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The 3rd Conference of the International Association for Biomonitoring of Environmental Pollution

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Book of Abstracts

Editors

Nenad Zarić - Faculty of Biology, University of Belgrade, Serbia
Mira Aničić Urošević- Institute of Physics Belgrade, University of Belgrade, Serbia

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IABEP

INTERNATIONAL ASSOCIATION FOR BIOMONITORING OF ENVIRONMENTAL POLLUTION

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Nenad Zarić - Faculty of Biology, University of Belgrade, Serbia

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Preface

After the successful launch of the International Association for Biomonitoring of Environmental Pollution (IABEP) in Trieste (October 2023) and the 2nd conference in Lisbon (November 2024), the 3rd IABEP Conference (IABEP 2025) was held in Belgrade, Serbia, from 13th to 15th October 2025.

The IABEP conference gathered scientists, students, policymakers, and professionals in the field of biomonitoring of environmental pollution across terrestrial, freshwater, and marine habitats. It featured insightful presentations, engaging discussions, and networking opportunities focused on the latest advancements, challenges, and solutions in biomonitoring. For the first time, the conference also embraced topics related to mitigation and removal of pollutants from the environment through bioremediation and phytoremediation.

The main topics of IABEP 2025 included:

- Biomonitoring techniques to assess spatial and temporal patterns of pollution and its impact on biodiversity, pollutant accumulation, molecular markers, and magnetic analysis
- Novel biomonitoring methodologies
- Emerging pollutants and their toxicity
- Advances in bioindicators and biomarkers
- Bioremediation and phytoremediation approaches
- Integration of biomonitoring data into risk assessment and policy-making

The local organizer of IABEP 2025 was the **Institute of Physics Belgrade**, a National Institute of the Republic of Serbia, University of Belgrade (IPB), with support from the **Faculty of Biology** and the **Faculty of Chemistry**, University of Belgrade.

The scientific program of IABEP2025 included plenary, invited, and honorary lectures, as well as oral presentations and poster sessions. Special attention was given to promoting young researchers through short talks and awards.

The first conference day was dedicated to mosses and lichens as biomonitors of air pollution and began with a plenary lecture by Prof. Marko Sabovljević, who provided a comprehensive overview of *“Bryophytes as biomonitors – from laboratory research to field application.”* It was followed by remarkable examples of long-term biomonitoring programs, including *“Past, present and future of the UNECE ICP Vegetation”* by Prof. Marina Frontasyeva and *“50 years of moss biomonitoring in Sweden”* by Dr. Marta Segura Roux. In addition, Prof. Linda Geiser emphasized *“Mapping air quality and climate vulnerabilities of epiphytic macrolichens across North America.”* The afternoon session concluded with a critical evaluation of lichen and moss biomonitoring data presented by Prof. Stefano Loppi.

The second conference day focused on the potential of vascular plants as biomonitors of inorganic and organic pollutants, with special emphasis on microplastics research. The day began with an inspiring plenary lecture by Prof. Roeland Samson on *“One Health concept, citizen science approach, and bio(magnetic)monitoring of vegetation.”* The afternoon session highlighted *“Phytoremediation perspectives: metal hyperaccumulation in Noccaea caerulescens”* presented by Prof. Mark Aarts.

The third day explored animal biomonitoring, particularly of PFAS (the “forever chemicals”) in marine and terrestrial environments. A memorable plenary lecture by Prof. Joerg Feldmann opened the session, followed by *"Legacy and emerging pollutants monitored by birds"* presented by Prof. Silvia Lacorte. Other contributions showcased the use of algae, mollusks, and fish as biomonitors of emerging pollutants, and bioavailability of contaminants from food of animal origin presented by Dr. habil. Stefan Jurjanz.

The IABEP Awards were presented to recognize outstanding scientific achievements and to encourage early-career researchers. The Best Oral Presentation Awards were granted to Tomica Mišljenović ("*Decoding survival: A synthesis of studies on Viola species from Allchar*") and Tijana Milićević ("*The human milk as a biomonitor of potentially toxic elements – bioaccessibility and infant health risk assessment*") while the Best Short-talk Awards were granted to Milica Popović *("Application of nonlinear optical imaging and Nile Red staining in microplastic analysis"*) and Mirko Legnaro Diamanti (*"Biomonitoring of airborne elements and microplastics: a comparison of terricolous and epiphytic lichens"*).

This *Book of Abstracts* represents a concise summary of the scientific contributions presented at IABEP 2025 highlighting current advances, challenges, and perspectives in biomonitoring of environmental pollution. We hope it inspires future collaborations and innovations in this vital field.

Dr. Mira Aničić Urošević
Chair of the 3rd IABEP Conference

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Plenary Speakers

Bryophytes as biomonitors – from laboratory research to field application

Sabovljević, Marko S.[1,2]

[1]Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Belgrade, Serbia
[2]Department of Plant Biology, Institute of Biological and Ecological Sciences, University Pavol Jozef Šafárik in Košice, Košice, Slovakia

E-mail of the presenting author: marko@bio.bg.ac.rs; marko.sabovljevic@upjs.sk

Bryophytes, the second largest group of terrestrial plants, are widely used in environmental monitoring due to their unique features not found in flowering plants. However, high diversity and long evolutionary history in bryophytes, provide a wide range of mechanisms and strategies for coping with harsh environments. Many of these are not universal across the bryophyte group, or even consistent among different populations of the same species.

Bearing in mind that the evolutionary and phylogenetic distance between two random bryophyte species can be significantly greater than that between any pair of vascular plants, it is clear that many features of bryophytes remain unknown or poorly understood up to date.

Here we present the advantages and disadvantages of using bryophytes as model organisms in environmental monitoring, along with case studies that highlight the strengths and limitations of conducting experiments in environmental studies.

Both laboratory and field tests are welcomed and expected in the future, as they may generate new insights into the role of bryophytes both as model organisms and as monitoring tools. In general this opens many spaces for further research and cooperation as well as novel and unique dicoveries.

One Health and bio(magnetic)monitoring of vegetation

Samson, R.[1]

[1]*Department of Bioscience Engineering, Research group Biobased Sustainability Engineering (SUSTAIN), University of Antwerp, Antwerp, Belgium*

E-mail of the presenting author: roeland.samson@uantwerpen.be

Since the rise of mankind, the situation of life on earth has never been more critical. Human appropriation of the Earth is enormous, taking the majority of the annually produced natural resources for its own benefit. For nature and our environment this appropriation goes hand in hand with loss and pollution of natural habitats. Meanwhile mankind changed the composition of the Earth's atmosphere so drastically, that climate is seriously disrupted, so that remaining habitats are further endangered and our environment is continuously further put under pressure. But the more the natural functioning of our living planet is endangered, the less people – and even more seriously our politicians - seems interested in solving any environment-related problem.

To illustrate how the organisms on our planet depend on, and are linked to, each other, the concept of One Health was recently launched, reflecting the holistic approach that recognizes the interconnectedness of human, animal, plant and environmental health. Many international organisations, like the European Union, have the ambition to use this One Health concept as a guiding star for their policies.

This lecture wants to focus of the opportunities of the One Health concept for reconnecting society with its environment, and in the role of bio(magnetic) monitoring in this reconnection. As plants, from cryptogams to vascular plants, form the cornerstone of each ecosystem, as well aquatic as terrestrial, this lecture will deal with potentials of plants to be used in biomonitoring of our environment in relation to humans and animals. We will also investigate also how plant bio(magnetic)monitoring can help in reconnecting citizens to their environment.

Understanding the bioaccumulation and transformation of PFAS – the forever chemicals in the marine and the terrestrial environment

Feldmann, J.[1], Müller, V.[1], Preihs, M.[1], Matic, E.[1], Al Zbedy, A.[1], Raab, A.[1]

[1]*TESLA–Analytical Chemistry, Institute of Chemistry, University of Graz, 8010 Graz, Austria*

E-mail of the presenting author: joerg.feldmann@uni-graz.at

Per- and polyfluoroalkyl substances (PFAS) comprise a vast group of more than 12,000 synthetic organofluorine compounds that have been produced and used for over seven decades in industrial and consumer applications due to their unique surfactant and stability properties. These same properties, however, render PFAS highly persistent in the environment, leading to their accumulation in soils, waters, wildlife, and humans. As a result, PFAS are now often referred to as *"forever chemicals."* Despite increasing public and scientific attention, the comprehensive analytical characterization of PFAS in environmental and biological samples remains a formidable challenge.

Currently, state-of-the-art targeted analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) enable the quantification of approximately 50 PFAS compounds. Even when employing high-resolution mass spectrometry (LC–HRMS) for non-targeted screening, the vast chemical diversity of PFAS means that only a small fraction of the total organofluorine burden can be identified. Consequently, conventional targeted approaches may underestimate the true extent of PFAS contamination and transformation in environmental systems.

To overcome these analytical limitations, we employ a complementary fluorine mass balance approach combining extraction of organofluorines with methanol and determination of total fluorine by combustion ion chromatography (CIC) or inductively coupled plasma mass spectrometry (ICP–MS). The difference between extractable organofluorine (EOF) and the sum of identified PFAS reveals the presence of "hidden" PFAS or other unidentified organofluorine compounds. This integrated strategy provides a more complete picture of PFAS occurrence and transformation in environmental and biological matrices, bridging the gap between targeted and non-targeted screening [1].

The effectiveness of this approach will be demonstrated through a series of biomonitoring case studies in both **terrestrial** and **marine** environments. In terrestrial ecosystems, livers from wild boar [2] (*Sus scrofa*), roe deer (*Capreolus capreolus*), and chamois (*Rupicapra rupicapra*), as well as honey bees [3] (*Apis mellifera*) and their collected pollen, have been analysed to assess PFAS bioaccumulation, seasonal trends, and possible exposure pathways. In marine environments, the focus is on shrimps [4] —both farmed and wild species—and pilot whales [5] (*Globicephala melas*) to evaluate PFAS burdens across trophic levels and to identify potential transformation processes in marine food webs.

These comprehensive studies collectively demonstrate that combining targeted LC–MS/MS and LC–HRMS analysis with total fluorine measurements by CIC and ICP–MS significantly enhances the understanding of PFAS bioaccumulation and transformation processes. The findings underscore the necessity of integrating multiple analytical platforms to achieve robust PFAS mass balances in environmental biomonitoring and to identify unidentified or novel PFAS species. This integrated analytical strategy represents a crucial step toward closing the fluorine mass balance gap and improving the assessment of PFAS exposure and risks to ecosystems and human health.

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Honorary Speaker

Monitoring long term and large-scale deposition of air pollutants based on moss analysis: past, present and future of the UNECE ICP Vegetation

Frontasyeva, M.[1]

[1]Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russia,

E-mail of the presenting author: marina@nf.jir.ru

This brief historical review outlines the development and milestones of the moss biomonitoring technique, which is utilized to study atmospheric deposition of various pollutants in Europe. Heavy elements and trace elements (monitored since 1990), Nitrogen (monitored since 2005); Persistent Organic Pollutants (POPs) (monitored since 2010), and Radionuclides (both technogenic and natural origin) (monitored since 2015). The significance of these studies is emphasized in relation to the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). Examples from the long-term activities of the ICP Vegetation (International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops), established in 1987, illustrate trends in air pollutant behavior. In alignment with the LRTAP Convention's long-term strategy to enhance participation and improve air quality in Eastern Europe, the Caucasus, Central Asia, and Southeastern Europe, efforts are underway to extend the program to Asia and the Pacific Region. As of now, 36 countries have expressed interest in participating in the 2025–2026 moss survey in Europe, along with 14 countries from Asia and the Pacific. This review also covers analytical methods and approaches to data interpretation related to the moss biomonitoring technique. For many heavy metals the lowest concentrations were generally found in the north and west of Europe and higher concentrations in the south-east of the region. For some metals the influence of point sources within the region could be identified (*e.g.* antimony and cadmium), whereas others had either more dispersed sources within the region (*e.g.* lead), or more homogenous distribution (*e.g.* zinc) suggesting long-range transboundary sources from outside of the region. Many (but not all) of the measured heavy metals have shown a decline in concentration within the moss tissue since 1990. In some cases, such as lead, this has mirrored the decline in emissions within the EU 27 countries, however, in many cases the decline in concentration in moss tissue has been more modest (*e.g.* chromium and nickel). The reductions in metal concentration in moss tissue between 1990 and 2020 (based on all countries that participated in four or more surveys since 1990) were 83.2% for lead, 61.9% for cadmium, 51.0% for vanadium, 30.4% for nickel, 25.6% for copper, 20.1% for chromium, 18.1% for zinc, 15.0% for iron. For arsenic and mercury there has been very little change. For arsenic there was an increase of 6% since 1995. For mercury the concentration in moss increased by 4.8%. Aluminum and antimony concentrations in moss tissue have only been measured since 2005 and have shown a reduction of 36.4% and 41.5% respectively between 2005 and 2020. A small increase in nitrogen concentration in moss tissue of 3.1% was observed over the period 2005–2020. Highest concentrations of nitrogen in moss tissue were generally found in central Europe.

Reference

United Nations Economic Commission for Europe International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops. http://icpvegetation.ceh.ac.uk/

Invited Speakers

Metal concentrations in moss in comparison to deposition and air particles PM10 measured at EMEP background stations, Sweden – 50 years of moss monitoring

Segura Roux, M.[1]

[1]*IVL Swedish Environmental Research Institute, Aschebergsgatan 44, 411 33 Gothenburg, Sweden*

Email of the presenting author: marta.seguraroux@ivl.se

A number of moss species (for example *Pleurozium schreberi* and *Hylocomium splendens)* have no root system and consume nutrients via atmospheric deposition. This property was used as a basis of using mosses in the biomonitoring of atmospheric pollution. The estimation of the metal deposition via moss samples gives a significantly geographically detailed picture of the deposition of these metals and enables the mapping of places with a local impact. For this reason, results of the moss surveys are important for evaluation of long-term trends of heavy metals and have been carried out in Sweden since 1975.

Over the past 45 years (1975 - 2020), the metal concentrations in moss for Sweden decreased most for lead (-112%Pb), followed by chromium (-98%Cr), vanadium (-96%V), nickel (-88%Ni), cadmium (-77%Cd), arsenic (-63 %As), copper (-52%Cu) and zinc (-44%Zn). The analysis also showed significantly decreasing levels of all metals in all regional areas of Sweden.

For example, the addition of lead in petrol was banned in the mid-1990s, which is one explanation for the large reduction. Arsenic content in moss has decreased in line with the European emission reduction until 2010. The reduction of cadmium from 1975 onwards is mainly due to better purification equipment at metal smelters and steelworks, but also because in the mid-1990s a tax on cadmium was introduced, although this tax was abolished in 2010. The reduction of cadmium in certain fertilizers may also have contributed. For arsenic, lead, copper and chromium, the reduction of levels in moss has been in good agreement with the emission reductions presented for Sweden and for the EU27 + UK from 1990. The levels of cadmium, zinc and mercury in moss, on the other hand, decreased less when compared to the emission reductions.

In general, a south-north gradient could be seen for most of the analyzed metals in moss, with the highest concentrations in southern and southwestern Sweden and the lowest in northern Sweden and the mountain areas.

In the Swedish background stations (Hallahus, Råö, Norunda and Bredkälen) and at one station in northern Finland (Pallas), metals (As, Cd, Co, Cr, Cu, Hg, Ni, Mn, Pb, V and Zn) in air particles PM10 and precipitation are measured monthly. The stations are part of the EMEP (European Monitoring and Evaluation Programme) network and the aim of the measurements is for evaluating long-range Transmission of Air Pollutants in Europe.

Temporal and geographic patterns in metal concentrations will be presented in moss, background deposition and ambient PM10 particles at EMEP stations.

Interpreting lichen and moss biomonitoring data: *hic sunt leones*

Loppi, S.[1,2]

[1]*Department of Life Sciences, University of Siena, Italy*
[2]*National Biodiversity Future Center, Palermo, Italy*

E-mail of the presenting author: stefano.loppi@unisi.it

Although biomonitoring of air pollution with lichen and moss has a long history, there are still many unclear aspects regarding the interpretation of the data.

First of all, the object of biomonitoring, i.e. what we are measuring, should be made clear, otherwise we would have to answer “biological data”. But at very least we should answer “the biological effects of (undefined) pollutants” or of “air pollution” (skipping about air quality).

Defining which pollutant(s) we are measuring is of key importance is we want that lichen/moss biomonitoring is used in legal scenarios (e.g. environmental justice), especially when these organisms are used as bioindicators.

The diversity of epiphytic lichens is a well know indicator of SO_2 pollution, but after this pollutant has dropped, it was unclear what was primary driving the lichen diversity, especially in a rapidly changing scenario (e.g. nitrogen pollution), to the extent that European biomonitoring guidelines were provided for “assessing the epiphytic lichen diversity” without any attempt to interpret the data in terms of air pollution. Recently, the standard LBI (lichen biodiversity index) method has been re-calibrated to monitor, separately, primary pollutants (SO_2, NOx, PM), ammonia/ammonium pollution, and ozone pollution.

The use of lichen and moss as bioaccumulators is more intuitive: they provide an estimate of a given element that is (more or less) proportional to its (more or less) recent environmental availability. However, the first concern is whether we measure element concentrations or element deposition, as this makes a big difference when using biomonitoring surveys as ground for epidemiological studies. There is compelling evidence that our estimates refer to bulk (wet and dry) atmospheric deposition, so how we express the data is of paramount importance. Estimating the data based on the surface area rather then classical dry weight, provides a more realistic picture and also allows deposition rates to be estimated.

The level of variability and uncertainty is poorly considered in bioaccumulation studies: even in remote areas, where disturbances are stochastic, there is great variability that often prevents accurate estimates and predictions from being made, particularly in dry areas (e.g. the Mediterranean) due to the deposition onto the biomonitors of airborne dust.

Finally, attempts to correlate biomonitoring data with pollutant emissions (frequently seen in the literature) are misleading, as emissions are only local, and are not a surrogate for concentrations neither for deposition.

Phytoremediation perspectives: The metal hyperaccumulation phenomenon in *Noccaea caerulescens*

Yamjabok, J.[1], Wang, Y.[1], Duan, S.[1], van Oostrom, L.[1], Schat, H.[1], van den Heuvel, J.[1], **Aarts, M.G.M.**[1]

[1]*Laboratory of Genetics, Wageningen University, Wageningen, The Netherlands*

E-mail of the presenting author: Mark.Aarts@wur.nl

Especially contamination with cadmium is one that could be remediated using metal hyperaccumulator plants. There are not many cadmium hyperaccumulators known, and even less that could be developed into a cadmium phytoremediation crop. Few of them being members of the *Noccaea* genus. Of these species, *Noccaea caerulescens* is best studied. Next to cadmium, this species accumulates nickel and zinc. Three major ecotypes are distinguished, typical for serpentine/ultramafic soils, for calamine soils and for non-metallicolous soils. Over the years we made a genetic and genomic toolbox with which to further examine the molecular genetic basis of metal hyperaccumulation. Examples of applications of this will be presented and discussed, as inspiration on how to make your favourite metal phytoremediation crop

Birds as biomonitors of legacy and emerging contaminants

Lacorte, S.[1], Zapata-Corella, P[1]., Vidal-Colomer, P[1]., Monclús, L.[1], Oró-Nolla, B.[1], Dulsat-Masvidal, M.[1], Santos, F.J.[2], Bertolero, A.[3]

[1]*Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain*
[2]*Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain*
[3]*Associació Ornitològica Picampall de les Terres de l'Ebre, Deltebre, Spain*

E-mail of the presenting author: silvia.lacorte@idaea.csic.es

Birds are effective biomonitors of legacy and emerging environmental pollutants due to their wide distribution, trophic diversity, and sensitivity to contaminants. They reflect ecosystem health through pollutant accumulation. The ability to accumulate organic contaminants depends on species-specific physiology, type of diet and habitat use, and apex avian predators exhibit the highest contaminant burdens due to prolonged exposure and trophic biomagnification. This study evaluates the use of different matrices (blood, eggs and feathers) in biomonitoring studies to determine pollution patterns of several bird species linked to different exposure scenarios. For that, several analytical methods were developed to determine a wide number of contaminants, spanning from POPs (polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), perfluoroalkyl substances (PFAS), polybromodiphenyl ethers (PBDEs)) and polycyclic aromatic hydrocarbons (PAHs) to emerging (pharmaceuticals, phthalates, organophosphate esters, in-use pesticides). These compounds originate from the past use in agriculture and industry that still persist in the environment or from newly produced chemicals that are discharged from domestic, industry, tourism or other activities. In this talk, several examples will be presented:

- Blood of flamingos chicks (*Phoenicopterus roseus*). The results indicate a multi-exposure of flamingo's chicks from the Ebro delta with ∑PFASs ranging from 9.34 to 576 ng/mL, being PFOA, PFOS and PFHxS detected in all samples. ∑PAHs ranged from 0.19 to 423 ng/mL, followed by PCBs and OCs. Pharmaceuticals, OPEs and in-use pesticides were not detected. The most plausible source of PFAS is the pesticides used in rice-fields of the area [1].
- Eggs of gulls (*Larus michahellis* and *Larus audouinii*). Eggs have been sampled annually since 2009 in the Ebro Delta, a wetland of international importance for waterbird conservation but severally affected by toxic waste discharges from a past chloro-alkali industry. Marker PCBs ranged from 0.03 to 700 ng/g ww, OC pesticides as DDE, dieldrin, mirex and hexachlorobenzene from 140 to 500 ng/g ww, PBDEs and PFAS up to 300 ng/g ww. Temporal trends were observed associated to the extraction of contaminated sediments from the chloro-alkali industry [2, 3].
- Feathers of free-living and captive red kites (*Milvus milvus*). Body feathers from the interscapular region were used as a minimally-invasive and integrative matrix. Compounds detected were 4,4′-DDE (6.10 ± 1.56 ng/g dw feather) followed by PCB-153 (3.10 ± 0.63 ng/g dw feather) and PCB-180 (2.43 ± 1.08 ng/g dw feather) in free-living kites and somehow lower concentration in captive kites [4]. Concentrations detected were associated to endocrine stress.

Through biomonitoring, birds provide critical insights into pollutant bioaccumulation, biomagnification, and spatial-temporal trends, ecological risk assessment, and support environmental policy and conservation efforts in vulnerable ecosystems affected by human footprint.

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Food of animal origin as a bio indicator of exposure to environmental pollutants

Jurjanz, S.[1]

[1]*Laboratory Animal and Agroecosystems (L2A), University of Lorraine, Nancy, France*

E-mail of the presenting author: Stefan.Jurjanz@univ-lorraine.fr

Different sanitary scandals showed that pollutants might reach very high levels in Food |1,2] what let suppose an important presence in the environment of the food-producing animals. Nevertheless, this link is very complex and depends on several parameters as the type of pollutant, the exposure (i.e. the contaminated environmental matter and its intake by the animals, the bioavailability of the pollutant) and the type of foodstuff (i.e. excretion product or body tissue). This synthesis focuses on the different families of Persistent Organic Pollutants (POPs) as the transfer of toxic trace elements (TTE) has been largely studied. Chemical characteristics (molecular weight, volatility, lipophilicity) and their metabolic sensitiveness make vary their transfer patterns and the risk to recover high concentrations in Foodstuffs. Among the different environmental matters on which the pollutants may be deposited, soil deserves special attention by its ability to store deposited pollutants over very long periods, mainly in its organic fraction, and several degradation mechanisms are slowed down in soil. Therefore, the pollutants may accumulate during decades reaching very high concentrations. Although some exceptions, the transfer soil-plant of lipophilic organic pollutants is generally weak. Moreover, vegetation would accumulate such deposits only during one growing period and polluted grass can be evacuated by simple cutting. Superficial water may also receive notable emissions of environmental pollutants, but its low affinity for hydrophobic POPs limits generally this exposure pathway even if some exceptions exist (PFAS, light PAHs or some pesticides). Free-ranged animals may ingest notable amounts of soil [3-5], which would represent their main exposure source. Although huge differences exist between animal species (herbivorous versus burrower) and rearing systems (intensive versus extensive), there is a minimum level of involuntary soil intake which has to be integrated in any risk assessment for any specie. Contrarily to TTEs, ingested POPs are generally highly bioavailable in animals [6], while a supply of sequestrants can reduce the bioavailability of soil bound POPs [7]. However, notable carry over rates have been shown for POPs, especially for the most toxic ones with a special focus on eggs [8]. Indeed, free-ranged laying hens appear very sensitive to ingest, extract and transfer POPs into eggs. Body fat and liver seem to be the most sensitive edible body tissues bioconcentrating ingested POPs. As the contamination risk for Food from free-ranged animals in polluted areas is notable, European Member States ensure the Food safety of consumers by a consequent device, which monitor on the one hand the contamination situation by random sampling of Feed and Food and control on the other hand sensitive products by target sampling.

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Oral Speakers

Mapping Air Quality and Climate Vulnerabilities of Epiphytic Macrolichens Across North America

<u>Geiser, Linda H.</u>[1,2], Moses, J.[3], Bell, M.[4], Velázquez Román, L.[5]

[1]Air Resource Management, US Department of Agriculture-Forest Service, Washington, DC; [2]Oregon State University Department of Botany and Plant Pathology, Corvallis, Oregon, USA
[3]Oak Ridge Institute for Science & Education, US Department of Energy, Seattle, Washington, USA
[4]Air Resources Division, Department of the Interior-National Park Service, Lakeview, Colorado, USA
[5]Air Resource Management, US Department of Agriculture-Forest Service, Ponce, Puerto Rico, USA

Email of the presenting author: linda.geiser@usda.gov

Epiphytic macrolichens contribute to North American biodiversity, play integral ecological roles, and are useful biomonitors of environmental change. We wished to understand the risks posed to the distribution of 359 common epiphytic macrolichens in North America from current levels of air pollution and future climate change. Our dataset consisted of 574,756 records from the Consortium of North American Lichen Herbaria and the USDA-Forest Service Lichens and Air Quality biomonitoring database collected between 1960 and 2024. To map current air pollution vulnerability, we calculated current critical load exceedances for acidity and nutrient nitrogen for each species historic range using 2021 data from the US Environmental Protection Agency Community Multiscale Air Quality v.5.4 and the North American Climate Integration and Diagnostics – Nitrogen Deposition v.1 models. To map climate variability, we used a machine learning method for presence-only data, Maximum Entropy (MaxEnt), to model the probability of the occurrence of each species to historic climates across the US, Canada, and Mexico, integrating 30-year normal for 11 climate variables from the University of British Columbia's ClimateNA. We then input future climate values under the IPCC AR6's Shared Socioeconomic Pathway 5 (+ 8.5 W/m^2 warming by 2100) and mapped species probabilities of occurrence in 2041-2070. Air pollution threats were greatest in the eastern US and urban corridors of the western US. Climate change poses a greater risk to specialized species with narrower climate tolerances compared to generalists, but even generalists may experience less suitable climates over much of their historic ranges within the next 16-45 years. These maps will be linked to the USDA PLANTS database, a nationally standardized source of conservation information about vascular plants, bryophytes, and lichens.

Thiol-peptides for Metal detoxification and homeostasis in Lichens and Bryophyes

Sanità di Toppi, L.[1]

[1]*Department of Biology, University of Pisa, Pisa, Italy*

E-mail of the presenting author: luigi.sanita@unipi.it

For nearly thirty years, my research has focused on the response of photoautotrophic organisms to heavy metals [1]. This contribution for IABEP 2025 specifically addresses the response of lichens and bryophytes to heavy metals, with emphasis on thiol-peptides such as glutathione (GSH, a γ-glutamyl-cysteinyl-glycine tripeptide) and phytochelatins. These compounds detoxify toxic heavy metals and metalloids (e.g., Cd, Pb, Cr(VI), Hg, As(III)/(V), etc.) primarily at the intracellular level, and maintain homeostasis of some metal micronutrients (Fe, Cu, Zn, Mn, etc.) [1]. GSH can chelate some metal(loid)s to reduce their intracellular toxicity or chemically transform them into less toxic forms. Phytochelatins, enzymatically derived from GSH, are typically more effective chelators of metal(loid)s. They have the general structure (γ-glutamyl-cysteinyl)n-glycine, with "n" indicating 2 to 5 repeats of the γ-glutamyl-cysteine dimer [2]. Due to their cysteine thiol groups, GSH and phytochelatins form complexes with metal(loid)s, sequester them in vacuoles, and/or distribute essential micronutrients intracellularly [2]. Given lichens and bryophytes' wide distribution, high surface-to-volume ratio, elevated cation exchange capacity, absence of strong hydrophobic barriers, and poikilohydric nature, they effectively adsorb and detoxify metal(loid)s [3]. Consequently, they serve as key biological systems for monitoring environmental metal pollution. However, the functional and molecular bases of their biomonitoring efficiency remain somewhat underexplored.

From studies conducted by myself and my collaborators, the following key findings have emerged:

1.Lichens: We demonstrated for the first time that phytochelatin biosynthesis in response to Cd, Pb, and Zn occurs in *Xanthoria parietina*, *Physconia grisea*, and *Physcia adscendens* [4]. This process is restricted to photobiont microalgae cells (*Trebouxia* spp.), while the mycobionts do not produce phytochelatins, despite having equal or even higher GSH levels than the whole thallus [4]. Thus, in lichens, phytochelatin production appears confined to the algal photobiont. Similar findings were reported for *Hypogymnia physodes* and *Cladonia* spp. exposed to As(V) [5] or Cu [6].

2.Bryophytes: Most tested bryophytes (liverworts, mosses, and hornworts) synthesize phytochelatins, mainly in the epidermal and chlorenchymatic cells of the gametophyte [7][8]. Liverworts (e.g., *Conocephalum conicum*, *Pellia epiphylla*, *Marchantia polymorpha*, *Lunularia cruciata*) are the primary producers, followed by hornworts (e.g., *Phaeoceros laevis*, *Anthoceros punctatus*) and mosses (e.g., *Sphagnum palustre*, *Polytrichastrum formosum*, *Hypnum cupressiforme*, *Fontinalis antipyretica*, *Leptodictyum riparium*), which rely more on GSH for protection [7][8][9]. We thus developed genome-edited mutants of *M. polymorpha* that are selectively hypersensitive to Cd, and that could serve as a highly specific, low-cost, visual bioindicator of Cd pollution in the environment [10]. Finally, recent data indicate that phytochelatins and GSH in *M. polymorpha* also play a role in Cd detoxification and the homeostatic control of Fe, Cu, and Zn concentrations at the extracellular level [11] [12]. This suggests a novel function for thiol-peptides in the regulation of fluxes of toxic or essential metal ions within the apoplastic environment, possibly mediated by pH-dependent mechanisms.

