. The free radical concentration in the leaves and stalks that were not irradiated is less than in the rhizoids, and is less for moss A than for moss B. With the increase of absorbed dose, the radical concentration increases, the change is not linear – there are rapid changes of the concentration between 50 and 100 mGy. In the ESR spectra of moss A, a signal for an inorganic radical was found that also matches the signal found in the sand the moss was taken from. Non irradiated moss species contain two types of organic radicals with similar parameters, these both radicals react differently to the absorbed dose which is concluded by the changes in signal forms, radical recombination are different for A and B moss. The moss could be as bio-indicator of absorbed dose.

Keywords: moss, free radical accumulation, ESR spectrometry, ionizing radiation, absorbed dose

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References

1. Steelink C. (1987) Review of ESR spectroscopy of humic substances. In: NMR of humic substances and coal. Techniques, problems and solutions. (Ed. R. L. Weshaw). Lewis publishers, USA, pp. 47-70
2. Līga Strazdiņa, Ligita Liepiņa, Anna Mežova (2015) Sūnu ceļvedis dabas pētniekiem. LU Akadēmiskais apgāds, pp. 66, 71
3. D. Savchenko, A. Vasin, Sh. Mutom E Kalabukhova, A. Nazarov. EPR study of Porous Si:C and SiO_2:C Layers. Phys. Status Solidi B, 2018, 1700559 (1-6). DOI: 10.1002/pssb.20170055

Quality Control Optimization in Novel Wood Fractionation Demoplant

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Abstract: Fibenol OÜ is 100% private equity-based company focused on creating sustainable biomaterials. Fibenol is using cutting-edge processes to develop a new generation of sugars, high purity hydrolysis lignin, and unique microcrystalline cellulose from hardwood. The raw material which is used is forestry and wood industry leftovers. The innovative flagship plant is giving new life to secondary-use wood with limited value in the industry and turn it into high-value biomaterials. Furthermore, the plant will convert more than 90% of biomass into high-value products with a minimal environmental impact by using 100% renewable energy and minimizing use of chemicals and water [1]. Since it is innovative technology there is no standard methods to analyse feed and product composition, process efficiency as fast as the plant needs. For determining feed, lignin and microcrystalline cellulose composition there is used two-step acid hydrolysis. It fractionates the biomass into forms that are more easily quantified. The lignin fractionates into acid insoluble material, which must be accounted for during gravimetric analysis, acid soluble material, which is measured by UV-Vis spectroscopy, and polymeric carbohydrates are hydrolyzed into the monomeric forms, which are measured by HPLC from hydrolysis liquid [2]. This procedure takes few days to be completed and finding faster method is still in progress. Moreover, in the production process pre-treatment takes roughly 20 seconds however, sugars and sugar by products analysis takes approximately 2 hours by analysing HPLC. For in-line measurement there is suggested MIR (mid-infrared) devices. At present the plant is testing IRmadillo by Keit Ldt [3]. Assessment of MIR suitability for production process quality control shall be assessed by the end of summer 2023.

Keywords: Biomass, lignin, wood sugars, microcrystalline cellulose, wood fractionation

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References

1. Fibenol OÜ website: www.fibenol.com (22.06.2023)
2. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker Determination of Structural Carbohydrates and Lignin in Biomass. NERL Laboratory Analytical Procedure, USA, 2008 (revision 2012); pp. 4.
3. Keit Ldt. Website: www.keit.co.uk (25.06.2023)

Interaction between Extracellular Polymeric Substances from diatom *Cyclotella meneghiniana* and Citrate Coated Silver Nanoparticles

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Abstract: Opposite to the significant knowledge about the toxicity of AgNPs to phytoplankton species, rather limited knowledge is available about the role of phytoplankton secretions such as extracellular polymer substances (EPS) in NPs fate [1]. This study, therefore, explores the interaction between 20 nm citrate-coated AgNPs (Cit-AgNPs) and the EPS derived from the diatom *Cyclotella meneghiniana* and in particular their colloidal stability and transformations. To this end a combination of different state-of-the-art techniques was employed to characterize the AgNPs-EPS interactions: Asymmetric Flow Field-Flow Fractionation (AF4) coupled with ICP-MS, Dynamic Light Scattering (DLS), Zeta Potential, and Surface Plasmon Resonance (SPR) absorbance spectroscopy. The changes in the size distribution, surface properties and stability of Cit-AgNPs (4 mg/L) in the presence of various concentrations of EPS (130, 65, 32.5, 13 mg-C/L) were studied in both short-term (0-2 h) and long-term (72 h) experiments. The results showed that EPS stabilizes Cit-AgNPs, presumably through the formation of an ecocorona. The interaction occurred rapidly in the short term leading to a long-term stabilization, as revealed by the changes in the SPR-UV-Vis spectrum characterized by the appearance of a typical shoulder associated with AgNPs aggregation/agglomeration and alterations in the hydrodynamic diameter. In the presence of EPS, the surface charge (zeta potential) of Cit-AgNPs also shifted towards values similar to those of EPS alone, indicating an interaction. The EPS reduced the dissolution of Cit-AgNPs after 24h. AF4-MD-ICP-MS provides relevant information with respect to DLS and UV-Vis spectroscopy confirming the changing in size of Cit-AgNPs, the stabilization in the long-term and the interaction with the EPS. Taken together, the results of this study demonstrates that the EPS derived from the diatom *Cyclotella meneghiniana* modifies the surface properties and stability of 20 nm Cit-AgNPs. Therefore, such interactions have to be taken into consideration for predicting the fate and effects of Cit-AgNPs in the environment.

Keywords: silver nanoparticles, EPS, diatom

Author Contributions: Conceptualization, R.G. and V.S.; methodology, R.G, I.W. and V.S.; software, R.G, V.I. and V.S.; validation, R.G and V.S.; formal analysis, R.G.; investigation, R.G.; resources, V.S.; data curation, R.G, I.W. and V.S.; writing—original draft preparation, R.G and V.S.; writing—review and editing, R.G and V.S.; visualization, R.G and V.S.; supervision, V.S.; project administration, V.S.; funding acquisition, V.S. All authors have read and agreed to the published version of the manuscript.

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References

1. Chetwynd, A. J.; Lynch, I. The Rise of the Nanomaterial Metabolite Corona, and Emergence of the Complete Corona. *Environ. Sci. Nano* 2020, 7 (4), 1041–1060. https://doi.org/10.1039/C9EN00938H

Revisiting Nanotoxicology Tests – Miniaturised Approaches of Nanotoxicity Tests in Daphnids

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Abstract: The great increase of nanotechnology in the last 20 years led to an alarmingly presence of nanomaterials in the environment as a new category of pollutants. Given the fact that legislation on nanomaterials is not concrete, the monitoring of their toxicity responses remains central. Focusing on tests in the aquatic environment, daphnids are commonly employed as a bioindicator species for experiments with nanomaterials. However, till now there is not a unified and agreed approach in nanotoxicity testing, while research among different laboratories is performed with significant different setups which can affect the reproducibility of the results. In this study, daphnids were exposed to silver nanoinks and the impact of surface to volume was assessed comparing shallow vessels such as Petri dishes with deeper exposure vessels. Furthermore, in an attempt to assess whether the tests can be performed in smaller volume, thus in miniaturized versions, experiments compared larger and smaller volume setups. Finally, another parameter explored was the crowding of animals in exposure, therefore, their absolute number. Mortality was affected by both the surface to volume and the miniaturization, and significantly with crowding, supporting the implication of number of animals in the tests. Further investigation with molecular and phenotypic endpoints confirmed these changes.

Keywords: molecular ecotoxicology; *Daphnia*; metabolomics; feeding; nanoink, nanotoxicology

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Frass Analysis and Usage Perspectives

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Abstract: Meeting an ever-increasing food demand while reducing agriculture’s negative environmental Impact is one of the greatest challenges of the twenty-first century. With the demand for nutritionally crucial proteins and meat products which is expected to increase from current levels by more than 75% in 2050, we need to search for alternatives to conventional protein sources if we want to restrict agricultural land use and avoid biodiversity losses and environmental degradation [1]. Rearing insects for mass consumption is increasingly sparking interest due to their high nutritional value and, most importantly, their resource efficiency when converting organic matter, especially waste, into protein. Insect production is a rapidly growing industry worldwide, as it presents a promising solution for the effective recycling of organic waste. The most abundant by-product of insect production is insect feces, scientifically known as “frass” [2]. The current trends in insect breeding, which are gaining wider applicability and legalization for use in the food industry, also create prerequisites for the development of possibilities for processing waste products [3].

Under laboratory conditions were carried studies of frass by the company UAB "Divaks" of the suitability for fertilizer production. The chemical composition of frass and the composition of different extracts were also analyzed. Because frass contains a large amount of organic matter, carbon concentration studies were conducted by several methods. The possibility of extracting the plant nutrients contained in frass using different solvents was also investigated. The samples were prepared under different conditions and for different experimental durations. The results are useful for the development of new fertilizing products.

The use of insects for food, feed and fertilizer is expected to continue to grow. Because it has the potential to help reduce the amount of waste, increase food reserves and increase yields.

Keywords: frass; insect; waste, fertilizer

References

1. NYANZIRA, Abnezer, et al. Analysis of Frass Excreted by Tenebrio molitor for Use as Fertilizer. 2023.
2. HOUBEN, David, et al. Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. Scientific Reports, 2020, 10.1: 4659.
3. Poveda, J. Insect frass in the development of sustainable agriculture. A review. Agron. Sustain. Dev. 41, 5, 2021.

Characterization of Natural Zeolites of Armenia, Georgia and Kazakhstan and their Thermally Modified Forms

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Abstract: The objects of our study are the natural zeolites from the Nor Kokhb deposite, Armenia (calcium (56%) and sodium (25%) clinoptilolite also containing kanemite (7%), quartz (5%), natrolite (4%) and sigma-2 zeolite(3%)), the Rkoni plot of the Dzegvi-Tedzami deposite, Georgia (high-silica sodium heulandite mixed with ≈10% of chabazite), and the Chankanay deposite, Kazakhstan (mixture of heulandite, chabazite and quartz containing relatively high content of calcium ions), selected for the preparation of new bactericidal zeolite filter materials for purification and disinfection of water from various sources [1]. Thermal treatment results in slight dealumination, dehydration, amorphization and structural changes. Thermal analysis data and powder XRD patterns show the stability of the Armenian clinoptilolite crystalline structure up to 720 °C, dehydration of most of the water occurs from the crystal lattice, but continues both during the amorphization process and after its completion; surface area of micropores has a maximum value after calcination at 200 °C and decreases monotonically with increasing calcination temperature; sample has nanometric (3-4 nm) and larger (15-60 nm) pores, both systems are sensitive to the calcination temperature. Amorphization of the Georgian heulandite begins at temperatures above 200 °C, the transition to the metastable heulandite B at ≈340 °C is not fixed, but at high temperatures wairakite or another mineral of the 9.GB.05 group is formed, complete dehydration is achieved at ≈800 °C; the BET surface area decreases, while diameter of mesopores increases with increasing calcination temperature. Thermally stable Kazakhstani zeolite loses a small amount of water, the dehydration process occurs in four stages up to 1000 °C, the BET surface area increases, the total volume of pores varies insignificantly, diameter of mesopores reaches a maximum after annealing at 700 °C, but then sharply decreses; samples calcined at 400 and 800 °C have high water adsorption capacity.

Keywords: heulandite-clinoptilolite; chabazite; thermal treatment; dehydration; amorphization; micro- and mesopores.

Author Contributions: Conceptualization, Vladimer Tsitsishvili; Investigation, Nato Mirdzveli, Lusine Harutyunyan, Hakob Sargsyan, Alla Manukyan, Lena Tangamyan, Manana Nijaradze and Nagima Dzhakipbekova; Methodology, Nanuli Dolaberidze and Gulaim Seisenbaeva; Project administration, Nato Mirdzveli. All authors have read and agreed to the published version of the manuscript.

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References

1. Tsitsishvili,, V.; Panayotova, M.; Miyamoto, M.; Dolaberidze, N.; Mirdzveli, N.; Nijaradze, M.; Amiridze, Z.; Klarjeishvili, N.; Khutsishvili, B.; Dzhakipbekova, N.; Harutyunyan, L. Characterization of Georgian, Kazakh and Armenian natural heulandite-clinoptilolites. *Bull. Georgian Natl. Acad. Sci*. **2022**, *16(4)*, 115-122. http://science.org.ge/bnas/vol-16-4.html.

Improving the Luminescence Properties of Lutetium Aluminum Garnets by Doping Different Amounts of Boron

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Abstract: In order to convert high-energy radiation, such as gamma or X-rays, into visible light scintillators are needed. Cerium or praseodymium doped lutetium aluminum garnets have high density, thermal stability, rather efficient luminescence processes which are needed for a good scintillator. However, further optimization of short decay time is needed. Luminescence decay is important because if it is very short then more signals can be measured within a defined timeframe, resulting in a better resolved and higher quality image in CT devices. One way to improve materials' properties is to doping compounds with different elements. One of these elements is boron. Boron can be used as a flux, also B^{3+} has a suitable neutron capture cross section and can help absorb gamma radiation [1-3]. Lutetium aluminum scandium garnets doped with $Ce^{3+}/Pr^{3+}/B^{3+}$ were obtained as a result. These garnets are synthesized and studied for the first time. In this work, the phase purity and morphology of the samples were analyzed with X-ray diffraction, SEM. Photoluminescence properties such as emission, excitation spectra, decay curves, quantum efficiency and temperature dependency spectra have been investigated. Radioluminescence was also measured. The positive impact of boron addition into the garnet structure on the luminescence properties will be discussed in detail.