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Active biomonitoring of potentially toxic elements in urban air by two distinct moss species and two analytical techniques: a pan-Southeastern European study

P. Lazo[1*], M. Aničić Urošević[2*], T. Stafilov[3*], M. Nečemer[4], K. Bačeva Andonovska[5], B. Balabanova[6], G. Hristozova [7], S. Papagiannis[8], V. Vassilatou[9], C. Stihi[10], M. Suljkanović[11], Z. Špirić[12], K. Vogel-Mikuš[4], F. Qarri[1]

[1] Faculty of Natural Sciences, Dept. of Chemistry,University of Tirana, Blv. "Zog I', PC. 1001, Tirana, Albania
[2] Institute of Physics Belgrade, University of Belgrade,Pregrevica 118, 11080 Belgrade, Serbia
[3] Institute of Chemistry, Faculty of Science, Ss. Cyriland Methodius University, 1000 Skopje, North Macedonia
[4] Department of Low and Medium Energy Physics, Jožef Stefan Institute, 1000 Ljubljana, Slovenia
[5] Research Center for Environment and Materials, Macedonian Academy of Sciences and Arts, Skopje, North Macedonia
[6] Faculty of Agriculture, Goce Delčev University, Štip, North Macedonia
[7] Faculty of Physics and Technology, Plovdiv University "Paisii Hilendarski", 4000, Plovdiv, Bulgaria
[8] Institute of Nuclear and Particle Physics, N.C.S.R. Demokritos, Ag. Paraskevi, 15310 Athens, Greece
[9] Environmental Radioactivity Laboratory, INRASTES, NCSR Demokritos, Ag. Athens, Greece
[10] Faculty of Sciences and Arts, Valahia University of Targoviste, 13004 Targoviste, Romania
[11] Faculty of Natural Sciences and Mathematics, University of Tuzla, 75000 Tuzla, Bosnia and Herzegovina
[12] Green Infrastructure, Fallerovo šetaliste 22, 10000 Zagreb, Croatia

E-mail of the presenting author: pranveralazo@gmail.com, mira.anicic@ipb.ac.rs, tstafilov@gmail.com

Biomonitoring uses living organisms to evaluate the presence and effects of pollutants in different ecosystems. By utilizing the capacity of biological organisms to absorb, adsorb, or accumulate pollutants, it offers important information about the health of the environment and possible risks. Due to the capacity to absorb and retain pollutants, lichens, bryophytes, and other higher plants are usually used as biomonitor.

Transplants of two moss species, *Hypnum cupressiforme* and *Sphagnum girgensohnii*, were used to investigate the airborne potentially toxic element (PTE) pollution in urban areas of ten Southeast European (SEE) countries. Moss bags were exposed at five sites in each selected urban areas with respect of different land use classes (typical urban, residential, urban background), and at one rural site. The moss bags were exposed for two months during the winter season (2019/2020). The concentrations of 35 PTEs (Ag, Al, As, B, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Ge, Hg, In, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sc, Se, Si, Sr, Th, Tl, U, V, Y, and Zn) were determined in the samples using inductively coupled plasma mass spectrometry (ICP-MS). This study is the first attempt to apply a uniform biomonitor technique and moss species for active biomonitoring PTEs on a regional scale.

Low content elements (Ag, Ba, Be, Bi, Ga, Sb, and U) showed high variation (CV% > 75%) in both moss species indicating the data are affected from high uncertainty of the analysis. No significant differences were found between average concentrations of elements in *H. cupressiforme* and *S. girgensohnii* moss bags ($p = 0.488 > 0.05$); confirmed by linear regression analysis ($R^2 = 0.94$) by indicating cimilar trends of variations. The element concentrations found in the moss bags exposed at the rural site were at the level of the initial element content, confirming the background status of the rural site. It means that moss bag method works properly and gives logical results.

The differences between the accumulation capacities of elements in both moss species were tested by RAF values and linear correlations of the data of *H. cupressiforme* and *S. girgensohnii* mosses. High RAFs were found for Cr, Cu, Fe, Ni, Sb, and V in moss bags, indicating these elements are the most abundant in most sampling sites. Similar accumulation capacities were found for Zn, Ti, As, Ni, and Fe in both moss species. Both moss species exhibited identical contamination levels for the elements, as indicated by the contamination factor (CF) data, with the exception of arsenic (As) and antimony (Sb). The median CF values for both moss species varied from 1.2 to 2.17%, indicating that the sites of exposure exhibit no contamination to slight contamination (C1 to C2 scales). Higher CFs were found in different exposure sites for selected PTEs. Ljubljana and Zagreb resulted with lower pollution compared to other sites. The roadside soils are heavily affected by historical total metal concentrations, so, the soil dust emission could be considered a strong factor affecting the level of total metal content in moss bag samples. It is clearly shown by high correlations between the crustal elements Fe, Ti, Cr and Co. Besides, the tires are the main sources of Zn and Cd, while brake linings are shown to be the main source for Cu and Sb in a heavy traffic environment. Given the present findings, an accurate standardization study will be a primary goal for the forthcoming years.

Urban and Rural PAH Contamination: A Comparative Moss Bag Biomonitoring Study in Serbia and Montenegro

Aničić Urošević M.[1], Relić D.[2], Pergal M.[2], Popović A.[2]

1Environmental Physics Laboratory, Institute of Physics Belgrade, University of Belgrade, Belgrade, Serbia
[2]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

E-mail of the presenting author: mira.anicic@ipb.ac.rs

Moss bag biomonitoring has been applied for the investigation of organic pollutants in the air [1, 2]. In this study, two moss species, *Hypnum cupressiforme* and *Sphagnum girgensohnii*, were exposed in bags for two months at nine urban and one rural site in two countries (Serbia−RS, Montenegro−ME) during the winter period 2019/2020. The bags were placed at the air quality monitoring stations, except at the rural site, to be representatively positioned and the biomonitoring data possibly compared with the instrumental measurements of polycyclic aromatic hydrocarbons (PAHs). After exposure, 16 EPA PAHs, naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Acp), fluorene (F), phenanthrene (Phen), anthracene (Ant), fluoranthene (Fl), pyrene (Pyr), benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-c,d]pyrene (I[cd]P), dibenzo-[a,h]anthracene (DB[ah]A) and benzo[ghi]perylene (B[ghi]P), were determined in the moss samples by gas chromatography−mass spectrometry (GC-MS). To identify of PAH pollution sources, the moss PAH diagnostic ratios were calculated.

The concentrations of all PAHs were significantly higher (up to 23 times) in the exposed moss bags than in the initial (unexposed) moss tissue. Total PAHs (∑PAHs) varied in the range 1163−2291 µg kg^{-1} in RS and 62−3187 µg kg^{-1} in ME in moss *H. cupressiforme* while the range 610−1561 µg kg^{-1} in RS and 136−2269 µg kg^{-1} in ME characterised *S. girgensohnii*. Both moss species recognised the most polluted sites and those with the least PAH content in the air. Specifically, at the rural studied site (far away from any pollution source), the content of PAHs was substantially lower than at the urban sites, at the level of the initial PAH concentrations in the unexposed mosses. At the typical urban sites, the mosses were mostly enriched by Phen, Fl and Pyr, a low- and two medium-molecular-weight PAHs, respectively. This enrichment rate was followed by high-molecular-weight PAHs: B[k]F, B[a]P, I[cd]P, B[ghi]P, etc. The mentioned PAHs represent so-called combustion PAHs (∑COMPAHs), which represented about 70% of ∑PAHs in the moss samples. Markedly higher concentrations of medium- and high-molecular-weight PAHs in the moss samples could be attributable to both, meteorological conditions, and increased emissions from residential heating during the winter season. The instrumental measurements for the studied period are available only for ME, and according to particulate matter (PM10), ∑PAHs and B[a]P measurements, the moss bag biomonitoring indicated the same order of air pollution at the studied sites.

To conclude, both moss species show sensitivity to changes in air concentrations of PAHs between rural, low-polluted, and heavily polluted sites. The mosses enriched their tissue with PAHs comparable to the air pollution level recorded by the instrumental measurements in ME, and prior knowledge about the site pollution in RS.

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Species-specific metal accumulation in moss bags exposed to urban and industrial pollution: a case study Tula, Russia

Chaligava, O.[1,3], Zinicovscaia, I.[1,4], Gorelova, S.V.[2], Vergel K.[1].

[1]Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russian Federation
[2]L.N. Tolstoy Tula State Pedagogical University, Tula, Russia
[3]Andronikashvili Institute of Physics, I. Javakhishvili Tbilisi State University, Tbilisi, Georgia
[4]Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, Magurele, Romania

E-mail of the presenting author: chaligava@jinr.int

Atmospheric contaminants from industrial sources present measurable risks to ecosystems and humans, requiring robust biomonitoring solutions. Active moss biomonitoring technique utilizing *Pleurozium schreberi*, *Dicranum polysetum*, and *Sphagnum fallax* was applied to assess atmospheric deposition of chemical elements and associated ecological risks as well as to evaluate species-specific differences in elements accumulation in moss samples exposed near metallurgical enterprises and urban highways in Tula, Russia. Over a three-month exposure period (November-February), moss bags were deployed within 200-600 m of the Kosogorsk metallurgical plant, Imperial Tula Arms Plant, Tulachermet, Evraz Vanadium Tula, and Polema facilities (producing ferromanganese, steels, cast iron, alloyed steels, ferrovanadium, vanadium pentoxide, high-purity chromium, molybdenum, tungsten, metal powders, and composites), and within 2-5 m of central city highways.

Content of Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, S, V, and Zn was determined using ICP-OES, while Hg was analyzed using DMA-80. The Kruskal-Wallis test revealed no significant differences in elemental content between species, except for Ba and Hg between *Dicranum polysetum* and *Sphagnum fallax*, as well as Zn between *Pleurozium schreberi* and *Sphagnum fallax*. The median Ba value was the highest in *Sphagnum fallax*, while the content of Hg and Zn was the lowest.

The Relative Accumulation Factor values showed higher *Sphagnum fallax* enrichment in most of the elements, and lower leakage compared to other species. *Sphagnum fallax* showed a particularly strong enrichment in Cd in samples exposed near Kosogorsk metallurgical plant and Tulachermet. In contrast, *Dicranum polysetum* showed enrichment only near Kosogorsk metallurgical plant, while *Pleurozium schreberi* showed no enrichment at all. *Sphagnum* exhibited Pb enrichment in most samples, whereas other species accumulated Pb only near direct pollution sources. The samples exposed near Imperical Tula Arms Plant showed the highest RAF score for Pb in the decreasing order *Pleurozium schreberi* (10.4) > *Sphagnum fallax* (8.6) > *Dicranum polysetum* (6.6). Barium and Zn were slightly enriched only in case of *Sphagnum fallax*, while S, Cu, Hg, and Ni were less enriched in case of *Dicranum polysetum*.

A very high Potential Ecological Risk score was observed for V in samples exposed close to enterprises such as Tulachermet and Evraz Vanadium Tula. Among the species analyzed, *Sphagnum fallax* exhibited the highest PER score (1462), while *Dicranum polysetum* (676) and *Pleurozium schreberi* (693) also consistently fell into the very high ecological risk category.

The findings demonstrate that *Sphagnum fallax* possess superior metal accumulation capacity compared to other species, particularly for Cd and Pb. Vanadium emissions from Tulachermet and Evraz Vanadium Tula pose the highest ecological risk, in case of all moss species indicating "very high risk" pollution levels. These results highlight the effectiveness of moss biomonitoring for urban-industrial pollution assessment and underscore the need for emission control measures in the studied industrial zones.

Spatial distribution of ^{7}Be atmospheric deposition measured during three years using terrestrial mosses

Krmar, M.[1], Radnović, D.[2], Ilić, M.[2], Arsenić, I.[3], Jovančević, N.[1]

1Physics Department, Faculty of Science, University Novi Sad, Novi Sad, Serbia
[2]Department of Biology and Ecology, Faculty of Science, University Novi Sad, Novi Sad, Serbia
[3]Faculty of Agriculture, University Novi Sad, Novi Sad, Serbia

E-mail of the presenting author: krmar@df.uns.ac.rs

Radionuclide ^{7}Be, produced by cosmic radiation is frequently used as a tracer in studies of atmospheric transport of aerosols. Air concentration or deposition rate of ^{7}Be are usually measured by air samplers or deposition collectors. In this way, temporal variations of the presence of ^{7}Be in the atmosphere at a selected location can be measured. The limited number of air samplers and deposition collectors does not allow for an overview of the spatial deposition of ^{7}Be over a wider area. By using mosses, this problem can be overcome and high-resolution deposition maps can be obtained.
To determine if some patterns in the ^{7}Be deposition can be observed in the selected area, a periodic moss sampling and measurement of ^{7}Be activity was carried out two times per year, in spring and autumn, during three years. Moss sampling was performed at 70 locations in the northern part of Serbia. Dried samples were measured in anti-Compton NaI detector adapted for this purpose to serve as a high efficiency well-counter.
The obtained ^{7}Be activity values (in Bq/kg) are in relative wide range: in each set of selected samples, the smallest and largest values differ by an order of magnitude. A higher content of ^{7}Be activity was observed in samples collected in autumn than in those collected in spring. Six maps of the spatial deposition of ^{7}Be have been created. All measured spatial distributions of the atmospheric deposition of ^{7}Be differ significantly from each other and no pattern is observed. The observed spatial distributions cannot be interpreted by the distribution of measured values of standard meteorological parameters, such as temperature, precipitation, wind direction and intensity, etc.

Bioaccumulation of potentially toxic elements in mosses: a case study of cities in the Perm Region, Russia

Gatina, E.[1], Zinicovscaia, I.[2], Sannikov, P.[1], Chetyrkina, V.[1], Kirshina, A.[1], Bronnikova, P.[1]

[1]*Perm State University, Perm, Russia*
[2]*Joint Institute for Nuclear Research, Dubna, Russia*

E-mail: suslovael@mail.ru

Air pollution is directly related to urbanization and is one of the most important environmental problems [1]. Potentially toxic elements (PTEs), one of the main air pollutants, may originate in the environment from a mixture of natural and technological emissions sources.

The economy of the Perm region has an industrial character. The region is situated on the eastern part of the Russian Plain (80%) and the western slope of the Urals (20%). Its climate is primarily influenced by westerly and northwesterly atmospheric processes. A key feature of the atmospheric circulation in Perm region is the higher frequency of cyclones (74%) compared to anticyclones (26%) throughout the year [2].

In the structure of industrial production in the urban areas of Perm Region, an important place is occupied by mechanical engineering, oil and gas processing, petrochemistry, electric power, metalworking, wood processing, paper industries and the fertilizers industry. The cities of Perm, Berezniki, Solikamsk and Gubakha form the core of the region's industrial framework [3].

Two sampling campaigns were carried out during the summer of 2025, which yielded 268 sites sampled for the moss (*Pleurzium schreberi*), mainly in cities, in urban forests. Moss sampling was performed according to manual developed by the ICP Vegetation program [4]. For analysis green and green-brown moss segments corresponding to three-year growth were selected. The moss was thoroughly cleaned of foreign debris and soil residues.

The elemental composition of moss samples was determined using inductively coupled plasma optical emission spectrometry and DMA-80 Milestone direct mercury analyzer.

The median values for all elements, except mercury and cadmium, were higher in Perm. The detected mercury levels in mosses were similar across all urban areas, with the highest level observed in Berezniki near the sludge storage facility. The median values of cadmium were twice as high in Gubakha as compared to other cities. A comparison of the median values of element contents in mosses revealed that the contents of aluminum, sulfur, phosphorus, vanadium, iron, lead, strontium, and copper were higher in the samples from Perm than in those from Berezniki, Berezniki, Solikamsk, and Gubakha. The content of chromium and barium was higher in samples of moss from Perm and Berezniki. The samples from Berezniki and Gubakha showed the highest nickel content. The zinc values are comparable in all cities, except for Solikamsk, where they were lower. The median values for cobalt and manganese were higher in Perm and Solikamsk.

The spatial distribution of potentially toxic elements for the territory of Perm [5] and other urban areas was done.

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Interactions between mosses and substrate soils in the assessment of trace elements in mosses

Qarri F[1], Lazo P[2], Bekteshi[3], Kane S[2]

1 Department of Chemistry, University of Vlora, Vlore, Albania
2 Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Tirana, Albania
3 Department of Chemistry, University of Elbasan, Elbasan, Albania

Email of the presenting author: flora.qarri@gmail.com

This study investigates whether substrate soils influence concentrations of chromium (Cr), nickel (Ni), and cobalt (Co) in *Hypnum cupressiforme* moss used for monitoring atmospheric trace metal deposition in Albania, a country with diverse geology and topography. Paired moss and topsoil samples were collected from multiple sites nationwide to distinguish atmospheric deposition from soil-derived inputs. Descriptive statistics (coefficient of variation, skewness, kurtosis) and geochemical normalization were applied to account for natural background levels. Moss samples showed greater variability in metal concentrations (higher CV, skewness, kurtosis) than soils, indicating a stronger influence from anthropogenic sources, such as industrial emissions or dust from mineral-rich soils. Spatial mapping revealed Cr anomalies in moss in Bulqiza and Shebenik, near mineral-rich areas, and Ni anomalies along Albania's western coastline, likely linked to shipping and oil/gas activities. Moss and soil anomaly patterns rarely overlapped, except in areas with high soil metal content and significant dust production, suggesting minimal metal uptake by moss from underlying soils in most cases. Spearman-Rho correlation analysis showed strong, significant correlations among metals within moss samples and within soil samples ($r > 0.8$, $p < 0.001$), but non-significant correlations between moss and soil for the same metals ($r < 0.4$, $p > 0.05$). Factor analysis confirmed that moss and soil metal contents are driven by distinct sources. Overall, substrate soils have a negligible effect on moss metal concentrations in Albania, except in mineral-rich areas with thin or absent humus layers and sparse vegetation, where windblown dust may contribute. These findings support moss biomonitoring as an effective, low-cost tool for assessing atmospheric trace metal deposition, provided local geochemical backgrounds and dust influences are considered, particularly in mineral-rich regions. Continuous monitoring is recommended, as moss concentrations of Cr, Ni, and Co in Albania exceed many European reference levels and exhibit geographic variability.

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Influence of light pollution on photosynthetic performance and elemental uptake in lichens

Paoli, L.[1], El Horri, H.[2], Agostini, M.[1], Bellini, E.[1], Fačkovcová, Z.[3], Aldreotti, F.[4,5], Beltrami, S.[4], Brunetti, C.[4,5], D'Asaro, L.[2], Gori, A.[4,5], Lauria, G.[2], Lo Piccolo, E.[5], Landi, M.[2]

[1] *Department of Biology, University of Pisa, Pisa, Italy*
[2] *Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy*
[3] *Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia*
[4] *Institute for Sustainable Plant Protection, National Research Council of Italy, Florence, Italy*
[5] *Department of Agriculture, Food, Environment and Forestry, University of Florence, Italy*

E-mail of the presenting author: luca.paoli@unipi.it

Nocturnal artificial lighting may interfere with the natural light that regulate circadian rhythms in living organisms, including animals and land plants (a phenomenon referred to as "light pollution"). Streetlamp illumination (SI), using light-emitting diode (LED) technology, can disrupt photoautotrophic metabolism, affecting processes such as plant growth, germination timing, flowering, bud opening and dormancy, and leaf senescence.

Lichens are known as effective biomonitors in urban environments due to their capacity to accumulate pollutants and their sensitivity to the fluctuation of environmental factors (including light irradiance). It is therefore conceivable that SI could also influence their physiological responses and, eventually, their monitoring efficiency.

This study aimed to assess the impact of LED-based SI on the photosynthetic performance of lichen photobionts (*Trebouxia*) and on the element-uptake capacity of the lichen thallus. Samples of two commonly used biomonitors, the fruticose lichen *Evernia prunastri* and the foliose *Xanthoria parietina*, were exposed for six months in an open stand to different nocturnal LED treatments: neutral white (White), warm white (Red), and a custom lamp enriched in green light (Green). Dark conditions (no nocturnal light) served as the control. Photosynthetic performance and chlorophyll content were measured monthly. After three months, chlorophyll content in *X. parietina* showed slight increases under all treatments, though not significantly different from the control. By six months, chlorophyll levels were significantly reduced in the Red and White treatments (by 34% and 28%, respectively) with respect to the control. Monthly analyses of *E. prunastri* revealed fluctuating chlorophyll content, but no significant differences were detected between the Green and control treatment in both lichens at the end of the experiment. After three months, PSII quantum yield efficiency (Fv/Fm) in Green treated *X. parietina* remained comparable to the control, while reductions of 11% and 8% were observed under White and Red treatments, respectively. In *E. prunastri*, Fv/Fm decreased by 10–16% during early exposure to nocturnal light. Over the final three months, *E. prunastri* samples exposed to the Green treatment maintained stable Fv/Fm values, similar to the control. These findings suggest that enhancing the spectral composition of LED street lamps with green light does not adversely affect the photosynthetic activity of lichen photobionts.

In a second step, element uptake was assessed after six months of exposure. Furthermore, *E. prunastri* was treated under controlled laboratory conditions with Cd, Cu, and Zn solutions (100 μM) to verify differences in its uptake capacity. The first results obtained will be presented.

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Tracking trace element pollution in Wallonia through lichen biomonitoring: past trends and spatial contrast

Counoy, H.[1], Matthée, Z.[1], Ferreira Lerche, G.[1], Turcati, L.[2], Agnan, A.[1]

[1]*Earth and Life Institute, Université de Louvain, 1348 Louvain-la-Neuve, Belgium*
[2]*OSU Ecce Terra, Sorbonne Université, 75252, Paris, France*

E-mail of the presenting author: hugo.counoy@uclouvain.be

Air pollution by trace elements (TE) remains insufficiently characterized at regional scales, particularly in urbanized and industrialized contexts. Wallonia, a densely populated region of Belgium with a strong history of steel and mining industries, provides a relevant case study for that purpose. In this context, biomonitoring with lichens offers an integrated and cost-effective approach to assess background concentrations of TE and to identify areas where populations may face higher exposure, complementing conventional physico-chemical sensors.

This study aimed to (1) assess long-term changes in TE pollution in Wallonia and (2) identify the main sources of trace elements contamination and areas at risk. To evaluate temporal trends in background pollution, we analyzed 42 herbarium specimens of *Xanthoria parietina* collected between 1960 and 1970 in rural areas across Wallonia, which serve as reliable indicators of diffuse atmospheric deposition. These samples were compared with new collections made at the same locations in 2023. To complement this dataset, additional samples were collected in underrepresented rural areas as well as in major residential and industrial cities. In total, 14 TE were quantified in *Xanthoria parietina* from 132 sampling sites.

Concentrations of Pb, Zn, As, and Cd have declined markedly since the 1970s, following the reduction of Walloon heavy industry and the ban on leaded gasoline in the early 2000s. Nevertheless, background contamination by Zn and Cd remains elevated (mean enrichment factors >10), and hotspots persist in industrial cities such as Charleroi and Liège, where local influences—including metallurgical activities and road traffic—drive enrichment factors up to 100.

These results demonstrate the effectiveness of lichen biomonitoring for assessing both temporal trends and spatial patterns of TE contamination at the regional scale.

Rare earth elements in lichens to trace atmospheric deposition: insights from historical and contemporary samples

Agnan, Y.[1], Caucheteux, B.[1], Counoy, H.[1]

[1]*Earth and Life Institute, Université de Louvain, 1348 Louvain-la-Neuve, Belgium*

E-mail of the presenting author: yannick.agnan@uclouvain.be

Lichens are well known for their ability to accumulate trace elements from the atmosphere, making them effective biosensors for air pollution. Among these elements, rare earth elements (REEs) are particularly valuable chemical tracers for identifying sources and processes. This is especially useful in heterogeneous environments where multiple pollution sources (traffic, industry, agriculture, etc.) and diverse geographical contexts coexist. The aim of this study was to assess the relative contributions of REEs accumulated in lichens (*Xanthoria parietina*) collected from contrasting urban and rural sites. To this end, we sampled lichens at 132 sites across Wallonia, southern Belgium, covering an urbanization gradient from rural areas to large cities (Namur, Liège, and Charleroi). At 42 of these sites, we analyzed samples from two time periods: a recent collection and historical specimens (1955–1986) from university herbaria. Results revealed regional patterns likely influenced by lithology, although lichens did not always reflect the same geochemical signatures (e.g., Eu and Ce anomalies, La/Yb and La/Sm ratios). Local land cover also appeared to influence dust deposition, with factors such as proximity to urban roads or the extent of permanent plant cover in agricultural landscapes playing a role. Historical samples displayed similar spatial patterns to their modern counterparts, but with a relative enrichment in light REEs over time, suggesting changes in global dust deposition. Overall, REEs show potential for discriminating between environmental sources across landscapes and for improving trace element studies by helping to characterize natural dust backgrounds.

The Black Box of Environmental Biomonitoring

Pacín, C.[1], Fernández, J.A. [2], Boquete, M.T[3], Aboal, J.R.[2]

[1]*CIQUS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain*
[2]*CRETUS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain*
[3]*Department of Botany, Universidad Autónoma de Madrid, Madrid, Spain*

E-mail of the presenting author: mcarme.pacin@usc.es

Biomonitoring is a widely used methodology for assessing environmental contamination by measuring chemicals, their metabolites, or the biological alterations they induce in organisms. Despite decades of refinement and application, a persistent challenge remains: the relationship between ambient contaminant levels and their accumulated concentrations in biomonitors is inherently complex, non-linear, and often unpredictable. This uncertainty stems from the fact that the core process of bioaccumulation functions as a "black box." While the inputs—environmental contaminant concentrations—and the outputs—chemical levels within the organism—are measurable, the mechanisms governing uptake, internal storage, biotic transformation, and excretion are largely obscured, thereby complicating any direct causal interpretation of the resulting data.
This presentation addresses this fundamental gap by introducing a comprehensive theoretical framework designed to open this black box and elucidate the hidden mechanisms within. Using a combination of observational and experimental data, we synthesize how a multitude of interdependent factors converge to dictate observed concentrations in biomonitors. These include environmental physico-chemical variables such as pH, temperature, and humidity; organism-specific characteristics like morphology, physiology, and metabolic status; the intrinsic properties of the pollutant, including its solubility, persistence, and bioaccumulation potential; and the profound influence of inherent spatial and temporal variability within ecological systems. A central theme of our discussion is the acknowledgment that the individual contribution of each factor, along with the nature of their complex interactions, is nearly impossible to disentangle or predict.
We critically conclude that absolute environmental concentrations cannot be reliably or universally back-calculated from organism concentrations alone. However, we argue that biomonitors remain an indispensable tool for accurately assessing the impacts, bioavailability, and ultimate ecological threat of pollutants, which direct environmental measurements alone cannot achieve.

Working group on sampling protocols: mission and opportunities for IABEP

<u>Giordani, P.</u>[1], Counoy, H.[2], Agnan, Y.[2]

[1]*DIFAR, University of Genova, Genova, Italy*
[2] *Earth and Life Institute, Université catholique de Louvain, Louvain-la-Neuve, Belgium*

E-mail of the presenting author: paolo.giordani@unige.it

At the last IABEP meeting, the decision was made to set up a working group to develop biomonitoring protocols. The IABEP Working Group on Sampling Protocols is dedicated to advancing and standardising biomonitoring practices for assessing air pollution. The group addresses a wide range of methods and promotes the adoption of best practice within the IABEP community.

Its work centres on three key objectives: 1) Endorsing validated protocols by supporting their dissemination and encouraging their wider use; 2) reviewing alternative sampling methods to assess their strengths, limitations and potential improvements; 3) identifying knowledge gaps and open questions in emerging techniques.

This presentation will provide a brief overview of the current state of art with some practical examples and propose steps to initiate the group's activities.

Chemical and magnetic biomonitoring with lichens and vascular plants for the preservation of cultural heritage: a case study at two museums in a megacity (Buenos Aires, Argentina)

Grifoni, L.[1,2], Winkler, A.[2], Chaparro, M.A.E.[3], Di Lella, L.A.[1], Marte, F.[4], Sgamellotti, A.[5], Spagnuolo, L.[2], Tascon, M.[4], Buitrago Posada, D.[3,6], Marié, D.C.[3,6], Scoccimarro, M.[4], Loppi, S.[1,7]

[1]*Department of Life Sciences, University of Siena, Siena, Italy*
[2]*Istituto Nazionale di Geofisica e Vulcanologia, Rome, Italy*
[3]*CIFICEN, CONICET-UNCPBA, Argentina*
[4]*Universidad Nacional de San Martin, Buenos Aires, Argentina*
[5]*Accademia Nazionale dei Lincei, Rome, Italy*
[6]*Facultad de Ciencias Exactas, IFAS, Tandil, Buenos Aires, Argentina*
[7]*National Biodiversity Future Center, Palermo, Italy*

E-mail of the presenting author: l.grifoni2@student.unisi.it

Biomonitoring of air pollution with lichen transplants is well known. In this study, this technique has been applied to monitor particulate matter (PM) and potentially toxic elements (PTEs) for the preservation of cultural heritage. Chemical and magnetic biomonitoring has been run to the Museo Nacional de Bellas Artes (MNBA) and the Museo Histórico Nacional (MHN), two museums situated along very busy roads in Buenos Aires, Argentina. The lichen species *Evernia prunastri* (L.) Ach. was selected as biomonitor and exposed for three months at both sites. A mixed sampling approach was followed, which included four outdoor exposure sites at MNBA and three sites at MHN, together with three indoor sites at both museums. Along with the lichen deployment, plant leaves (*Jacaranda mimosifolia* and *Fraxinus americana* at MNBA and MHN, respectively) were sampled at the beginning and the end of the lichen exposure. Lichen transplants aimed at assessing the diffusion of metallic PM and PTEs from outdoors to inside the museum's halls, while leaf analysis aimed at evaluating their suitability for providing ecosystem services. Lichen and leaf samples were analysed for magnetic susceptibility and for the magnetic hysteresis properties. Chemical analyses of samples were performed by ICP-MS to quantify the content of PTEs related to traffic, i.e. Al, Ba, Cr, Cu, Fe, Mn, Sb, Zn. The magnetic properties of leaves and lichen transplants highlighted a significant accumulation of metallic and coarse particles, typically from brake abrasion, in the area facing the museums; indoor transplants, on the other hand, showed no accumulation of metallic particles. Both leaves and outdoor lichen transplants accumulated relevant concentrations of PTEs, showing vehicular emission as the main pollution source (especially for Sb and Cd, mainly derived from brakes). Lichens did not bioaccumulate PTEs inside the museums, whose concentrations were comparable to the unexposed samples. In this experiment, lichen was verified to be a viable biomonitor of airborne PM and PTEs, indicating clearly that PTEs accumulation was insignificant indoors, although the outdoor accumulation of the same elements was significant. Moreover, *J. mimosifolia* leaves showed a higher accumulation capacity compared to *F. americana* leaves.

Biomonitoring of airborne trace elements in forest ecosystems: the use of *Tillandsia usneoides* and the case study of Monte Amiata, Central Italy

<u>Scimone, G.</u>[1], Cotrozzi, L.[1], Fačkovcová, Z.[2], Merlini, C.[1,3], Nali, C.[1], Paoli, L.[3], Pisuttu, C.[1], Pellegrini, E.[1]

[1]*Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy*
[2]*Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia*
[3]*Department of Biology, University of Pisa, Pisa, Italy*

E-mail of the presenting author: giulia.scimone@phd.unipi.it

Tillandsia usneoides L. (also known as Spanish moss) is an epiphytic flowering plant from the bromeliad family that grows on trees in humid, warm climates, absorbing moisture and nutrients from the air. So doing, it is a well-recognized bioaccumulator of trace elements capable of reflecting the intrinsic characteristics of different sampling areas, by providing quantitative responses to air pollution and detecting differences related to various emission sources. In 2024, a field campaign was conducted using the Spanish moss as a biomonitor of airborne trace elements in the southern Tuscany Antiapennines (Monte Amiata, Grosseto province, Central Italy). Six sites were selected, differing in vegetation cover (i.e., oak, chestnut, and beech) and forest management practices (wood or coppice). Samples of *T. usneoides* (purchased from a Tuscan nursery) were then placed facing north at a height of 2 m on four trees per station, located at 10–15 m distance, according to the territory morphology. A control site was set in San Piero a Grado (Pisa province) while plants were maintained under charcoal-filtered air. At the end of the exposure period (that lasted 3 months, from June to September), the Spanish mosses were collected, along with soil samples taken from depths of both 10 and 30 cm. Plant and soil samples were then analysed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to determine the content of 21 trace elements. Differences among the sampling sites, in terms of element concentration, were evaluated by a one-way analysis of variance, which was followed by a Tukey's HSD *post hoc* test. Additionally, to estimate the relative contribution of crustal contamination to the bulk element distribution in *T. usneoides*, the enrichment factor (EF) was calculated by normalizing each trace element concentration to aluminium. Data from ICP-MS showed that the plants were able to accumulate 1 metalloid and 8 metals (i.e., boron, calcium, iron, magnesium, manganese, mercury, potassium, sodium, vanadium) compared to those located at the control site, however no specific differences were observed in relation to vegetation cover and/ or the forest management practices at the exposure sites. Notably, all sites reflected elevated levels of boron, iron and sodium (averagely 855-fold, +81 and +73%, respectively, compared to control). Although the EF for iron was below 10 (thus not considered enriched), the elevated levels observed are aligned with the geological history of the studied area, as Monte Amiata primary volcanic morphologies are still distinguishable due to the combined effect of the vegetation cover and organic material, which has preserved soil from erosion. Both boron and sodium had EFs greater than 100, suggesting their presence was due to an atmospheric enrichment. The proximity of geothermal power plants was reflected in the high enrichment of both boron and sodium, further confirming the potential of *T. usneoides* as a reliable indicator of those elements. Calcium concentration was significantly higher only in oak wood and chestnut coppice sites (+48 and +46%, respectively), while potassium levels increased in all sites (more than 2-fold compared to control) except in the beech coppice. For both elements, EFs were up to 100, indicating high enrichment. By contrast, although *T. usneoides* accumulated magnesium (in both oak and chestnut sites; +18 and +32%, averagely) and manganese (in all the exposure sites, except the oak coppice; 3-fold higher than control), their EFs ranged between 10-100. Mercury was found statistically high and enriched only in the chestnut wood (more than 2-fold; EF up to 100), whereas vanadium concentration was higher than the control only in the oak wood (+58%). However, due to an EF below 10, vanadium presence was influenced by the soil rather than atmosphere. Antimony was not detected at foliar level but found in traces in soil samples. Lastly, considering most of the studies which applied the Spanish moss were conducted in tropical areas, the present work confirms the reliability of *T. usneoides* as a biosensor, capable of detecting differences among sites even in forest ecosystems placed in mountain environments, located in the Mediterranean basin.

Current challenges in biomonitoring for ozone impacts on vegetation

Hayes F.,[1] Sharps K.[1]

[1]ICP Vegetation Coordination Centre, UK Centre for Ecology & Hydrology, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK

E-mail of the presenting author: fhay@ceh.ac.uk

Visible leaf-injury caused by ozone has often been used as a bioindicator of the spatial location of where impacts of ambient ozone are occurring. This was particularly successful when peaks of ozone were high and symptoms were prevalent.

On-the-ground evidence of where ozone impacts are occurring remains an important topic, and is desirable to supplement and complement the information and predictions that can be gained from models. However, there are currently many challenges for this, particularly where the ozone profile is changing and peaks of ozone concentration are lower.

Here we outline some of the current challenges to biomonitoring for ambient ozone pollution and suggest some potential solutions. We use information and examples from experimental working including in the UK solar domes, and experiences from within the Convention on Long-range Transboundary Air Pollution and the ICP Vegetation.

Biomonitoring of airborne anthropogenic microfibers by *Pittosporum tobira* leaves: Effects of season and rainfall

Spagnuolo V.[1], Gaglione A.[1], Granata A.[1], Sorrentino M.C.[1], Rallo A.[1], Capozzi F.[1], Giordano S.[1]

[1]*Department of Biology, University di Napoli Federico II, Campus Monte S. Angelo, Via Cinthia 4, 80126*

E-mail of the presenting author: valeria.spagnuolo@unina.it

Plastic pollution presents a significant and persistent environmental challenge [1]. The ubiquitous and durable nature of plastic means its degradation into micro- and nanoplastics (MPs and NPs) takes decades to millennia, leading to pervasive contamination across all environmental matrices, including some of the planet's most remote locations. While ocean and sediment contamination has been extensively studied, airborne MPs remain largely underestimated. These airborne particles, mainly fibers, are prevalent in both urban and pristine environments and can travel long distances. Key sources include road traffic, sea spray, and synthetic fabrics, with their transport and distribution heavily influenced by meteorological conditions and human activities.

Current monitoring methods for airborne MPs often rely on expensive passive samplers that require specialized expertise. This study evaluates the use of plant leaves as a fast and accessible biomonitoring system for airborne microfibers (MFs) along a suspected pollution gradient. Based on previous research that established a repeatable four-steps sequential extraction procedure for isolating MFs from *Pittosporum tobira* leaves [2], this work further investigates the influence of sampling season and rainfall on MF accumulation. A biomonitoring survey was conducted along an urban pollution gradient, with *P. tobira* leaves sampled during both dry (summer) and rainy (winter) seasons to assess seasonal variations. Leaf traits were examined by SEM. Additionally, *P. tobira* transplants were exposed both sheltered and unsheltered to heavy rain to analyse the direct impact of precipitation on MF accumulation. This research highlights the good performance of *P. tobira* in passive and active biomonitoring of airborne MFs. Also, it supports the potential of plant biomonitoring as a reliable and fast method to be used both for understanding airborne plastic pollution dynamics, and in citizen science approaches for people sensibilization and participation.

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Biomonitoring the present and the past using macrobenthic invertebrates: a case study from the eastern Italian Alps

Bertoli, M.[1], Salvi, G.[2], Baratto, A.[1], Sala, A. [1], Gabetti, A. [3], Maganza, A. [3], Mossotto, C. [3], Prearo, M.[3], Pastorino, P.[3], Pizzul, E.[1]

[1]*Life Science Department, University of Trieste, Trieste, Italy*
[2]*Department of Mathematics, Informatics and Geosciences, University of Trieste, Trieste, Italy*
[3]*The Veterinary Medical Research Institute of Piemonte, Liguria and Valle d'Aosta, Torino, Italy*

E-mail of the presenting author: marbertoli@units.it

Lacustrine ecosystems are widely spread over mountain and high-altitude landscapes and are highly sensitive to anthropogenic impacts, despite their location (generally remote or far from urban areas), as they can be heavily affected by chemical-physical, biological, and even morphological alterations, both on local and global scale [1]. Therefore, alpine and mountain lakes are considered as "environmental sentinels" for the whole mountain area [2], and the effects of anthropogenic impacts can be assessed via analysis of proper bioindicators. Among the freshwater communities, macrobenthic invertebrates are one of the most used in biomonitoring [3], and especially in lacustrine ecosystems non-biting midges (Diptera Chironomidae) represents a pivotal taxonomical group: they can reach 50% of secondary production in lakes, being an important prey item for fish, they can be affected by different features, include different Functional Feeding Guilds and can provide a good picture of the ecological status in lakes. [4,5]. Finally, due to their larval chitinous head capsules, subfossils chironomids are abundant and well preserved in lake sediments, becoming an excellent proxy for paleolimnological investigations [6]. Due to these reasons, these organisms have been largely used in the monitoring of present and past conditions in lakes, providing useful information both on actual conditions and allowing to reconstruct ecological changes through time.

In the present study, we analysed the modern and subfossil chironomid communities of a Mountain Lake in Friuli Venezia Giulia (Lower Fusine Lake), to assess its present and past ecological conditions

Our results highlight the changes in subfossil assemblages during the past 1500 years, mainly related to temperature variations and especially to global warming. Despite the observed changes, the communities in the Lower Fusine Lake can be still associated with oligotrophic conditions and the present impacts are mainly related to anthropogenic modifications of the territory and tourism.

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Transplants of *Pseudevernia furfuracea* to assess the deposition of anthropogenic microfiber: refinement of the method

Varela Z.[1], Gaglione A.[2], Spagnuolo V. [2], Bouso M.[1], Giordano S.[2], Fernández J.A.[1], Capozzi F.[2].

[1] *CRETUS and Functional Biology Department (Ecology Unit), University of Santiago de Compostela, 15782 Santiago de Compostela, Spain*
[2] *Department of Biology, University di Napoli Federico II, Campus Monte S. Angelo, Via Cinthia 4, 80126 Napoli, Italy*

The ubiquitous presence of anthropogenic microfibers (AMFs) in various environmental compartments has emerged as a significant global concern [1]. These microscopic fragments, originating predominantly from synthetic textiles, packaging and waste disposal, are readily transported through atmospheric pathways, contributing to air pollution and ultimately impacting ecosystems and human health. Biomonitoring offers a complementary approach to assess their load in the environmental and accumulation. Among biomonitors, cryptogams, particularly transplant of epiphytic lichens, have proven to be excellent bioaccumulators of airborne pollutants due to the lack of well-developed cuticle and root system, relying solely on atmospheric deposition for nutrient uptake. This inherent characteristic makes them highly sensitive to atmospheric fallout, rendering them ideal passive samplers for a wide range of contaminants, including particulate matter. Despite their proven utility in monitoring various pollutants, their application in assessing AMF deposition is still a relatively novel and developing field [2]. Further research is needed to refine methodologies and establish their efficacy as reliable biosensors for this emerging contaminant.

This study aimed to refine the biomonitoring methodology for AMFs using transplanted thalli of the epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf. A comprehensive exposure campaign was conducted across four distinct sites in Italy and Spain, chosen to represent a gradient of anthropogenic impact: semi-natural, rural, urban, and industrial. This strategic site selection allowed us to evaluate the capacity of *P. furfuracea* transplants to distinguish among various levels of AMF deposition linked to different human impacts.

Furthermore, the experimental design incorporated a rigorous investigation into the influence of two critical factors on AMF accumulation: exposure duration and rain-washing effect. Transplants were exposed for three different time periods: 3, 6, and 9 weeks. This temporal assessment allowed us to determine the optimal exposure duration required for significant and representative AMF accumulation within the lichen thalli, identifying potential saturation points or steady-state conditions. To address the potential for rain-induced washout of deposited AMFs from the lichen surface, a subset of the exposed thalli was sheltered by protective aluminium covers. These protections of the bags were designed to allow for air circulation and pollutant deposition while simultaneously mitigating the direct impact of rainfall. By comparing AMF accumulation in sheltered versus unsheltered samples, we aimed to quantify the contribution of rain-washing to the overall AMF load and to assess whether bag coverage could serve as a valuable methodological refinement to ensure more accurate measurements of atmospheric deposition. The collected lichen samples underwent meticulous laboratory analysis for AMF extraction, quantification, and characterization. This comprehensive approach, combining a diverse range of exposure sites with controlled variables of exposure time and rain protection, provides valuable insights into the efficacy and reliability of *P. furfuracea* as a biosensor for AMFs. The findings of this study contribute significantly to the development of standardized and effective biomonitoring protocols for these persistent airborne contaminants, offering a valuable tool for environmental assessment and policy development.

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Detection of small microplastics in the atmosphere using fluorescent tagging and moss biomonitoring

Jafarova, M.[1], Awada, A.[2], Potter, M.[2], Rondeau-Gagné, S.[2], Aherne, J.[1]

[1]School of the Environment, Trent University, Peterborough, Canada
[2]Department of Chemistry and Biochemistry, University of Windsor, Windsor, Canada

E-mail of the presenting author: mjafarova@trentu.ca

There is growing interest in the use of biomonitoring for the assessment of atmospheric microplastics (plastic particles < 5 mm). During the past five year, studies across Europe and North America have demonstrated that mosses can be effectively used as biomonitors of microplastics [1,2,3]; however, these studies have primarily used optical microscopy, which limits microplastic detection to particles > 50 µm, leaving smaller sized fractions largely unquantified. It is these smaller sized fractions that are of most concern for human health, given their ability to penetrate deep into the human lungs. While quantification is possible using spectrometric techniques, such as Raman spectroscopy or Fourier Transform Infrared spectroscopy, they are time consuming, expensive, and require additional specialized equipment such as a focal plane array. To effectively use mosses for biomonitoring of atmospheric microplastics, we need a rapid, inexpensive, and accessible technique.

Emerging analytical techniques based on fluorescent tagging have shown promising results in the identification and quantification of microplastics below 50 µm [4,5]. Coupled with imagine analysis they can potentially provide a rapid and accessible method. In this study, we applied conjugated polymer nanoparticles, which selectively bind to microplastics and fluoresce upon attachment, to detect particles smaller than 50 µm. These microplastic-specific tags were prepared from the nanoprecipitation of a semiconducting polymer and a fluorescein-tagged hyaluronic acid, an amphiphilic polymer. Further, we tested the effectiveness of this approach against Fourier Transform Infrared spectroscopy using transmission mapping.

Here we analyzed a subset of sites from the MADAME project using this method, to assess the abundance of microplastics < 50 µm in the atmosphere across Europe. MADAME (Microplastic Atmospheric Deposition Assessment using Moss in Europe) is a multi-institutional project facilitated by ICP Vegetation that has assessed microplastics across 33 counties (94 sites) in Europe. This novel approach allowed us to quantify the proportion of microplastics below detection limits for optical microscopy, providing a more complete understanding of microplastic contamination of the atmosphere and their implications to human health.

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Enhancement of low- to medium-quality digital images towards reliable microplastic particle counting

<u>Ilić, A.Ž.</u>[1], Popović, M.[1], Milošević, I.[1], Aničić Urošević, M.[1]

[1]Institute of Physics Belgrade, Belgrade, Serbia

E-mail of the presenting author: andjelijailic@ieee.org

With the exponential increase in plastics production over time, and the potential of environmental factors to cause its fragmentation, micro- and nanoplastic particles have become environmental contaminants of a serious concern [1-3]. A significant part of current environmental pollution research efforts is devoted to monitoring the microplastics (MP) pollution, as there are few long-term studies on its spatial and temporal variability necessary to fully understand the extent and effects of such pollution. Pretreatment of samples is typically required, followed by MP staining, microscopic, imaging, and analytical confirmation (FTIR, microRaman) analyses [4,5]. In biomonitoring of airborne MPs, atmospheric deposits in urban as well as rural areas can be collected by being washed-off from the vegetation (vascular plant leaves or cryptogams) [6]. The next step after microscoping of stained MPs is particle counting and classification based on their size and shape, typically followed by analytical characterization by microRaman spectroscopy.

As a part of broader study, we obtained the photographs of the filters with the washed-off MP deposit using a microscope at 50x magnification. No particular care was taken about lighting conditions or uniformity of colours in different image parts, therefore the medium-quality digital images were obtained. The counting and sizing of entities in such images is labor-intensive, mainly due to low contrast and uneven lightness of pixels, and as a consequence, the results are not entirely reliable. As a remedy to the observed problems, we propose the transfer of images from the red-green-blue (RGB) colour space representation to the hue-saturation-value (HSV) colour space representation, detailed analysis of colour histograms, and the sequence of steps for digital image enhancement and contrast improvement. The counting of particles belonging to different subsets of HSV image data was performed using regular particle size and shape classification routines. The obtained results were therefore classified by their colour, size, and shape properties. We describe in detail the applied procedures, show the obtained results for a typical example photograph, and emphasize the importance of image colour space analysis and contrast enhancement towards the more reliable particle counting.

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An efficient, cost-effective, and environmentally friendly protocol for extracting microplastics from soil samples

Grujić, T.[1] Saljnikov, E.[2], Stefanović, S.[3], Lazović, V.[1], Belanović Simić, S.[4] **Marjanović, Ž[2]**

[1]Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia
[2]University of Belgrade–Institute for Multidisciplinary Research, Kneza Višeslava 1, 11000 Belgrade, Serbia
[3]Faculty for Applied Ecology "Futura", Metropolitan University, Požeška 53a, 11000 Belgrade, Serbia
[4]Faculty of Forestry, University of Belgrade, Kneza Višeslava 1, 11000 Belgrade, Serbia

E-mail of the presenting author: zaklina@imsi.bg.ac.rs

As land plastic pollution has accumulated over recent decades, small fragments of its degradation, microplastics (MPs) [1], have become an increasingly urgent problem in soil environments. Monitoring and reporting soil MPs will be essential in the future, but existing literature describes many protocols, none of which appear to be efficient for this purpose. The initial step in MP analysis from soils is extracting MP particles, which is particularly challenging due to the substrate [2]. Challenges include aggregate formation and the presence of other organic particles of similar size, complicating the development of efficient and reliable extraction protocols. Density separation methods are commonly used, and finding solutions that are effective, affordable, and environmentally friendly is crucial, with data comparability being vital for advancing knowledge on this topic. Here, we introduce an environmentally friendly protocol based on density separation using a sucrose solution. Its effectiveness for extracting MPs from soil was tested and validated. Validation results showed that this method efficiently recovered polyethylene (PE) for all tested MP sizes (>32 µm) and PVC for particles >500 µm, with high recovery rates. Extraction of 125–500 µm PVC is justified, despite a lower recovery rate of 64.04 ± 1.73%, because sucrose solution is practically free and environmentally safe, unlike previously used more expensive and toxic media. Validation results suggest that the proposed protocol has strong potential for extracting MPs from challenging soil samples. This method can be effectively used in any soil science laboratory, including those responsible for monitoring.

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Decoding survival: A synthesis of studies on *Viola* taxa from Allchar

Jakovljević, K.[1], **Mišljenović, T.[2]**, Salinitro, M.[3], Bačeva Andonovska, K.[4], Brueckner, D.[5], van der Ent, A.[3]

[1]Department of Ecology, Institute for Biological Research Siniša Stanković´, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia
[2]Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Belgrade, Serbia
[3]Laboratory of Genetics, Wageningen University and Research, Wageningen, The Netherlands
[4]Research Center for Environment and Materials, Macedonian Academy of Sciences and Arts, Skopje, Macedonia
[5]Deutsches Elektronen-Synchrotron DESY, Germany

E-mail of the presenting author: tomica.m@bio.bg.ac.rs

The abandoned Allchar mine site in North Macedonia is one of the world's most extreme metalliferous habitats, with exceptionally high concentrations of thallium (Tl) and arsenic (As) in the soil (up to 5,750 and 12,800 mg kg^{-1}, respectively). Despite these toxic conditions, the site supports a specialised *Viola* flora, including stenoendemics *V. arsenica* and *V. allchariensis* and the widespread Balkan metallophyte *V. tricolor* subsp. *macedonica*. All three taxa display extraordinary tolerance - and in some cases hyperaccumulation - of Tl and As, making them excellent models for studying plant adaptation to extreme edaphic stress. Initial field surveys combining inductively coupled plasma–atomic emission spectrometry (ICP–AES) with synchrotron-based micro-X-ray fluorescence (µXRF) showed that all three taxa accumulate exceptionally high concentrations of Tl (up to 58,900 mg kg^{-1} in *V. arsenica*) in their aboveground tissues. In contrast, As accumulation patterns were more variable - *V. arsenica* exhibited significant endogenous accumulation (up to 381 mg kg^{-1}), while *V. tricolor* subsp. *macedonica* and *V. allchariensis* showed much lower foliar concentrations of As, likely influenced by external contamination. Notably, As hotspots in *V. arsenica* were co-localised with calcium oxalate deposits and bromine, suggesting complex compartmentalisation mechanisms. Building on these findings, synchrotron µXRF mapping of hydrated plant tissues provided high-resolution insights into elemental partitioning. Leaves were the principal sinks for Tl and As, with Tl enrichment near stomatal regions. In *V. arsenica*, Tl was predominantly sequestered in mature leaves, whereas *V. tricolor* subsp. *macedonica* showed restricted translocation to shoots and possible Tl excretion through stomata. Root tissue analysis further indicated epidermal localisation of both elements in *V. arsenica* and As in *V. tricolor* subsp. *macedonica*, while Tl accumulated in the root cortex. Hydroponic dosing experiments (up to 20 µM As and 50 µM Tl) confirmed the capacity of these taxa to tolerate and accumulate increasing concentrations of both elements. Patterns observed under controlled conditions mirrored those in field-grown plants, underscoring the adaptive significance of their elemental handling strategies. The integration of field surveys, synchrotron imaging, and experimental dosing reveals that *Viola* species from Allchar have evolved diverse and highly specialised strategies to survive in one of the most toxic natural environments on Earth. These include selective uptake, differential translocation, tissue-specific sequestration, and possible foliar excretion of metals. The hyperaccumulation of Tl across all taxa, and the unusual As compartmentalisation in *V. arsenica*, highlight the evolutionary potential of metallophytes as model systems for understanding metal tolerance and for potential applications in biogeochemistry and phytotechnology.

Assessing tree response to soil contamination in a Milan urban park to support phytoremediation planning

Russo, C.[1], Vegini, E[1]., Casati, E[1]., Caronni, S[1]., Citterio S[1].

[1]*Department of Earth and Environmental Sciences, University of Milan-Bicocca, Milan, Italy*

E-mail of the presenting author: claudia.russo1@unimib.it

Urbanization and increasing anthropogenic activities have led to a progressive degradation of urban soil quality, with direct consequences on the balance of green urban ecosystems and their usability.

Among the available solutions to bring back these areas, phytoremediation offers a socially, economically, and environmentally sustainable approach. However, its application in urban parks presents challenges due to the presence of established tree vegetation, which limits the planting of species specifically suited for soil remediation. Therefore, assessing the health status of existing trees and their potential role in soil remediation is essential to define the most effective phytoremediation strategy, particularly in deciding whether to preserve or replace current tree populations.

To this end, this study investigated the impact of soil contamination on arboreous vegetation in an urban park in Milan over a 15-year period. Height, trunk diameter, and crown size were measured on 348 geo-referenced trees within the 5-hectare study area. In addition to morphological traits, photosynthetic efficiency (PEA) and leaf metal concentrations were assessed on a subset of 54 trees from 11 different species. These variables were then correlated with the type and concentration of pollutants present in the soil near each tree. Furthermore, a comparison between the current and historical morphological measurements collected 15 years earlier by the Municipality of Milan was performed to assess growth trends over time, using Linear Mixed Effects Models (LME) to relate changes to pollutant levels.

Results showed that soil contamination negatively affected springtime photosynthetic efficiency in trees growing in areas where at least one pollutant (regardless of type) exceeded Italian legal thresholds. Among all contaminants, benzo(a)pyrene emerged as the most critical: concentrations above 0.1 $mg \cdot kg^{-1}$ were significantly associated with reduced photosynthetic efficiency. Over the 15-year period, benzo(a)pyrene was also significantly linked to reduced tree growth, as indicated by smaller trunk diameters.

Additionally, the combined presence of lead and zinc was associated with reduced crown diameter. In contrast, the presence of other pollutants (hydrocarbons, copper, arsenic, cadmium, and mercury) either alone or in combination, did not significantly affect any of the morpho-functional parameters measured.

Anyway, despite the observed impacts on growth and photosynthesis, the trees demonstrated notable resilience to environmental stress. In addition, contaminant analyses in leaves indicated that the trees actively contributed to metal removal from the soil and microbiological analyses showed that they supported the presence of a soil microbial community, including hydrocarbon-degrading bacteria and fungi.

Based on these findings and considering the key ecosystem services provided by arboreous vegetation in urban environments, the phytoremediation strategy should preserve the existing tree species and complement them with selected shrub and herbaceous species.

CO_2-activated biochar–chitosan blend for (bio)monitoring potentially toxic element deposition in urban and industrial environment

Vannini, A.[1], Sidoli, M.[2], Costa, M.[3], Barrulas, P.[3], Carbognani, M[1], Pontiroli, D.[2], Barrocas Dias, C.[3], Petraglia, A.[1]

[1]*Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, Parma, Italy*
[2]*Department of Mathematical, Physical and Computer Sciences, University of Parma, Parma, Italy*
[3]*Department of Chemistry, University of Évora, Évora, Portugal*

E-mail of the presenting author: andrea.vannini@unipr.it

Biochar is a functional waste-derived product that can limit the concentration and mobility of pollutants in water and soil and act as an air-filtering agent [1]. Its performance is mainly governed by high porosity, alkalinity, and surface area. For inorganic compounds such as potentially toxic elements (PTEs), removal is driven by electrostatic interactions, complexation, precipitation, and ion-exchange capacity.

Wood-derived biochar has recently been proposed as a tool for biomonitoring the environmental availability of ionic Cd in water and air, thus providing a novel application [2]. A subsequent study showed that two Italian wood-derived biochars (BCH1 and BCH2) performed as well as or better than the lichen *Evernia prunastri* (L.) Ach. in accumulating PTEs from atmospheric deposition in both outdoor and indoor environments. This result was attributed to surface unevenness rather than surface area or carbon structure [3].

This study investigated whether activated wood biochar can outperform *E. prunastri* in PTE accumulation under indoor and outdoor conditions. BCH2, which previously showed the highest accumulation capacity [3], was selected, activated with a CO_2 stream, and combined with chitosan (50:50) after being dissolved in water, ethanol, and acetic acid. The mixture was cast onto glass Petri dishes and left to dry at 60 °C overnight, producing thin discs (12 cm diameter). Lichen samples were collected from a remote area of the Tuscan-Emilian Apennines.

Both materials were exposed for nine weeks (April 7–June 9) at i) the University Park of Parma, near a major ring road (~100 m), and ii) the mechanical workshop of the University campus. To prevent predation, all samples were placed inside plastic mesh cubes (10×10×10 cm; 1 cm² mesh).

After retrieval, chlorophyll *a* fluorescence was used to assess lichen viability, and all samples were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Instrumental calibration was performed with NIST 610 ("*Trace elements in glass*"), while quantification was carried out using NIST 1563a ("*Tomato leaves*"). Preliminary findings show that Mg, Al, Ca, Cr, Fe, Ni, Cu, Zn, As, Cd, Sb, and Ba accumulated to higher levels in the activated biochar than in the lichen, suggesting that biochar–chitosan blends may represent a promising tool for environmental (bio)monitoring of potentially toxic elements.

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Biochar-assisted phytoremediation of Cd and Cu in lettuce: effects on metal uptake and plant stress responses

Fedeli, R.[1], Desideri, S.[1], Loppi, S[1].

[1] *BioAgry Lab, Department of Life Sciences, University of Siena, Italy*

E-mail of the presenting author: riccardo.fedeli@unisi.it

Biochar has gained attention as an effective soil amendment to reduce heavy metal contamination in agriculture [1]. It is a carbon-rich material produced by the pyrolysis of organic biomass under limited oxygen conditions, and is known for its high porosity, large surface area, and capacity to retain nutrients and contaminants [2].

This study explores how biochar (2 and 5% w/w) affects the absorption of cadmium (Cd) and copper (Cu), plant development, oxidative stress, and physiological responses in lettuce (*Lactuca sativa* L.) exposed to varying metal levels (5 and 15 mg kg for Cd - 120 and 600 mg kg for Cu). The findings show that biochar significantly lowered the availability of Cd in the soil, reducing its accumulation in plant tissues by up to 31.9% and easing oxidative stress, with notable decreases in malondialdehyde and proline levels (up to 51.0% and 60.2%, respectively), especially at higher biochar rates (5%). Lettuce plants treated with biochar under Cd stress showed increased fresh weight (+22.6%), reduced oxidative markers, and greater chlorophyll content (rising by 14.9% to 24.1%) compared to untreated plants. The Cd bioaccumulation factor also declined by up to 31.8%, while the immobilization index rose, confirming biochar's role in limiting Cd mobility in soil.

On the other hand, Cu uptake remained low in all treatments, with a significant drop (up to −34.2%) only at higher contamination levels. Biochar also helped immobilize Cu, as indicated by increased immobilization index values, and promoted biomass and chlorophyll content under Cu exposure (increases of 15.4% and up to 24.1%, respectively), suggesting a partial reduction in Cu toxicity. Overall, these results highlight biochar's potential to enhance plant-based remediation of heavy metal-contaminated soils—particularly for Cd—by reducing metal bioavailability and improving plant resilience, supporting its integration into sustainable phytoremediation approaches.

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Removal of pollutants in blue-green infrastructures

Morais J.[1], Ribeiro, A.R.[2], Alves A.[1], Homem, V.[1], **Ratola, N.**[1]

[1]*LEPABE, ALiCE –Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal*
[2] *LSRE-LCM, ALiCE –Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal*

E-mail of the presenting author: nrneto@fe.up.pt

There is currently insufficient knowledge on the assessment and potential for removal of micropollutants and their potential hazardous effects related to blue-green infrastructures (BGIs). This gap should be decreased to support the environmental friendliness (and consequent implementation) of these constructions, under a smart cities framework. Project Springiness is a European initiative under the Water4All programme, which brings together five partners that include professionals from the water sector, specialists in BGI design, researchers, water quality experts, and risk assessment professionals, all with strong expertise in their areas. At the Faculty of Engineering - University of Porto (FEUP), the team will analyse proxy compounds found in water, soil, and vegetation using fast-track methods aligned with the principles of Green Analytical Chemistry and Green Sample Preparation [1,2]. Parameters related to human exposure and potential risks will also be estimated. The project began with an in-depth review of existing literature on the topic, particularly as it relates to BGIs. Nevertheless, from almost 50 documents in literature it was possible to obtain a list of the most common organic (*e.g.*, PAHs) and inorganic (*e.g.*, heavy metals) chemicals studied so far (mostly in water matrices), with the respective concentration ranges and removal efficiencies. Widely variable values were obtained depending on the BGI and the family of compounds. While the mean removal of PCBs was just below 90%, for PFAS only about 30% overall was obtained. Interestingly, there was a tendency to increase the concentration of background metals in most cases. This review is the first step to address the analysis of water, soil and vegetation matrices from the BGI installations of the project, with the ultimate goal of obtaining proxy compounds for the most relevant families of contaminants to perform the pertinent risk assessment. This review serves as a stepping stone towards the analysis of water, soil, and vegetation samples from the project's BGI installations. The main objective is to identify proxy compounds representing key contaminant groups, allowing a comprehensive risk assessment.