Keywords: Garnet, Scintillator, Sol-Gel

Author Contributions: Conceptualization, G.I. and R.S.; methodology, G.I.; formal analysis, G.I, J.K., T.J. R.S.; investigation, G.I., J.K; writing—original draft preparation, G.I.; writing—review and editing, J.K, T.J. R.S.; supervision, R.S.; All authors have read and agreed to the published version of the manuscript."

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References

1. Foster C., Wu Y., Koschan M., et. al. Boron Codoping of Czochralski Grown Lutetium Aluminum Garnet and the Effect on Scintillation Properties. *J. Cryst. Growth* **2018**, 486, 126–129.
2. McGregor D. S., Materials for Gamma-Ray Spectrometers Inorganic Scintillators. *Annu. Rev. Mater. Res.* **2018**, 48, 254–277.
3. Inkrataite G., Kemere M, Sarakovskis A., Skaudzius R. Influence of boron on the essential properties for new generation scintillators. *J. Alloys Compd* **2021**, 875, 160002.

Towards the Development of Sustainable Antimicrobial Surface Coatings

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Abstract: With the increasing trend of hard-to-treat microbial infections including multiresistant nosocomial infections, food-related outbreaks and rapid spread of potentially infectious microbes in the common spaces of densely populated areas, awareness on the importance of proper systemic hygiene practices has increased. One of the main routes of potential pathogen transmission to vulnerable hosts is via contaminated surfaces. Therefore, introduction of antimicrobial surface materials may be considered as a potential preventative solution in infection hot spots. Similarly to disinfectants and other hygiene products, the global market of antimicrobial surface coatings is increasing with an annual rate of 10% and is projected to reach 7 billion US dollars by 2027 [1]. Although other experimental formulations have been used in antimicrobial surfaces, silver, copper, titanium dioxide and zinc are still the most frequently used active agents [2]. Compared with traditional antibiotics, such metal-based antimicrobial agents have a broad mode of action, which should theoretically prevent the emergence of antimicrobial resistance – a process that has been detected very frequently in case of antibiotics. Yet various types of metal resistance mechanisms in microbes have been described in association to polluted industrial areas and metal mining sites [3]. Furthermore, recent evidence suggests that appearance of metal resistance may also be linked to the emergence of antibiotic resistance [4] and that such resistant phenotypes may be selected in the presence of sublethal levels of stressors, including various antimicrobial agents [5]. Therefore, ensuring the safety of antimicrobial formulations and their specific applications in terms of reducing their potential to induce antimicrobial resistance or tolerance, is of great importance when developing sustainable antimicrobial materials. In this work, we propose a strategy to determine the potential of antimicrobial surfaces to induce resistance or tolerance either by enhanced mutation frequency and subsequent selection of resistant mutants or by exchange of genetic material. Along with the fact that such information is required to commercialize biocidal products in the European Union [6], we believe that the proposed framework can be used to ensure long-term safety and sustainability of antimicrobial surfaces.

Keywords: antimicrobial materials, surface coatings, antimicrobial resistance, safety, sustainability

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Author Contributions: Conceptualization, A.I., V.K., M.R.; methodology and results, A.I, M.R, V.K. project administration, A.I.; funding acquisition, A.I, V.K. All authors have read and agreed to the published version of the manuscript."
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References

9. Markets and Markets. Antimicrobial Coatings Market. Available online: https://www.marketsandmarkets.com/Market-Reports/antimicrobial-coatings-market-1297.html (accessed on 17.08.2023).
10. Yılmaz, G.E.; Göktürk, I.; Ovezova, M.; Yılmaz, F.; Kılıç, S.; Denizli, A. Antimicrobial Nanomaterials: A Review. *Hygiene* **2023**, *3*, 269-290. doi: 10.3390/hygiene3030020
11. Haferburg, G., Kothe, E. Microbes and metals: interactions in the environment. *J Basic Microbiol*. **2007**, *47(6)*, 453-67. doi: 10.1002/jobm.200700275
12. Vats, P., Kaur, U.J., Rishi, P. Heavy metal-induced selection and proliferation of antibiotic resistance: A review. *J Appl Microbiol.* **2022**, *132(6)*, 4058-4076. doi: 10.1111/jam.1549
13. Andersson, D.I., Hughes, D. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug*

Resist Updat. **2012,** *15(3)*, 162-72. doi: 10.1016/j.drup.2012.03.005

14. Biocidal Products Directive (Directive 98/8/EC). Available online: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A31998L0008 (accessed on 17.08.2023).

Synthesis of Molybdenum Oxide and Sulfide Nanoparticles at Room Temperature Using Organometallic Approach

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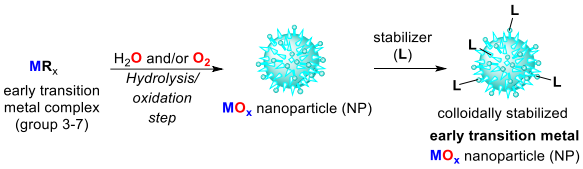
Abstract: In the early 1990s, the seminal work by B. Chaudret and J. S. Bradley led to the establishment of metal nanoparticles (NPs) synthesis from organometallic precursors. Since then, this methodology has attracted the attention of many research groups, ours included, and offered possibilities to selectively control the shape and size of these nano-objects under mild reaction conditions. Clever design of substrate and stabilizing ligand systems has allowed the synthesis of late transition metal and their oxide NPs (group 8-12) from precious metal (Ru, Rh, Pd, Pt, Ag, Au) and from first-row (Fe, Co, Ni, Cu, Zn) metal complexes [1,2]. However, to this day, the development of suitable early transition metal complexes (group 3-7) for the preparation of corresponding oxide, sulfide, etc. NPs has attracted only sporadic attention.

This presentation will focus on the use of highly reactive Mo(0) complex for the synthesis of molybdenum oxide and sulfide NPs at room temperature. These results will illustrate how the choice of the substrate, stabilizing ligand and solvent system can lead to stable colloidal NPs with a diameter of <10 nm in size (Scheme 1) [3].



Scheme 1. Synthesis of early transition metal oxide NPs using organometallic approach.

Keywords: organometallic approach; nanoparticles; mild conditions; size control; colloidal stability; molybdenum oxide, molybdenum sulfide

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References

1. Amiens, C.; Chaudret, B.; Ciuculescu-Pradines, D.; Collière, V.; Fajerwerg, K.; Fau, P.; Kahn, M.; Maisonnat, A.; Soulantica, K.; Philippot, K. Organometallic approach for the synthesis of nanostructures. *New J. Chem.* 2013, *37*, 3374-3401.
2. Cerezo-Navarrete, C.; Lara, P.; Martínez-Prieto, L.M. Organometallic Nanoparticles Ligated by NHCs: Synthesis, Surface Chemistry and Ligand Effects. *Catalysts* 2020, *10*, 1144.
3. Jakoobi, M.; Carnide, C.; Vendier, L.; Bijani C.; Mingotaud, A.-F.; Clergereaux, R.; Kahn, M. L. *Manuscript in preparation.*

Neonicotinoids: Agrochemicals with Toxic Impact on Reproductive Functions in Males

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Abstract: In general, agrochemicals are compounds used to control weeds, and diseases in crops during many agronomic practices, and they have become an essential tool in crop protection [1]. The group of neonicotinoid pesticides are highly effective against some destructive crop pests, and their occurrence in aquatic ecosystems could represent a relevant risk. Especially Acetamiprid N-[(6-chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide and thiacloprid (2Z)-3-[(6-chloro-3-pyridinyl)methyl]-1,3-thiazolidin-2-ylidenecyanamide) are frequently used agrochemicals with a wide spectrum efficacy [2]. Currently, exact knowledge about the impact of neonicotinoid exposure on the reproductive system is limited as well as inconsistent. The scientific environment does not provide a relevant background for this problem-solving. The objective of our in vitro study was to examine the potential effect of selected neonicotinoids on mice Sertoli cells. TM4 cells were treated with experimental doses of acetamiprid (10 to 500 µM) and thiacloprid (7.8 to 500 µM) for 48 hours of exposure. The metabolic activity and cell membrane integrity were examined to determine the potential toxicity. The results from alamarblue assay revealed that higher experimental doses of acetamiprid (200 - 500 µM) significantly ($p<0.0001$) decreased the metabolic activity of exposed TM4 Sertoli cells. A similar tendency was confirmed after thiacloprid exposure when the significant ($p<0.0001$) cytotoxicity started from 125 to 500 µM. The cell membrane integrity evaluated by CFDA-AM assay showed a significant ($p<0.01$) decrease at 250 or 300 µM followed by significant ($p<0.001$; $p<0.0001$) inhibition at 350 and 500 µM of acetamiprid. In the case of thiacloprid, the presented parameter was significantly ($p<0.01$) inhibited at 125 and 250 µM, while the highest concentrations 300 and 500 µM caused significant changes at ($p<0.001$; $p<0.0001$). A considerably more detailed and systematic research in thiacloprid toxicology is definitely required for a better understanding of risks associated with reproductive health**.**

Keywords: acetamiprid, thiacloprid, Leydig cells, Sertoli cells, toxicity,

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Author Contributions: For abstracts with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, T.J. and N.L.; methodology, T.J. and H.G.; validation, N.L. and A.K.; formal analysis, N.K.; original draft preparation, T.J. and L.Z.; writing—review and editing, N.L.; supervision, N.L. and A.K.; project administration, T.J., A.K. and N.L; funding acquisition, N.L. All authors have read and agreed to the published version of the manuscript."

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References

1. Gupta, S.; Khanna, R. Agrochemicals as a Potential Cause of Ground Water Pollution: A Review. Int. J. Chem. Stud. 2018, 6 (3), 985-990.
2. Renaud, M.; Akeju, T.; Natal-da-Luz, T.; Leston, S.; Rosa, J.; Ramos, F.; Sousa, J. P.; Azevedo-Pereira, H. M. V. S. Effects of the Neonicotinoids Acetamiprid and Thiacloprid in Their Commercial Formulations on Soil Fauna. Chemosphere 2018, 194, 85–93. DOI: 10.1016/j.chemosphere.2017.11.102

Liquid Complex Fertilizers with Bio Additives

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Abstract: Every year, about 2.5 billion are generated in the European Union tons of waste. Due to these reasons and the limited amount of raw materials, the integration of the circular economy is encouraged [1]. The fertilizer industry is one of the industries that is characterized by the recycling of by-products into new products. One of the by-products in the fertilizer industry is the liquid phase, which is obtained as a secondary product after the crystallization and filtration of the solid, primary product. Liquid fertilizers have many advantages: are easily compatible with trace elements, fungicides, physiologically active substances or other important additives [2]. It is very important to choose the right fertilizer, because the plants need to be given the required amount of nutrients. If at least one element is missing, the plant may rot or even die [3]. During this work, the possibility of using the post-crystallization solution for the production of liquid complex fertilizers was examined. To achieve this goal, the concentrations of plant nutrients and chlorine in the post-crystallization solution and its chemical-physical properties were determined. Considering the need to increase concentration of nitrogen in the post-crystallization solution, studies were conducted during which influence of different nitrogen compounds to the crystallization temperature of the post-crystallization solution were observed. Studies have also been carried out in which solutions (as organic nitrogen additives) were extracted from the lupine (*lupinus polyphyllus*) that would be easily compatible with the post-crystallization solution. After the chemical analysis, liquid complex fertilizers were found to contain: 1.4%–P_2O_5, 4%–Cl and about 13.5%–K_2O. Depending on the nitrogen additions, the concentration of nitrogen in the liquid fertilizers varied from 1.02% to 3.78% N. Four liquid complex fertilizers of different brands were obtained and used: 1-2-14; 3-2-14+ME;
4-2-14+ME+mineralized lupine leaves; 4-2-14+ME+lupine leaf extract.

Keywords: liquid fertilizers, nitrogen, bio fertilizers, blue-pod lupin

References

1. European Parliament (EP). Circular economy: definition, importance and benefits. Available online: https://www.eumonitor.eu/9353000/1/j9vvik7m1c3gyxp/vknegugz7hwu?ctx=vjxzjv7ta8z1 (accessed 2023-02-27).
2. Bommaraju, V.T.; Barnicki, D. S.; Kent, A. J. Handbook of Industrial Chemistry and Biotechnology: Thirteenth Edition, 2017; pp. 784-785.
3. Paleckienė, R.; Sviklas, M. A. Trąšų agrochemija: metodinė priemonė, Vilnius, 2012; pp. 32-107.

Selective Capture of Small Environmental Pollutants by Cyclohexanohemicucurbit[n]urils

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Abstract: Persistent organic pollutants that occur in different environmental matrices create a need for their sensing, removal and remediation.

Cyclohexanochemicucurbit[*n*]urils (cycHC[*n*], *n* = 6 or 8) are chiral macrocyclic cavitands with a well-defined electron-deficient cavity suitable for accommodation of various electron-rich guests [1–3]. The current work describes the application of cycHC[8] as a molecular container for encapsulation of small neutral organic molecules, including environmental pollutants (mustard gas degradation products, haloalkanes) and biologically active compounds. Formation of inlcusion complexes in solid state was confirmed by SC-XRD. Complexation studies in solution by ^{1}H NMR and ITC revealed host-guest interactions between cycHC[8] and hydrophobic S-and O-containing neutral heterocycles, as well as small haloalkanes, in methanol and methanol-water media, resulting in stronger binding upon increase of solvent polarity. Since the macrocycle is insoluble in water, it was further utilized for selective capture of the neutral guests from aqueous solutions [3].

Keywords: hemicucurbituril; heterocycle; haloalkane; pollutant; inclusion complex; solid-phase extraction; sorbent recycling

Author Contributions: Conceptualization, supervision and methodology, R.A.; validation, T.J.; formal analysis and investigation, T.J., K.M.L., S.K. All authors have read and agreed to the published version of the manuscript.

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References

1. Öeren, M.; Shmatova, E.; Tamm, T.; Aav, R. Computational and Ion Mobility MS Study of (*All-S*)-Cyclohexylhemicucurbit[6]uril Structure and Complexes. *Phys. Chem. Chem. Phys* **2014**, *16* (36), 19198–19205. https://doi.org/10.1039/c4cp02202e.
2. Kaabel, S.; Adamson, J.; Topić, F.; Kiesilä, A.; Kalenius, E.; Öeren, M.; Reimund, M.; Prigorchenko, E.; Lõokene, A.; Reich, H. J.; Rissanen, K.; Aav, R. Chiral Hemicucurbit[8]uril as an Anion Receptor: Selectivity to Size, Shape and Charge Distribution. *Chem. Sci. J.* **2017**, *8* (3), 2184–2190. https://doi.org/10.1039/c6sc05058a.
3. Shalima, T.; Mishra, K. A.; Kaabel, S.; Ustrnul, L.; Bartkova, S.; Tõnsuaadu, K.; Heinmaa, I.; Aav, R. Cyclohexanohemicucurbit[8]uril Inclusion Complexes With Heterocycles and Selective Extraction of Sulfur Compounds From Water. *Front. Chem.* **2021**, *9*, 1036. https://doi.org/10.3389/fchem.2021.786746

Interactions of Diatom *Cyclotella meneghiniana* and Citrate Coated Silver Nanoparticles

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Abstract: Silver nanoparticles (AgNPs) are extensively utilized engineered nanomaterials that inevitably find their way into the aquatic environment [1]. A significant amount of research has been conducted to assess their potential toxicity to aquatic biota [2]. However, the underlaying cellular mechanisms involved in the toxicity and tolerance of diatoms to AgNPs are still poorly understood. The present work aims to get better insight into the response to AgNPs exposure of diatom *Cyclotella meneghiniana*, as a representative model for the lower trophic organisms in freshwater environment, and the underlaying mechanisms. *C. meneghiniana* was exposed to various concentrations of Cit-AgNPs (ranging from 0.001 mg/L to 5 mg/L) up to 72h and the biological responses were compared with those induced by dissolved Ag^+. The response of diatoms to Cit-AgNP and Ag^+ was characterized in terms of diatom growth, membrane permeability, photosynthesis alterations, and morphological changes. The stability of the Cit-AgNPs in the exposure medium was also investigated by determining their dissolution, surface charge and hydrodynamic size. DLS and SPR-UVvis results showed a shift in size distribution of cit-AgNPs towards higher values related to aggregation/agglomeration processes. Dissolution of cit-AgNP in exposure media increased over time and was concentration dependent.

The calculated 72h-EC50 values, based on growth inhibition, were 0.348± 0.038 mg/L and 0.019± 0.001 mg/L for cit-AgNP and Ag^+ respectively, suggesting higher toxicity of Ag^+ than cit-AgNP for *Cyclotella meneghiniana*.

Short-term exposure (24h) to Cit-AgNPs and Ag^+ resulted in reduced chlorophyll autofluorescence and impaired membrane integrity in *C. meneghiniana*. Furthermore, the photosystem II was affected, as indicated by a decrease in quantum yield (Fv/Fm) and an increase in non-photochemical quenching (NPQ). Cells exposed to Cit-AgNPs and Ag^+ exhibited higher levels of proline accumulation compared to control implying an activation of antioxidant mechanisms in diatom since proline plays a role in ROS scavenging [3]. Additionally, SEM-EDS analysis revealed the presence of polyphosphate bodies (PPB) in both Cit-AgNP and Ag^+ treated cells, in response to metal toxicity and stress. Indeed, polyphosphates are known as chelator of cations and their accumulation is linked to abiotic stress [4].

This study demonstrates synergistic mechanisms adopted by *Cyclotella meneghiniana* to deal with toxic levels of silver in both its ionic and nanoparticulate forms.

Keywords: nanoparticles, toxicity, diatom

Author Contributions: Conceptualization, A.K. and V.S.; methodology, A.K and V.S.; software, A.K. and V.S.; validation, A.K and V.S.; formal analysis, A.K.; investigation, A.K.; resources, A.K. and V.S.; writing—original draft preparation, A.K and V.S.; writing—review and editing, A.K and V.S.; visualization, A.K and V.S.; supervision, V.S.; project administration, V.S.; funding acquisition, V.S. All authors have read and agreed to the published version of the manuscript.

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References

1. 1. McGillicuddy E, Murray I, Kavanagh S, Morrison L, Fogarty A, Cormican M, et al. Silver nanoparticles in the environment: Sources, detection and ecotoxicology. Sci Total Environ. 2017;575: 231–246. doi:10.1016/j.scitotenv.2016.10.041
2. 2. Kalantzi I, Mylona K, Toncelli C, Bucheli TD, Knauer K, Pergantis SA, et al. Ecotoxicity of silver nanoparticles on plankton organisms: a review. J Nanoparticle Res. 2019;21: 65. doi:10.1007/s11051-019-4504-7
3. 3. Torres MA, Barros MP, Campos SCG, Pinto E, Rajamani S, Sayre RT, et al. Biochemical biomarkers in algae and marine pollution: A review. Ecotoxicol Environ Saf. 2008;71: 1–15. doi:10.1016/j.ecoenv.2008.05.009
4. 4. Sanz-Luque E, Bhaya D, Grossman AR. Polyphosphate: A Multifunctional Metabolite in Cyanobacteria and Algae. Front Plant Sci. 2020;11: 938. doi:10.3389/fpls.2020.00938

Hydrogenase-Based Electrode for Hydrogen Sensing in a Fermentation Bioreactor

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Abstract: Global climate change forces people to search for alternative energy sources. Meanwhile, the hydrogen-based economy proposes an effective decarbonisation solution: using green H2 as an energy carrier to replace fossil fuels. The fermentation process by bacterium/a consortium of several bacteria could be the most sustainable way to generate H_2. However, its production process remains unstable; another obstacle to H_2-economy (gas storage and transportation) is H_2 flammability in the range of 4-75%. The main limitations of the commercially available H_2-sensors are the use of expensive rare metals and security risks as they often require elevated temperatures. Thus, developing (bio)sensors that can operate under mild conditions is highly demanded. In this study, a third generation amperometric biosensor that consists of carbon nanomaterials with immobilised enzymes was developed. Hydrogenase (*Aa* Hase) from the hyperthermophilic bacterium *Aquifex aeolicus* was used as it has unique properties such as thermal stability and high O_2- and CO-tolerance. The electrode fabrication process was optimised regarding the amount of carbon nanotubes, enzyme concentration, and absorption time. Effects of the pH and temperature of the medium and dispersion degree of the nanomaterials on H_2 oxidation were investigated. The sufficiently high current was observed at room temperature with the enzyme concentration of 0.5 μM. Linear biosensor response was obtained in the range of 1-20% of H_2, with H_2 sensitivity of 4 μA cm^{-2} per % H_2 using the pulsed strategy. It was proved that the main co-products of the fermentation process in a bioreactor (CO_2 and H_2S) do not interfere with the H_2 quantification. The produced biosensor was successfully applied for the in-situ measurements of the amount of H_2 produced through the dark fermentation process in the bioreactor in a steady-state.

Keywords: bioelectrode; Hydrogenase; enzymatic sensor, amperometric sensor, hydrogen detection, fermentation bioreactor

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Synthesis and Antibacterial Efficiency of Chitosan-Copper Oxide Nanocomposites

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Abstract: Antimicrobial resistance is among the most serious global healthcare problems today. In Europe, a third of the estimated 8.9 million yearly hospital-acquired infections in 2016 and 2017 were caused by antibiotic-resistant bacteria [1]. One possible source for novel, efficient antimicrobials is nanotechnology. Some metallic nanoparticles (NPs) like silver, CuO and ZnO are already commercially available for biomedical applications. NPs are often coated with different polymers to improve their characteristics. An intriguing biopolymer to combine with metal NPs is the antimicrobial, biocompatible and immunomodulating chitosan, presently used in bandages and wound dressings.

In this study, novel chitosan-CuO nanocomposites (NCs) were synthesized via the precipitation of copper acetate by sodium hydroxide in the presence of chitosan (50–190 kDa, Sigma Aldrich). Varying copper to chitosan weight ratios (1:0.3, 1:1 and 1:3) were used. The NCs were characterized by DLS, EDX, FTIR, SEM, XPS and XRD. The minimum bactericidal concentrations (MBC) against clinically relevant gram-negative (*Escherichia coli* ATCC 25922, *E. coli* MG1655, *Pseudomonas aeruginosa* ATCC 27853) and gram-positive (*Staphylococcus aureus* ATCC 6538) bacteria were determined by the Spot test [2]. Flow cytometry and confocal laser scanning microscopy were used to reflect the interactions between NCs and bacteria.

The zeta potential of the synthesized NCs was >40 mV. The NCs hydrodynamic diameter and polydispersity index increased with higher chitosan content, varying between 90–180 nm and 0.24–0.33, respectively. Based on the XRD analysis the CuO portion of the NCs had a crystalline structure.

The NCs were similarly effective against gram-negative and –positive bacteria displaying MBC values of 0.13–0.25 mg Cu/L after 24 hours of exposure. Interestingly, after a 1-hour period of exposure the NCs were more toxic against gram-negative bacteria than Cu ions suggesting that chitosan may facilitate the interaction of NCs and bacterial cells, enabling the immediate shedding of Cu ions alongside the bacteria. Furthermore, after a 1-hour exposure the NCs with higher chitosan content were up to 2 times more biocidal against gram-negative bacteria than NCs with lower chitosan concentration.

Keywords: antimicrobial resistance; bacteria; chitosan; copper; copper oxide; nanocomposites; nanoparticles; nanotechnology.

Author Contributions: Conceptualization, J.L. and K.K.; methodology, formal analysis and investigation J.L., M.S., M.O., K.K.; writing J.L.; review and editing, M.S. and K.K.; supervision, project administration, and funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

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References

1. European Centre for Disease Prevention and Control. Available online: https://www.ecdc.europa.eu/en/publications-data/infographic-healthcare-associated-infections-

threat-patient-safety-europe (accessed on 22.06.2023).
2. Suppi, S.; Kasemets, K.; Ivask, A.; Künnis-Beres, K.; Sihtmäe, M.; Kurvet, I.; Aruoja, V.; Kahru, A. A novel method for comparison of biocidal properties of nanomaterials to bacteria, yeasts and algae. *J Hazard Mater* **2015**, *286*, 75–84. (DOI: 10.1016/j.jhazmat.2014.12.027)

Multi-Element Profile Characterization in Monofloral Honey

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Abstract: Apiculture, in general, is an important sector of the national economy due environmental benefits of pollination. Honey is the main product of apiculture and is regarded as a product of health promoting properties, therefore often used for medical purposes [1]. The high demand of honey raises concerns of honey quality available in the market because of possible counterfeited or fraudulent product presence, thereby highlighting the need for modern instrumental analysis. Monofloral honey is gathered from the majority of single floral source, thus leading to unique taste, organoleptic or visual properties, which consumer might find more attractive than regular polyfloral honey. The macro- and trace element profile seems valuable information, serving purpose to consumers, manufacturers and researchers as well. The 83 honey samples of different floral origins were used, and floral origins were confirmed by melissopalynology analysis. Macro and trace element profile was determined using inductively coupled plasma-mass spectrometry (ICP-MS). Results were processed using chemometric methods – principal component analysis (PCA) and dendrogram of hierarchical clustering. Significant differences were determined using ANOVA one-way analysis Fisher test. 30 different elements were found in natural honey of Latvian origins and 18 of them could be used as floral markers for buckwheat, clover, heather, linden, rapeseed and willow honey. Heather honey showed the most diverse element profile with increased concentrations of As, Ba, Ca, Cs, Fe, K, Mn, Rb and Tl. Comparing to other studies, preliminary results of ICP-MS show one of the most versatile evaluations of floral origins determination [2]. The preliminary results of macro and trace elements show promising use as biomarkers for honey floral origins evaluation and evaluation of concentrations of elements harmful to health.

Keywords: honey; ICP-MS; floral origins, macro elements, trace elements, chemical profile

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Author Contributions: Conceptualization, K.D.L. and A.V.; methodology, K.D.L., K.K. and V.R.; software, K.D.L.; validation, A.B., K.D.L. and M.B.; formal analysis, A.B. and M.B.; investigation, K.D.L., A.B. and A.V.; resources, K.D.L. and A.V.; data curation, K.D.L., M.B., A.B. and V.R.; writing—original draft preparation, K.D.L.; writing—review and editing, K.D.L., A.V., K.K. and V.R.; visualization, K.D.L. and V.R.; supervision, K.D.L., A.V., K.K. and V.R.; project administration, K.D.L.; funding acquisition, K.D.L. All authors have read and agreed to the published version of the manuscript."