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From plastic to biomass: the role of *Tenebrio molitor* in waste valorization

Ilijin, L.[1], Nikolić, M.V.[2], Vasiljević, Z.[2], Popović, D.[3], Živković, M.[4], Vlahović M.[1], Mrdaković, M.[1], Todorović, D.[1] and Perić-Mataruga, V.[1]

[1]*Department of insect physiology and biochemistry, Institute for Biological Research „Siniša Stanković“, National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia*
[2]*Center for Green Technologies, Department of Material Science,Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia*
[3]*Department of Ecology, Institute for Biological Research „Siniša Stanković“, National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia*
[4]*Institute of Molecular Genetics and Genetics Engineering, University of Belgrade, Belgrade, Serbia*

E-mail of the presenting author: lararid@ibiss.bg.ac.rs

Due to the rapid growth of the human population and increased food consumption, the generation of plastic municipal waste has become both substantial and persistent. Polystyrene (PS) and low-density polyethylene (LDPE), mainly from food packaging and plastic bags, are among the most prevalent single-use plastic (SUP) materials in today's "throwaway society." Plastic waste poses a major environmental challenge, motivating our research on the potential of *Tenebrio molitor* larvae to biodegrade PS and LDPE and their role in a circular economy. Over the past five years, we have established self-sustaining populations of *T. molitor* larvae reared on wheat bran (control group) or wheat bran supplemented with PS or LDPE. ATR-FTIR spectroscopy confirmed the absence of plastic residues in larval tissues (guts, fat body, heads, and exoskeletons) and frass across all groups.

T. molitor larvae can utilize PS and LDPE as carbon sources, degrading them into CO_2 and H_2O, supported by their diverse gut microbiota. Sequencing revealed a stable core of OTUs, mostly from *Bacilli*, *Clostridia*, and *Bacteroidia*, representing over 75% of the core. Yet, 229 OTUs were unique to the control group (27.7%) and 191 to the PS group (24.1%). The PS group microbiota closely resembled the control, suggesting shared bacterial species capable of degrading lignocellulose and PS, whereas LDPE consumption reduced *Bacteroidota* and *Actinobacteriota* compared to control and PS groups, and *Campylobacterota* compared to PS. These findings indicate that *T. molitor* gut microbiota adapts to dietary changes while maintaining a functional core [1].

Nutritionally, PS and LDPE larvae had higher protein (C: 27.69%, PS: 33.73%, LDPE: 29%) and carbohydrate levels (C: 3.18%, PS: 5.68%, LDPE: 6.55%) per 100 g dry weight. Fat content was similar between C and LDPE (C: 20.53%, LDPE: 20.65%), slightly higher in PS (23.27%). Energetic values followed the trend: C: 1284 ± 90 kJ / 306 ± 21 kcal, PS: 1531 ± 107 kJ / 366 ± 26 kcal, LDPE: 1382 ± 97 kJ / 330 ± 23 kcal.

Finally, larvae reared on PS and LDPE provide a sustainable source of chitin. Yields ranged 5.29–6.78%, slightly lower in plastic-fed larvae despite heavier exoskeletons. Extracted chitin showed high deacetylation (98.17–98.61%) and α-chitin structure with 62–66.5% crystallinity [2].

These results demonstrate that *T. molitor* larvae efficiently biodegrade PS and LDPE while producing nutrient-rich biomass and valuable biopolymers, highlighting their potential as a multifunctional component in sustainable waste management and circular bioeconomy systems.

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Greenspaces can reduce the level of airborne microplastic contamination in urban environments: Evidence from a lichen biomonitoring study

Stringa Basile C.[1,5], Jafarova M.[2,3], Aherne J.[2], Wyonch J.[2], Ruffini Castiglione M.[1], Spanò C.[1], Fačkovcová Z.[4], Loppi S.[3], Paoli L.[1]

[1] *Department of Biology, University of Pisa, Pisa, Italy*
[2] *School of the Environment, Trent University, Peterborough, Canada*
[3] *Department of Life Science, University of Siena, Siena, Italy*
[4] *Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia*
[5] *Department of Pharmacy, University of Genoa, Genova, Italy*

E-mail of the presenting author: carolina.stringabasile@edu.unige.it

Plastics, microplastics (MPs), and their associated chemicals are an emerging global concern due to their potential impact on living organisms and human health. Microplastics have been detected in a wide range of environments, yet their presence in the atmosphere remains understudied. This work assessed airborne MP contamination using transplants of the fruticose lichen *Evernia prunastri* placed in urban environments. Lichen samples were exposed for a seven-week period (April–June 2023) in the city of Pisa (Tuscany, Central Italy), specifically in parking lots (n = 9) and urban parks (n = 9). In parallel, native lichen samples collected from rural sites (n = 4) were also investigated. The study aimed to characterize MPs based on number, shape, size, and polymer type under differing environmental conditions. Further, the positive role of green urban areas in buffering atmospheric MPs was assessed.

Microplastics, including fibres, fragments, and tyre wear particles, were found in all sites. The average number of MPs per gram of dry weight of lichen significantly increased along a rural-to-urban gradient: from 2 ± 0.4 MPs/g dw in rural sites to 7 ± 1.1 MPs/g dw in urban parks and 16 ± 4.1 MPs/g dw in parking lots. Estimated average daily deposition rates in urban areas ranged from 12 to 143 MPs/m²/day, suggesting that inhabitants are exposed to different levels of airborne MPs. No significant differences in fibres length were observed between parks and parking lots, though fragments tended to be longer and tyre wear particles shorter in parking lots. Polyethylene terephthalate emerged as the most common polymer detected.

Post-exposure analysis of chlorophyll *a* fluorescence revealed no significant decline in lichen vitality, suggesting that short-term exposure did not notably affect physiological performance. Overall, our results support the suitability of lichen transplants as effective biomonitors for airborne MPs in urban settings and highlight the buffering role of urban greenspaces in mitigating atmospheric MP contamination.

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Biodiversity indicators for national reporting in Serbia: integrating biomonitoring and environmental risk assessment

Popović, S.[1], Džoljić, J.[2]

[1] Serbian Environmental Protection Agency, Belgrade, Serbia
[2] Academy of Applied Technical and Preschool Studies-Vranje Department, Niš, Serbia

Email of the presenting author: slavisa.popovic@sepa.gov.rs

Biodiversity and ecosystem services are fundamental for human development, yet their unsustainable use and environmental degradation increasingly threaten socio-economic progress. These pressures highlight the urgency of sustainable development strategies and policies that integrate multidisciplinary and cross-sectoral cooperation to ensure the conservation and long-term viability of biodiversity. Integrating biodiversity data into policy and development strategies is essential to ensuring a sustainable future and addressing the most pressing global challenges [1].

The UN Convention on Biological Diversity (CBD) requires Parties to monitor their biodiversity state and to provide reports on progress toward global targets through national reports. These reports are based on reliable indicators that translate biodiversity data into policy-relevant knowledge. Integrating biomonitoring data into this process strengthens the scientific basis of national reporting and supports evidence-based biodiversity tracking and governance.

In Serbia, the national Environmental Protection Agency (SEPA) systematically collects environmental data and uses indicators for national and international reporting. Some of these indicators have been specifically adapted to assess environmental and climate change impacts on biodiversity for the 6th National Report to the UN CBD [2]. Biodiversity indicators, as part of this official reporting framework, serve as key measures for tracking progress toward both national and global biodiversity targets. Each of the national indicators additionally has an indicator data sheet fact with all relevant basic information such as sources, institution, author, geographic coverage, etc.

Among the others, the main biodiversity indicators for assessing environmental impacts used by SEPA are Forest health conditions, Forest damage, Forest fires, Biomonitoring of air pollution, Aquatic macrophytes of water pollution biomonitoring, red algae population trend, Trend of concentration of allergenic pollen of ambrosia, Wintering aquatic-habitat birds population trends, Common birds' population trend and Common butterfly population trend [3].

Time-series analysis of biodiversity monitoring data reveals critical trends, including increases or decreases that show positive or negative environmental impacts. These indicators also provide insights into the adaptive responses of plants and animals to changing environmental conditions. Serbia is strongly committed to establishing a national biodiversity monitoring network for the 7th National Report to the UN CBD, with a particular focus on continuous data collection for taxonomic groups and habitats threatened by environmental pollution or climate change.

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Distinguishing between anthropogenic and natural sources of carbonaceous aerosol in Belgrade

Petrović, B[1], Živković, M[1], Jovanović, M[1], Davidović, M[1], Platt S.M.[2], Yttri, K.E [2], Bartonova, A[2], Jovašević-Stojanović, M[1]

[1]VIDIS Centre, Vinča Institute of Nuclear Sciences, National Institute oth the Republic of Serbia, University of Belgrade,Mike Petrovića Alasa 12-14, 11307 Vinča, Belgrade, Serbia
[2]NILU, Instituttveien 18, N-2007 Kjeller, Norway

bojana.petrovic@vin.bg.ac.rs

Air pollution is a major global challenge due to its well-documented impacts on human health and climate. While emission controls have reduced emissions of inorganic aerosol precursors, attention has increasingly turned to carbonaceous aerosol, which contributes 10 to 50% of PM_{10} mass and comprises components with varying toxicity [1]. Carbonaceous aerosol consists of elemental carbon (EC), organic carbon (OC), and occasionally inorganic carbon (IC, typically carbonate). EC is emitted from fossil fuel and biomass combustion, whereas OC represents a complex mixture of carbon-based compounds associated with hydrogen, oxygen, nitrogen, and sulphur, originating from both natural and anthropogenic sources.

In the Western Balkans, knowledge of ambient carbonaceous aerosol levels and sources is limited. To address this, the WEBASOOP project conducted OC/EC and organic tracer measurements at the urban background site Ada Marina in Belgrade (Serbia). OC sources were quantified using organic tracer concentrations and established emission ratios to OC and EC. Levoglucosan, mannosan, and galactosan were used as biomass burning (BB) tracers; fructose, glucose, trehalose, arabitol, and mannitol as tracers of primary biological aerosol particles (PBAP); and 2-methylerythritol and 2-methylthreitol as tracers of biogenic secondary organic aerosol (BSOA) from isoprene oxidation. EC served as a general combustion tracer. By applying a set of equations, EC and OC from biomass burning and fossil fuel (FF) combustion were derived, as well as OC associated with PBAP and isoprene oxidation ($OC_{ISOPRENE}$). The residual obtained when subtracting OC_{BB}, OC_{FF}, and OC_{PBAP} from total OC, was considered a proxy for the sum of anthropogenic and biogenic SOA.

Carbonaceous aerosol was the major fraction of PM_{10} at Ada Marina, contributing 42% annually [2]. Primary organic aerosol from residential wood combustion (RWC) ($OC_{BB} \times 1.8 + EC_{BB}$) contributed 65% to carbonaceous aerosol in winter, but only 4% in summer. Fossil fuel contributions showed little seasonality, contribution 22%, annually. The wintertime RWC estimate is considered conservative, as a high correlation ($R^2 = 0.78$) between levoglucosan and SOA suggests RWC was a major SOA source. In summer, biogenic precursors likely dominated SOA. Mean 2-methyltetrol concentrations increase from <0.1 ng m^{-3} in winter to >10 ng m^{-3} in summer, yet isoprene-derived BSOA contributed no more than approximately 5% of total SOA. The applicability of chamber-derived emission ratios [3] to ambient conditions is uncertain, highlighting the need for updated, region-specific data. Additional biogenic VOCs and tracers such as 3-methylbutane-1,2,3-tricarboxylic acid and pinic acid, could help close this gap. PBAP contributed > 20% to carbonaceous aerosol in spring and summer, but < 2% in winter. An upper estimate indicates that more than 40% of the annual PBAP contribution (11%) may be attributed to fungal spores.

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Mapping urban ecosystem health with bioindicators in Verona

Greco, R.[1], Latella, L.[2], Sommaggio, D.[3], Tiritelli, R.[4], Marcon, A.[5], Tagliaferro, S.[5], Guzzo, F.[1], Avesani, L.[1]

[1]*Department of Biotechnology, University of Verona, Verona, Italy*
[2]*Narìtural History Museum, Verona, Italy*
[3]*Department of Life Science, University of Modena and Reggio Emilia, Reggio Emilia, Italy*
[4]*Research centre for Agriculture and Environment, CREA, Bologna, Italy*
[5]*Department of Diagnostics and Public Health, University of Verona, Verona, Italy*

E-mail of the presenting author: riccardo.greco@univr.it

The *èVRgreen* project is a multidisciplinary environmental monitoring initiative developed through collaboration between the University of Verona, the Municipality of Verona, and the University of Padua. Its primary objective is to assess and enhance urban ecosystem services, with particular focus on air quality, urban heat islands (UHI), and biodiversity. This is achieved by integrating remote sensing, citizen science, and ecological bioindicators—specifically epiphytic lichens and hoverflies (Syrphidae).

A network of temperature, humidity, and particulate matter (PM) sensors has been deployed across Verona, and spatial analysis is performed using the iTree Canopy tool to correlate PM concentrations and surface temperature with green cover percentage. Complementing this, a lichen-based biomonitoring protocol—adapted with guidance from Stefano Martellos and Stefano Loppi—was used to evaluate air quality. Lichens are highly effective bioindicators due to their direct dependence on atmospheric inputs for nutrients and water, their absence of cuticles, and their sensitivity to pollutants such as SO_2, NO_x, and nitrogen-based particulates. Their presence, abundance, and community composition can signal levels of air pollution, eutrophication, and habitat continuity.

In the field, collaborating with 542 students, we identified and recorded lichen species using a standardized 10 × 50 cm sampling grid placed on urban trees at cardinal orientations. Tree species are selected based on bark characteristics and location, and sampling takes place across a network of mapped 1 km² grid cells. The resulting data allowed for the calculation of a Lichen Diversity Value (LDV), a semi-quantitative index used to assess atmospheric quality. Areas with high LDV values indicate more favourable environmental conditions, while a scarcity or absence of lichens often correlates with high pollution or ecological degradation.

In parallel, Syrphidae were used to monitor ecosystem quality. With support from Professor Daniele Sommaggio and the Natural History Museum of Verona, Malaise traps were deployed in both revegetated urban plots and semi-natural meadows. Hoverflies serve as powerful ecological indicators because of their dual ecological roles: adults as pollinators and larvae as aphid predators, detritivores, or saproxylic decomposers. Their sensitivity to changes in habitat structure, floral availability, and environmental stress makes them valuable in understanding ecosystem function and restoration outcomes [1, 2, 3].

Additionally, the same two areas were monitored for soil invertebrate biodiversity using pitfall traps. Collected data are used to calculate the Soil Biological Quality Index (IQBS), which is based on the diversity and presence of key soil macroinvertebrate groups (e.g., Carabidae, Isopoda, Diplopoda). This metric provides insight into ecological integrity and land management effects, further supporting a multi-taxa approach to urban ecosystem assessment.

By integrating multiple bioindicators and involving students and local institutions in scientific research, *èVRgreen* strengthens both the ecological knowledge base and public engagement in sustainability. This approach demonstrates the value of lichens and syrphids in urban ecological monitoring and offers a replicable model for linking biodiversity, air quality, and environmental policy in urban contexts.

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Nonlinear laser imaging for unravelling biomonitor potential: from insect down to fungi

Tanja Pajić[1], Katarina Stevanović[1], Nataša Todorović[2], Svetlana Savić Šević[3], Miroslav Živić[1], Mihailo Rabasović[3] and **Aleksandar Krmpot[3]**

[1] *Faculty of Biology, University of Belgrade, Serbia*
[2] *Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia*
[3] *Institute of Physics Belgrade, University of Belgrade, Serbia*

E-mail of the presenting author: krmpot@ipb.ac.rs

Nonlinear laser scanning microscopy (NLSM) is an advanced powerful technique for deep tissue high resolution imaging. It is based on interaction of ultrashort (femtosecond) laser pulses with specimen material. The image is made upon detection of nonlinear optical effects, two photon excitation fluorescence (TPEF), second or third harmonic generation (S/THG) referred as three imaging modalities. Each of the modalities provide different information on specimen: TPEF on fluorescent molecules (both, exogenous and endogenous), SHG on ordered structures such as starch, collagen and myosin, while THG, as the most versatile, but yet the least selective, on distribution of indices of refraction changes. While NLSM is indispensable technique for *in vivo* imaging on large laboratory animals (mice and rats), there is plenty of literature on the various applications, from animal cells and tissues to plants [1]

In this work we present the application of NLSM for imaging and laser processing on the insects and hyphae as the specimens that are potentially interesting for biomonitoring field.

Insects are mostly built of chitin, polysaccharide which exhibit strong blue fluorescence when excited in near UV region by conventional single photon excitation. The same spectroscopic properties are valid for chitosan. This feature might be used for imaging, for instance by confocal microscopy under 405 nm excitation, but due to strong absorption of laser light by chitin structures, the imaging is limited only to outer morphology and shape of the specimen without deep insight and structural details. This is overcome by TPEF imaging since the excitation is achieved by 700-800 nm femtosecond laser pulses, penetrating deeply into specimen without interaction except in the tiny focal volume. Utilizing this advantage high resolution images form in depth structures and 3D models are reported [2].

Since chitin, along with chitosan, is the main constituent of the hyphal cell wall imaging possibilities by TPEF are utilized for hyphae, along with THG modality which provides more details with less selectivity [3]. Since THG is produced at steep changes of index of refraction, it is used mostly to visualise lipids (e.g. lipid droplets) that has significantly different index of refraction from the surrounding cytoplasm. These two techniques in combination have been employed for studying lipid metabolism in live hyphae of *Phycomyces blakesleeanus* and proved to be reliable, providing more details than conventional biochemical methods. In addition to TPEF signal form chitinous cell wall, other endogenous structures like NADH and FAD can produce detectable signal that might be used for imaging, as well.

NLSM as a microscopic technique is not limited to imaging only, but for sample processing as well, in terms of laser cell surgery of hyphal cell wall. This method in combination with TPEF imaging has been utilized to enable patch clamping on the bare hyphal membrane and electrophysiological measurements on adult hyphae in controlled and reproducible manner [4]. This will open the possibility for a golden standard method such as patch clamp to be applied to whole kingdom of live organisms, which was not possible so far.

Since both, insects and fungi are sensitive to pollution and might be used as biomonitors, utilization of NLSM and its modalities might be a turnover in their characterization by advanced techniques. The results shown in the literature and this presentation might be considered as the first step for utilization of NLSM's modalities in the biomonitoring and in the environment protection in general.

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Challenges for the use of ecological indicators in small ponds and streams

Pinho, P.[1], Antunes, C.[1], Martelo, J.[1], Rocha, B.[1], Dekan-Carreira, V.[1], Rebelo, R.[1]

[1]cE3c - Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa
[2]FCT-NOVA…Department, Organisation, City, Country (Arial 9, italics)

E-mail of the presenting author: ppinho@fc.ul.pt

The use of ecological indicators to quantify the effects of environmental drivers in land ecosystems is becoming well established — for example, using lichens and bryophytes in atmospheric pollution studies. However, applying ecological indicators in small aquatic habitats, including ponds and streams, remains more challenging. The aim of this presentation is to depict such challenges and suggest ways forward.

One of the main challenges lies in the small size of these aquatic freshwater habitats, which often leads to them being overlooked in ecological assessments. As a result, their value in supporting biodiversity and providing ecosystem functions and services is greatly underestimated. The small size also poses challenges to their characterization, as many methods measuring aquatic biodiversity were originally designed for large water bodies Thus standardization of methods for smaller habitats is lacking. While dedicated earth observation methods and citizen science can improve mapping of these habitats, characterization of their biodiversity must be further validated to become closer to a standard method, akin to those of the Water Framework Directive.

The selection of the target variable must also be considered. While the use of aquatic macroinvertebrates as indicators of water quality is well developed, small water ecosystems have their own set of ecological drivers whose effects remain poorly known, such as climate – including total drying and freezing, habitat fragmentation, and sound and noise pollution in shallow water. Some promising variables include water oxygen and hydrogen isotopes (to measure water residence time) and the presence of taxa susceptible to light and sound to complete their life cycle (amphibia and macroinvertebrates).

As for all ecosystems, environmental drivers are likely to influence biodiversity and associated ecosystem functions and services in a non-linear and interactive way, but these relationships and associated thresholds remain major knowledge gaps on these small habitats. These can be tackled by machine learning methods but require large number of samples while working over large environmental gradients.

Overcoming these challenges can improve the protection, restoration and building aquatic ecosystems, including nature-based solutions, which are especially valuable for both biodiversity support and provision of ecosystem services, including water quality and cycle regulation, critical to future proof both cities and rural areas.

Biomonitoring of potentially toxic elements in the Venice Lagoon (Italy): a century-long comparison using natural history collection macroalgal specimens

Martellos S.[1], Seggi L.[1], FedeliR.[2] Trabucco R.[3], Falace A.[1], Metalli A.[1], Loppi S.[2,4]

[1]*Department of Life Sciences, University of Trieste, Italy*
[2]*Department of Life Sciences, University of Siena, Italy*
[3]*Fondazione Musei Civici di Venezia, Natural History Museum of Venice Giancarlo Ligabue, Venezia, Italy*
[4]*National Biodiversity Future Center, Palermo, Italy*

E-mail of the presenting author: martelst@units.it

The Venice lagoon is a peculiar highly biodiverse environment, which, however, underwent remarkable anthropogenic pressure and modifications, especially in the last century. In the years 1930-1932, Aristocle Vatova carried out a comprehensive survey of the lagoon's algal flora and collected specimens from ca. 70 sites. They were subsequently identified, mainly by Victor Schiffner, and are now preserved in the Natural History Museum of Venice (1). This historical collection provides an unprecedented basis for the assessment of environmental change over a century-long scale through comparative biomonitoring, at the same time serving as a valuable case study for the broader scientific potential of natural history collections in other research fields. It was therefore decided to compare the elemental content of *Ulva* spp. specimens from this collection with that of samples collected in 2025. In a first step, 22 sampling sites were identified where at least 10 samples of *Ulva* spp. had been collected during the 1930s. A new sampling campaign was then carried out in May 2025 at the same sites or close to the historical locations if they were neither accessible nor suitable for sampling. Large thalli of *Ulva* spp. were collected at each site to obtain at least 10 replicate samples of dry material for elemental analysis. Since historical material can be neither damaged nor destroyed, a non-invasive portable X-ray fluorescence device was used for the analyses. Comparison between of the content of potentially toxic elements accumulated by *Ulva* in the two periods showed a notable decrease for all elements analyzed, except Mg, with median old/new ratios ranging from 1.2 for Ba to 7.4 for Zn. High (>10) ratios were found at some sampling sites for P (11.6), Cr (10.6), Mn (17.0), Fe (14.5) and Zn (21.5). In a few locations Cu and Cr showed higher concentrations in 2025, which for Cu were limited to the urban areas of the lagoon (Venice and Chioggia) and for Cr to the vicinity of the airport and some settlements. However, further investigations are needed to link the sampling sites to possible sources.

This study proves that macroalgae can be used to investigate the occurrence of potentially toxic elements in the marine environment, and that the methodological approach adopted in this research, comparing specimens from historical collections with fresh samples by a non-destructive technique, is robust and can be replicated in other areas. Furthermore, this study suggests that conditions in the Venice lagoon, at least with regard to the elements investigated, have greatly improved over the last century.

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Application of fish and bivalves in biomonitoring of microplastic impact on the aquatic environment

Filipović Marijić, V.[1], Kuštro, M.[1], Šariri, S.[1], Ugrinović, M.[1], Faraguna, F.[2], Kučić Grgić, D.[2], Kralj, T.[1], Valić, D.[1], Pikelj, K.[3]

[1]Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia
[2] Faculty of chemical engineering and technology, University of Zagreb, Zagreb, Croatia
[3]Department of Geology, Faculty of Science, University of Zagreb, Zagreb, Croatia

E-mail of the presenting author: vfilip@irb.hr

Microplastics (MP), defined as particles smaller than 5 mm, are widely recognized as emerging pollutant in the aquatic environment. Large amounts of MP enter and persist in marine and freshwater ecosystems worldwide and, due to their small size, present the threat for aquatic organisms [1]. Besides causing physical damage and other physiological disturbances in biota, MPs also serve as carriers for other contaminants and microorganisms. Data on the presence of MPs in different tissues of bivalves and fish are still scarce, and depend on the tissue type, physiology and their role in the organism [2]. In this biomonitoring study, we isolated and compared the shapes, size, colour and type of microplastics in brown trout (*Salmo trutta*) from the Krka River and in sea bream (*Sparus aurata*) and mussels (*Mytilus galloprovincialis*) from the Šibenik Bay (Adriatic Sea). Bivalves and fish represent common bioindicator organisms in aquatic ecosystems, which are also used in human diet, therefore allowing estimation of the possible effects on human health. Additionally, to get the comprehensive overview of MP presence in the surrounding area, the isolation and analyses of MPs in water and sediment were also performed.

Intestine and gonads of fishes and digestive gland and mantle (overgrown with gonads) of mussels were digested using 10% KOH at 60 °C for 48 hours in the ultrasonic bath, followed by vacuum filtration over polyester and polycarbonate filters coated with gold of a pore size of 0.8 µm. Additionally, gut content of fishes was acid digested using combination of HNO_3 and HF at 85°C for 3.5 hours in the dryer. After drying the filters at room temperature, the presence of MP was analyzed by ATR-FTIR spectroscopy (IRTracer-100, AIM-9000, Shimadzu). A total of 1000 L of seawater was filtered through sieves to collect the following size fractions: >1 mm, 1 mm – 500 µm, 500 – 125 µm, 125 – 63 µm, <63 µm. MP particles were rinsed from each sieve with MQ water and isolated by vacuum filtration. Sediment was separated from MP with NaCl solution and MP was extracted for further analysis by filtration. For all samples, the procedure involved working in a protective cabinet to prevent environmental contamination, and application of a "blank sample" to check the cleanliness and accuracy of the work.

The results confirmed the presence of MPs in water, sediment and analyzed tissues of fish and bivalves. Generally, MP particles were rare in tissues, showing differences in relation to the type of tissue, with higher occurrence in fish intestine as a site for uptake from the environment. In the fish gut content different shapes and colours of MPs were found, confirming their uptake by dietary route. The most common shapes were fibres of different colours, and detected polymers included polystyrene, polyethylene, polypropylene, polyamide, poly(ethylene-co-acrylic acid), poly(ethylene-co-vinyl acetate). Presented findings confirmed the exposure of freshwater (Krka River) and marine organisms (Adriatic Sea) to MP, and highlighted the importance of biomonitoring accumulation and impact of MP to aquatic organisms. By assessing the occurrence, abundance, accumulation, and impacts of MPs in various organisms and environmental compartments, biomonitoring provides valuable data for understanding potential risks and developing strategies to protect aquatic ecosystems, but also human health.

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Interspecies variability of heavy metals and other microelements in mussels from the selected marine zones of South Africa

Kravtsova, A.[1], Nekhoroshkov, P. [1], Rikus le Roux[2], Bezuidenhout Jacques [2], Zinicovscaia, I. [1, 3], Yushin, N. [1]

[1]Laboratory of Neutron Physics, Joint Institute for Nuclear Reseasrch, Dubna, Russia
[2]School for Science and Technology, Faculty of Military Science, Stellenbosch University, South Africa
[3]Horia Hulubei National Institute for Physics and Nuclear Engineering (IFIN-HH), Bucharest - Magurele, Romania

E-mail of the presenting author: alexkravtsova@yandex.ru

The neutron activation analysis is successfully used for determination of wide spectrum of macro and microelements in mussels collected from South African coast during last 10 years. To analyse the species differences in elemental accumulation four species of mussels belonging to Mytilidae family (*Aulacomya atra*, *Mytilus galloprovincialis*, *Choromytilus meridionalis*, *Semimytilus patagonicus*) were collected from a farm in Saldanha Bay (South Africa) in March 2020.

The mass fractions of Na, Mg, Al, Cl, K, Ca, Sc, V, Cr, Mn, Fe, Co, Ni, Zn, As, Se, Br, Rb, Sr, Sb, I, Cs, Th, and U were determined in soft tissues and shells of 56 adult mussels using neutron activation analysis.

The *Aulacomya atra* (AA) and *Choromytilus meridionalis* (CM) are considered as indigenous mussels for west coast of South Africa [1], while *Mytilus galloprovincialis* (MG) and *Semimytilus patagonicus* (SP) were introduced on the west coast in 1980s and 2000 respectively [1,2].

The highest content of Na, Mg, and Cl were observed in the alien SP and MG. In mussel tissues, the levels of these elements, the major ions of the marine environment, are influenced by their levels in the surrounding waters (which is related to salinity) and biological adaptation mechanisms of the organisms. In contrast, Al and Ca are usually associated with terrigenous sources, such as sediments. Their content was also elevated in the alien mussels compared to the native ones. The shell/soft tissue ratio revealed high content of such elements as V and I in the shells of AA, which is probably related to the species-specific biological regulation.

The condition indices (CI) [3] expressed in the weight of soft tissues and length of shells reflected the mussels' response to local hydrochemical conditions. The calculated CI showed the better functional states in the native species AA and CM in comparison to MG and SP. This is also consistent with the lower Na and Cl content found in the soft tissues of AA and CM, indicating an adaptive potential to salinity fluctuations compared to the alien MG and SP, which accumulated the elements in higher quantities. It is important to note that natural environmental factors such as storms, runoffs, local geology could affect elemental ratios in mussels' soft tissues and shells. The adaptive potential and functional state of the selected local species can be assessed by analysing the elemental ratios in soft tissues and shells, in conjunction with the Condition Index.

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Soil-dwelling insects, order Coleoptera, could be indicators of microplastic pollution of soils

Krnjajić, S.[1], Vukajlović, F.[2], Knežević, G.[3], Stanković, S.[4], Stefanović, S.[3], Marjanović, Ž.[1]

[1]University of Belgrade–Institute for Multidisciplinary Research, Kneza Višeslava 1, 11000 Belgrade, Serbia [2]University of Kragujevac, Faculty of Science, Radoja Domanovića 12, 34111 Kragujevac, Serbia
[3]Faculty for Applied Ecology "Futura", Metropolitan University, Požeška 53a, 11000 Belgrade, Serbia
[4] Institute for Science Application in Agriculture, Belgrade, Blv. despota Stefana 68b, 11000 Belgrade, Serbia

E-mail of the presenting author: slobodan.krnjajic@imsi.bg.ac.rs

Environmental pollution is generally expected to reduce insect diversity, but the studies on how soil plastic pollution affects soil-dwelling insects are scarce. To evaluate plastic waste's influence on insect diversity, we collected soil-dwelling insects using pitfall traps in natural forests exposed to illegal waste dumping and agroecosystems exposed to greenhouses in the alluvial plains of the Danube, Sava, and Morava in Serbia over two years. Pitfall traps were used to collect insects, while MP was isolated using the new protocol described in [1] . We focused on the diversity of dominant Coleoptera and used multiple biodiversity indices: Shannon-Weaver (H'), Simpson (1-D), Margalef (d), and Pielou's evenness (J') to evaluate if the presence of MP and its amounts could be influencing Coleoptera populations in polluted sites. The diversity and especially the abundance of Coleoptera in agricultural sites were much lower than in natural forest sites. Furthermore, Coleoptera were strongly affected by the waste dumping. Measured MP was significantly correlated with Coleoptera diversity and abundance in natural forest sites, and especially in agricultural sites. Pollution sometimes increased insect diversity, sometimes had no effect, and sometimes caused delayed negative impacts depending on the location and habitat type. These findings demonstrate that pollution-biodiversity relationships are highly site-specific and temporally variable, supporting the intermediate disturbance hypothesis at some locations while revealing delayed negative impacts at others. Plastic pollution effects on insects are highly location-specific and can take time to develop, highlighting the need for long-term monitoring across multiple sites to truly understand environmental impacts and develop effective monitoring and conservation strategies.

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Determination of *in vitro* gastrointestinal bioaccessibility of organochlorine pesticides in poultry

Akhatzhanova, A.[1,2], Amutova, F.[1,2], Konuspayeva, G.[1,2], Jurjanz, S.[3]

[1]Faculty of biology and biotechnology, al -Farabi Kazakh National University, Almaty, Kazakhstan
[2]Laboratory of physicall-chemical research methods, Antigen Co LTD, Almaty, Kazakhstan
[3]L2A, University de Lorraine, Nancy, France

E-mail of the presenting author: ahatzhana@gmail.com

Organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDTs and its metabolites o,p'-p,p'-DDT, DDD and DDE) and hexachlorocyclohexane isomers (HCHs including α-HCH, β-HCH, γ-HCH) are persistent organic pollutants (POPs) that are well-known for their persistency, bioaccumulation, lipophilicity and long-range transport [1]. Especially in soil, their half-life lasts several years, and they can enter the food chain by being transferred from soil to living organisms. For example, laying hens may ingest contaminated soil (3.6-7.2 g/day in summer, 15-30 g/day in spring) [2] while pecking for feed, leading to the ingestion of soil-bound OCPs and potential accumulation in edible tissues and eggs. This route of soil ingestion is a known source of OCP exposure in chicken and a mean of these pesticides being introduced into the human diet. It is important to distinguish between bioavailability and bioaccessibility when assessing the pesticide uptake. In this context, *in vivo* determined bioavailability is the fraction of contaminant that reaches the central compartment and can be distributed throughout the body's tissues. Bioaccessibility, on the other hand, is the portion of compound that released from the ingested matrix into the gastrointestinal tract and is potentially available for absorption [3] and generally evaluated *in vitro*. The examination of bioaccessibility considered as a central element of the risk assessment of POPs.

The aim of this study was to assess *in vitro* the bioaccessibility of organochlorine pesticides in the gastrointestinal tract of poultry. The *In vitro* digestion (IVD) method [4] with Tenax as adsorptive sink was used to reproduce the main conditions of digestion (gastric and intestinal phases with corresponding enzymes, pH and temperature) in birds. OCP compounds from two families were tested: DDTs and HCHs.

The results showed that the bioaccessible fractions of these OCPs in soil were specific to each compound and ranged from 25% to 67%. Among the DDTs, p-p′ DDT showed the highest bioaccessibility (67%), which significantly exceeds the values for its degradation products p-p' DDD (34%), o-p′ DDD (37%), and p-p′ DDE (25%). Among HCHs, β-HCH showed the highest bioaccessibility (55%), while bioaccessibilty of α-HCH and γ-HCH (lindane) was only at 26%. These bioaccessibilities suggest that a significant portion of soil-bound OCPs may become available for absorption in the gastrointestinal tract of poultry. Birds do indeed ingest and retain most of the OCPs they consume, i.e., in feeding studies, only 12.8% of HCH and 3.3% of DDT were excreted, with the rest accumulating in the body [5].

In vitro assays are powerful tools for predicting the bioavailability of contaminants without using model animals, but careful consideration of their limitations is needed. However, *in vitro*-*in vivo* correlations (IVIVCs) need to be conducted to validate the use of *in vitro* methods as prediction tools.

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Phthalate Contamination in Cow Milk: Age-Dependent Risk Assessment

Baizhuma, Zh.[1,3], Turgambek, A.[1], Chuvashova, Ye.[1], Dronova. K.[1], Akhmetsadykov, N.[1], Amutova, F. [1,2]

[1] *Antigen LLP, Scientific and Production Enterprise 040905, Almaty region, Kazakhstan*
[2] *Al-Farabi Kazakh National University, Faculty of Biology and Biotechnology, 050040 Almaty, Kazakhstan.*
[3] *Satbayev University, Department of Chemical and Biochemical Engineering, 050012, Almaty, Kazakhstan*

E-mail of the presenting author: bbaizhumazh@gmail.com

Phthalate esters (PAEs) are widely used in the production of food packaging materials. Phthalates are known to disrupt the endocrine system, negatively affect reproductive function and fetal development, and cause disturbances in hormonal balance and metabolic processes [1]. However, despite their widespread use, systematic studies on their effects on human health, particularly across different age groups, remain limited [2].

In this study, we assessed the health risks associated with dietary exposure to phthalates through the consumption of commercial cow's milk, considering five age groups: i) 2–6 years: 17 kg body weight, 0.4 L milk/day; ii) 7–11 years: 32 kg, 0.43 L milk/day; iii) 12–16 years: 57 kg, 0.32 L milk/day; iv) 17–21 years: 72 kg, 0.26 L milk/day; and v) >22 years: 80 kg, 0.12 L milk/day. A total of 24 commercial cow milk samples (3.2% fat) were analyzed using the QuEChERS extraction method followed by gas chromatography–mass spectrometry (GC–MS) [3]. Seven target phthalates (DMP, DEP, DPrP, DIBP, DBP, BBP, and DEHP) were determined. Samples were collected from supermarkets in Almaty, Kazakhstan [4]. DBP, BBP, and DEHP were the most frequently detected compounds in milk samples, with maximum concentrations of 1362.4 µg/L (DBP), 641.6 µg/L (BBP), and 368.6 µg/L (DEHP), respectively. Much higher concentrations were found in packaging materials: up to 9935.1 µg/kg (DBP), 1611.5 µg/kg (BBP), and 4941.3 µg/kg (DEHP), confirming the migration of phthalates from packaging into food.

For quantitative risk assessment, estimated daily intake (EDI, µg/kg body weight/day) and Hazard Index (HI) were calculated for each age group. The HI is an integrated risk indicator used to evaluate the potential adverse effects of mixtures of chemicals. It is calculated as the sum of individual Hazard Quotients (HQ) for each compound with a similar mechanism of toxicity or acting on the same organ/system. Reference doses (RfD) established by the U.S. Consumer Product Safety Commission were used as toxicological benchmarks [4].

Estimated daily intake (EDI) values for DBP in children aged 6–11 years (≈ 6.0 µg/L/day), adolescents (12–16 years; ≈ 2.0 µg/L/day), and adults (>22 years; ≈ 0.5 µg/L/day) were all below the established toxicological reference values. The highest exposure was observed in children aged 2–6 years, where at a maximum DBP concentration of 1362.4 µg/L, the EDI reached ≈ 10.7 µg/L/day, slightly exceeding the threshold value of 10 µg/L/day. For BBP and DEHP, EDI values in all age groups remained below benchmarks.

The highest hazard index (HI) values were observed in children aged 2–6 years, where 7 out of 24 samples exceeded the threshold level of 1, including one sample that reached 3.5, indicating a considerable risk. Among children aged 6–11 years, HI values ranged from 0.4 to 0.9, with six samples exceeding 1. In adolescents (11–21 years), HI values did not surpass 0.5, whereas in adults they remained below 0.3, suggesting a low risk level.

The findings highlight the need for regular monitoring of phthalate levels in food products, stricter quality control of packaging materials, and the implementation of safer alternatives with low migration potential. This is especially important for children's nutrition, where even low levels of contamination may exceed toxicological thresholds.

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The human milk as a biomonitor of potentially toxic elements – bioaccessibility and infant health risk assessment

Milićević T.[1], Dojčinović B.[2], Mitić J.[1], Relić D.[3], Matek Sarić M.[4],Popović A.[3], Herceg Romanić S.[5]

[1] *Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*
[2] *Institute of Chemistry, Technology and Metallurgy, Centre for Chemistry, Belgrade, Serbia*
[3] *University of Belgrade - Faculty of Chemistry, Belgrade, Serbia*
[4] *Department of Health Studies, University of Zadar, Zadar, Croatia*
[5] *Institute for Medical Research and Occupational Health, Zagreb, Croatia*

E-mail of the presenting author: tijana.milicevic@ipb.ac.rs

Human breast milk is the main source of nutrients for newborns, providing immunological benefits. However, when mothers are exposed to different pollution sources in the prenatal and lactation periods, their breast milk may accumulate potentially toxic elements (PTEs). This study focuses on the research of PTEs in human milk, concentration evaluation comparing to mother's age, number of births, smoking habits, and other environmental factors (e.g. proximity to industry or chemical use in their living and working environments). The 50 milk samples were collected from healthy mothers who live in urban and semi-urban areas of Zadar city (Croatia). Participants provided informed consent with their self-reported data on exposure to environmental pollutants.
Human milk samples were prepared for analysis by microwave digestion using HNO_3 and H_2O_2. The concentrations of PTEs were analyzed by inductively coupled plasma–optical emission spectrometry (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS). In this study, we presented the concentrations of 16 PTEs (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, P, Pb, Sr, and Zn). The quality control and assurance were done by using blanks, analysing triplicates and using certified reference material of milk (powder milk). *In vitro* bioaccessibility assays simulating the gastrointestinal tract (GIT) of an infant — using gastric (GE) and gastrointestinal (GIE) fluids — were performed at 37°C to determine the bioaccessible PTE concentrations from human milk. The UBM bioaccessibility assay was adopted and adapted to the infant GIT [1]. The infant risk assessment has been assessed by models developed by the U.S. Department of Energy (DOE), Oak Ridge Operations Office (RAIS).
The highest pseudo-total concentrations of PTEs were observed for Zn > Fe > Cu. Elevated levels of As, Cd, and Pb were determined in some of the samples, regardless of maternal smoking status, indicating multiple exposure sources. Some outliers can be noticed for specific PTE (As, Cd, Pb). Anyway, the distribution is not clearly indicated, probably due to the influence of indirect exposure to PTE, such as passive exposure to cigarettes or dust. Also, exposure to other sources has an influence on PTE concentrations in human milk.
Observing the assessed infant health risk (the worst-case scenario; fed by human milk for 2 years) showed no adverse health effects (THQ <1). The most significant contributors to the THQ are in the decreasing order Li > As >Cu > Zn >Cd >Mn, and then other elements. Cu, Zn and Mn, besides beneficial effects on the infant, may also, in high concentrations, be harmful. Contrary, As and Cd should be strictly monitored, and the risk should be assessed in the case of prolonged breastfeeding for mothers who are exposed to PTE pollution. There are some variations of THQ values for As, which may indicate the environmental factor influence on mothers and their breast milk.
Bioaccessibility results showed that Ba, Cd, Cu, Fe, Mn, and Zn were most bioaccessible in the stomach (GE), whereas Cu, Mn, and Zn were more bioaccessible in the small intestine (GIE) compared to the other investigated PTEs. These findings pointed to the importance of monitoring maternal exposure to environmental pollutants during pregnancy and lactation.

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Short-talk Speakers

Magnetic and Chemical Biomonitoring at Cultural Heritage sites: In Remembrance of Antonio Sgamellotti

Winkler, A.[1], Grifoni, L.[1, 2], Lapenta, V.[3], Spagnuolo, L.[1], Loppi, S.[2,4]

[1]*Istituto Nazionale di Geofisica e Vulcanologia, Rome, Italy*
[2]*Department, of Life Sciences, University of Siena, Italy*
[3]*Accademia Nazionale dei Lincei, Rome. Italy*
[4]*National Biodiversity Future Center, Palermo, Italy*

E-mail of the presenting author: aldo.winkler@ingv.it

Antonio Sgamellotti left us on August 20nd, 2025. He was professor emeritus of inorganic and theoretical chemistry at the University of Perugia, and National Member of the Accademia Nazionale dei Lincei, one of the oldest scientific institutions in Europe. He was a promoter of the dialogue between science and art, conceiving the Molab, the mobile laboratory for research on artwork. In the framework of CERIF, the Lyncean Cultural Heritage Research Center of Villa Farnesina, Antonio Sgamellotti inspired the application of Nature-based solutions for monitoring the impact of vehicular particulate matter and for the preventive conservation of Cultural Heritage.

He started a fruitful season of intercultural cooperation among rock-magnetists, life scientists, museum directors and curators, taking magnetic biomonitoring to previously unexplored and prestigious worldwide known contexts.

This short talk will recall the lichen exposure and vascular plant sampling performed at Villa Farnesina [1] and Archeological Park of the Colosseum [2] in Rome, at Peggy Guggenheim Collection in Venice [3], at Metropolitan Museum of Art in New York, at Nacional Museums in Buenos Aires [4]. The innovative results brought intensive coverage at congresses and media, disseminating magnetic biomonitoring into unusual scientific and cultural environments.

With his insights and ability to create interdisciplinary scientific networks, Antonio Sgamellotti left a precious legacy that will continue along the path he traced, through the projects CHIOMA (Cultural Heritage Investigations and Observation: a Multidisciplinary Approach), BIO4CBC (Preventive conservation of cultural, artistic, and architectural heritage from air pollution) and a new PhD program at Tor Vergata University.

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Application of nonlinear optical imaging and Nile Red staining in microplastic analysis

Popović, M.[1], Aničić Urošević, M.[1], Popović, A.[2], Ćurčić, M.[1], Pajić, T.[3], Rabasović D. M.[1], Krmpot, A.[1]

[1]Institute of Physics Belgrade, Belgrade, Serbia
[2]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia
[3]University of Belgrade, Faculty of Biology, Belgrade, Serbia

E-mail of the presenting author: pmilica@ipb.ac.rs

Microplastic (MP) pollution has become a global issue worldwide, but its qualitative and quantitative analysis remain a major challenge [1]. It largely relies on visual inspection which often leads to overestimation or underestimation of MP particle numbers in samples [2]. In addition, commercial plastics often contain different additives and, during fragmentation, undergo chemical structure changes that complicate the standardization of MP identification methodologies.

In this study, we examined three types of reference plastic materials: high-density polyethylene (HDPE), polystyrene (PS), and polypropylene (PP) using nonlinear laser scanning microscopy with two modalities: two-photon excitation fluorescence (TPEF) and third harmonic generation (THG). In addition to imaging, fluorescence spectra were recorded upon two photon excitation at three different wavelengths: 730, 800 and 1040 nm. Ti:Sa (Mira900, Coherent Inc.) and Yb:KGW (YbGLX Time Band width Products AG) mode locked lasers were used as the sources of femtosecond pulses for two photon excitation, while the spectra were recorded utilizing USB portable fiber spectrometer with cooled linear CCD array (Glacier X, B&W Tek). Subsequently, reference plastics were stained with Nile Red (NR), a fluorescent dye that has been proposed as a fast and reliable method for MP identification in various media [3]. A working solution of NR was prepared by dissolving the dye in methanol to a concentration of 1 μg ml^{-1}. Reference plastics were immersed in this solution and incubated for 15 min at 60 °C in the dark to enable dye adsorption. Stained plastics were also analysed by TPEF paired with the spectral measurements.

To demonstrate the applicability of this approach to MPs derived from packaging products, we examined the same polymer types in the form of MPs (<50 μm), prepared by grinding the following materials: HDPE from a transparent plastic bottle, PS from white styrofoam packaging and PP from a transparent plastic cup. This analysis aimed to identify spectral variations and changes in optical characteristics arising from the particle size, presence of different additives and structural modifications.

The results showed that none of the examined reference plastics exhibited detectable autofluorescence upon any of the excitation wavelengths, whereas NR-stained plastics displayed strong fluorescence across all three wavelengths, even at low laser power (≈4 mW). Contrary to the reference plastics, almost all MPs derived from packaging products emitted measurable autofluorescence: HDPE and PP exhibited autofluorescence upon shorter excitation wavelengths (730 and 800 nm), while the signal remained weak upon longer wavelength (1040 nm).

Polystyrene depicted a weak fluorescence signal at 730 nm, whereas no autofluorescence was observed at 800 and 1040 nm. Unlike the reference plastics, the application of NR staining on MPs did not enhance the fluorescence signal, indicating that further investigation is needed to determine to which extent additives and structural modifications affect dye binding to commercial plastics. An encouraging result is that all studied samples, including pure polymers and product derived MPs, exhibited strong THG signals, highlighting the potential of this modality to enable MPs detection in environmental samples without the need for staining.

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Polystyrene microbeads affect seed germination, plant growth and nutrient uptake in two cultivars of *Capsicum annuum* L.

Gaglione, A.[1], Granata A.[1], Giordano S. [1], Capozzi F. [1], Spagnuolo V. [1],

[1] *Department of Biology, University di Napoli Federico II, Campus Monte S. Angelo, Via Cinthia 4, 80126 Napoli, Italy*

E-mail of the presenting author: anna.gaglione@unina.it

The ubiquitous presence of microplastics (MPs) in terrestrial environments poses a significant threat to agricultural ecosystems. This study investigates the effects of polystyrene microbeads (PS-MPs) on seed germination, plant growth, and nutrient uptake *in Capsicum annuum* L., a globally important crop. With plastic production projected to reach 600 million metric tons by 2050 [1], the environmental accumulation of persistent microplastics, particularly in agricultural soils, is a critical concern. Previous research indicates that MPs, including polystyrene (PS), can negatively impact plant development, affecting germination rates, growth, and nutrient absorption, with species - and cultivar - specific responses.

This research hypothesized that two landraces of *C. annuum* (namely sassaniello and papaccella), typical of southern Italy and characterized by a different intrinsic antioxidant production, would exhibit different morpho-physiological responses to PS-MPs. Seeds were germinated and seedlings grown in the presence of 1 μm diameter PS-MPs at concentrations of 0, 0.5, 1, and 1.5 mg L^{-1}. Our experimental design focused on evaluating the impacts on seed germination, plantlet biometry and architecture, and oxidative stress in both leaves and roots. Furthermore, chemical analyses were conducted to assess nutrient accumulation, and gas exchange in the sassaniello cultivar. The findings of this study aim to elucidate cultivar-specific responses to PS-MP contamination, thereby contributing to the broader understanding of microplastic pollution and their ecological implications in agriculture.

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Biomonitoring of airborne elements and microplastics: acomparison of terricolous and epiphytic lichens

Legnaro Diamanti, M.[1], Fedeli, R.[1], Fiaschi T.[1], Grifoni, L.[1,2], Angiolini, C.[1], Loppi, S.[1,3]

[1]*Department of Life Sciences, University of Siena, Siena, Italy*
[2]*National Institute of Geophysics and Volcanology (INGV), Rome, Italy*
[3]*National Biodiversity Future Center, Palermo, Italy*

E-mail of the presenting author: m.legnarodiamanti@student.unisi.it

The aim of this study is to compare the capacity of terricolous and epiphytic fruticose lichens to accumulate airborne elements and microplastics. To this purpose, triplicate samples of epiphytic (*Evernia prunastri* (L.) Ach. and *Ramalina* spp.) and terricolous (*Cladonia rangiformis* Hoffm.) lichens were collected from 10 remote sites in Tuscany (Central Italy). Evernia and Ramalina were collected from Quercus trees in edge forest environments, while Cladonia was collected from the soil in open stands.

For each sample, about 1 g was used for the elemental analysis by X-ray fluorescence (XRF), and about 0.5 g were used to detect microplastics by the wet peroxide method in combination with the hot needle test. A Mann-Withney U-test was used to check for differences between epiphytic and terricolous lichens.

The results showed that terricolous lichens accumulated much higher concentrations of soilborne elements, namely Al, Fe and Si, while epiphytic lichens accumulated higher amounts of elements such as Ba, Ca, Cl, Cr, Cu, K, Mn, P, S and Zn; As, Rb and Sr were accumulated in a similar way. It remains to be investigated if foliar leaching contributes to the elemental content of epiphytic lichens. As far as microplastics are concerned, differences did not emerge between epiphytic and terricolous lichens, with comparable average values: 4.9 ± 2.5 MPs/g for the cladonia and 4.0 ± 1.5 MPs/g for the epiphytic lichens.

The dominant microplastic type are microfibers in every area, and number and length similar in both types, with the fibers under 2 mm of length dominant.

From this study it can be concluded that epiphytic lichens are much more efficient for monitoring airborne elements, while the use of terricolous lichens may be a suitable alternative to epiphytic lichens for monitoring airborne microplastics.

“Moss wars”: *Hypnum* vs. *Sphagnum* in biomonitoring of urban trace element atmospheric deposition – a case study in Novi Sad, Serbia

Ilić, M.[1], Aničić Urošević, M.[2], Arsenov, D.[1], Ćuk, M.[1], Vlku, A.[1], Šikuljak, T.[1], Vukov, D[1].

[1] *Department of biology and ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia*
[2] *Environmental Physics Laboratory, Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*

E-mail of the presenting author: milos.ilic@dbe.uns.ac.rs

Human activities including industry and traffic, directly contribute to increased levels of pollutants in the environment, particularly in urban areas. Active biomonitoring with mosses (“moss-bag” technique) is a cost-effective and reliable method to assess atmospheric deposition of trace elements, yet the choice of moss species may strongly influence the interpretation of pollution sources.

In this study, two widely explored moss biomonitors—*Hypnum cupressiforme* and *Sphagnum girgensohnii*—were exposed for 60 days at ten urban sites in Novi Sad City. Concentrations of 15 trace elements (B, Al, V, Cr, Fe, Co, Ni, Cu, Zn, As, Sr, Cd, Sb, Ba, Pb) were determined by ICP-MS to evaluate species-specific differences in atmospheric deposition patterns. Moss material was collected from pristine sites, dried, packed into mesh bags, and deployed on street-light poles at ~3 m height to capture urban fallout.

A suite of univariate and multivariate statistical approaches was applied. Paired t-tests and Wilcoxon signed-rank tests with false discovery rate correction revealed consistent interspecific differences. *S. girgensohnii* accumulated significantly higher levels of typical anthropogenic trace elements (Pb, Zn, Cu, Cd, Ni), highlighting its sensitivity to traffic- and industry-related emissions. *H. cupressiforme* showed stronger retention of mineral-derived elements (Fe, Mn, partly Ba, and Al), reflecting its affinity for geogenic dust. Linear mixed-effects models confirmed these patterns while accounting for site variability.

Multivariate analyses reinforced species separation. PERMANOVA detected significant differences in overall chemical profiles. PCA ordination showed *S. girgensohnii* clustering with anthropogenic metals along PC1, while *H. cupressiforme* grouped with mineral elements along PC2. Heat-map clustering emphasized the complementary accumulation strategies. Spatial mapping further revealed urban hotspots of Pb and Zn in *S. girgensohnii*, whereas Fe and Mn were more evenly distributed and consistently higher in *H. cupressiforme*.

To capture overall pollution loads, the Metal Pollution Index (MPI) and Enrichment Factors (EF, normalized to Fe background) were calculated. *S. girgensohnii* exhibited higher MPI values across sites and strong enrichment for Pb and Zn, confirming anthropogenic origin, whereas *H. cupressiforme* displayed EF values closer to unity for crustal elements. Spearman correlation matrices highlighted distinct co-occurrence patterns: Pb–Zn associations in *S. girgensohnii* versus Al–Cr groupings in *H. cupressiforme*.

The “moss war” does not crown a single winner but emphasizes complementarity. *S. girgensohnii* serves as a sentinel of anthropogenic emissions, while *H. cupressiforme* reflects mineral background. Used together, they provide a robust dual biomonitoring system capable of disentangling the complexity of urban atmospheric deposition.

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Climate change as a driver of the range expansion of the invasive diatom *Skeletonema potamos* (C.I.Weber) Hasle in Serbian lowland rivers

Pećić, M.[1], Knežević, J.[2], Jakovljević, O.[1], Karadžić, V.[2]

[1]*Department of Algology and Mycology, Faculty of Biology, University of Belgrade, Belgrade, Serbia*
[2]*Department of Laboratory for ecotoxicology, Institute of Public Health of Serbia "Dr Milan Jovanović Batut", Belgrade, Serbia*

E-mail of the presenting author: marija.pecic@bio.bg.ac.rs

Climate change and invasive alien species are widely recognized as serious threats to biodiversity, ecosystems and human well-being, primarily due to their strong and complex mutual connections. *Skeletonema potamos* (C.I.Weber) Hasle is small, chain-forming, scarcely perceptible centric freshwater diatom. It is broadly distributed in the temperate zone in Europe, mainly in large rivers and their reservoirs, but also considered invasive in several regions, including the Great Lakes of the United States, the River Loire in France, and the River Elbe in the Czech Republic [1].

As part of the mandated biomonitoring program implemented by the Institute of Public Health of Serbia "Dr Milan Jovanović Batut", phytoplankton studies were performed in four major lowland rivers in Serbia between 2018 and July 2025. The Danube (at 12 localities distributed along its entire course) and the Velika Morava (at its confluence with the Danube) were investigated during 2018-2024, whereas the Sava (at Obrenovac and the confluence with the Danube) and the Tisza (at Kanjiža and the confluence with the Danube) were surveyed from 2018 to July 2025. Phytoplankton sampling was performed in different seasons in accordance with the standard method EN 16698:2016 [2]. Microscopic analysis was performed using an AxioObserver Z1 inverted microscope. A total of 148 phytoplankton samples were processed during this study.

The survey results confirmed the occurrence of *S. potamos* throughout the entire investigated period along the whole Danube course, with a maximum share of 31% in the total cell abundance and, at the same time, a comparatively lower contribution (around 7%) to the total biomass recorded in September 2021. Its low abundance was also detected in the Velika Morava. In contrast, the share of this diatom in the Sava phytoplankton was markedly higher, reaching 83% of the total cell abundance and 62% of the total biomass in August 2018, and 57% and 23%, respectively, in early July 2025. *S. potamos* was also recorded for the first time in the phytoplankton of the Tisza River in Serbia during this continuous monitoring, contributing significantly to both total cell abundance and biomass (≈20% in June 2019 and early July 2025). Our findings are in agreement with earlier studies, confirming the permanent presence of *S. potamos* in Danube phytoplankton [1]. On the other hand, although the occurrence of this species in the Sava has been previously recorded [3], our results revealed the eightfold increase in its biomass compared to the initial record.

As previously reported, *S. potamos* is a warm stenothermic and photophilic diatom whose increases in abundance and biomass within aquatic ecosystems are closely associated with elevated water temperatures. This is consistent with our results that revealed seasonal peaks during the warmer period of the year, with the highest share recorded in the Sava River (at Obrenovac) at a water temperature of 30º C and a depth of 0.5 m. The first record of *S. potamos* in the Tisza River in Serbia, accompanied by its abundant development and significant increase leading to undoubted dominance in the Sava phytoplankton, appear to support the predictions of Duleba et al. (2014). They indicated the possible expansion of its geographic range and/or an increase in seasonal duration within existing habitats as a response to surface water warming driven by global climate change [1]. Overall, these results highlight that global warming exerts a profound influence on phytoplankton community composition by facilitating the proliferation and range expansion of invasive taxa. Such alterations not only reshape biodiversity patterns but also pose a serious threat to the stability and health of aquatic ecosystems.

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Screening soil microbial isolates for PTE tolerance and solubilisation traits

<u>Mamirova, A.</u>[1], Baubekova, A.[1], Batykova, Zh.[1], Akhmetalina, N.[1]

[1]*Department of Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan*

E-mail of the presenting author: aigerim.mamirova@mail.com

Considering the aim of this research, to isolate microbial cultures capable of tolerating elevated concentrations of potentially toxic elements (PTEs) and solubilising target PTEs, bulk soil samples were collected from contaminated sites. It was hypothesised that soils differing in origin and type of contamination would provide a broader and more diverse range of microbial groups with the desired traits.

For this purpose, four sites in the Almaty and Zhetysu regions of Kazakhstan were selected, each named after the corresponding village: 1) Talgar (TA): background soil collected at the foot of Talgar peak (GPS: 43°16'36.0" N 77°12'37.0" E); 2) Kyzylkairat (K): soil collected near a former pesticide storage facility (GPS: 43°17'58.7" N 77°11'39.6" E), representing a site complexly contaminated with organochlorine pesticides (OCPs), persistent organic pollutants (POPs), and PTEs; 3) Saimasai (S): soil collected near another obsolete pesticide storage facility (GPS: 43°27'19.5" N 77°18'51.5" E), serving as a second model site for OCP-, POP-, and PTE-contaminated soil; 4) Tekeli (TE): soil collected from the territory of the operating KazZinc JSC mining complex (GPS: 44°53'15" N 79°37'34" E), representing a site heavily contaminated by PTEs.

All soils contained Ni, Zn, and As at concentrations exceeding maximum permissible levels, while Cr levels were elevated in TA, K, and TE soils. TE soil was the most contaminated with respect to PTEs, with Zn and Pb concentrations reaching 1.08 ± 0.01 and 1.04 ± 0.03 g kg^{-1}, respectively. In contrast, K soil showed the highest organic contamination (∑ POPs = 3.77 ± 0.26 mg kg^{-1}), followed by S soil (∑ POPs = 2.09 ± 0.08 mg kg^{-1}).

Using the serial dilution method on five agar media (NA, TSA, PA, GYM, and YM), 242 microbial isolates were obtained: 73 from K soil, 65 from S soil, 63 from TA soil, and 41 from TE soil. All isolates were purified and subsequently screened for Zn-solubilising ability on solid medium (per L: glucose, 10 g; $(NH_4)_2SO_4$, 1 g; KCl, 0.2 g; K_2HPO_4, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; agar, 15 g; supplemented with 0.1% ZnO). Based on the solubilisation index (SI), 27 isolates were selected for further analysis. Zn solubilisation was categorised as weak (SI < 1.5), moderate (1.5 < SI < 2.5), or strong (SI > 2.5). Four isolates (S32, S37, S47, and TE32) showed strong solubilisation potential, 19 - moderate, and 4 - weak. Notably, despite the high number of isolates from K soil, only two cultures exhibited Zn solubilisation, whereas the highest proportion was found in S soil (15 isolates), followed by TE soil (7 isolates). Ultimately, 15 isolates (S12, S20, S21, S25, S32, S36, S37, S39, S40, S47, S56, TA5, TA15, TA33, and TE32) consistently demonstrated strong or moderate solubilisation potential and were selected for subsequent evaluation.

The next screening step assessed tolerance to Zn at concentrations of 0.07% and 0.14% (w/v) using Muller–Hinton broth. Growth was evaluated spectrophotometrically after 24 h of incubation at 28 °C and 140 rpm. At 0.07% Zn, eight isolates exhibited growth enhancement relative to the control (by 1.3–57.5%). At 0.14% Zn, seven isolates maintained growth inhibition below 50%, namely S21, TA5, S32, S36, S56, S39, and TE32, all of which also performed better at the lower Zn concentration.