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References

1. Skadiņš, I.; Labsvārds, K.D.; Grava, A.; Amirian, J.; Tomsone, L.E.; Ruško, J.; Viksna, A.; Bandere, D.; Brangule, A. Antimicrobial and Antibiofilm Properties of Latvian Honey against Causative Agents of Wound Infections. *Antibiotics* **2023**, *12*(5), 816. doi.org/10.3390/antibiotics12050816.
2. Labsvards, K.D.; Rudovica, V.; Kluga, R.; Rusko, J.; Busa, L.; Bertins, M.; Eglite, I.; Naumenko, J.; Salajeva, M.; Viksna, A. Determination of Floral Origin Markers of Latvian Honey by Using IRMS, UHPLC-HRMS, and 1H-NMR. *Foods* **2022**, *11*(1), 42. doi.org/10.3390/foods11010042.

Future Perspective of *In-Situ* Soil Analysis

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Abstract: Soil health monitoring is becoming progressively more important to meet the needs of sustainable agriculture and climate policy goals. Portable capillary electrophoresis (CE) devices could be used to monitor micro and macro nutrients available to plants in soil real-time as anions and cations to make informed decisions about the need for fertilization. These devices could be considered as a precise, cheap and fast alternative to time-consuming laboratory analyses and sensors that are currently on the market that can determine only N, P and K and not other important macro or micro elements [1-5]. Method development for determining cations in soil samples were carried out using commercial CE device coupled to contactless conductivity detector (C4D) with intent to later implement developed method on portable CE. Separation of seven cations (NH_4^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+}) was obtained using capillary with effective length of 47.5 cm and inner diameter of 50 μm, 6 M acetic acid solution as background electrolyte and applied voltage 15 kV. Analysis time was 20 minutes (including 3 minutes of pre-wash). Linearity for analytes was determined in the range of 1-10 mM, limit of detection (LoD) (0.2-0.8 mM) and limit of quantification (LoQ) (0.4-0.9 mM) for the analytes. The extraction of cations was tested from 1 minute up to 24 hours using distilled water and 0.01 M $CaCl_2$ [6, 7]. No remarkable differences in extraction recoveries were observed within the tested timeframe. Therefore, 1-3 minutes was suggested as an optimum extraction time for in situ extraction procedure. Developed analysis method is suitable for qualitative and quantitative analysis of seven cations extracted from soil and could be implemented on portable CE device. Further optimization of simple and quick sample preparation must be carried out so that it could be easily used directly on the field prior analysis.

Keywords: soil health, soil fertility, nutrients, portable capillary electrophoresis, GHG emissions, fertilization of soils, over fertilization

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References

1. Environmental XPRT, Renke - Model RS-NPK-TR - Soil NPK Sensor. Available online: https://www.environmental-expert.com/products/model-rs-npk-tr-soil-npk-sensor-784865 (accessed 31 May 2023).
2. Teralytic Soil Probe. Available online: https://teralytic.com/soil-probe/ (accessed on 31 May 2023).
3. Paul-Tech Soil Station service. Available online: https://paul-tech.com/paul-tech-soil-station-service/ (accessed on 31 May 2023).
4. AgroCares Nutrient Scanner. Available online: https://www.agrocares.com/scanners/ (accessed on 31 May 2023).
5. Bruker, Application Note N526, Online Quality Control of Mineral Fertilizers using FT-NIR Spectroscopy. Available online:https://www.bruker.com/en/meta/forms/bopt-form-pages/thank-you-an-n526-

form/_jcr_content/root/contentpar/twocolumns_copy_copy/contentpar-1/calltoaction.download-asset.pdf/primaryButton/AN_N526_Fertilizer_online_EN.pdf (accessed on 31 May 2023).

6. Pontes, F. V. M., Carneiro, M. C., et. al. Fast and simultaneous ultrasound-assisted extraction of exchangeable-NH4+, NO3- and NO2- species from soils followed by ion chromatography determination. *Chem. Speciation Bioavailability*, **2012**, *24(4)*, p. 229. DOI: 10.3184/095422912X13488406544054
7. Stanišić, S. M.; Ignjatović, L. M., et. al. The comparison of sample extraction procedures for the determination of cations in soil by IC and ICP-AES. *Cent. Eur. J. Chem*, **2011,** *9(3)*, pp. 481-484. DOI : 10.2478/s11532-011-0031-9

Optimising ZnO Nanostructures for Label-Free Electrochemical Immunosensor Used for Antibody Detection Against Prostate-Specific Antigen

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Abstract: Recent studies have extensively investigated semiconducting metal oxides, with a particular interest in one-dimensional ZnO nanostructures. These nanostructures have shown great potential in influencing the detection capabilities of electrochemical and photoluminescence-based biosensors. Their affordability and suitability as a scaffold make them a promising option for cancer detection, offering high sensitivity and selectivity. Consequently, the development of point-of-care immunosensors for early cancer detection could revolutionize malignancy treatment and patient care through real-time monitoring [1]. However, achieving these objectives requires meeting certain criteria, including rapid label-free and selective identification of cancer markers, compact sensor size, accessibility, and portability. To fulfil these requirements, we utilized inexpensive, reproducible, and disposable screen-printed carbon electrodes [2] in combination with various ZnO nanostructures, which improved the electrical properties of the system. To evaluate the affinity interaction between prostate-specific antigen (PSA) and monoclonal antibodies against PSA (anti-PSA), we employed ZnO nanostructures mixed with Nafion solution. The mixture was drop-casted onto screen-printed carbon electrodes (SPCE). Subsequently, the PSA was immobilized onto the modified electrodes. We utilized the differential pulse voltammetry technique to assess the interaction. Through this analysis, we determined the limit of detection and limit of quantification of anti-PSA [3]. Our research aimed to create a novel tool for early cancer detection by comparing and analysing the structural, morphological, and photoluminescent properties of ZnO nanostructures. Furthermore, we investigated the feasibility of employing popular electrochemical techniques such as differential pulse voltammetry and cyclic voltammetry in conjunction with ZnO-modified electrodes to establish a label-free immunosensing platform.

Keywords: zinc oxide nanostructures, cyclic voltammetry, differential pulse voltammetry, electrochemical methods, immunosensors.

Author Contributions: Conceptualization, A.P., M.T.G. and A.R. (Almira Ramanaviciene); methodology, V.L., D.K., B.B., A.P., M.T.G. and A.R. (Almira Ramanaviciene); software, V.L., B.B. and M.T.G.; validation, V.L., D.K., B.B., A.P., M.T.G. and A.R. (Almira Ramanaviciene); formal analysis, V.L., D.K., B.B. and A.P.; investigation, V.L., D.K., B.B., resources A.R. (Arunas Ramanavicius), M.T.G. and A.R. (Almira Ramanaviciene); data curation, V.L., D.K., B.B., M.T.G., A.P. and A.R. (Almira Ramanaviciene); writing—original draft preparation, V.L., D.K., B.B., A.P., M.T.G. and A.R. (Almira Ramanaviciene); writing—review and editing, V.L., D.K., B.B., A.P., A.R. (Arunas Ramanavicius), M.T.G., D.E. and A.R. (Almira Ramanaviciene); visualization, V.L., D.K. and B.B.; supervision, A.P., M.T.G. and A.R. (Almira Ramanaviciene); project administration A.R. (Almira Ramanaviciene); funding acquisition, D.E. and A.R. (Almira Ramanaviciene).

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References

1. Popov A., Brasiunas B., Kausaite-Minkstimiene A., Ramanaviciene, A. Metal nanoparticle and quantum dot tags for signal amplification in electrochemical immunosensors for biomarker detection. *Chemosensors* **2021,** *9, no. 4* 85.
2. Drobysh, M., Liustrovaite, V., Baradoke, A., Viter, R., Chen, C. F., Ramanavicius, A., Ramanaviciene, A. Determination of rspike protein by specific antibodies with screen-printed carbon electrode modified by electrodeposited gold nanostructures. *Biosensors* **2022**, *12(8)*, 593.
3. Liustrovaite, V., Karoblis, D., Brasiunas, B., Popov, A., Katelnikovas, A., Kareiva, A., Ramanavicius, A. et al. Electrochemical Immunosensor for the Determination of Antibodies against Prostate-Specific Antigen Based on ZnO Nanostructures. *International Journal of Molecular Sciences* **2023**, *24*, *no. 6*, 5803.

"Hook & Loop" Interactions Between Fibrous Microplastics and Zooplankton

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Abstract: Nowadays, microplastics (1 μm – 5 mm) have been found accessible to organisms ranging from small invertebrates to large mammals [1-3]. However, fibrous microplastics, the dominant shape of microplastics in the environment, are still lacking research attention [4]. Previous studies indicated that microfibers caused more behavior toxicity of organisms than pellets and fragments because of the entanglement of the organisms [5,6]. Herein, the overarching research objective is to gain more information about the behavior effects of fibrous microplastics to freshwater zooplankton *Daphnia magna*. *D. magna* were exposed to fibrous polyester microplastics sized 300 ± 192 μm at different concentrations (10, 10^2, 10^3 items/mL) in the presence of algal food. The behavior responses, particularly the swimming speed and swimming trajectory were recorded and analyzed via Tracker for 7 days. The frequency of hop and sink behavior decreased when *D. magna* were exposed to microfibers. Moreover, a special phenomenon like "hook & loop" was noticed between fibrous microplastics and *D. magna*, especially in 10^2 items/mL group. Microfibers can easily twin the antennae and tail claw of *D. magna*, which was because of the faint pectinate spines. Strong pull of intertwined microfibers with algae or some impurity caused *D. magna* to not move freely, which caused the decrease of swimming speed and the swimming trajectories. More interestingly, this phenomenon was not a dose-dependent effect on organisms. *D. magna* exposed to much higher concentration (10^3 items/mL) swam freely because of the aggregation of microplastics themselves, which indicated the importance of number concentration of microplastics. Overall, this work pointed out "hook & loop" interactions between fibrous microplastics and zooplankton would be the direct reasons causing the organism behavior changes, which indicated that the interactions of microplastics and organisms played a key role in understanding the behavior, fate, and effects of microplastics in the aquatic environment.

Keywords: Hook & Loop; microfibers; *Daphnia magna*

Author Contributions: Conceptualization, M.C. and V.S.; methodology, M.C and V.S.; software, M.C and V.S.; validation, M.C and V.S.; formal analysis, M.C.; investigation, M.C.; resources, M.C and V.S.; data curation, M.C and V.S.; writing—original draft preparation, M.C and V.S.; writing—review and editing, M.C and V.S.; visualization, M.C and V.S.; supervision, V.S.; project administration, V.S.; funding acquisition, V.S. All authors have read and agreed to the published version of the manuscript.

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References

1. Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T.S. Microplastic ingestion by zooplankton. *Environ Sci Technol* **2013,** *47(12)*, 6646–6655. DOI: 10.1021/es400663f.
2. Rebolledo, E.L.B.; Van Franeker, J.A.; Jansen, O.E.; Brasseur, S.M.J.M. Plastic ingestion by harbour seals (*Phoca vitulina*) in the Netherlands. *Mar Pollut Bull* **2013,** *67(1-2)*, 200–202. DOI: 10.1016/j.marpolbul.2012.11.035.
3. Sanchez, W.; Bender, C.; Porcher, J.M. Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics: preliminary study and first evidence. Environ Res. **2014**, *128*, 98–100. DOI: 10.1016/j.envres.2013.11.004.

4. Rebelein, A.; Int-Veen, I.; Kammann, U.; Scharsack, J.P. Microplastic fibers — Underestimated threat to aquatic organisms? *Asci Total Environ* **2021**, *777*, *1*. 146045. DOI: 10.1016/j.scitotenv.2021.146045.
5. Gray, A.D.; Weinstein, J.E. Size- and shape-dependent effects of microplastic particles on adult dagger blade grass shrimp (*Palaemonetes pugio*). *Environ Toxicol Chem* **2017**, *36*, 3074-3080. DOI: 10.1002/etc.3881.
6. Ziajahromi, S.; Kumar, A.; Neale, P.A.; Leusch, F.D.L. Impact of microplastic beads and fibers on waterflea (*Ceriodaphnia dubia*) survival, growth, and reproduction: Implications of single and mixture exposures. *Environ Sci & Technol* **2017**, *51*, 13397-13406. DOU: 10.1021/acs.est.7b03574.

Synthesis and Antibacterial Properties of Lignin-based Quaternary Ammonium and Phosphonium Salts

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Abstract: Lignin, a naturally occurring aromatic polymer, possesses a range of biological functions. Notably, plants have harnessed lignin's properties as a defense mechanism against invading pathogenic microbes. Consequently, the utilization of isolated lignin as an eco-friendly antimicrobial agent holds great promise for enhancing the value of lignin. Furthermore, given lignin's green and sustainable origin through plant photosynthesis, its integration into the antimicrobial industry has the potential to reduce carbon emissions. Numerous studies have explored the utilization of lignin for the development of antimicrobial agents tailored to various applications. However, lignin is a highly heterogeneous polymer, characterized by variations in monomer composition, linkages, molecular weight, and functional groups. Consequently, the relationship between lignin's structure and properties, as well as its mechanism of action as an antimicrobial agent, remains unclear[1]. To address these gaps in knowledge, we conducted a study in which we prepared forty-two quaternary Ammonium/phosphonium organosolv lignin samples from Aspen, Pine, and Barley straw (representing hardwood, softwood, and grass sources, correspondingly) using a versatile intermediate known as chloromethylated lignin developed recently[2]. These samples were then assessed for their potential antibacterial properties against clinical isolates of Gram-positive (*Staphylococcus aureus, MRSA*) and Gram-negative (*Klebsiella pneumonia*) pathogenic bacterial strains. Our findings suggest that the antibacterial activity of these lignin samples exhibits an increase with the length of the hydrophobic chain, up to C14, after which it begins to decline. Additionally, ongoing research is being conducted to investigate the antimicrobial activity of ammonium and phosphonium surfactant materials, both individually and in combination. In this research, the chemical modification has significantly boosted the antimicrobial efficacy of lignin, and incorporating extra chemical structures, e.g., cationic functional groups through chemical modification, presents a viable strategy to enhance the future potential of lignin's antimicrobial activity.