Finally, tolerance to Zn, Cu, and Ni was tested in liquid medium at five concentrations (0.03, 0.06, 0.12, 0.24, and 0.48% w/v). Based on optical density results, three isolates demonstrated the most promising performance. S21 exhibited the highest tolerance to Zn, maintaining 39.8% growth relative to the control at 0.48% Zn. S56 showed strong growth at 0.12% Zn (85.9%) and 0.03% Cu (85.2%), while TE32 also grew under 0.03% Cu (51.4%), though less effectively than S56. Both S56 and TE32 displayed weak tolerance to Ni (0.02–0.06%), the most toxic element tested.

Further investigations will focus on evaluating the efficiency of immobilising selected microbial strains on the surface of waste-derived biochars, particularly those produced from unconditioned post-phytoremediation plant biomass, and on assessing the broader interactions between them.

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Proteomic Insights into the Adaptive Response of *Pseudomonas* sp. to PFOA Exposure

Medić, A.[1], Lješević, M.[2], Minić, Z.[3], Izrael Živković, L.[1], Gojgić Cvijović, G. [2], Karadžić, I.[1], Beškoski, V.[4]

[1]*Department of Chemistry, Faculty of Medicine, University of Belgrade, 11000 Belgrade, Serbia*
[2] *Institute of Chemistry, Technology and Metallurgy, University of Belgrade, 11000 Belgrade, Serbia*
[3] *John L. Holmes Mass Spectrometry Facility, Faculty of Science, University of Ottawa, Ottawa, Ontario, Canada*
[4] *Department of Biochemistry, Faculty of Chemistry, University of Belgrade,11000 Belgrade, Serbia*

E-mail of the presenting author: ana.medic@med.bg.ac.rs

Per- and polyfluoroalkyl substances (PFAS), especially perfluorooctanoic acid (PFOA), popularly known as "forever chemicals", represent a group of highly persistent environmental pollutants due to the stability of the carbon–fluorine bond [1]. As emerging contaminants, PFAS are increasingly detected in soil, water, and biota, raising concerns due to their bioaccumulation, toxicity, and resistance to natural degradation processes. Their long-term presence in the environment has been linked to adverse effects on microbial community structure, reduced ecosystem function, and disruptions in nutrient cycling. Despite their recalcitrance, certain microbial species, including *Pseudomonas* sp., have demonstrated the ability to degrade PFOA [2]. In this study, we investigated the proteomic response of a *Pseudomonas chlororaphis* subsp. *aurantiaca* isolated from PFAS-contaminated sediment during its growth on PFOA. The aim was to explore microbial mechanisms of adaptation and specificity of response to PFOA using a proteomics approach based on liquid chromatography–tandem mass spectrometry (LC-MS/MS), combined with bioinformatics analysis.

Shotgun proteomic approach based on comparative analysis of proteomes of cells exposed to PFOA versus non-exposed revealed significant changes in protein expression. Proteome analysis reflects profound changes in membrane and outer membrane structures, and is related to the altered lipid metabolism. Redox homeostasis, the tricarboxylic acid cycle, followed by respiration and energy metabolism, indicates fine metabolic adjustments in response to PFOA contamination. Downregulation of DNA-associated proteins suggested a shift towards stress adaptation at the expense of cellular replication and maintenance. Numerous down-regulated transporters suggest changes in the metal ion homeostasis and reduced metal transport systems. Proteomic changes at the level of virulence factors indicate changes in the mechanisms of both defense and protection. Besides, the changed activities of catalase, superoxide dismutase, laccase, lipase and peroxidase from *P. chlororaphis* indicate a strong influence of PFOA on vital enzyme activities.

This study provides new insights into the molecular mechanisms underlying microbial adaptation to PFAS contamination and highlights potential biomarkers of exposure.

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Seasonal dynamics of metal accumulation in *Chlorella sorokiniana* biomass from the urban photobioreactor LIQUID3

Dimitrijević, M.[1], Santrač, I.[1], Ćurić, V.[2], Kovačević, S.[1], Stanić, M.[1], Stojiljković, D.[1], Spasojević, I.[1]

[1]*Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia*
[2]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*

E-mail of the presenting author: milena.dimitrijevic@imsi.rs

Urban environments with high population density and intensive traffic are characterized by elevated particulate matter levels, which hinder conventional greening approaches. As an innovative solution, the urban photobioreactor LIQUID3 has been developed, integrating biotechnological functions of microalgae (*Chlorella sorokiniana*) in CO_2 sequestration, O_2 release, and particulate matter removal, with urban functions such as a bench, solar charger, and street lighting [1,2]. In addition to improving air quality, LIQUID3 produces algal biomass that can serve as an indicator of environmental quality. In this study, we analyzed the metal content in *C. sorokiniana* biomass from LIQUID3 photobioreactors located in different urban areas of Belgrade (Terazije and Makedonska Street) and Užice city center between April and July 2025. Samples were digested and analyzed by ICP-OES, and concentrations were expressed in µg/g dry biomass. The results revealed pronounced regional and seasonal differences. The concentrations were higher in Belgrade than in Užice, which is related to more intensive traffic, industrial surrounding and lower altitude of Belgrade. The concentrations in Belgrade were drastically higher in April compared to any other month, which may be a consequence of local heating system that still heavily relies on heating oil and coal. In June, Belgrade concentrations were still high probably in relation to traffic (Serbian legislation does not accommodate EU regulations for particle emission by vehicles). The concentrations dropped in July due to lower intensity of traffic during Summer. The findings indicate that *C. sorokiniana* biomass from LIQUID3 can serve as a bioindicator of seasonal and spatial dynamics of metal pollution in urban environments, while retaining the primary role of the system in CO_2 mitigation and biomass production. This dual functionality highlights the potential of LIQUID3 as a sustainable and multifunctional solution for improving environmental quality in cities.

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Potential of crosslinked P(MAA-co-PEGMA) hydrogel for pharmaceutical (fluoxetine) removal from aquatic environment

Aćimović, J.[1], Spasojević, P.[2,3], Popović, A.[4], Milićević, T.[1]

[1] University of Belgrade - Environmental Physics Laboratory, Institute of Physics Belgrade, National Institute of the Republic of Serbia, Belgrade, Serbia
[2] Innovation Center of Faculty of Technology and Metallurgy, Belgrade, Serbia
[3]University of Kragujevac - Faculty of Technical Sciences, Čacak, Serbia
[4]University of Belgrade - Faculty of Chemistry, Belgrade, Serbia

Email of the presenting author: acimovic@ipb.ac.rs

Pharmaceuticals are increasingly recognized as emerging pollutants due to their persistence, bioactivity, and potential to disrupt aquatic ecosystems [1]. Among them, the antidepressant fluoxetine is of particular importance because of its widespread use and reported ecotoxicological effects on fish, invertebrates, and algae. In addition, in silico toxicity assessment (ProTox II 3.0) predicted fluoxetine to be hepatotoxic, neurotoxic, and capable of inducing respiratory and immunotoxic effects, demonstrating its relevance as a pollutant of concern for both environmental and human health. Effective and sustainable removal strategies are therefore required to limit its environmental impact. In this study, we investigated a crosslinked poly(methacrylic acid-co-poly(ethylene glycol) methacrylate) [P(MAA-co-PEGMA)] hydrogel as a sorbent for fluoxetine remediation from water. The hydrogel structure combines ionizable carboxylate groups and flexible PEG chains, enabling both electrostatic interactions with the protonated amine of fluoxetine and hydrophobic association with its aromatic groups. Swelling studies confirmed favorable water uptake, while sorption experiments demonstrated near-complete removal of fluoxetine (~93%) from aqueous solution, starting from an initial concentration of 100 ppm.

These results indicate that P(MAA-co-PEGMA) hydrogels are a promising remediation material for mitigating fluoxetine contamination and contribute to the development of sustainable strategies for managing pharmaceutical pollutants in aquatic ecosystems.

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Wasp and Hornet Nests as Biomonitoring Tools: Elemental Composition and Temporal Trends

Pavlović, R.[1], Dolašević, S.[2], Goessler, W.[1]

[1] *Institute of Chemistry, Analytical Chemistry for Health and Environment, University of Graz, Graz, Austria*
[2] *Institute for Animal Husbandry, Belgrade, Serbia*

E-mail of the presenting author: ratko.pavlovic@stud.bio.bg.ac.rs

The rapid expansion of industrial and agricultural activities, combined with urbanization and traffic emissions, is leading to increased pollution [1]. Toxic metals are of particular concern, as they persist in ecosystems and pose risks to biodiversity and human health [2]. While social insects are increasingly used as bioindicators, their nests remain unexplored as biomonitoring matrices despite clear advantages as nests can be collected non-destructively after colony decline. Hornet *(Vespa crabro*) nests, which are built layer by layer during the active season, are of particular interest as they may capture temporal variations in contamination.

In this study, we compared the elemental composition of German wasp (*Vespula germanica*) and hornet nests collected at the same site, to determine which nests accumulate more potentially toxic metals and whether hornet nests can serve as seasonal recorders of environmental change. Element concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS). Wasp nests were analyzed as whole samples, while hornet nests were broken down into their structural components: base (B), outer envelope (OE), and successive comb layers (L1–L10).

Our results showed clear differences between species. Wasp nests contained significantly higher concentrations of most major and trace elements, including Li, Mg, Al, P, S, K, Ca, V, Fe, Co, Ni, Zn, As, Se, Rb, Mo, Ag, Cd, and Cs, suggesting a strong accumulation capacity. In contrast, hornet nests had lower overall concentrations but clear stratification between layers. For example, Na concentrations were higher in the late comb layers (L9) than in the early ones (L2–L4), Mn and Cu were more enriched in L1–L4 than in the outer envelope, Cd was elevated in L2 compared to the envelope, and Tl showed peaks in L5–L6. Boron was the only element that was consistently more abundant in hornet nests than in wasp nests, with particularly high values in L8.

These inter- and intraspecific differences are likely due to differences in nest construction materials [3-5], prey selection, and the accumulation of larval remains such as exuviae and feces. Both *V. crabro* and *V. germanica* collect carbohydrate-rich resources (nectar, honeydew, sap, ripe fruit) and protein- and lipid-rich prey (arthropods), but their dietary preferences and feeding behavior differ, which in turn affects nest composition.

Taken together, our results show that while wasp nests have a higher absolute load of metals, hornet nests provide a unique temporal resolution, by recording seasonal variations in environmental contamination. This dual value, high accumulation in wasp nests and temporal stratification in hornet nests, suggests that social insect nests provide an ethical, practical, and highly informative resource for biomonitoring toxic elements in terrestrial ecosystems.

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Bats as bioindicators: First insights into seasonal metal exposure and immune stress in bats from mining area

Arsenijević, S.[1], Spariosu, K.[2], Subotić, S.[1], Kostić, J.[3], Dimitrijević, M.[3], Višnjić-Jeftić, Ž.[3], Kovačević Filipović, M.[2], Burazerović, J.[1]

[1]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*
[2]*Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia*
[3]*Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia*

E-mail of the presenting author: e3001_2023@stud.bio.bg.ac.rs

Bats play a crucial role in ecosystems, and their long lifespan and preference for specific habitats make them effective bioindicators of environmental quality [1]. Interactions between metal exposure, immune response, and parasitism in bats are complex and species-specific, where metals can simultaneously weaken immunity and influence the risk of infection by various pathogens [2]. This study aimed to investigate the impact of seasonal variation and metal exposure on the immune status of bats, with a particular focus on the role of parasitic infections in modulating these relationships in a mining area. In Eastern Serbia, a region renowned for its rich geological heritage and diverse mining activities, we conducted a study using *Miniopterus schreibersii* as a model species. Bogovinska Cave is located 2 km from the Bogovina coal mine, while Sesalačka Cave served as a control site distant from direct mining activities. Non-lethal methods were used to collect 35 hair samples (~0.01 g) for metal analysis and blood from the intrafemoral vein for the preparation of blood smears. Romanowsky-stained blood smears were assessed microscopically, evaluating: (1) total leukocyte count, (2) leukogram including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and (3) presence of parasitic inclusions. Five metals, Cr, Fe, Li, Mn, and Zn, were detected in *M. schreibersii* hair using Inductively coupled plasma–optical emission spectrometry (ICP-OES). We used generalised linear models (GLM) to determine seasonal differences as an effect of metal concentrations and parasites on leukocyte count/distribution, and consequently, immune status. The mean concentrations of detected metals at the investigated sites were: Cr 5.763 mg/kg dw, Fe 143.382 mg/kg dw, Li 0.209 mg/kg dw, Mn 8.329 mg/kg dw and Zn 49.996 mg/kg dw. Although metal concentrations did not differ between sites, parasite composition varied: bats from the mine-exposed site harboured haemosporidians, while those from the control site carried microfilariae. The season had a significant effect on changes in the concentration of Cr, Fe, and Mn in hair, so the highest concentration of these metals was in spring. Significant positive correlations between metals (Cr-Fe, Cr-Mn, Fe-Mn, and Li-Zn) indicate potential common sources or accumulation mechanisms. Seasonal variations in neutrophils, lymphocytes, and monocytes depend on the presence of blood parasites and locality. An increase in the Cr concentration negatively affected the number of lymphocytes, which may indicate the immunosuppressive effect of this metal and the reduced ability of the body to respond to infections. Eosinophil counts did not differ between sites or between infections alone. However, when considering both metal concentrations and parasite type, eosinophils increased with rising metal levels (Cr, Fe, Mn, and Zn) in bats with haemosporidians (Bogovinska Cave), but decreased in bats with microfilariae (Sesalačka Cave), showing a significant interaction effect. These results do not demonstrate a direct causal link between metals and immune modulation, but indicate that bat immune responses reflect a complex interplay of environmental and biological factors. Future studies should adopt a multifactorial approach, integrating metal exposure, parasitism, and other ecological variables to better identify drivers of immune variation in wildlife. This first-of-its-kind study shows that *M. schreibersii* can serve as a seasonally sensitive bioindicator of metal exposure, with combined hair and leukocyte analyses providing insight into the long-term accumulation and potential health effects of mining pollution.

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Fish as bioindicators of reservoir pollution

Nikolić, D.[1], Skorić, S.[1], Tasić, A.[2], Smederevac-Lalić, M.[1], Rašković, B.[3]

[1]Department of Biology and Inland Waters Protection, University of Belgrade – Institute for Multidisciplinary Research, Belgrade, Serbia
[2]Department of Chemistry and Biochemistry and Drug Testing, Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia
[3]Institute of Animal Science, University of Belgrade – Faculty of Agriculture, Belgrade, Serbia

E-mail of the presenting author: dusan@imsi.rs

In this study we investigated the bioindication potential of the European perch (*Perca fluviatilis*) [1,2,3], European chub (*Squalius cephalus*) [4], and common roach (*Rutilus rutilus*) from reservoirs in Serbia with different anthropogenic influences and uses: water supply (Garaši) and power generation (Vlasina, Perućac, Zaovine, and Međuvršje). We determined the concentrations of 26 elements - PTEs (Ag, Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Sr, and Zn) in the muscle, gills, and liver using inductively coupled plasma optical emission spectrometry (ICP-OES), and 17 organochlorine pesticides – OCPs (Aldrin, α-HCH, β-HCH, γ-HCH, δ-HCH, 4.4′-DDD, 4.4′-DDE, 4.4′-DDT, Dieldrin, Endosulfan I, Endosulfan II, Endosulfan sulfate, Endrin, Endrin aldehyde, Heptachlor, Heptachlor epoxide, and Methoxychlor) in muscle using gas chromatography–mass spectrometry (GC-MS). These results were compared with histopathological (HP) analyses in gill and liver, and the potential health risks to humans from the consumption of fish meat were assessed. The target hazard quotient (THQ) and target cancer risk factor (TR) are used to calculate the human health risk associated with the non-carcinogenic and carcinogenic contaminants, respectively [7]. The results showed that the accumulation of PTEs varied by reservoir, species and tissue. Reservoirs used for power generation were under the highest anthropogenic pressure, and those at lower elevations were more polluted compared to reservoirs at higher elevations. Chub and roach were not good indicators of Pb pollution. In addition, roach were not good indicators of Cd and Cr pollution. The muscle tissue of all species showed the lowest metal contamination, while the highest was found in the gills. The concentrations of 14 OCPs were below the detection limit in all studied reservoirs. Heptachlor, 4.4′-DDD and 4.4′-DDE were seldom detected in the sampled fish. In all species, low to moderate HP alterations were detected in both the gills and the liver, which were not due to the accumulation of PTEs in these tissues. The concentrations of As, Cu, Pb, Zn, 4.4′-DDD, 4.4′-DDE, 4.4′-DDT, heptachlor, and heptachlor epoxide were below the maximum allowable concentrations in food [5,6]. The THQ and TR values showed a low and acceptable risk to consumers. The reservoir designated as a drinking water source can be considered the safest in terms of fish consumption.

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Determination of LD_{50} and sublethal effects of cypermethrin in *Myathropa florea (Linnaeus, 1758)*

Radenković M.[1], Kojić D.[1], Krivokuća N.[1], Purać J.[1], *WP2 WildPosh Consortium*, Skendžić T.[1], Vujić A.[1]

[1]Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

E-mail of the presenting author: milicar.0505@gmail.com

Pollinator populations are experiencing a decline in numbers, with pesticide use recognized as one of the main contributing factors. Hoverflies are rarely included in ecotoxicological studies [1]. In this study, we investigated the acute contact toxicity of cypermethrin in the hoverfly *Myathropa florea* by determining LD_{50} values and monitoring sublethal effects (paralysis, unusual behavior, reduced coordination) at 4, 24, 48, and 96 h post-treatment. Six doses were applied (0.414, 0.188, 0.085, 0.039, 0.018, 0.008 µg/fly). Males and females were tested separately, each individual treated by applying 2 µL of solution to the dorsal thorax. Lethal dose values (LD_{50}) were determined by probit and logit regression analyses using IBM SPSS Statistics 27.0. Probit and logit analyses gave similar LD_{50} values for *M. florea*: females 0.127 µg/fly (24h) and 0.035 µg/fly (48h), males 0.033 µg/fly (24h) and 0.010 µg/fly (48h). Males were more sensitive than females. Paralysis of individuals was observed even after 48 h post-treatment.

When compared to *Apis mellifera* (0.023 µg/bee) and *Bombus terrestris* (0.119 µg/bee) [2], *M. florea* was found to be more sensitive than both. The results demonstrate high susceptibility of *M. florea*, indicating its potential as an important bioindicator of pesticide toxicity. These findings highlight the need for further research on non-target insects and their integration into standardized risk assessment procedures.

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Towards the validation of an *in vitro* method to predict the bioavailability of soil organic contaminants

Mailfert, N.[1,2], Pelfrêne, A.[3], Louzon, M.[1], Soligot-Hognon C.[2], Huguet-Cizo, M.[2], Jurjanz, S.[2], Collas, C.[2]

[1]*Pôle Ecotoxicologie, ENVISOL, 38000 Grenoble, France*
[2]*L2A, Université de Lorraine, INRAE, F-54000 Nancy, France*
[3]*LGCgE, Université de Lille, IMT Nord Europe, Université d'Artois, Junia, 59000 Lille, France*

E-mail of the presenting author: n.mailfert@envisol.fr

For several decades, the presence of organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) or pesticides such as chlordecone (CLD) in ecosystems has raised significant concerns regarding environment and human health. Humans may be exposed to these substances through unintentional ingestion of soil (hand-to-mouth contact particularly common in children [1]), potentially leading to health hazards. Considering the total contaminant concentration in soil may lead to an overestimation of the risk, as only the bioaccessible fraction released into the digestive tract can be absorbed. Predicting the bioavailable fraction by estimating oral bioaccessibility is relevant for accurate risk assessment [2]. Bioaccessibility can be estimated by *in vitro* methods simulating human digestion, already available for trace metal elements (for ex. Unified Bioaccessibility Method). The PBET (Physiology Based Extraction Test) method simulates a stomachal digestion following by an intestinal digestion. Recently, an adaptation for hydrophobic organic contaminants has been suggested by adding Tenax (absorbent sink) in the intestinal phase, improving the extraction of organics liberated from soils (for ex. DDT pesticide [3], PCBs [4], PAHs [5]). The Ti-PBET (Tenax-improved PBET) method shows promising results, but its use may be limited by the high cost of Tenax. The general objective of our work was therefore to improve the Ti-PBET test in terms of both practicability and cost. We compared different adsorption supports for extracting PAHs and PCBs, and found XAD-2 performed comparably to Tenax while being more cost-effective; XAD-2 was therefore selected for subsequent test improvements. To simplify resin recovery after intestinal digestion, we tested two ways of incorporating XAD-2 into the *in vitro* assay: (1) enclosed in a tea bag; (2) freely dispersed within the flask. No significant differences were observed between the two types of control samples ($p > 0.05$), although certain compounds such as PCB 28 and fluorene showed higher chromatogram signals with the tea bag method. Although more convenient, this option was abandoned as it increased the risk of control contamination and compromised the accuracy of bioaccessibility estimates. We finally tested the Xi-PBET (XAD-2–improved PBET) method by spiking PCBs at 1, 5, 10, 50, and 200 ng/mL ($n = 3$) to assess the recovery rates of six congeners (PCB 28, 52, 101, 138, 153, 180) and GC–MS/MS quantification accuracy across this concentration range. Recovery rates ranged from 87–132% (CV ≤ 12%) between 5 and 200 ng/mL, while the 1 ng/mL concentration showed higher overestimation and variability (120–188%; CV ≤ 69%) likely due to low spiking volumes and background noise. These results confirm that 500 mg of XAD-2 effectively captures the entire free PCB fraction between 5 and 200 ng/mL without saturation of the resin, and that GC–MS/MS is reliable for this concentration range. This work has demonstrated that XAD-2 resin is a good alternative to Tenax for reducing the cost of bioaccessibility testing without compromising its effectiveness, making such testing more accessible to the contaminated sites and soils sector. However, *in vitro* bioaccessibility methods still require improvement to provide reliable estimates. Therefore, our future work will compare Xi-PBET against other *in vitro* assays using a wide range of soils contaminated with different pollutant families, and validate results against bioavailability measurements obtained from *in vivo* experiments on piglets (the best model to simulate children's physiology). The *in vitro* methodologies are expected to provide a valuable tool for site and soil managers in health risk assessment.

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Indirect human health risk assessment of consuming agricultural products influenced by PAHs from illegal landfills in agricultural areas

Mitić, J.[1], Relić, D.[2], Kojić, I.[3], Popović, A.[2], Vizi, A.[3], Milićević, T.[1]

[1] *Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*
[2] *Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*
[3] *Innovative Centre of the Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*

E-mail of the presenting author: jelenadj@ipb.ac.rs

Illegal landfills are widespread throughout Serbia, often found in agricultural areas. In addition to direct contact with contaminated soil, people can also be indirectly exposed through the consumption of agricultural products grown on such land. Elevated concentrations of pollutants directly in the soil and indirectly in the products may indicate a risk to human health. The most common pollutants originating from landfills are polycyclic aromatic hydrocarbons (PAHs), well known for their carcinogenic, teratogenic and mutagenic properties [1].

The soil organic layer was collected near 11 illegal landfills in agricultural areas. From each landfill, three samples were collected, approximately 5 m apart. Soil samples were prepared for PAH analysis using Soxhlet extraction (10 hours) with an azeotropic mixture of dichloromethane and methanol. Following extraction, the samples were purified on a silica gel column deactivated with 5% water, after which the aromatic fraction was analysed by Gas Chromatography-Mass Spectrometry (GC–MS). Sixteen polycyclic aromatic hydrocarbons (PAHs), identified as priority pollutants by the US EPA, were analysed [2].

The measured PAH concentrations were used to evaluate the indirect human health risk associated with the consumption of agricultural products cultivated on soil near illegal landfills. Due to the absence of Reference Dose (RfD) and Slope Factor (SF) values for some PAHs, the non-carcinogenic risk was estimated for acenaphthylene, anthracene, benzo[a]pyrene, fluoranthene, fluorene, naphthalene, and pyrene. Carcinogenic risk was assessed for benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene, and naphthalene. The non-carcinogenic risk to human health from consuming agricultural products grown in soil contaminated by illegal landfills is in the range 9.18×10^{-3} – 1.24×10^{-1}. These values were generally below 0.1, indicating a negligible level of concern. However, at one sampling location the value exceeded 0.1, suggesting a relatively higher exposure that may indicate further monitoring. Benzo[a]pyrene was the most significant contributor to the non-carcinogenic risk in all samples. The calculated carcinogenic risk ranged from 1.49×10^{-6} to 1.87×10^{-5}, indicating an acceptable carcinogenic risk according to commonly used health risk benchmarks [2]. Benzo[a]pyrene was also the predominant contributor to the carcinogenic risk, and along with dibenz[a,h]anthracene, showed elevated values in the majority of samples. The results of the indirect risk assessment indicated that one sampling site (*Kotež*) has a higher non-carcinogenic and carcinogenic risk compared to the others, suggesting potential higher contamination of agricultural products in that area. Although these values indicate a low or acceptable risk to human health from the consumption of agricultural products from the landfill area, they also highlight the need for further monitoring. It should be noted that the calculated risk may be underestimated due to the absence of RfD and SF values for certain PAHs. The current risk estimate considers only PAH concentrations in the analysed soil, while inclusion of metals and other POPs in future studies will enable a more precise overall risk assessment.

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First assessment of environmental genotoxicity using the the long-fingered bat *Myotis capaccinii* (Bonaparte 1837) as a bioindicator

Kostić, J.[1], Subotić, S.[2], Arsenijević, S.[2], Burazerović, J.[2], Dimitrijević, M.[1], Višnjić-Jeftić, Ž.[1]

[1]*University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia*
[2]*University of Belgrade, Faculty of Biology, Belgrade, Serbia*

E-mail of the presenting author: jkostic@imsi.rs

Coal remains a major energy source around the world, although it contains radioactive elements, metals, and organic compounds that can adversely affect the DNA of exposed organisms [1]. Bats, with their widespread distribution, are frequently impacted by environmental pollution, taking in contaminants through their diet, water, skin, and even by inhalation. They act as sensitive bioindicators, making them valuable for assessing environmental quality [2]. One such species, the long-fingered bat *Myotis capaccinii* (Bonaparte 1837) is recognized as endangered in Europe and it is strictly protected by the Serbian legislation. The aim of this study was to investigate the genotoxic response in *M. capaccinii* blood cells using the alkaline comet assay. It also sought to identify variations in response related to the age groups and sex of the bats, as well as the sampling season. Additionally, follow-up analyses were conducted to measure the levels of 11 elements (As, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, and Zn) in the bats' hair. The sampling was conducted in two caves in eastern Serbia, Bogovinska Cave and Sesalačka Cave, natural monuments and protected natural resources of III category, both located near coal mining activities. In 2024 during summer and autumn, 24 bats were studied and categorized into groups based on sex (male/female), age (juveniles/adults), and season (summer/autumn). For the analysis of elemental concentration, a total of 24 hair samples were collected from the dorsal side of the specimens and subsequently transferred into vials for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis. For the comet assay, 15 samples of capillary blood were obtained through a non-lethal technique from the intrafemoral vein and stored in 1 mL of cryopreservation medium in liquid nitrogen until analysis. The findings indicated that DNA damage was significantly higher in bats sampled during summer compared to their autumn counterparts. There were no statistically significant differences detected between sexes or age groups. A significant positive correlation was found between chromium concentrations and DNA damage, suggesting a potential link to environmental contamination, likely resulting from mining activities. Conversely, a significant negative correlation was observed between zinc concentrations and DNA damage, which may support its role as a protective agent against DNA damage and oxidative stress. This study is the first to utilize *M. capaccinii* as a bioindicator, and the results suggest that this species could be a useful indicator of the genotoxic potential in the environment.

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Badger (*Meles meles*) hair as a bioindicator of metals in the environment – the importance of methodological approach (washed vs. unwashed)

Ćosić N.[1], Kovačević, S.[2], Santrač, I.[2], Ćurić, V.[3], **Dimitrijević, M.[2]**

[1]Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade
[2]Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia
[3]Faculty of Biology, University of Belgrade, Belgrade, Serbia

milena.dimitrijevic@imsi.bg.ac.rs

Mammals inhabiting diverse habitats represent valuable bioindicators of heavy metal pollution, with hair as a non-invasive biological material offering special potential for biomonitoring [1,4]. In this study, we analyzed hair samples of road-killed badgers (*Meles meles*) from different regions of Serbia. For each animal, washed and unwashed hair samples were separated, and metal concentrations were determined using ICP-OES. Results showed that unwashed samples generally contained higher concentrations of metals such as Al, Fe, and Pb, indicating the dominant impact of external contamination (dust, traffic particles, soil) [2,3]. In contrast, washed samples better reflected internal metal accumulation, with Cu and Zn showing the smallest differences between treatments, suggesting their biological incorporation. Moreover, differences between sampling sites suggested the potential to identify local sources of contamination through this approach. This study highlights the importance of a carefully defined methodological protocol in mammalian biomonitoring. Comparing washed and unwashed samples allows differentiation between external contamination and biological accumulation, thus increasing data reliability. Therefore, badger hair can be considered a suitable biomonitoring material for assessing metal load and tracking environmental quality across regions.

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Seasonal Variability of PM_{10} Oxidative Potential: Insights from Nine Months of AA and DTT Measurements at Two Urban Locations in Serbia

Jovanović, M[1], Petrović, B[1], Živković, M[1], Davidović, M[1], Jovašević-Stojanović, M[1]

[1]VIDIS Centre, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11307 Vinča, Belgrade, Serbia

E-mail of the presenting author: majaj@vin.bg.ac.rs

Particulate matter (PM) represents the atmospheric contaminant most strongly associated with different adverse health outcomes. Accordingly, air quality monitoring networks routinely quantified PM_{10} and $PM_{2.5}$, as they have been metrics for regulatory standards. Nevertheless, such metrics do not capture the toxicological properties of the particles, which are crucial in understanding negative health effects. In this context, oxidative potential (OP) has been proposed as a promising indicator, reflecting the ability of PM to induce oxidative reactions within the respiratory tract and formation of reactive oxygen species (ROS). OP of PM, acknowledged as a new air pollutant metric relevant to human health, is now being incorporated into EU legislation on air quality monitoring and will be routinely reported from supersites starting in 2030 (European Parliament, 2024).

In this study, 24 h PM_{10} samples were collected using low-volume reference pumps at two source-specific locations: an urban background site in Belgrade (Ada Marina station) and an urban industrial site in Bor (Gradski Park station). Sampling was conducted simultaneously from June 2023 to February 2024 (117 samples at both sites). The oxidative potential of PM_{10} was quantified by applying dithiothreitol (OP^{DTT}) and ascorbic acid (OP^{AA}) assays (Dominuti et al., 2025; Calas et al., 2018).