Keywords: Lignin; Quaternisation; Antibacterial

Author Contributions. Conceptualization, methodology, investigation, original draft preparation: Mahendra K Mohan, Yevgen Karpichev; investigation: Harleen Kaur, and Ella Duvanova; validation: Merilin Rosenberg; resources, supervision, project administration, funding acquisition: Angela Ivask, Tiit Lukk, Jean-Manuel Raimundo, and Yevgen Karpichev.

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References

1. Chen, M. *et al.* High value valorization of lignin as environmental benign antimicrobial. *Mater Today Bio* **18**, 100520 (2023).
2. Mohan, M. K. *et al.* Chloromethylated lignin as a precursor of sustainable catalytic material for cross coupling and click reactions (unpublished results).

Application of Fly Ash of Lignite Combustion in Air and Water Purification

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Abstract: The study was aimed at evaluating the catalytic and photocatalytic properties of lignite fly ash sample S1 and S2 from the Pilsen Power Station (Teplárna Plzeň) collected by electrostatic precipitators with relatively high contents of Fe_2O_3 (6.0-7.4%) and TiO_2 (4.6-4.8%). Iron oxides are used often as heterogeneous Fenton(-like) reaction catalysts in certain compositions, having iron oxides and oxo-hydroxides attached to catalyst supports with developed contact surface, such as, for example, zeolites [1]. TiO_2 is the most studied photocatalyst for the photocatalytic oxidation of gaseous volatile organic compounds (VOCs) [2]. Experimental tests of the catalytic oxidation of the textile dye Acid Orange 7 in aqueous solutions by the heterogenous Fenton-like system (H_2O_2/fly ash) and the photocatalytic oxidation of acetone vapors in the UV-A/fly ash system were carried out. In water treatment trials, adsorption and Fenton-like experiments were carried out in parallel under similar treatment conditions. Adsorption of the VOC was characterized by its concentration in the reactor's effluent growing with the adsorbent saturation with the acetone vapors. Once the studied sample of fly ash accumulated certain amount of acetone, the UV-A light was switched on to start the photocatalytic oxidation reaction. The results showed moderate catalytic and negligible photocatalytic properties of S1 and S2 in the studied systems, although exhibiting certain adsorption properties. Surprisingly, S2 showed a noticeably stronger catalytic ability in the Fenton-like system compared to S1 despite almost 2.6 times lower surface area at a similar chemical composition. The fly ashes were also used for zeolite synthesis [3] with their subsequent testing in ion exchange in respect to ammonium cations, showing ability close to a commercial zeolite specimen.

Keywords: heterogenous catalyst; adsorption; advanced oxidation processes; fly ash; reuse

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Author Contributions: Conceptualization, J.B., N.D. and M.K.; methodology, J.B., N.D. and M.K.; formal analysis, J.B., N.D., L.P. and M.K.; investigation, D.N., J.B., N.D. and M.K.; resources, L.P., M.V. and S.P.; writing—original draft preparation, N.D. and M.K.; writing—review and editing, N.D. and S.P.; visualization, J.B., N.D. and M.K.; supervision, N.D. and S.P.; funding acquisition, M.V. and S.P. All authors have read and agreed to the published version of the manuscript.

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References

1. Ioffe, M.; Kundu, S.; Perez-Lapid, N.; Radian, A. Heterogeneous Fenton catalyst based on clay decorated with nano-sized amorphous iron oxides prevents oxidant scavenging through surface complexation. *Chem. Eng. J.* **2022**, 433, 134609. DOI: 10.1016/j.cej.2022.134609
2. Jang, W.-S.; Pham, V.N.; Yang, S.-H.; Baik, J.; Lee, H.; Kim, Y.-M. Enhanced selective photocatalytic oxidation of a bio-derived platform chemical with vacancy-induced core-shell anatase TiO_2 nanoparticles. *Appl. Cat. B: Environ.* **2023**, 322, 122140. DOI: 10.1016/j.apcatb.2022.122140
3. Imbert, F.E.; Moreno, C.; Montero, A.; Fontal, B.; Lujano, J. Venezuelan natural alumosilicates as a feedstock in the synthesis of zeolite A. *Zeolites.* **1994**, 14, 374-378. DOI: 10.1016/0144-2449(94)90112-0

Crystal and Magnetic Structure Transitions in Doped Lu and Fe Based Perovskite Oxides

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Abstract: The possibility to control physical properties via chemical doping is particularly important when concerning the formation of both electrical and magnetic orderings in the same compounds, which are commonly referred as multiferroics [1]. However, for the most part, due to the conflicting nature of these properties, the coupling between the electrical and magnetic properties is relatively weak**.** Recently a new class of hexagonal rare earth ferrite perovskite compounds has been found to exhibit multiferroic ordering, with a mechanism and structure similar to that of hexagonal manganites, making them a new avenue for potential research. This new family of room temperature multiferroic compounds is based on $LuFeO_3$ with a hexagonal structure (space group $P6_3cm$). It has been discovered that $LuFeO_3$ in the hexagonal state has both ferroelectric and weak ferromagnetic orderings [2,3]. As such, in this work we adapted an ethylene glycol based sol-gel synthesis procedure for the preparation of bulk Sc doped hexagonal lutetium ferrite powders. The crystal structure was investigated using XRD and Raman spectroscopy at room temperature. The temperature driven crystal structure transitions were analyzed by means of in-situ high temperature XRD while the magnetic transitions were investigated by means of low temperature magnetization measurements. The obtained results revealed that at room temperature polar hexagonal phase can be stabilized in a quite narrow doping range that depends on the sintering temperature of the samples [4]. Additionally, samples with higher Sc doping content showed lower phase transition temperature from ferroelectric to paraelectric phases. While Sc itself is not magnetic the Sc doping caused substantial changes to the magnetization of the samples as well. Overall, the results indicate that $LuFeO_3$ can be successfully synthesized by means of the ethylene glycol based sol-gel procedure. The desired phase composition, magnetic and electric properties can be optimized by means of doping content and sintering temperature.

Keywords: multiferroic; perovskite; magnetization.

Author Contributions: Conceptualization, A.K. and R.S. D.K.; methodology, A.P., S.C.; formal analysis, A.P. D.K. G.R. G.N.; investigation, A.P. G.R. S.C. G.N.; resources, P.R., A.K., T.Y.; data curation, R.S. P.R.; writing—original draft preparation, G.R., A.P., D.K.; writing—review and editing, D.K., A.P.; visualization, A.P, S.C., D.K., G.R.; supervision, R.S. G.R. P.R.; project administration, A.K.; funding acquisition, A.K., T.Y., P.R. All authors have read and agreed to the published version of the manuscript.

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References

1. Fiebig, M.; Lottermoser, T.; Meier, D.; Trassin, M. The evolution of multiferroics. *Nat. Rev. Mater.* **2016**, 1, 16046. https://doi.org/10.1038/natrevmats.2016.46
2. Wang, W.; Zhao, J.; Wang, W.; Gai, Z.; Balke, N.; Chi, M.; Lee, H.N.; Tian, W.; Zhu, L.; Cheng, X.; et al. Room-temperature multiferroic hexagonal $LuFeO_3$ films. *Phys. Rev. Lett.* **2013**, 110, 237601. https://doi.org/10.1103/PhysRevLett.110.237601

3. Cao, S.; Zhang, X.; Paudel, T.R.; Sinha, K.; Wang, X.; Jiang, X.; Wang, W.; Brutsche, S.; Wang, J.; Ryan, P.J.; et al. On the structural origin of the single-ion magnetic anisotropy in $LuFeO_3$. *J. Phys. Condens. Matter* **2016**, 28, https://doi.org/10.1088/0953-8984/28/15/156001
4. Pakalniškis, A.; Alikin, D.O.; Turygin, A.P.; Zhaludkevich, A.L.; Silibin, M.V.; Zhaludkevich, D.V.; Niaura, G.; Zarkov, A.; Skaudžius, R.; Karpinsky, D.V.; Kareiva, A. Crystal Structure and Concentration-Driven Phase Transitions in $Lu_{(1-x)}Sc_xFeO_3$ ($0 \leq x \leq 1$) Prepared by the Sol–Gel Method. *Materials* **2022,** 15, 1048. https://doi.org/10.3390/ma15031048

Influence of Black Alder Bark Extractives as Integral Building Blocks on the Susceptibility to Biodegradation of Resilient Polyether Polyurethanes

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Abstract: In response to the increasing demand for environmentally friendly materials and the necessity of effective end-of-life management [1], this study investigates the susceptibility of innovative polyurethanes (PUs) that incorporate plant extractives to biodegradation. The PUs was synthesized using polymeric methylene diphenyl diisocyanate, polyol PEG 400, and tetrahydrofuran as a solvent, with NCO/OH ratios of 1.0, 0.8, and 0.5 [2]. Partial or complete substitution of the conventional PEG 400 polyol with black alder bark extracts as bio-polyols was performed. Degradation tests were conducted in sewage water and compost-enriched soil [3,4]. The susceptibility to biodegradation was assessed through weight loss measurements, FTIR spectroscopy, and biological oxygen demand (BOD) analysis [5–7]. After 2 months, conventional PUs exhibited weight losses of 9.6% in soil and 12.4% in water. Complete replacement of PEG 400 with bark-sourced polyol increased weight loss to 15.6% in soil and 15.7% in water. The reference biodegradable polylactic acid (PLA) film displayed weight losses of 35.6% in soil and 15.0% in water. BOD measurements indicated that PUs, particularly those containing bio-based building blocks, supported microbial metabolism, corroborating previous findings [8]. The incorporation of bark extractives significantly enhanced the susceptibility of PUs to biodegradation, resulting in a reduction of up to 45% in the intensity of FTIR spectral peaks associated with the urethane functional group (-NHC(=O)-O-), compared to a maximum reduction of 11% for conventional PUs. Moreover, it increased peak intensities for O-H and N-H stretches after biodegradation, exhibiting a notable negative correlation with changes in peak intensities for N-CO-O, C-O-C, and C-N vibrations following PU biodegradation. This indicates the formation of hydroxyl and amino groups resulting from the hydrolysis of various chemical bonds within the polyurethane network, facilitated by the integration of bark extractives. This work emphasizes the potential of bark-derived building blocks in the design of PU materials suitable for biological recycling while recognizing the need for further investigation into the optimal agents and conditions for the biological conversion of the latter.

Keywords: polyurethanes; bio-based; bark extractives, polyols, biological degradation.

Author Contributions: Conceptualization, J.P. and A.A.; methodology, A.A.; validation, J.P., A.A., M.P. and O.B.; formal analysis, J.P., M.P, O.B.; investigation, J.P.; data curation, J.P.; writing—original draft preparation, J.P.; writing—review and editing, J.P., A.A., M.P. and O.B.; visualization, J.P., A.A., M.P. and O.B.; supervision, A.A.; project administration, A.A.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

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References

1. Magnin, A.; Pollet, E.; Phalip, V.; Avérous, L. Evaluation of Biological Degradation of Polyurethanes. *Biotechnol Adv* **2020**, *39*, 107457, doi:10.1016/j.biotechadv.2019.107457.
2. Arshanitsa, A.; Krumina, L.; Telysheva, G.; Dizhbite, T. Exploring the Application Potential of Incompletely Soluble Organosolv Lignin as a Macromonomer for Polyurethane Synthesis. *Ind Crops Prod* **2016**, *92*, 1–12, doi:10.1016/j.indcrop.2016.07.050.
3. Pischedda, A.; Tosin, M.; Degli-Innocenti, F. Biodegradation of Plastics in Soil: The Effect of Temperature. *Polym Degrad Stab* **2019**, *170*, 109017, doi:10.1016/j.polymdegradstab.2019.109017.
4. Nabeoka, R.; Suzuki, H.; Akasaka, Y.; Ando, N.; Yoshida, T. Evaluating the Ready Biodegradability

of Biodegradable Plastics. *Environ Toxicol Chem* **2021**, *40*, 2443–2449, doi:10.1002/etc.5116.
5. Kim, J.-H.; Choi, S.H.; Park, M.G.; Park, D.H.; Son, K.-H.; Park, H.-Y. Biodegradation of Polyurethane by Japanese Carpenter Bee Gut-Associated Symbionts Xanthomonas Sp. HY-71, and Its Potential Application on Bioconversion. *Environ Technol Innov* **2022**, *28*, 102822, doi:10.1016/j.eti.2022.102822.
6. Gogoi, S.; Karak, N. Biobased Biodegradable Waterborne Hyperbranched Polyurethane as an Ecofriendly Sustainable Material. *ACS Sustain Chem Eng* **2014**, *2*, 2730–2738, doi:10.1021/sc5006022.
7. Zuliani, A.; Rapisarda, M.; Chelazzi, D.; Baglioni, P.; Rizzarelli, P. Synthesis, Characterization, and Soil Burial Degradation of Biobased Polyurethanes. *Polymers (Basel)* **2022**, *14*, 4948, doi:10.3390/polym14224948.
8. Gunawan, N.R.; Tessman, M.; Zhen, D.; Johnson, L.; Evans, P.; Clements, S.M.; Pomeroy, R.S.; Burkart, M.D.; Simkovsky, R.; Mayfield, S.P. Biodegradation of Renewable Polyurethane Foams in Marine Environments Occurs through Depolymerization by Marine Microorganisms. *Science of The Total Environment* **2022**, *850*, 158761, doi:10.1016/j.scitotenv.2022.158761.

Hazard Evaluation of Novel Plasticizer, Di(2-propylheptyl) Phthalate, to Aquatic Ecosystems

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Abstract: Plastics differ in chemical composition due to numerous chemicals (additives) used to produce plastic for specific applications. Plastic additives may enter the environment not only from the plastic waste during its weathering, but also at all stages of plastic products' life cycle [1]. Plastic additives can be hazardous to living organisms and play a significant role in the adverse effects of plastic contamination [2,3]. However, there are knowledge gaps concerning the ecotoxicity of plastic additives [4]. Plasticizers are one group plastic additives of which phthalates are the most used but also known for their toxic potential. As the use of phthalate plasticizers has been increasingly regulated since the beginning of the 21st century, alternative plasticizers have appeared in their stead. The current study was conducted to obtain new experimental data on potential ecotoxicity of high molecular weight phthalate plasticizer DPHP (Di(2-propylheptyl) phthalate) in comparison to DEHP (Di-2-ethylhexyl phthalate), a former dominant but now restricted [5] low molecular weight plasticizer. For hazard evaluation, long-term (21 day) effects were studied in life cycle tests with Daphnia magna, representative of microcrustacea and important link of the freshwater food-web. D. magna was exposed to the plasticizers via spiked sediment (sand).The tests were conducted in lake water to increase environmental relevance of the hazard data. Alarmingly, preliminary results showed that DPHP may be more hazardous to aquatic organisms than DEHP. Mortality of the organisms, exposed to DPHP plasticizer, was higher than with DEHP. D. magna reproduction (the average number of offspring/organism) was lower than in the unexposed control and comparable for both DPHP and DEHP. However since fewer offspring were also recorded for the used solvent (ethyl acetate) control, the affected reproduction was not necessarily attributable to the plasticizers.

Keywords: microcrustacean, ecotoxicity, DPHP, DEHP, *Daphnia magna*

Author Contributions: conceptualization, M.H., I.B. and A.L.; methodology, I.B., A.L. and H.V.; investigation, A.L., I.B., A.P., and H.V.; resources, M.H.; data curation, A.L., I.B. and H.V.; writing—original draft preparation, I.B. and A.L.; writing—review and editing, I.B., A.L. and M.H.; project administration M.H.; funding acquisition, M.H. All authors have read and agreed to the published version of the manuscript.

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References

1. Hahladakis, J.; Velis, C.; Weber, R.; Iacovidou, E.; Purnell, P. An overviez of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 2018 344(5), 179–199. DOI 10.1016/j.jhazmat.2017.10.014
2. Beiras, R.; Verdejo, E.; Campoy-López, P.; Vidal-Liñán, L. Aquatic toxicity of chemically defined microplastics can be explained by functional additives. J. Hazard. Mater. 2021 406, 124338. https://doi.org/10.1016/j.jhazmat.2020.124338
3. Gunaalan, K.; Fabbri, E.; Capolupo, M. The hidden threat of plastic leachates: a critical review on their impacts on aquatic organisms. Water Res. 2020 184, 116170. DOI 10.1016/j.watres.2020.116170
4. Barrick, A.; Champeau, O.; Chatel, A.; Manier, N.; Northcott, G.; Tremblay, L.A. Plastic additives:

challenges in ecotox hazard assessment. PeerJ. 2021 9, e11300. DOI: 10.7717/peerj.11300
5. ECHA (European Chemicals Agency) (2022a) Substances restricted under reach—Annex XVII Entry 51. https://www.echa. europa.eu/substances-restricted-under-reach

Buckwheat Husks, Ash and Biomass for Sustainable Plant Fertilization and Soil Improvement

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Abstract: Nowadays, it is difficult to imagine agriculture without the use of fertilizers, because plants cannot absorb the required amount of nutrients from the soil. The only way to provide plants with all the nutrients they need is to fertilize them. Depending on the properties of the soil, the type of plant, climatic conditions, etc., different amounts of nutrients are required for effective plant growth [1]. However, the intensive and unbalanced use of concentrated mineral fertilizers reduces the reserves of fertilizer raw materials, exhausted the soil, and harms the ecosystem. Meanwhile, in some industries (food, energy, etc.), increasing amounts of production by-products are generated, which could be processed into high-quality organic fertilizers. The decomposing matter from organic fertilizers, break down naturally and would provide nutrient and minerals to the soil [2, 3]. One such material is waste from the buckwheat groats industry: uncleaned buckwheat biomass (UBM), buckwheat husks (BH) and buckwheat husk ash (BHA). These wastes contain many different nutrients that plants need, it becomes possible to use them as fertilizers. It is difficult to directly use biofuel ash, buckwheat hulls or biomass for soil fertilization, because due to the improper shape, non-uniformity and high dustiness of the particles, maximum fertilization efficiency is not achieved, and it is necessary to granulate them. A drum granulator was used to produce a mixture of raw materials containing various wastes: BH, BHA and UBM. Depending on the composition of the starting materials, it was possible to obtain up to 60 % of the productive fraction. The moisture content of such granules varied between 2-10 % and they have relatively low bulk density (between 430–480 kg/m^3). The pH values of the 10 % solution of the produced granules ranged from 9.7 to 12.0, which indicates that the fertilizer can act as a lime agent, it is recommended to fertilize acidic soils. It should also be mentioned that the obtained pellets are quite weak and plastic. Granular fertilizers are non-hygroscopic and retain their shape when stored at 21–23°C and 70-75% humidity. In summary, it can be said that buckwheat groat waste can be used in the production of environmentally friendly fertilizers in order to reduce environmental pollution, slow down soil degradation, and increase the amount of soil organic matter.

Keywords: buckwheat; organic fertilizers; granulation.

References

1. J., E. A. et al. Soil Fertility: Factors Affecting Soil Fertility, and Biodiversity Responsible for Soil Fertility. *IJPAES* **2022,** *12*, 021-033.
2. S. A., S. T. The Principal Role of Organic Fertilizer on Soil Properties and Agricultural Productivity - A Review. *ARTOAJ* **2019**, *22(2)*, 0046-0050.
3. Ye, L., Z., X., B., E. et al. Bio-organic fertilizer with reduced rates of chemical fertilization improves soil fertility and enhances tomato yield and quality. *Sci Rep* (**2020**), *10*, 177.

Norwegian National Plan for Cleaning Contaminated Seabeds - Cooperation Possibilities Between the Public Sector and the Academia

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Abstract: The Norwegian Parliament passed an action plan in 2006-2007 that they called «Working together towards a non-toxic environment and a safer future – Norway’s chemicals policy» (1) with the aim of setting back and removing chemicals from the natural environment that can be harmful for human health and the environment. One of the goals was also to be an internationally active initiator for political discussions on ecotoxicology and environmental pollution. The remediation plan listed national prioritized sites - 17 harbour and fjord areas that had been polluted by many decades of extensive traffic and industrial activity. The polluted sediments are analysed for heavy metals and organic pollutants (e.g. TBT, PAH, PCB), followed by dredging of the polluted areas and replacing or covering them with clean masses as the most favored clean-up methodology according to the project coordinator Norwegian Environment Agency. These types of projects and locations are a perfect arena for „before and after“ environmental research as within only a few years, polluted seabed areas are remediated. Unfortunately, the results and lessons learned from these projects remain mostly locally (nationally) communicated whereas such large-scale initiatives are an untapped potential for research yet only one Master´s thesis from the Norwegian University of Life Sciences is available on the subject (2). This presentation aims to highlight the potential of „before and after"-type research in these environment-altering nationally prioritized projects.

Keywords: dredging, polluted seabed, environmental pollution, remediation, contaminated sediments

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References

1. The Norwegian Ministry of Climate and Environment, Stortingsmelding. nr.14 (2006-2007) «Sammen for et giftfritt miljø», Norway, 2006.
2. The Norwegian Environment Agency, Guidelines for handling of sediments, report, 2018
3. Uleberg, Embla Vildalen, Short-term effects of substrate type on the growth and survival of transplanted eelgrass (Zostera marina): an experimental case study in Horten harbor, Master’s thesis, Norwegian University of Life Sciences, Ås, 01.06.2021

Effect of MgO Templating on the Synthesis and Properties of Dissolved Lignin-Based Hard Carbon for Na-Ion Battery Applications

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Abstract: The increasing use of wind and solar energy creates an enormous need for intermittent storage of electrical energy in batteries. Today, Li-ion batteries are the state-of-the art in mobile applications, such as electric vehicles. However, due to the limited sources of lithium, there is a growing need to replace Li-ion batteries with more sustainable alternatives, such as Na-ion batteries. This development involves replacement of graphite with alternative anode materials, such as hard carbon. Lignin, a naturally abundant biopolymer, has shown promising potential as a carbon precursor for electrical energy storage applications, particularly in the synthesis of hard carbon anode for Na-ion batteries [1]. In this study, we investigate the synthesis of lignin-based hard carbon using a MgO-template technique, where lignin is dissolved using NaOH. The effect of the synthesis process on the morphology, porous structure, and electrochemical properties of the resulting hard carbon material is investigated. The synthesis process involves carbonization of freeze-dried solutions containing dissolved lignin and magnesium gluconate [2]. Through subjecting the mixture to preheat-treatment at 600°C, nano-sized domains of Mg and Na crystals form within the carbon matrix. Acid leaching of the resulting particles is subsequently carried out, followed by high temperature postheat-treatment at 1100-1500°C. These lead to the formation of a hierarchical porous hard carbon structure for Na-ion batteries applications. The findings from this research have the potential to contribute to the development of sustainable and high-performance energy storage systems.

Keywords: lignin; hard carbon; Na-ion battery; porous material; template synthesis

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References

1. Matei Ghimbeu, C.; Zhang, B.; Martinez de Yuso, A.; Réty, B.; Tarascon, J.-M. Valorizing Low Cost and Renewable Lignin as Hard Carbon for Na-Ion Batteries: Impact of Lignin Grade. *Carbon* **2019**, *153*, 634–647. https://doi.org/10.1016/j.carbon.2019.07.026.
2. Kamiyama, A.; Kubota, K.; Igarashi, D.; Youn, Y.; Tateyama, Y.; Ando, H.; Gotoh, K.; Komaba, S. MgO-Template Synthesis of Extremely High Capacity Hard Carbon for Na-Ion Battery. *Angew. Chem. Int. Ed.* **2021**, *60 (10)*, 5114–5120. https://doi.org/10.1002/anie.202013951.

Solid Solution Formation in Xanthone – Thioxanthone Binary System: Experimental Investigation

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Abstract: Solid solutions (or mixed crystals) are phases consisting of at least two components arbitrarily located at equivalent sites in the crystal structure. The composition of such phases can be varied continuously over a certain range [1]. Solid solutions of inorganic compounds are long known as metal alloys, and many of the minerals are solid solutions. Furthermore, it has been demonstrated that such phases enable fine-tuning of material structure and properties [2,3]. Properties and rules of the formation of solid solutions in organic compound systems, however, are notably less explored [4]. Nevertheless, in the past decades, the number of publications on molecular solid solutions has increased significantly. For example, such systems have gained increased attention as a promising tool to develop or test the rules of crystal engineering [5,6]. In this study the formation of solid solutions in binary systems formed by thioxanthone and xanthone was explored. Crystallization experiments were performed to characterize the formation of solid solutions, The obtained crystalline phases were characterized using powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) methods in order to construct respective phase diagrams. The binary system of xanthone−thioxanthone has been explored, showing that two solid solutions (formed based on xanthone and thioxanthone parent structures, respectively) exist for this system. One of the solid solutions shows miscibility of both molecules in a large composition range (>0−80 mol % of xanthone). A structure of thioxanthone : xanthone (75 : 25 mol %) solid solution is also presented.

Keywords: Solid solution; thermal analysis; binary phase diagram

Author Contributions: Conceptualization, T.R.; methodology, T.R., A.B., and K.S.; software, T.R., A.B., and K.S.; validation, T.R.; formal analysis, K.S.; investigation, T.R., and K.S.; resources, T.R.; data curation, T.R., and K.S.; writing—original draft preparation, T.R., A.B., and K.S.; writing—review and editing, T.R., A.B., and K.S.; visualization, T.R., A.B., and K.S.; supervision, T.R.; project administration, A.B., and K.S.; funding acquisition, A.B., and K.S. All authors have read and agreed to the published version of the manuscript.

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References

1. Barbour, L. J.; Das, D.; Jacobs, T.; Lloyd, G. O.; Smith, V. J. *Concepts and Nomenclature in Chemical Crystallography*; 2012. (DOI: 10.1002/9780470661345.smc108).
2. Vujovic, D.; Raubenheimer, H. G.; Nassimbeni, L. R. One-Dimensional CdII Coordination Polymers: Solid Solutions with NiII, Thermal Stabilities and Structures. *Eur. J. Inorg. Chem.* **2004**, *2004*(14), 2943–2949. (DOI: 10.1002/ejic.200300794)
3. Krishnan, R.; Swart, H. C. Luminescence Properties of Octahedrally and Tetrahedrally Coordinated Mo6+ in the Solid Solutions: Judd–Ofelt Investigation. *J. Phys. Chem. Solids* **2020**, *144*, 109519. (DOI: 10.1016/j.jpcs.2020.109519)
4. Lusi, M. A Rough Guide to Molecular Solid Solutions: Design, Synthesis and Characterization of Mixed Crystals. *CrystEngComm* **2018**, *20*(44), 7042–7052. (DOI: 10.1039/C8CE00691A)
5. Lusi, M. Engineering Crystal Properties through Solid Solutions. *Cryst. Growth Des.* **2018**, *18* (6), 3704–3712. (DOI: 10.1021/acs.cgd.7b01643)
6. Braga, D. Crystal Engineering, Where from? Where To? *Chem. Commun.* **2003**, *3* (22), 2751–2754. (DOI: 10.1039/b306269b)

Toxicity of Silver-Chitosan Nanocomposites to Aquatic Species

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Abstract: According to the World Health Organization, antimicrobial resistance (AMR) is one of the top ten global public health threats justifying the need for new effective antimicrobials for biomedical applications. For the successful commercialization of any new material data on its environmental safety are obligatory. Silver (Ag) compounds are widely used in wound dressings as well as disinfectants in health care due to their antibacterial activity and assumingly low risk of the development of AMR.