The average concentrations of PM_{10} in Belgrade and Bor during the study period were very similar, 21.64 ± 13.72 μg m^{-3} and 24.31 ± 14.11 μg m^{-3}, respectively. Although the difference in the average values of PM_{10} was negligible between sites, the two applied assays exhibited different trends throughout the examined period. Namely, both OP^{DTT} and OP^{AA} values were higher during the cold, heating, period in Belgrade. Still, in Bor, the assays showed the opposite trend; OP^{DTT} had higher values during the cold, and OP^{AA} had twice the higher values in the warm, non-heating period. Additionally, the measured OP^{DTT} values were similar at both tested sites, while the OP^{AA} values were significantly higher in Bor. The examined OP^{DTT} and OP^{AA} datasets showed significant intercorrelation between the responses of the two assays ($r = 0.78$, $p < 0.01$) in Belgrade during the cold period and during both periods in Bor ($r = 0.49$ for cold, $r = 0.46$ for warm, $p < 0.01$). Based on Spearman correlation coefficients, it was noticed that the number of strong correlations for both assays and PM_{10} chemical species was lower during the warm than during the cold period for Belgrade. However, in Bor, an opposite situation was observed: a stronger correlation with chemical species was noted for DTT during the cold period and for AA during the warm period. During the cold period, strong correlations (above r=0.70) were observed with K, V, Rb, Cd, Sb, As, OC, and EC in Belgrade, the species mostly related to the traffic and biomass burning. On the other hand, in Bor, both assays showed moderate to strong (r=0.43-0.74) correlations with Cu, Zn, As, Se, Cd, Sb, P, OC, and EC, which indicates an influence from industrial activities combined with combustion and traffic-related sources.

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Poster presentations

Clean Air in Abruzzo? A Three-Year Overview of Monitoring and Biomonitoring Systems

Rosoni, D.[1], Bianco, S.[1], Bianchi, G.[2], Cotrozzi, L.[2], **Merlini, C.**[2], Nali, C.[2], Pedrelli, A.[2], Pellegrini, E.[2], Pisuttu, C.[2], Ricci, G.P.[2], Risoli, S.[2], Scimone, G.[2], Tonelli, M.[2], Lorenzini, G.[2]

[1]Regional Agency for the Protection of the Environment of the Abruzzo Region, Pescara, Italy
[2]Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

E-mail of the presenting author: c.merlini1@studenti.unipi.it

Traditionally, the evaluation of regulated ambient air pollutants relied exclusively on the use of physico-chemical analyzers. While these instruments are indispensable for accurately quantifying pollutant concentrations, they do not provide information regarding the biological impact of them. Their capacity to elicit engaging responses is markedly limited. Furthermore, their high acquisition, operation, and maintenance costs limit the feasibility of deploying a sufficiently dense monitoring network. In the case of the Abruzzo Region (Central Italy), which extends over 10,832 km^2 and comprises 305 municipalities with a population of 1,270,000, routine monitoring of tropospheric ozone (O_3) is currently conducted in only 16 municipalities. Given these limitations, the use of biological monitoring systems, particularly plant-based indicators, offers a cost-effective and scalable approach for assessing both the spatial distribution and potential biological impact of airborne pollutants. In this study, we discuss the results of a three-year integrated monitoring campaign conducted in the cities of Pescara and Chieti, and their surrounding areas, involving the use of miniaturized kits based of two-week-old O_3-hypersensitive tobacco germlings (*Nicotiana tabacum* L. cultivar Bel-W3) [1], along with four calibrated automatic analyzers from the regional air quality monitoring network managed by Regional Agency for the Protection of the Environment of the Abruzzo Region in accordance with European Directive 2024/12881/EU. All monitoring stations complied with the O_3 target value threshold for the protection of human health, defined as no more than 18 exceedances per calendar year (averaged over three years) of the maximum daily 8-hour mean (M8) O_3 concentrations above 120 µg m^{-3}. Conversely, all stations recorded O_3 concentrations exceeding the long-term objective for the protection of human health, regardless of the calendar year. During the study period, the M8 exceeded 100 µg m^{-3} on more than 3 days at all stations. At the rural background station of Sant' Eufemia a Maiella (SEM, Pescara province; 900 m a.s.l.), the long-term objective was exceeded on 18, 25 and 17 days in 2023, 2024, and 2025, respectively. These exceedances were already recorded within a single month, underscoring the severity of local O_3 levels. All cotyledons and the first true leaves of the Bel-W3 seedlings exhibited typical O_3-induced lesions [1] - when exposed to ambient air, at all monitoring stations and throughout the entire study period. The percentage of necrotic area was estimated by comparison with reference standards and expressed using two indices: the Cotyledonar Injury Index (CII) and the Leaf Injury Index (LII), both based on a 0-to-4 scale (0: no lesions; 4: more than 50% of surface covered by necrosis) [2]. The responses of the cotyledons and first leaves showed a linear relationship ($Y = 0.51X + 0.74$, $R^2 = 0.67$, $n = 274$). This implies that the threshold for visible injury to Bel-W3 seedlings - which is generally associated with 4-5 hours of exposure to O_3 concentrations above 25-30 µg m^{-3} [3] - was systematically exceeded, confirming that the observed O_3 levels were potentially capable of inducing visible damage to sensitive biological targets. The spatial distribution of the mean CII and LII values revealed that the biological effects of O_3 followed a clear northwest-to-southeast gradient. In the city center of Pescara, the lowest levels of O_3 phytotoxic effects were detected (mean CII +LII = 1.25 ± 0.49, averaged over three years). This pattern is consistent with what is typically expected for O_3 in urban areas, where O_3 depletion by nitrogen monoxide (directly emitted by vehicles) is higher in the most polluted zones (i.e., the central ones). The highest CII + LII values (1.50 ± 0.21, averaged over three years) were observed at the SEM station. This is noteworthy considering that Laffray et al. [4] documented that altitude (approximately 900 m a.s.l.) and climate induced variations in the responsiveness to O_3 of mature Bel-W3 leaves.

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Lichen collections for tracking environmental change and the problem of residual Hg contamination in herbaria

Guttová A.[1,*], Fačkovcová Z.[1], Petrini R.[2], Ghezzi L.[2], Di Dio M.[3], Agostini M.[3], Paoli L.[3]

[1] *Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia*
[2] *Department of Earth Sciences, University of Pisa, Pisa, Italy*
[3] *Department of Biology, University of Pisa, Pisa, Italy*

E-mail of the presenting author: anna.beresova@savba.sk

In this work lichen collections (*Lobaria pulmonaria*) kept in SAV and BRA were used to reconstruct six decades (1960–2022) of air quality changes in remote forests of Central Europe (the Western Carpathians). A retrospective analysis of major and trace elements (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, S, Sb, Zn) highlighted both historical and current background concentrations. The results revealed peaks of Potentially Toxic Elements (As, Fe, Hg, Mn, Ni, S, and Sb) up to 1989, with a progressive decline observed thereafter, particularly for Pb following the introduction of unleaded gasoline. Background element concentrations from up to 1989 were at least four times higher than actual levels. On the other hand, during the study we also considered the oldest collections (specimens from the 1920s), which were found contaminated with extremely high levels of Hg (up to 10 μg/g dw), and hence excluded from further analysis, as they likely reflected treatment with mercuric chloride ($HgCl_2$), commonly used in the past as an insecticide to protect herbarium collections. Such observations stimulated a further study of residual Hg indoor contamination inside herbaria. To deepen this topic, the fruticose lichen *Evernia prunastri* has been exposed for up to six months in the Herbarium of the University of Pisa (*Herbarium Horti Botanici Pisani*) and in parallel at the Natural History Museum, Bratislava (BRA). Mercury uptake, photosynthetic performances and chlorophyll content in the transplants were evaluated, together with real-time measurements of atmospheric Hg gathered by a multifunctional mercury analyzer (RA-915M), comparing outdoor and indoor environments.

Machine learning prediction of heavy metal accumulation in transplanted *Hylocomium splendens*: case study from Upper Silesia

Kosior Grzegorz[1], Ziembik, Z.[1], Dołhańczuk-Śródka A.[1], Kłos A[1],

[1]*Institute of Environmental Engineering and Biotechnology, University of Opole, ul. kard. B. Kominka 6, 45-032 Opole, tel: +48/781797137*

E-mail of the presenting author: grzegorz.kosior@uni.opole.pl

Moss biomonitoring is a widely used tool for assessing atmospheric pollution, yet the integration of advanced computational methods remains limited. In this study, we applied machine learning (ML) algorithms to predict the accumulation of trace elements (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn) in transplants of *Hylocomium splendens* exposed at 45 sites in Upper Silesia (southern Poland), one of the most polluted regions of Central Europe. The experimental data, originally collected through active biomonitoring and presented in [1], provided concentrations of trace metals in mosses, bulk precipitation, and dust deposition. We tested regression models including Random Forest and XGBoost with spatial cross-validation. Predictor variables included site category (industrial, urban, rural), relative accumulation factors (RAF), and deposition characteristics. Model performance was evaluated against empirical concentrations after 90 days of exposure. Explainable AI methods (SHAP values) were applied to identify environmental drivers of metal accumulation. The ML models successfully reproduced observed gradients of pollution, with the best predictions obtained for Pb, Cd, and Zn ($R^2 > 0.7$). Feature importance analysis highlighted site type and dust deposition as the most influential factors. The results demonstrate that machine learning can enhance the predictive power of moss biomonitoring, enabling the identification of "hotspots" of heavy metal deposition in regions lacking direct measurements. This approach provides a framework for combining traditional biomonitoring with modern data-driven techniques to improve environmental risk assessment and air quality monitoring strategies.

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Biomonitoring of microplastics with terrestrial plants – opportunities and challenges

Radojević, A.[1], Kalinović, T.[1], Kalinović, J.[1], Jordanović J.[1], Šerbula S.[1]

[1]*Department of Environmental Protection, Technical Faculty in Bor, University of Belgrade, Bor, Serbia*

E-mail of the presenting author: aradojevic@tfbor.bg.ac.rs

Due to the versatility and key role of plastics in various industries, including food packaging, their usage continues to increase yearly [1]. After using plastic products, they break down into small particles known as micro- (MPs) and nano-plastics (NPs). These particles have been detected in various environmental matrices, such as water, soil and air (including clouds) [2], which has raised global concerns regarding negative impacts on the environment and human health [3]. Because their degradation can take over 500 years [1], MPs are recognized as persistent anthropogenic contaminants [4]. Due to their unique characteristics, including a porous structure and a large specific surface [5], MPs play a significant role as carriers of pollutants, such as plasticisers, polycyclic aromatic hydrocarbons, and particularly potentially toxic elements (PTEs) [6]. As a significant environmental challenge, the focus of numerous studies is interaction of MPs and PTEs, and their biotoxic effects on various organisms [5]. While aquatic systems are considered the main sinks for plastic waste, researchers are highlighting the important role of terrestrial and soil systems in accumulating these pollutants [7]. Biomonitoring could provide a solution for long-term monitoring of MPs in the air as a simple, cost-effective, and non-invasive method [1]. However, one of the main problems is the lack of standardised sampling strategies and legislation concerning atmospheric MPs [5,8], compared to water sampling [9]. Additionally, a comprehensive base of biomonitors' application, bioavailability and bioaccumulation by plant in terrestrial systems need to be broaden [5,7]. Mainly, the studies focus on determining the number and shape of MPs, while mass-based information of polymers is less investigated, which are needed for assessing the fate of MPs in the environment [8]. Studies showed that in terrestrial plants, lichens are excellent biomonitors of atmospheric MPs in urban areas [10,11] as they have a high surface area to volume ratio and have a simple anatomy, as well as mosses [8]. Tree leaves are potential candidates for biomonitoring of MPs in the air [12]. Leaf characteristics, such as surface roughness, hydrophobicity, and the presence of a waxy coating, are important for the retention of MPs and concentration variability. Predicting MPs exposure hotspots is challenging due to degradation, organism ingestion, and biofouling that change characteristics and toxicity of MPs. Despite the significant increase in data regarding MPs occurrence across various environments/species, there is still a lack of experimental data on MPs uptake and effects in terrestrial ecosystems [13]. The lack of data for Serbia is also notable, indicating a need for extensive field research.

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Assessing air quality around a ceramic industry in Italy through vascular plant biomonitoring

Bianchi G.[1], Carli M.[1,2], **Cotrozzi L.[1,*]**, Pedrelli A.[1,3], Pellegrini E.[1], Pippi L.[1,4], Pisuttu C.[1], Ricci G.P.[1], Risoli S.[1], Scimone G.[1], Tonelli M.[1], Nali C.[1], Lorenzini G.[1]

[1]*Department of Agriculture, Food and Environment, University of Pisa, Italy*
[2]*Department of Agriculture Technologies, Food, Environment and Forestry, University of Florence, Italy*
[3] *Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy*
[4]*University School for Advanced Studies IUSS, Pavia, Italy*

**E-mail of the presenting author: lorenzo.cotrozzi@unipi.it*

Since autumn 2022, the Department of Agriculture, Food and Environment of the University of Pisa has been carrying out a research program commissioned to biomonitor air quality in the area surrounding a large-scale ceramic slab production plant located in Northern Italy. The study aimed to confirm over time the good air quality in the surrounding environment and the absence of harmful impacts on local ecosystems using standardized biological monitoring techniques.

A monitoring network was designed following the conceptual framework of the Italian ANPA guidelines and the international VDI standards. The network included both potentially impacted and reference sites: one station inside the plant (UCS7), one in the urban center (UCS6), one downwind extra-urban site (UCS8), one site exposed to vehicular traffic (UCS10), and two remote areas at different altitudes (UCS13 and UCS16; 481 and 840 m a.s.l., respectively). This systematic design ensured spatial representativeness and statistical robustness while allowing comparisons across environmental gradients.

Four biomonitoring methods were applied on a seasonal basis: (i) *Brassica oleracea* var. *acephala* (curly kale) for the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs), in accordance with VDI 3957/Part 3; (ii) *Gladiolus gandavensis* (gladiolus) for the assessment of fluoride (F) injury, according to VDI 3957/Part 14; (iii) *Lolium multiflorum* (Italian ryegrass) for the accumulation of F, sulfur (S), arsenic (As), cadmium (Cd), and lead (Pb), following VDI 3957/Part 2; and (iv) *Nicotiana tabacum* (tobacco) cvv. Bel-W3 and Bel-B for the assessment of ozone (O_3)-induced injury, following the conceptual framework of the Italian ANPA guidelines.

During the first year of the monitoring campaign, results showed that PAH accumulation in kale did not significantly differ among the six stations, with most values falling below detection limits or legal reference thresholds. Similarly, passive air sampling of volatile organic compounds and inorganic pollutants yielded concentrations largely below regulatory thresholds. F biomonitoring with gladiolus revealed significantly higher injury indices at the plant-internal station (UCS7) compared to remote sites. These localized effects are consistent with concentrations typical of the ceramic sector and do not indicate exceedances or widespread impacts. Ryegrass analysis indicated generally low levels of F and Cd, while S accumulation was higher at UCS7 and UCS8, pointing to potential plant responses linked to emissions or site-specific microclimatic conditions. Variability in As and F distribution suggested minor contributions from different anthropogenic sources.

Ozone monitoring with tobacco plants highlighted temporal rather than spatial variability, with injury symptoms peaking at the urban station (UCS6) during late-season exposures. However, measured O_3 concentrations remained well below European alarm thresholds. Meteorological data confirmed that variations in pollutant uptake were influenced by temperature, precipitation, and seasonality rather than by consistently high emission levels.

Overall, the integrated use of bioindicators confirmed the suitability of plant-based methods for characterizing air quality around industrial sites in complex landscapes. While most pollutants remained at low or negligible levels, the detection of localized signals for F and S warrants continued surveillance in the coming years. This approach demonstrates how standardized biomonitoring networks can provide reliable, spatially resolved information on air quality, supporting both environmental management and public health protection.

Ozone biomonitoring on top: a case study in the Italian Alps

Pisuttu, C.[1†], Desandré, C.[2†], Nali, C.[1], Panont, D.[2], **Pellegrini, E.**[1], Guarnieri, C.[2], Glarey, L.[2], Ferrarese, R.[2], Lorenzini, G.[1]

[1]*Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy*
[2]*Regional Environmental Protection Agency (ARPA) Valle d'Aosta, Saint Christophe, Italy*
† Equal contributions.

E-mail of the presenting author: elisa.pellegrini@unipi.it

Since 1962, *Nicotiana tabacum* L. cultivar Bel-W3 has been extensively employed as a reliable, highly sensitive and standardized bioindicator for tropospheric ozone (O_3), the most prevalent phytotoxic trace gases, also significant because of its impact on human health. This cultivar can be employed either at full maturity (about two months old) for two consecutive weeks, or at an early developmental stage with weekly replacement, as in Lorenzini's O_3 biomonitoring miniaturized kits [1]. However, this tobacco indicator system has not found application in areas located at certain altitudes because of the relatively little knowledge about plant response in harsh climatic and physiologic conditions; in particular, it was also found that at altitudes over 1,000 m a.s.l., the tobacco responses were highly reduced [2]. As most of these investigations were conducted nearly two decades ago, the present work re-examines the suitability of *N. tabacum* as a biomonitor for tropospheric O_3 at high altitudes, within the broader context of contemporary climate change.

With this purpose, a field campaign was conducted in Aosta Valley, a region in Northwestern Italian Alps, one of the main routes for trans-European transport between France and Italy. The valley also includes some industries, such as, metallurgy, food processing and tourism-related services. In accordance with the Regional Agency for Environmental Protection of Aosta Valley (ARPA–Aosta Valley), four biomonitoring sites were selected in the municipalities of Aosta and Courmayeur (urban sites; 580 and 1200 m a.s.l., respectively), Donnas and La Thuile (rural/remote sites, 300 and 1600 m a.s.l., respectively). Each station was equipped with a calibrated automatic analyzer (to monitor the distribution of ground-level O_3) and a meteorological unit. Between July and mid-August 2025, three consecutive cycles of weekly exposures were performed using miniaturized kits (n = 3, per site, each week). The O_3-resistant tobacco Bel-B was used to confirm the cause of injury on the sensitive Bel-W3.

Due to the valley's complex orography, each site showed distinct microclimatic conditions. Nevertheless, a clear increase in average daily maximum (T_{max}) and minimum (T_{min}) temperatures was observed. During the third week of exposure, T_{max} and T_{min} reached 30.0±3.3 °C and 15.1±1.8 °C in Courmayeur and 26.2±3.1 °C and 13.9±2.1 °C in La Thuile, significantly exceeding the previous two-decade averages (i.e., 24.4±0.6 °C and 11.9±0.3 °C°, 20.6±0.5 °C and 10.3±0.3 °C, respectively).

The maximum daily 8-hour mean (M8) O_3 concentrations increased over time: 42–46 ppb in the first week, 42–53 ppb in the second, and 53–59 ppb in the third. The highest daily averages (M1) peaked at 59 ppb in Donnas and 55 ppb in La Thuile, indicating that O_3 levels were higher in rural areas.
Cotyledon and first-leaf mortality was highest in Aosta and Courmayeur (9%), moderate in Donnas (5%), and lowest in La Thuile (<5%). Typical pathognomonic bifacial necrotic symptoms appeared within a few days in all the stations. Significant site differences in the Cotyledonous Injury Index (CII) emerged in the third week, with Courmayeur, Donnas and La Thuile showing values over twice those of Aosta. In contrast, the Leaf Injury Index (LII) remained consistently lower in Aosta and Courmayeur (–50% on average *vs.* Donnas and La Thuile). A strong CII–LII correlation was found only in La Thuile (R^2 = 0.68). CII showed strong correlations with O_3 metrics (M1 and M8) in Aosta and Courmayeur, while LII correlated well with M1 and M8 in Courmayeur (R^2 > .65), and La Thuile (R^2 > *.75*).

Based on these findings, this pilot study confirms that tobacco Bel-W3 remains a reliable bioindicator of tropospheric O_3 pollution even at elevations up to 1600 m a.s.l., demonstrating consistent sensitivity despite local microclimatic variability and current climate conditions. Overall, this biomonitoring approach represents a valuable tool for regional agencies to evaluate environmental health, including in Alpine settings. The educational, emotional, and engaging value of biomonitoring can therefore be leveraged even under these peculiar conditions.

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Biomonitoring of Toxic Metals Using Selected Conifer Species

Roganović, J.[1], Ilijević, K.[1]

[1] *Faculty of Chemistry, University of Belgrade, Studentski trg 12-14, 11000 Belgrade, Serbia*

E-mail of the presenting author: jovanaorlic@chem.bg.ac.rs

Conifer trees are particularly favorable for biomonitoring studies due to their morphological and physiological characteristics. Their needle-like leaves provide a large surface area relative to their volume, enabling the interception and accumulation of airborne pollutants. Toxic metals can be absorbed directly through the stomata, but they are also efficiently retained on the waxy leaf surfaces by deposition of aerosol particles containing trace metals. Because conifers are evergreen and do not shed their foliage annually, they allow the integration of contaminant loads over extended periods, making them especially suitable for long-term monitoring of atmospheric pollution. In addition, their wide geographical distribution and adaptability to different environmental conditions further enhance their value as biomonitoring species. Numerous studies have confirmed that conifer needles can serve as reliable bioindicators of both local and regional metal pollution, reflecting not only airborne deposition but also broader spatial and temporal patterns of environmental contamination [1,2].

Our research focused on three coniferous species most commonly found in the urban area of Belgrade: *Pinus*, *Abies*, and *Taxus*. Leaf samples were collected in spring, with the aim of obtaining both current-year shoots and one-year-old needles from the same trees. To better assess the influence of traffic-related pollution, sampling was carried out on trees located in close proximity to roads, as well as on individuals situated at greater distances from traffic. This approach enabled us to compare the extent of toxic metal deposition and accumulation between roadside and background sites, thus providing insight into the spatial variability of urban air pollution.

For ICP-OES analysis, conifer powder samples were digested a microwave oven ETHOS 1 (*ETHOS 1, Advanced Microwave Digestion System, Milestone, Italy*). Analysis of digested plant samples was carried out with an ICP-OES instrument (*iCAP 6500 Duo; Thermo Scientific, Loughborough, United Kingdom*). In total, 19 elements (Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Ag, Cd, In, Tl, Bi, and Pb) were quantified.

The results indicate clear differences in the accumulation patterns among the three conifer species. Pinus showed the highest affinity for sodium and silver, while Abies was characterized by pronounced accumulation of barium, strontium, and calcium. In contrast, Taxus showed accumulation of a wide range of elements, with noticeably elevated levels of manganese and consistently higher concentrations of various trace and toxic metals (e.g., Fe, Ti, Al, Cu, Zn, Cr, Sb, Bi). These findings suggest that pine can be considered a selective accumulator, fir a strong accumulator of alkaline-earth elements, whereas yew demonstrates the greatest potential as a general bioindicator of urban and traffic-related pollution.

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Assessment of atmospheric potentially toxic elements pollution in Kurchatov (former Semipalatinsk Test Site), Kazakhstan, using moss-bags technique

Nurkassimova, M.[1], **Omarova, N.**[1], Zinicovscaia, I.[2,3], Chaligava, O.[2,4*], Galustov, V.[2,5]

[1]L.N. Gumilyov Eurasian National University, Satpayev 2, 010008 Astana, Kazakhstan
[2]Joint Institute for Nuclear Research, Joliot-Curie 6, 141980 Dubna, Russia
[3]Horia Hulubei National Institute for RD in Physics and Nuclear Engineering, 30, Reactorului Str. 077125 Magurele (Ilfov), Romania
[4]Andronikashvili Institute of Physics, I. Javakhishvili Tbilisi State University, 6 Tamarashvili Str, 0162 Tbilisi, Georgia
[5]National Research Nuclear University MEPhI, 115409 Moscow, Russia

E-mail of the presenting author: chaligava@jinr.int

Active moss biomonitoring was applied to assess atmospheric pollution in Kurchatov Kazakhstan, an area with a long history of anthropogenic impact from nuclear testing and industrial operations. The content of 34 potentially toxic elements was determined in exposed and unexposed moss samples using a multi-method analytical approach employing instrumental neutron activation analysis, inductively coupled plasma atomic emission spectrometry, and a direct mercury analyzer. The content of elements in exposed moss samples was considerably higher compared to unexposed samples. The resulting data were used to calculate relative accumulation factor and contamination factor values, which showed significant enrichment of exposed mosses in Ce, Tb, La, Ta, Th, Sc, U, Fe, Cr, Co, Hf, Ba, V, Al, Mg, Na, Sb, W, Ni, Sr, Cu, Cl, Pb and Ca. Furthermore, the application of correlation analysis revealed distinct associations among determined elements and enabled the identification of their potential emission sources. This work establishes a baseline for atmospheric quality in a historically significant region and demonstrates the efficacy of moss bags for environmental monitoring in Central Asia

Effect of ferric ions on the UV-induced degradation of piroxicam in acidic aqueous solution

Petrović, A.[1], Milenković, M.[2], Korać Jačić J.[3].

[1] *Institute of Mining and Metallurgy, Bor, Serbia*
[2] *Department of General and Inorganic Chemistry, University of Belgrade - Faculty of Chemistry, Belgrade, Serbia*
[3] *Department of Life Sciences, University of Belgrade - Institute for Multidisciplinary Research, Belgrade, Serbia*

E-mail of the presenting author: aleksandra.petrovic@irmbor.co.rs

Piroxicam (PRX) is a nonsteroidal anti-inflammatory drug (NSAID) belonging to their oxicam class, widely recognized for its analgesic, antipyretic, and anti-inflammatory effects [1]. While PRX is effective in alleviating pain, inflammation, and stiffness associated with rheumatoid and osteoarthritis, its presence in environmental waters such as surface water, groundwater, drinking water, and effluents from wastewater treatment plants [2] become a growing ecological concern. Understanding the interactions between PRX and Fe^{3+} ions in aquatic environments is essential for developing effective removal strategies, particularly those based on advanced oxidation processes (AOPs) that can be initiated or enhanced by ultraviolet (UV) irradiation. In this study, the photodegradation of PRX in the presence of Fe^{3+} ions at pH 5 was investigated using UV-Vis spectroscopy and cyclic voltammetry (CV). Under acidic conditions, PRX is resistant to degradation by UVA and UVB irradiation. However, in the presence of Fe^{3+}, the formed Fe^{3+}-PRX complex becomes susceptible to oxidation by $HO^{\bullet}$ radicals generated through UV-induced photolysis of Fe^{3+}–OH^{-} complexes. These findings were confirmed by CV analysis, which implied that chelation with Fe^{3+} increases the oxidative susceptibility of PRX, as indicated by a shift in oxidation/anodic peak potentials to lower values. Finally, the toxicity of the photodegradation products was assessed using *Artemia salina*, providing insight into the potential ecological impact of the degradation process.

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Computational ecotoxicity assessment of fluoxetine as an emerging aquatic pollutant

Aćimović J.[1], Popović, A.[2], Obradović D.[1], Milićević T.[1]

[1] *University of Belgrade - Environmental Physics Laboratory, Institute of Physics Belgrade, National Institute of the Republic of Serbia, Belgrade, Serbia*
[2]*University of Belgrade - Faculty of Chemistry, Belgrade, Serbia*

E-mail of the presenting author: acimovic@ipb.ac.rs

Pharmaceuticals such as fluoxetine are increasingly recognized as emerging pollutants of concern due to their persistence in aquatic ecosystems and potential to disrupt biological processes [1]. In this study, ecotoxicity predictions were performed using QSAR computational models in VEGA (Fish Acute, *Daphnia* Acute, Algae Acute, Chronic NOEC, Zebrafish embryo) and ECOSAR class-based estimations. QSAR approaches aim to correlate molecular structure and physicochemical properties with biological activity or toxicity through statistical modelling [2].

QSAR VEGA models predicted acute toxicity to fish with LC_{50} values ranging from 0.8 to 4.1 mg/L (NIC model outlier: 18.6 mg/L). *Daphnia magna* was consistently identified as more sensitive, with acute LC_{50}/EC_{50} values between 0.07 and 0.33 mg/L, and one model giving 1.1 mg/L. Algal EC_{50} values were approximately 0.65 mg/L. Chronic NOEC predictions were 0.055 mg/L for fish and ~0.31 mg/L for daphnids and algae, indicating potential long-term risk. Zebrafish embryo predictions indicated an AC_{50} of ~0.9 mg/L.

ECOSAR results were in agreement, with fish LC_{50} values around 1.1 mg/L, Daphnia LC_{50} values of 0.18–0.77 mg/L, and mysid LC_{50} at 0.17 mg/L. A terrestrial endpoint (earthworm, 14 d LC_{50}) was far less sensitive at 286 mg/L, confirming aquatic organisms as the primary receptors of concern and potentially indicators of aquatic ecosystem pollution of pharmaceuticals. Based on these results, *Daphnia magna* would be the best bioindicator, followed by algae and zebrafish.

According to Globally Harmonized System (GHS) criteria, fluoxetine falls within the Category 1 - very toxic to aquatic life (LC_{50} ≤ 1 mg/L) based on multiple independent model outputs. These findings classify fluoxetine as a compound of ecotoxicological concern and identify Daphnia magna, followed by algae, zebrafish and fish models, as potential indicators for assessing pharmaceutical pollution in aquatic systems. This supports the prioritization of the experimental validation, and demonstrates the utility of computational approaches in early-stage environmental risk assessment of pharmaceuticals in the aquatic environment.

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Protective role of Fe^{3+} ions against the photodegradation of meloxicam

Petrović, A.[1], Milenković, M.[2], Korać Jačić J.[3].

[1] *Institute of Mining and Metallurgy, Bor, Serbia*
[2] *Department of General and Inorganic Chemistry, University of Belgrade - Faculty of Chemistry, Belgrade, Serbia*
[3] *Department of Life Sciences, University of Belgrade - Institute for Multidisciplinary Research, Belgrade, Serbia*

E-mail of the presenting author: aleksandra.petrovic@irmbor.co.rs

Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide), a nonsteroidal anti-inflammatory drug (NSAID) of the enolic acid class was selected for therapeutic use due to its high anti-arthritic activity and broad-spectrum of anti-inflammatory effects in animal and cells models [1]. The widespread use of meloxicam (MLX) has led to its notable accumulation as a pollutant in aquatic and terrestrial environments, highlighting the urgent need for effective water treatment strategies to ensure its degradation and removal [2]. A detailed understanding of the coordination behavior and photochemical properties of MLX in the presence of ferric ions can offer valuable insights for enhancing iron-based advanced oxidation processes (AOPs) in wastewater treatment. In this study, chelation of MLX with ferric ions and effect UV irradiation on MLX in the presence of ferric ions were examined using UV-Vis spectroscopy at pH 5. In the presence of Fe^{3+} ions at pH 5, a coordination reaction occurs between the Fe^{3+} ions and MLX, probably resulting in the formation of the Fe^{3+}-MLX complex. UV A and UV-B radiation can lead to the degradation of MLX. Additionally, the results show that in the presence of Fe^{3+} ions, the photodegradation effect is weaker compared to MLX alone. In other words, the Fe^{3+} ions form a complex with MLX and protect it from photodegradation. Therefore, the removal of MLX by Fe(III) photolysis is not the method of choice. The toxicity of MLX and its degradation products was ultimately evaluated using *Artemia salina* as a test organism, providing valuable insights into the potential environmental impact of the degradation process.