In this study, the potential environmental hazard of silver-chitosan nanocomposites (nAgCSs) was evaluated. nAgCSs were synthesized by the reduction of the AgNO3 with trisodium citrate and stabilized by coating with low molecular-weight chitosan (50–190 kDa). Chitosan (CS) amount in the nanocomposites was varied, resulting in three different types of nanocomposites with the weight ratio of Ag to CS 1:0.3 (nAgCS-0.3), 1:1 (nAgCS-1) and 1:3 (nAgCS-3). The toxicities of different Ag-CS nanocomposites and citrate-coated Ag nanoparticles were tested on the naturally luminescent bacterium Vibrio fischeri and microcrustaceans Daphnia magna and Thamnocephalus platyurus.

The primary size of the synthesized nAgCSs was ~50 nm. In deionized water, the average hydrodynamic sizes were in the nanoscale (≤100 nm) and the surface charges positive (16–26 mV). The toxicity of the studied Ag nanomaterials was evaluated by the bacterial kinetic bioluminescence inhibition and viability test [1, 2], and acute immobilization/mortality tests with crustaceans [3, 4]. The nAgCSs were about 10–500 fold more toxic to microcrustaceans D. magna (48-h EC50=0.044–0.077 mg Ag/L) and T. platyurus (24-h EC50=0.19–0.261 mg Ag/L) than to bacterium V. fischeri (30-min EC50=3–26 mg Ag/L). Taking into the account the data obtained with this multi trophic test battery the synthesized silver chitosan nanocomposites could be classified as "extremely toxic" [L(E)C50 ≤ 0.1 mg/L] [5]. Interestingly, the nanocomposites with the highest chitosan content (nAgCS-3) were the most toxic to bacteria V. fischeri, but the least toxic to the crustaceans. The data obtained show that the chitosan-silver nanocomposites may pose a hazard to aquatic organisms and must be handled accordingly.

Keywords: antimicrobial nanomaterials, ecotoxicity, regulatory tests, bioluminescent bacteria, crustaceans.

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References

1. ISO 21338:2010. Water quality — Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of Vibrio fischeri (kinetic luminescent bacteria test). Geneva, Switzerland: International Organization for Standardization (ISO), 2010.
2. Suppi, S., Kasemets, K., Ivask, A., Künnis-Beres, K., Sihtmäe, M., Kurvet, I., Aruoja, V., Kahru, A. A novel method for comparison of biocidal properties of nanomaterials to bacteria, yeasts and algae. J Hazard Mater 2015, 286: 75–84. DOI: 10.1016/j.jhazmat.2014.12.027
3. OECD guidelines for the testing of chemicals/section 2. Effects on biotic systems, test no. 202: Daphnia sp. acute immobilization test. Paris, France: Organization for Economic Cooperation and Development (OECD), 2004. https://doi.org/10.1787/9789264069947-en
4. ISO 14380:2011. Water quality — Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca). Geneva, Switzerland: International Organization for Standardization (ISO), 2011.

5. Bondarenko, O.M., Heinlaan, M., Sihtmäe, M., Ivask, A., Kurvet, I., Joonas, E., Jemec, A., Mannerström, M., Heinonen, T., Rekulapelly, R., Singh, S., Zou, J., Pyykkö, I., Drobne, D., and Kahru, A. Multilaboratory evaluation of 15 bioassays for (eco)toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID. Nanotoxicology 2016, 10: 1229-1242. DOI: 10.1080/17435390.2016.1196251

Silver Assisted Binding of Anions by Biotin[6]uril

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Abstract: Biotinurils are chiral macrocycles that consist of natural D-biotin building blocks linked by methylene bridges. The ability of normal biotin[6]uril to bind different inorganic anions into the cavity [1] was employed in anion transport in human transmembrane [2]. Our group developed a mechanochemical approach for amidation of all carboxylate groups on biotin[6]uril structure, resulting in hexa-amide conjugate with phenylalanine methylester (*Fig 1.*) [3].

I will present binding properties of the new derivative, which exhibited higher affinities to anions in methanol than regular biotin[6]uril ester. Previously, Pittelkow *et al.* [1] reported that cations do not influence the anion binding, but we found out that the strength of interaction can be enhanced in the presence of silver cation. We tested the interactions of the biotinuril derivative with inorganic silver salts as well as macrocycle and $AgPF_6$ 1-1 mixture by Na R-/S-camphorsulfonates and Na R-/S- Mosher acid salts. We state a weak enantioselectivity of camphorsulfonate enantiomers by various methods. Due to this phenomenon the macrocycle can be used in the making of chiral columns.

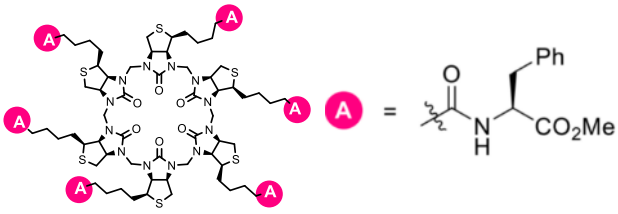

Fig.1. Biotin[6]uril hexa-amide

Keywords: supramolecular chemistry, chiral macrocycles, anion binding, enantioselectivity.

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References

1. Lisbjerg, M. *et al*. Org. Biomol. Chem, 2015, 13, 369-373. https://doi.org/10.1039/C4OB02211D
2. Valkenier, H. *et al. Chem*, **2019**, 5, 2, 429-444. https://doi.org/10.1016/j.chempr.2018.11.008
3. Dalidovich, T., Mishra, K.A., Shalima, T., Kudrjašova, M., Kananovich, D.G., Aav, R, 2020, ACS Sustain. Chem. Eng., 8, 41, 15703–1571. https://doi.org/10.26434/chemrxiv.12735377.v1

Silver Nanoparticles May Promote Antibiotic Resistance Gene Persistence in Wastewater Treatment Systems

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Abstract: Silver nanoparticles (AgNPs) are among the most widely used engineered nanomaterials. They can reach wastewater during their production, use, and disposal. Understanding how AgNPs affect wastewater treatment facilities, including constructed wetlands, is essential for public health. This study evaluated the impact of increased concentrations of AgNPs and Ag+ ions on the microbial community composition, system treatment efficiency, and removal of antibiotic resistance genes (ARGs) in a hybrid wastewater treatment system. Shotgun metagenomics was applied to assess the microbial community structure and antibiotic resistome diversity. In addition, the quantitative PCR was applied to determine the abundance of ARGs. The results showed that increased amounts of AgNPs and Ag+ ions had a modest effect on the prokaryotic community composition in the filter material biofilms and did not significantly impact the purification efficiency of the system [1]. AgNO3 induced a greater increase in the genetic potential of certain silver resistance mechanisms in vertical flow filters than collargol. This suggests that the microbial community composition in biofilms of VFs and HFs is largely resistant, resilient, or functionally redundant in response to silver nanoparticles. Higher Ag concentrations did affect the abundance and removal efficiency of ARGs in the wastewater, resulting in an elevated ARG discharge into the environment [2]. The accumulated Ag in the filters had a more profound effect on the absolute and relative abundance of ARGs in the treated water than on the Ag content in the water. The study recorded enhanced relative abundance values for tetracycline, sulfonamide, and aminoglycoside resistance genes, and elevated levels of plasmid and integron-integrase genes in the biofilms of the AgNP-treated system. The findings indicate that further investigation is needed to understand the impact of AgNPs on the nature and characteristics of prominent resistance genes in wastewater treatment systems.

Keywords: Wastewater treatment; Hybrid subsurface flow filter system; Silver nanoparticles; Microbial community structure; Silver resistance genes; Antibiotic resistome

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References

1. Truu; M.; Ligi; T.; Nõlvak; H.; Peeb; A.; Tiirik; K.; Devarajan; A. K.; Oopkaup; K.; Kasemets; K.; Kõiv-Vainik; M.; Kasak; K.; & Truu; J. (2022). Impact of synthetic silver nanoparticles on the biofilm microbial communities and wastewater treatment efficiency in experimental hybrid filter system treating municipal wastewater. *J. Hazard. Mater.*; *440*; 129721. DOI: 10.1016/j.jhazmat.2022.129721.
2. Nõlvak; H.; Truu; M.; Tiirik; K.; Devarajan; A. K.; Peeb; A.; & Truu; J. (2023). The effect of synthetic silver nanoparticles on the antibiotic resistome and the removal efficiency of antibiotic resistance genes in a hybrid filter system treating municipal wastewater. *Water Res.*, *237*, 119986. DOI: 10.1016/j.watres.2023.119986

Biocompatibility of Metal-Phenolic Network-Coated Nanoparticles

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Abstract: Metal-phenolic networks (MPNs) are novel adsorbent materials that have promising applications in the environmental remediation of organic pollutants and heavy metals. For efficient adsorption, the specific surface area of MPN materials can be increased by coating nanoparticle surfaces with MPN. Such MPN-coated nanoparticles may prove very efficient in various environmental applications; however, their safety needs to be tested at the early stages of material development. Here, free-living freshwater ciliated protozoa were used as model organisms for testing the biocompatibility of iron-tannic acid network-coated Au nanoparticles (Fe-TA@Au NPs). Viability after 24-h Fe-TA@Au NP exposure was measured using an ATP assay kit and intracellular reactive oxygen species (ROS) were quantified using H_2DCFDA [1]. Microscopy was used to qualitatively characterize the swimming behavior of protozoa as well as the uptake and depuration of Fe-TA@Au NPs in the ciliates. The results showed that Fe-TA@Au NPs were not lethal to protozoa at the maximum concentration tested (~10^{10} particles/mL). Despite MPN-coated NP phagocytosis by protozoa and accumulation in food vacuoles, the MPN did not affect the swimming behavior or viability during 24 h. To test the performance of the novel materials as heavy metal toxicity mitigating agents, co-exposures of protozoa to Fe-TA@Au NPs and toxic levels of copper salt ($CuSO_4$, LC_{50}~3 mg/L) were conducted. Fe-TA@Au NPs completely rescued protozoa from cell death induced by $CuSO_4$. The underlying mechanism of toxicity mitigation could have been removal of Cu ions by MPNs or tannic acid in MPN acting as an antioxidant as evidenced by reduced levels of ROS in protozoa. Thus, the study showed that the effective levels of Fe-TA@Au NPs were biocompatible with the unicellular freshwater model organism and have a potential for applications in metal contamination remediation in aqueous environment.

Keywords: metal-phenolic network (MPN), heavy metal, toxicity, protozoa, nanoparticles, remediation, environment

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References

1. Juganson, K., Mortimer, M., Ivask, A., Pucciarelli, S., Miceli, C., Orupõld, K., & Kahru, A. Mechanisms of toxic action of silver nanoparticles in the protozoan *Tetrahymena thermophila*: From gene expression to phenotypic events. *Environmental Pollution* **2017**, *225*, 481–489. https://doi.org/10.1016/j.envpol.2017.03.013

Characteristics of Landfill Leachates as a Microbial Resource for Plastic Degradation: A Study of Solid Waste Dumping Site of Getlini, Latvia

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Abstract: Global Municipal Solid Waste (MSW) landfills represent one of the largest plastispheres containing an average of 42% of plastic waste and thus serve as unique ecological niches for microbial communities [1]. Recent studies have demonstrated the presence of potential microbial genes in landfill leachates that are associated with the biodegradation of different types of plastics [2]. The aim of this work was to characterize the landfill leachate in terms of its chemical properties, as well as plastic degrading capabilities, i.e., metagenome, functional gene profiling, ecotoxicity, and others. Leachates were obtained at the Getliņi municipal solid waste landfill, which is managed by Getlini EKO Ltd. (Riga, Latvia). Landfill receives on average 500 thousand tons of waste from witch roughly half is landfilled. The physicochemical characteristics were tested using respective electrodes and kits (WTW Multi9620 IDS, Spectroquant® test kits, Merck, Germany) and various test results from national laboratories. DNA from the leachate samples was isolated using a FastDNA Spin Kit for Soil DNA Extraction (MP Biomedicals, USA) according to the manufacturer's guidelines. To explore microorganism community taxonomical and functional composition of leachate landfill, metagenomic sequencing analysis was performed using DNBSEQ-G400 sequencing platform (MGI Ltd., China). Metagenomic analysis of the leachate showed the dominance of Proteobacteria 54.7%, followed by Euryarchaeota 12.5%. Among bacterial genera, which are known as potent bioplastics-degraders, the most abundant were *Pseudomonas* and *Bacillus*. The most frequent gene families were connected to ATP binding and other essential elements for microorganisms. Landfill leachates were shown as an appropriate environment for biodegradation of plastics, as well as a source of microorganisms and respective gene families responsible for biodegradation.

Keywords: landfill leachate; plastic biodegradation; metagenome

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References

1. Lin, X.; Wang, S.; Ni, R.; Song, L. New insights on municipal solid waste (MSW) landfill plastisphere structure and function. *Sci. Total Environ.* **2023**, *888*, 163823, doi:10.1016/j.scitotenv.2023.163823.
2. Kumar, R.; Pandit, P.; Kumar, D.; Patel, Z.; Pandya, L.; Kumar, M.; Joshi, C.; Joshi, M. Landfill microbiome harbour plastic degrading genes: A metagenomic study of solid waste dumping site of Gujarat, India. *Sci. Total Environ.* **2021**, *779*, 146184. doi:10.1016/j.scitotenv.2021.146184.

Mitigation of Metal Oxide Nanotoxicity with Functional Fibrils

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Abstract: The toxicity of metal oxide nanoparticles has been a central research topic over the past two decades, owing to the domestic and industrial applications of this vast class of nanomaterials [1]. In literature, ion release has been implicated as a primary cause for metal oxide nanotoxicity, coupled with the distinct physicochemical properties (e.g., large surface area, ready diffusion and dissolution, and strong adsorption) of nanoparticles, in comparison with bulk materials [2,3]. However, few solutions have been proposed thus far for overcoming the toxicity of metal oxide nanoparticles *in vitro* and *in vivo*. In this study, we engineered functional amyloid fibrils [4] using beta lactoglobulin (blg), a major whey protein, and demonstrated a scheme of ion sequestration by blg amyloid fibrils co-incubated with CuO or ZnO nanoparticles, using inductively coupled plasma mass spectrometry (ICP-MS). Our computer modeling revealed that blg fibrils possessed multiple binding sites for Cu^{2+} and Zn^{2+}, while strong binding of the metal ions often occurred at the Cys-121 residues of the fibrils. In addition, our cell viability and reactive oxygen species assays implicated blg amyloid fibrils as a functional nanomaterial with minimal toxicity, a finding corroborated by zebrafish hatching and survival assays. This study offered a facile engineering strategy for remediating the toxicity of metal oxide nanoparticles for facilitating their safe biological and environmental applications.

Keywords: amyloid fibril; metal ion; nanoparticle; binding; toxicity

Author Contributions: Conceptualization, P.C.K.; methodology, Y.W., G.P., X.L.; simulation, H.T.; data collection and analyses, Y.W., X.L., F.H., H.T., N.A., Y.L., M.M., G.P.; writing, Y.W., N.A., F.H., G.P., M.M., P.C.K., supervision, G.P., P.C.K.; funding acquisition, G.P., P.C.K. All authors have read and agreed to the published version of the manuscript.

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References

1. A. Nel, T. Xia, L. Madler, N. Li, Toxic potential of materials at the nanolevel. *Science*, 2006, 311, 622-627 (DOI: 10.1126/science.1114397).
2. A. Ivask, K. Juganson, O. Bondarenko, M. Mortimer, V. Aruoja, K. Kasemets, I. Blinova, M. Heinlaan, V. Slaveykova, A. Kahru, Mechanisms of toxic action of Ag, ZnO and CuO nanoparticles to selected ecotoxicological test organisms and mammalian cells in vitro: a comparative review. *Nanotoxicology*, 2014, 8, 57-71 (DOI: 10.3109/17435390.2013.855831).
3. S. Lin, M. Mortimer, R. Chen, A. Kakinen, J.E. Riviere, T.P. Davis, F. Ding, P.C. Ke, NanoEHS beyond toxicity–focusing on biocorona. *Environmental Science: Nano*, 2017, 4, 1433-1454 (DOI: 10.1039/C6EN00579A).
4. P.C. Ke, R. Zhou, L.C. Serpell, R. Riek, T.P. Knowles, H.A. Lashuel, E. Gazit, I.W. Hamley, T.P. Davis, M. Fändrich, Half a century of amyloids: Past, present and future. *Chem. Soc. Rev.*, 2020, 49, 5473-5509 (DOI: 10.1039/C9CS00199A).

Optimization and Upscaling of Non-Thermal Atmospheric Plasma for Food Processing

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Abstract: Non-thermal plasma technology is recognized for its strong antimicrobial efficacy on food and food production environment associated microorganisms and ability to enhance food safety and food shelf-life [1]. However, the lack of scientific knowledge regarding the differences in physical and chemical processes at various upscaling levels hinders the successful transition of this technology from laboratory settings to industrial applications [2]. Hence, the aim of this thesis is to study the relation between plasma processed air (PPA) composition and its antimicrobial effects, as well as the underlying mechanism of action. This research focuses on three different air plasma torch devices, each representing a distinct stage of upscaling. A microwave-plasma torch operated with compressed air delivers PPA as antimicrobial acting process gas. Three air plasma torch devices are applied and will be optimized to study the relation between PPA composition and its antimicrobial effects. By employing advanced analytical methods like Fourier transform infrared spectroscopy (FTIR), a comprehensive understanding of PPA's characteristics will be achieved. Key reactive nitrogen species (RNS) in PPA have been discovered via spectroscopic measurements for the lab-size device MidiPLexc. From the spectrum, nitrogen monoxide (NO), nitrogen dioxide (NO_2) and dinitrogen pentoxide (N_2O_5) were detected, where NO_2 took up more than 70 % in quantity of the key reactive species [3]. Input power and relative humidity were found to have an impact on the species' concentration. The findings of this thesis will provide valuable insights into the physical and chemical factors that affect the scalability of non-thermal microwave plasma for antimicrobial processes. Knowledge obtained will accelerate the development and application of non-thermal atmospheric pressure plasma as a highly promising alternative for disinfection and cleaning purposes.

Keywords: non-thermal plasma; FTIR spectroscopy; reactive oxygen and nitrogen species; plasma processed air; microbial decontamination; food safety

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References

1. Ziuzina, D.; Patil, S.; Cullen, P.J.; Keener, K.M.; Bourke, P. Atmospheric cold plasma inactivation of Escherichia coli, Salmonella enterica serovar Typhimurium and Listeria monocytogenes inoculated on fresh produce. *Food Microbiol* **2014**, *42*, 109-116, doi:10.1016/j.fm.2014.02.007.
2. Liao, X.; Liu, D.; Xiang, Q.; Ahn, J.; Chen, S.; Ye, X.; Ding, T. Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control* **2017**, *75*, 83-91, doi:10.1016/j.foodcont.2016.12.021.
3. Weihe, T.; Yao, Y.; Opitz, N.; Wagner, R.; Krall, J.; Schnabel, U.; Below, H.; Ehlbeck, J. Plasma-Treated Water: A Comparison with Analog Mixtures of Traceable Ingredients. *Microorganisms* **2023**, *11*, doi:10.3390/microorganisms11040932.

Low-Temperature Synthesis and Characterization of Iron Whitlockite ($Ca_{18}Fe_2(HPO_4)_2(PO_4)_{12}$)

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Abstract: Calcium phosphates (CPs) represent the most widespread class of bioceramic materials used for bone regeneration purposes due to their excellent biological performance and similarity to the natural bone. Synthetic CPs substituted with other biologically active ions can be considered as a sub-group of the CP family while possessing specific biological or physical properties provided by incorporated foreign ions. Magnesium whitlockite (Mg-WH, $Ca_{18}Mg_2(HPO_4)_2(PO_4)_{12}$) is a Mg-substituted CP, which naturally occurs in humans. This material is assumed to be the second most abundant biomineral in human body. Although the ionic radius of Mg^{2+} is very similar to those of the first-row transition-metal ions (Zn^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}), reports on the synthesis of such materials are very rare [1-3]. At the same time transition-metal ions can provide some very specific properties (e.g. magnetic properties). To the best of our knowledge, there is only one work describing the synthesis and characterization of iron whitlockite (Fe-WH, $Ca_{18}Fe_2(HPO_4)_2(PO_4)_{12}$) [4]. The authors prepared Fe-WH by treating $Ca_9Fe(PO_4)_7$ with H_2 or D_2 at elevated temperatures. In our work, Fe-WH was synthesized at low temperature by dissolution-precipitation method in aqueous medium under hydrothermal conditions. Phase conversion from brushite to Fe-WH took place in slightly acidic medium in the presence of Fe^{2+} ions just in 1 h. The crystal structure of synthesized products was confirmed by XRD analysis, FTIR, Raman and Mössbauer spectroscopies. The magnetic ordering and oxidation state information were determined using magnetic susceptibility. Morphological features were studied by means of SEM analysis. Cytotoxicity experiments were performed with MC3T3-E1 cells.

Keywords: whitlockite; dissolution-precipitation; low temperature.

Author Contributions: Conceptualization, A.Z. and D.G.; methodology, A.Z. and D.G.; formal analysis, D.G., A.D., D.Z., V.T.; investigation, D.G., A.D., D.Z., V.T.; writing—original draft preparation, A.Z.; writing—review and editing, A.Z.; visualization, D.G.; supervision, A.Z.; funding acquisition, A.Z. All authors have read and agreed to the published version of the manuscript.

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References

1. Kizalaite, A.; Grigoraviciute-Puroniene, I.; Asuigui, D.R.C.; Stoll, S.L.; Cho, S.H.; Sekino, T.; Kareiva, A.; Zarkov, A. Dissolution–Precipitation Synthesis and Characterization of Zinc Whitlockite with Variable Metal Content. *ACS Biomater. Sci. Eng.* **2021**, *7*, 3586-3593, doi:10.1021/acsbiomaterials.1c00335.
2. Kizalaite, A.; Klimavicius, V.; Versockiene, J.; Lastauskiene, E.; Murauskas, T.; Skaudzius, R.; Yokoi, T.; Kawashita, M.; Goto, T.; Sekino, T.; et al. Peculiarities of the formation, structural and morphological properties of zinc whitlockite ($Ca_{18}Zn_2(HPO_4)_2(PO_4)_{12}$) synthesized via a phase transformation process under hydrothermal conditions. *Crystengcomm* **2022**, *24*, 5068-5079, doi:10.1039/D2CE00497F.
3. Heng, C.; Liu, W.; Zheng, X.; Ma, X.; Hui, J.; Fan, D. Dopamine and DNA functionalized manganese whitlockite nanocrystals for magnetic resonance imaging and chemo-photothermal therapy of tumors. *Colloids Surf. B Biointerfaces* **2023**, *222*, 113120, doi:10.1016/j.colsurfb.2022.113120.
4. Belik, A.A.; Izumi, F.; Stefanovich, S.Y.; Lazoryak, B.I.; Oikawa, K. Chemical and Structural Properties of a Whitlockite-like Phosphate, $Ca_9FeD(PO_4)_7$. *Chem. Mater.* **2002**, *14*, 3937-3945, doi:10.1021/cm020411+.

Pilot Study of Grassland Soil Soluble Organic Matter with High Resolution Mass Spectrometry

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Abstract: Soil is the largest terrestrial carbon pool and regulates carbon, water, and nutrient cycles [1,2]. The diversity of soil environment prevents it from having one certain parameter to assess the condition of soil. Instead, different physical, chemical, and biological parameters are used to assess soil health in general [1,3]. A non-targeted method was proposed for fingerprinting soil soluble organic matter (SOM) with high resolution mass spectrometry (HRMS) and making assessments on soil health from collected data. Soil samples were collected from a permanent grassland in Tõravere over the course of April 2022 to June 2022. Samples were dried with vacuum and extracted with organic solvents. Three solvents were compared for extraction – acetonitrile, methanol, and toluene. Extracts were analyzed with HRMS by using Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR MS). Flow infusion together with two ionization sources were compared – nano-electrospray ionization (nESI) and atmospheric pressure chemical ionization (APCI) [4]. Results demonstrated that mass spectra of methanol extracts were most abundant in peaks. Further data analysis of elucidated molecular formulas revealed, that identified compounds in each extract consisted mostly of various lipids, although some peptides were identified in extracts of all three solvents, and some carbohydrates were identified in methanol and toluene extracts. Results also suggest nESI to be more suitable for HRMS analysis since it used up smaller amounts of extract but gave more peaks on the mass spectrum [4]. Further research of more diverse sample types and variations of additional parameters (time-series for observation the effects of plants development cycle and soil microorganisms' population dynamics, changing weather conditions, etc) are needed for looking relationships between SOM chemical composition and soil health.

Keywords: soil; soil health; soil organic matter; high resolution mass spectrometry; FT-ICR MS

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References

1. Rinot, O.; Levy, G. J.; Steinberg, Y.; Svoray, T.; Eshel, G. Soil Health Assessment: A Critical Review of Current Methodologies and a Proposed New Approach. *Science of The Total Environment* **2019**, *648*, 1484–1491. https://doi.org/10.1016/j.scitotenv.2018.08.259.
2. Communication from The Commission to The European Parliament, The Council, The European Economic and Social Committee and The Committee of The Regions EU Soil Strategy for 2030 Reaping the Benefits of Healthy Soils for People, Food, Nature and Climate; 2021. Available online: https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=CELEX:52021DC0699 (accessed on 20.05.2023).
3. Bünemann, E. K.; Bongiorno, G.; Bai, Z.; Creamer, R. E.; De Deyn, G.; de Goede, R.; Fleskens, L.; Geissen, V.; Kuyper, T. W.; Mäder, P.; Pulleman, M.; Sukkel, W.; van Groenigen, J. W.; Brussaard, L. Soil Quality – A Critical Review. Soil Biology and Biochemistry 2018, 120, 105–125. https://doi.org/10.1016/j.soilbio.2018.01.030.
4. Zobel, A. Pilot study of grassland soil soluble organic matter with high resolution mass spectrometry. Bachelor's thesis, University of Tartu, Estonia, 31.05.2023.