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Phytoremediation Potential for Monitoring and Removing Perfluorooctanoic Acid from Aquatic Environments

Lugonja, N.[1], Lješević, M.[1], Lončarević, B.[1], Žeradjanin, A.[1],

Joksimović, K.[1], Kasalica, K.[1], Beškoski, V.[2]

[1]University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Belgrade, Serbia
[2]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia

E-mail of the presenting author: nikoleta.lugonja@ihtm.bg.ac.rs

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that pose a significant risk to ecosystems and human health [1,2]. Among them, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are the most studied due to their persistence, toxicity, bioaccumulative potential, and presence in the food chain. Conventional methods for PFAS removal from the environment are often expensive, energy-intensive, and not very effective, leading to growing interest in sustainable and eco-friendly techniques such as phytoremediation. Phytoremediation involves using plants to absorb, degrade, or stabilize pollutants in soil and water. This study investigates the potential of selected plants for monitoring and phytoremediation of PFOA contamination in aquatic environments. Hydroponic experiments were conducted using four different plant species cultivated in a nutrient solution containing 0.1 mg/L of perfluorooctanoic acid (PFOA). At the end of the cultivation period, stems and roots of the samples were measured, and the presence and concentration of PFOA in plants were determined. Chlorophyll content, the Normalized Difference Vegetation Index in the leaves, and the activity of oxidative stress-related enzymes were monitored to assess the physiological responses of plants to PFOA exposure. The results revealed species-specific responses, with notable changes in spectral properties and chlorophyll content, indicating their potential use as bioindicators for PFAS pollution. Elevated activity of antioxidant enzymes suggests the activation of defense mechanisms that may contribute to PFAS transformation or degradation. These findings support the dual role of selected plants in environmental monitoring and remediation, contributing to the development of sustainable strategies for PFAS management in aquatic environments.

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Heavy metals – environmental river water risk

Sekulić, Z.[1], Pisinov, B.[2], Keškić, T.[3], Špoljarević, M.[4], Milićević, T.[5]

[1]Department for Human Ecology and Ecotoxicology, Institute of Public Health of Belgrade, Belgrade, Serbia
[2]Department of Phytopharmacy and Environmental Protection, Institute for Plant Protection and Environment, Belgrade, Serbia
[3]Department of biomedicine, technology and food safety research and development, Institute for Animal Husbandry, Belgrade, Serbia
[4]Department of Agroecology and Environment Protection, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[5]Environmental Physics Laboratory, Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

E-mail of the presenting author: zoran.sekulic@zdravlje.org.rs

The constant threat of hazardous substances in water recipients caused by anthropogenic activities require continuous monitoring of river water quality in densely populated regions. In accordance, one of the obligations of the Belgrade City Institute for Public Health is the systematic monitoring of rivers within the Belgrade region.

The generated database spans many years in past; however, for the purposes of this study, a representative dataset was extracted, covering the period from the beginning of 2021 to June of 2025. A total of 896 water quality parameters were successfully analyzed for the observed period.

Specifically, by monitoring the Danube and Sava rivers and their tributaries at 30 measuring locations within the Belgrade region, over 200 environmental parameters were tracked. From these, 22 representative water quality indicators and 13 heavy metals (Al, As, B, Cd, Cr, Cu, Fe, Hg, Mn, Na, Ni, Pb and Zn) were selected per monitoring site, resulting in 990 annual measurements and a total of 3960 results.

These data were used to train artificial neural networks and subsequently applied to predict heavy metal pollution in 2025 [1-2]. For the purposes of this research, the most commonly used neural network models were GRNN (General Regression Neural Network) and BPNN (Backpropagation Neural Network), trained using monitoring data collected between 2021 and 2024 and were subsequently applied to predict future pollution trends.

The predicted results for the first half of 2025 were compared with actual measurements, achieving a matching index of 22% for GRNN and 26% for BPNN. Statistical analysis showed that additional descriptive input parameters should be included in the training process to improve prediction accuracy. Since the current findings are satisfactory, future research will focus on expanding the dataset to include additional groups of indicators, such as biological and microbiological parameters, to enhance the precision and robustness of the predictive models.

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Spruce (*Picea abies* L.) and Douglas-fir (*Pseudotsuga menziesii* Mirb.) as bioindicators: Field study of heavy metal uptake

<u>Milošević, Ivana R.</u>[1], Živković, Sanja[2], Momčilović, Miloš[2], Višnjić-Jeftić, Željka[3], Veselinović, Milorad[4], Marković, Ivana D.[5], Marković, Dragan M.[1]

[1]Institute of Physics Belgrade, University of Belgrade, Belgrade, Serbia
[2]Institute of Nuclear Sciences Vinča, University of Belgrade, Belgrade, Serbia
[3]Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia
[4]Institute of Forestry, Belgrade, Serbia
[5]Worldwild Clinical Trials, Belgrade, Serbia

E-mail of the presenting author: novovic@ipb.ac.rs

Urban growth and development have introduced many pollutants - such as trace elements, pesticides, and other compounds - that remain in soil and threaten ecosystems and human health. Trace elements are of particular concern because they are toxic, persistent, and tend to be built up in plants, animals, and the environment [1]. Vascular plants, particularly trees, are useful indicators of such pollution. Parts like leaves, bark, seeds, and tree rings are often studied to track contamination. With their large biomass, long lifespan, and strong roots, trees can take up and stabilize trace elements through phytoextraction, helping to keep them from moving deeper into soil and groundwater [2].

To explore this, we carried out two experiments (I and II) focusing on spruce (*Picea abies* L.) and Douglas-fir (*Pseudotsuga menziesii* Mirb.) seedlings. The seedlings were subjected to elevated amounts of lead, strontium, cobalt, and nickel by adding them to the soil. Our field-based approach enabled us to study how these metals accumulate under natural conditions. Using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES), we quantified the concentrations of these metals, along with manganese and zinc, in the soil, wood, and bark. The analysis revealed that both tree species accumulated the added metals, with particularly pronounced increases observed in Douglas-fir [3]. In Douglas-fir wood, lead levels rose to 7 times the control in experiment I and 4 times in experiment II, while nickel concentrations reached 18 and 10 times higher than the control in the respective experiments. These results highlight the capacity of Douglas-fir wood to accumulate and stabilize lead and nickel, indicating its potential role in phytostabilization strategies.

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Spatiotemporal Comparison of Environmental Dust and Apicultural Samples Across Urban and Industrial Landscapes

Roganović, J.[1], Zarić, N.[2], Pavlović, R.[1], Vujanović, D.[3], Orčić, S.[4], Purać, J.[4], Kojić, D.[4], Čelić, T.[4], Blagojević, D. P.[5], Ilijević, K.[1]

[1] Faculty of Chemistry, University of Belgrade, Studentski trg 12-14, 11000 Belgrade, Serbia
[2] University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia;
[3] University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia
[4] University of Novi Sad, Faculty of Sciences, Trg D. Obradovića 3, 21000 Novi Sad, Serbia
[5] University of Belgrade, Institute for Biological Research "Siniša Stanković", Bulevar despota Stefana 142, 11060 Belgrade, Serbia

E-mail of the presenting author: jovanaorlic@chem.bg.ac.rs

The study explored concentrations of 16 elements in samples collected from 3 different environments: rural (Golija mountain), urban (Belgrade city) and industrial (Zajača town) during summer and winter to evaluate seasonal variations and pollution-related differences. Macroelements (Ca, K, Mg, Na), microelements (Cu, Fe, Mn, Zn) and non-essential elements (Al, Ba, Cd, Co, Cr, Ni, Pb, Sr) were determined in environmental dust and various honey bee-related samples: honey bees, hemolymph, pollen, honey stomach, bee bread, larvae, pupae, honey, propolis, and wax.

The samples were digested in closed microwave digestion system (ETHOS 1, Advanced Microwave Digestion System, Milestone, Italy) using 5 to 8 ml of concentrated HNO_3 and 1 or 2 ml of concentrated H_2O_2. Concentrations of: Al, Ba, Cd, Co, Cr, Cu, Ca, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn were determined by ICP-OES (iCAP 6500Duo, Thermo Scientific). Very low concentrations of: Co, Cr, Cd and Pb, were additionally tested using ICP-MS (iCAP-Q-ICP-MS, Termo Scientific).

Dust samples exhibited higher concentrations of most metals during winter, which is related to increased fossil fuel combustion in this period [1]. Pb and Cd were particularly high in industrial dust across both seasons. Honey bee and hemolymph samples showed elevated levels of Na, K, Sr, Ca, Ba, Mg, Fe, Ni, Pb, and Al during summer, as a consequence of increased bee activity and exposure. Exceptions to this pattern were observed with: Zn, Mn, Cu, and Cd. These elements showed elevated concentrations in bees or hemolymph during winter, especially in industrial areas.

Relative concentrations of elements in different samples revealed that dust, propolis, and wax can accumulate metals at levels far higher than those in bees. Honey had the lowest concentrations of toxic elements, which is probably consequence of protective mechanisms in bees [2]. The industrial environment had high I/U (industrial-to-urban) ratios for toxic metals (Pb, Cd, Mn) and reduced bioelement content in honey, hemolymph, larvae, and pupae.

The results suggest impairment of physiological processes in bees as consequence of metal exposure [3]. Strong seasonal and environmental influences on metal accumulation patterns were observed. Bees and their products can serve as sensitive indicators of local pollution, while the distribution of bioelements versus toxic metals provides insight into sublethal stress and colony-level detoxification strategies.

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Evaluating the human health risk from consuming plants growing in the metal(loid)-contaminated mine waste site

Ranđelović D.[1], **Mlićević T.[2]**,

[1]Sector for metallurgical technologies and environmental protection, Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia
[2]Environmental Physics Laboratory, Institute of Physics Belgrade, University of Belgrade, Belgrade, Serbia

E-mail of the presenting author: tijana.milicevic@ipb.ac.rs

The area of the Bor mining complex is considered to be one of the most polluted areas in Serbia. Long-term mine activities left historical pollution and significant amounts of deposited mine wastes. The old mine tailing site in Bor was formed by applying non-selective disposal of extracted rocks, resulting in heterogeneity of physico-chemical conditions that hindered the full development of vegetation coverage.

In order to assess the health risk of As, Cd, Co, Cu, Mn, Ni, Pb, and Zn transfer to biota and further by the consumption at given area, their concentration from plant rhizosphere area of soils developed on three main distinguished lithological groups present at the old mine tailing site (andesite-A, altered andesite – AA, and fine-grained material – FM) is accessed. The plant bioavailable fraction of metal(loid) is considered to be a more reliable parameter for indirect health risk assessment by biota consumption than total metal(loid) concentration [1]. The non-carcinogenic (THQ) and carcinogenic (CR) risks were determined for consuming a biota grown on the investigated soil, based on the plant bioavailable soil fraction (Na_2EDTA extracted). The indirect models for biota consumption assessment based on the soil concentration have been developed by the U.S. Department of Energy (DOE), Oak Ridge Operations Office (RAIS). Results show that THQ_{sum} risks exceeded 1 for soils from all investigated lithological groups (median values: $THQ_{sum}A=29.02$, $THQ_{sum}AA=2.94$ and $THQ_{sum}FM=3.53$), indicating the existing non-carcinogenic risk during long-term consumption of plants grown on the investigated sites. Additionally, carcinogenic risk to biota is present for all investigated sites as CR >1 × 10^{-6} (median values: $CR_{sum}A$= 0.0023, $CR_{sum}AA$= 0.00018, and $Cr_{sum}FM$= 0.00018).

Results of health risk assessments applied to biota consumption in this area may be used in the decision-making process for evaluating the adequate reclamation route and for decision-making when switching to selective waste disposal practices. In this case, due to the increased health risk at all main lithological groups present at the investigation site, agricultural reclamation route involving growing of different plant categories for human and cattle consumption, such as crops, palatable and medicinal plants, should be avoided prior to soil remediation or use of metal-accumulating plants for lowering the soil metal(loid) content in this area.

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Accumulation of trace elements in the grain of two varieties of organically grown spelt (*Triticum spelta*)

Brdar, J.[1], Roje, V.[2], **Popović-Djordjević, J.[1]**, Kostić, A.Ž.[1], Roljević Nikolić, S.[3], Dolijanović, Ž.[1]

[1] University of Belgrade, Faculty of Agriculture, Belgrade, Serbia
[2] University of Zagreb, Faculty of Forestry and Wood Technology, Zagreb, Croatia
[3]"Tamiš" Research and Development Institute, 33 Novoseljanski Put, 26000 Pančevo, Serbia

E-mail of the presenting author: jelenadj@agrif.bg.ac.rs

Cultivation of alternative small grains, especially spelt, is the basis of organic field production. The areas are constantly increasing, and modernized cultivation technology, attributed primarily to the increased supply of organic and microbiological fertilizers, enables the achievement of ever-better yields, both in terms of quantity and quality. Spelt (*Triticum spelta*) is one of the old types of wheat, which nowadays is becoming interesting again due to its favorable agronomic and nutritional characteristics. Due to its chemical composition, resistance and good adaptation to wide cultivation areas, this species plays a leading role in organic field production, accounting for about 50% of the area under organic cultivation. There is an increasing demand for safe and high nutritional products. Trace elements like Co, Cu, Mn, Mo, Fe, and Zn are essential for organisms, and wheat is a good source of these elements [1].

In this study the potential of two spelt varieties (Ostro and Nirvana), that were organically grown, to accumulate trace elements was analyzed. The research was conducted as three-factorial experiment, based on a randomized complete block design with four replications. The first factor - two varieties Ostro and Nirvana; second factor - two sawing periods (1st - mid-October and 2nd -early November); third factor - NPK organic fertilizer of animal origin (Guanito) and microbiological fertilizers (UniMax and Slavol). Multi-elemental characterization of the spelt and soil samples was carried out by means of inductively coupled plasma – optical emission spectrometry (ICP-OES) after microwave-assisted wet digestion with acidic reagents. 13 trace elements (Al, As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Tl and Zn) were selected for quantification in both grain (20 samples) and soil (10 samples) [1]. Bioaccumulation factor (BF) was calculated as the ratio between the concentrations (expressed in mg/kg) of an element in the grain (C_G) and soil (C_S), using equation $BF=C_G/C_S$ [2].

As, Co, Cr, Pb, Se and Tl were found to be below their method detection limit (MDL) values in the grain samples, while As, Cd, Mo, Pb, Se, and Tl were below their MDLs in soil. The bioaccumulation factor was calculated for Al, Cu, Mn, Ni and Zn, as these elements were quantified in both grain and soil. Mn and Cd were quantified in the highest and lowest concentration in grain, while Al and Co had the highest and lowest concentrations in soil, respectively.

Cd was quantified in the grain of both varieties, but it was below MDL in soil. The highest concentrations of Al and Cu, in the grain of both varieties, were observed for samples sown in the early November; Al - Ostro treated with Guanito and Nirvana treated with combination of Guanito, UniMax and Slavol; Cu - in samples of both varieties treated with combination of all 3 fertilizers; For Mo and Zn opposite trend was observed. The samples of both varieties sown in the mid-October had highest concentrations of Mo and Zn these elements; Ostro treated with Unimax and Nirvana control had the highest concentrations of Mo, while Ostro control and Nirvana treated with Guanito had highest concentrations of Zn.

The highest values of bioaccumulation factor (BF) in both Ostro and Nirvana were observed for Zn, followed by Cu, Mn, Ni and Al. Ostro treated with Guanito (sown in the mid-October) had highest potential to accumulate Zn (1.337) Cu (0.391), Mn (0.111) and Ni (0.015). On the other hand, Nirvana (control and treated with different fertilizers) sown in the early November had highest BF values for Zn (0.709), Cu (0.268), Ni (0.011) and Al (0.006). Ostro treated with Guanito and sown in both sowing periods expressed highest accumulation potential for Zn (>1). The obtained results indicate that all applied factors have influenced the accumulation of trace elements in the grain of the investigated spelt varieties. Moreover, the varieties did not show tendency to accumulate toxic elements.

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Mitigating wastewater irrigation stress in maize: The synergistic role of biofertilizers and biochar

Shirzad H.[1], Siavash Moghaddam S.[2,1], Rahimi, A.[1], Rezapour, S.[3], Xiao, J.[4], **Popović-Djordjević, J.**[5]

[1] *Department of Plant Production and Genetics, Faculty of Agriculture, Urmia University, Urmia, Iran*
[2] *Department of Plant Production and Genetics, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran*
[3] *Department of Soil Science, Faculty of Agriculture, Urmia University, Urmia, Iran*
[4] *Department of Analytical Chemistry and Food Science, Faculty of Food Science and Technology, University of Vigo, Vigo, Spain*
[5] *University of Belgrade-Faculty of Agriculture, Belgrade, Serbia*

E-mail of the presenting author: (jelenadj@agrif.bg.ac.rs)

Toxic metals are significant environmental pollutants, posing serious threats to ecosystem health, food safety, and plant physiology [1]. This study investigated the potential of integrated soil amendments to mitigate the adverse effects of wastewater irrigation on maize (*Zea mays* L.), a globally vital cereal crop. A factorial pot experiment employed a randomized complete block design with three replications. The treatments consisted of two irrigation sources (regular water vs. wastewater) and fertilizer regimes: control, bacterial NPK, arbuscular mycorrhizal fungi (AMF), *Trichoderma harzianum*, biochar, bacterial NPK + AMF + *Trichoderma harzianum*, bacterial NPK + AMF + *Trichoderma harzianum* + biochar. All amendments were applied to the soil prior to sowing. Wastewater was contaminated with the toxic metal cadmium. The results demonstrated that wastewater irrigation significantly reduced biological performance and photosynthetic pigment content (chlorophyll a and b) compared to regular water irrigation. However, the application of organic and biofertilizers ameliorated these negative effects, enhancing plant growth and chlorophyll content under both irrigation conditions. Furthermore, wastewater irrigation decreased the grain content of oleic and linoleic acids. The fertilizer treatments differentially influenced fatty acid composition; for instance, under regular irrigation, biochar and AMF increased oleic acid content, while other treatments decreased it. Under wastewater stress, AMF and bacterial NPK reduced oleic acid levels, whereas the other amendments increased it. The application of bio- and organic fertilizers increased seed linoleic acid content compared to the control, under both regular and wastewater irrigation conditions. In conclusion, the application of biofertilizers and biochar presents a viable strategy to enhance maize resilience and improve key quality markers under toxic metal stress induced by wastewater irrigation.

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Metal exclusion and physiological plasticity shape orchid success across diverse geologies

Jakovljević, K[1]., **Mišljenović, T.[2]**, van der Ent, A[3]., Mutić, J.[4], Ranđelović, D.[5], Andrejić, G.[6], Šinžar-Sekulić, J.[2], Vesović, M.[2], Vujičić, M.[2], Djordjević, V.[2]

[1]Department of Ecology, Institute for Biological Research Siniša Stanković´, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia
[2]Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Belgrade, Serbia
[3]Laboratory of Genetics, Wageningen University and Research, Wageningen, The Netherlands
[4]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia
[5]Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia
[6]Institute for the Application of Nuclear Energy – INEP, University of Belgrade, Belgrade, Serbia

E-mail of the presenting author: tomica.m@bio.bg.ac.r)

Edaphic stress is a major factor influencing plant fitness, yet it remains insufficiently understood, especially in rare species like orchids. Orchids, with their complex ecological requirements and sensitivity to soil chemistry, provide valuable models for studying adaptations to contrasting geologies. We investigated the ecophysiological responses and elemental accumulation strategies of two terrestrial orchids — *Gymnadenia conopsea* and *Dactylorhiza sambucina* — growing on carbonate, ultramafic, and siliceous substrates in the Central Balkans. Despite differences in phylogeny and ecology, both species showed strikingly similar patterns of elemental partitioning. Macroelements (Ca, Mg, K, and P) consistently accumulated in the above-ground organs, particularly in leaves and inflorescences, while most trace elements (Cr, Co, Ni, Fe, and Mn) were retained in the roots, indicating an exclusion-based tolerance strategy. However, *D. sambucina* exhibited a notable capacity for Zn and Cu accumulation in shoots, suggesting species-specific flexibility in metal handling. Physiological analyses revealed that individuals from carbonate soils exhibited the lowest stress levels, as indicated by higher photosynthetic pigment concentrations, whereas plants from ultramafic sites showed elevated antioxidant activity and accumulation of phenolics and lipid peroxidation products, reflecting a stronger stress response. These findings demonstrate that exclusion remains the dominant metal tolerance mechanism in terrestrial orchids, but species-specific physiological adjustments and selective metal accumulation strategies contribute to their successful establishment on chemically contrasting substrates.

Monitoring Polar Pesticide Residues in Europe via Honeybee Matrices: Results from the INSIGNIA-EU Project

Tzanetou, E.N.[1], Manea-Karga E.[1], Van der Steen J.J.M.[2], Machera K.[1], Kasiotis K.M.[1] and the INSIGNIA-EU consortium1

[1] *Laboratory of Pesticides' Toxicology, Benaki Phytopathological Institute, Athens, Greece*
[2]*Alveus AB Consultancy, Oisterwijk, Netherlands*

E-mail of the presenting author: ev.tzanetou@bpi.gr

Honey bees have recently been recognized as effective bioindicators for environmental monitoring [1–3]. INSIGNIA-EU represents the first pan-European initiative, encompassing 27 EU countries, that established a standardized protocol for using honey bee colonies in large-scale environmental surveillance. Fresh honey collected from frame samples was selected as the most suitable colony-derived matrix for polar pesticide analysis. Eight target compounds were monitored: glyphosate, aminomethylphosphonic acid (AMPA), glufosinate, phosphonic acid, ethephon, fosetyl-Al, maleic hydrazide, and N-acetyl-glyphosate. Extraction and analysis were performed following the Quick Method for the Analysis of Highly Polar Pesticides in Food (QuPPe-PO Method, Version 12.2, 2023 – latest applicable version for honey matrices) [4], using acidified methanol extraction and liquid chromatography–electrospray tandem mass spectrometry equipped with a triple quadrupole mass spectrometer (LC-MS/MS) operated in negative ionization mode [5]. Out of 1164 honey samples analyzed, 73 (6.3%) contained at least one target analyte. Glyphosate was detected in 28 samples, AMPA in 37, phosphonic acid in 8, and N-acetyl-glyphosate in 1 sample, whereas glufosinate, ethephon, fosetyl-Al, and maleic hydrazide were not detected. Overall, the detection frequency of polar pesticide residues was relatively low. This large-scale monitoring study demonstrates the applicability of honey as a suitable matrix for detecting polar pesticide residues within a standardized European framework. Although the overall occurrence was limited, the detection of glyphosate and AMPA in multiple samples highlights the relevance of honeybee-based biomonitoring for environmental exposure assessment.

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Optimized extraction of neonicotinoids and their metabolites from honey bees for comprehensive environmental monitoring

Zarić, N. M.[1,2], Stanisavljević, Lj.[2], Pavlović, R.[3], Ruso, D[4], Schuhmacher, R.[1]

[1] Institute of Bioanalytics and Agro-Metabolomics, Department of Agricultural Sciences, BOKU University, Tulln, Austria,
[2] Faculty of Biology, University of Belgrade, Belgrade, Serbia
[3] Faculty of Chemistry, University of Belgrade, Belgrade, Serbia
[4] Core Facility Bioactive Molecules: Screening and Analysis, BOKU University, Tulln, Austria

E-mail of the presenting author: nenad.zaric@boku.ac.at

Neonicotinoid insecticides are extensively monitored in the environment and in biological matrices due to their effects on pollinators such as honey bees (*Apis mellifera*). In living organisms, these pesticides are rapidly metabolized: Within a few hours, more than 50% of the parent compound is metabolized, and within 24–48 hours the parent compound is almost completely degraded. For this reason, it is crucial to monitor not only the parent compounds but also their metabolites. While robust methods exist to detect the parent compound, its metabolites are rarely included in monitoring programs. Yet these transformation products, which are often polar or acidic, are essential to understanding the full environmental and physiological impact of neonicotinoids.

Here, we developed and optimized a QuEChERS-based extraction protocol for the simultaneous recovery of neonicotinoids and their known metabolites from honey bee homogenates. Three extraction procedures were compared under uniform solvent-to-matrix conditions. Cleanups using PSA and C18 significantly reduced the recovery of polar metabolites such as 6-chloronicotinic acid and desnitro-imidacloprid, whereas $MgSO_4$-only cleanup preserved a broader analytical spectrum with less matrix suppression. A single hexane dewaxing step efficiently removed the beeswax without analyte loss. Reconstitution in 50:50 water: methanol with 0.1% formic acid improved LC-HRMS performance (Orbitrap, polarity switching). The optimized method enabled the reproducible detection of neonicotinoids and their metabolites at concentrations below 10 ppb.

By extending the analytical coverage beyond parent compounds, this workflow provides a robust platform for environmental biomonitoring. It enables the inclusion of metabolites as reliable biomarkers of exposure, supports more accurate risk assessment, and lays the foundation for untargeted metabolomics with isotopically labeled analogues to discover new transformation products. Ultimately, this approach strengthens the use of honey bees as bioindicators of agricultural pollution and advances our understanding of the impact of pesticides on pollinator health.

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Centipedes (Myriapoda: Chilopoda) as bioindicators

Mitić, B.[1], Stojanović, D.[1], Aničić Urošević M.[2]

[1]*Institute of Zoology, University of Belgrade – Faculty of Biology, Belgrade, Serbia*
[2]*Environmental Physics Laboratory, Institute of Physics Belgrade, Belgrade, Serbia*

E-mail of the presenting author: bojan@bio.bg.ac.r)

One of the main objectives of bioindicator research is to identify species or other taxonomic units that reflect the abiotic or biotic state of the environment, represent the impact of environmental change on a habitat, community or ecosystem, or indicate the diversity of other species [1,2]. However, there is no perfect bioindicator, and the selection of the most suitable one depends largely on the specific objective of the survey. Here we analyse the suitability of centipedes (Chilopoda) — one of the two main lineages of myriapods — as bioindicators and consider their use in conservation planning.

Centipedes are an important group of predatory arthropods in many terrestrial habitats. Most species inhabit leaf litter and soil or are found under stones, bark or wood in forests. They usually feed on small arthropods and other invertebrates, although large species can also eat vertebrates [3]. In temperate deciduous forests, centipedes can occur in densities of more than 100 individuals per square metre [4] and are of great potential importance for the transfer of potentially toxic elements (PTEs) to the higher carnivores within the food chain. Centipedes fulfil all the requirements that useful bioindicators should have: they have a well-established taxonomy and ecology, a broad geographic distribution, specific habitat preferences and the ability to signal early environmental changes. They are also easy and inexpensive to sample, their data are relatively independent of sample size and they can distinguish between natural cycles or trends and those caused by anthropogenic influences.

The traditional approach of indicator studies (environmental indication), in which the indicator taxon is monitored to detect changes in environmental conditions, has been most commonly applied to soil invertebrates as bioindicators of pollutant levels [5]. Early studies on the banded centipede *Lithobius variegatus* Leach, 1814 have shown that the extent to which certain PTEs are assimilated depends both on the content in the diet and the degree of contamination of the site where the animals were collected [6-8]. In addition, centipedes are sometimes well suited to indicate the diversity of organisms restricted to terrestrial systems, such as the scorpions and some beetle taxa (Cicindelidae and Tenebrionidae) [5]. Our recent research on three centipede ecomorphotypes collected from a site polluted by car exhaust has shown that the zinc concentration in *Cryptops anomalans* Newport, 1844 corresponds to the concentration in its environment [9].

Centipedes constitute a substantial and functionally significant component of terrestrial biodiversity and are valuable indicators of environmental condition. Although consideration of centipedes has historically been neglected in conservation planning and management, substantial progress with surveys, systematics and bioindication, both globally and locally, means that it is now both feasible and advisable to incorporate them into protected area monitoring activities.

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Mussels as biomonitors of volatile methylsiloxanes: temporal trends and depuration

Ferreira S.[1], Rocha, F.[1], Coelho J.P.[2], Homem, V.[1], **Ratola, N.**[1]

[1]*LEPABE, ALiCE –Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal*
[2] *CESAM, Biology Department, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal*

E-mail of the presenting author: nrneto@fe.up.pt

Aquatic ecosystems are complex, dynamic and are globally becoming increasingly altered. The study of molluscs such as mussels is particularly interesting because they are present in diverse marine ecosystems and have a wide geographic distribution. In addition, they have a good tolerance to a wide range of pollutants, with similar bioconcentration factors throughout the monitored marine area, and the ability to reflect changes in the concentration of contaminants in the surrounding. Thus, they function as good global bioindicators of Persistent Organic Pollutants (POPs) - organic compounds resistant to chemical, biological and photolytic degradation processes and are therefore substances that tend to accumulate - such as volatile methylsiloxanes (VMSs) [1]. VMSs have been identified as emerging organic contaminants [2] with potential harmful effects to the environment. Considering the potential for the presence of these compounds in coastal areas, resulting from their high urbanization, industrial development, and the seasonal impact of tourist influx, improving the knowledge on the distribution of these pollutants in these delicate ecosystems is essential. The objective of this work was to analyse the presence of seven VMSs, four cyclic (D3-D6) and three linear (L3-L5), in mussels (*Mytilus galloprovincialis*) collected in Portugal and evaluate potential temporal trends and the effect of mussel depuration before commercial use on the VMS levels. The analysis was done by optimizing and validating a QuEChERS extraction protocol, with the quantification done by GC-MS. The method used, with *n*-hexane as the extraction solvent, showed high recoveries, with an overall mean considering all VMSs of 86 ± 6%. Intermediate repeatability and precision also led to good results, with a mean coefficient of variation of 11% in both tests. VMSs were detected in all the samples, and D5 and D6 were detected in higher concentrations, reaching a maximum level of (53 ± 12) ng/g_{ww} and (27.2 ± 0.8) ng/g_{ww}, respectively. However, there was no clear geographic or temporal trend. Depuration of mussel reduced VMSs levels by (69 ± 12)%, minimizing human exposure to these contaminants through ingestion. The annual intake of siloxanes, in the worst-case scenario, due to uncooked mussel consumption is at a vestigial level, (maximum of 1.77 mg/kg_{bw} for D5), so they are unlikely to threaten human health.

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Metal bioaccumulation in muscle tissue of the northern pike *Esox lucius* Linnaeus, 1758 from Tisza River

Jovičić, K.[1], Vranković, J.[1], Dimitrijević, M.[2], Pankov, N.[3], Djikanović, V.[1]

[1]Department of Hydroecology and water protection, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, Despot Stefan Blvd 142, 11108 Belgrade, Serbia
[2]Department of life sciences, , Institute for Multidisciplinary Research, University of Belgrade, Kneza Viseslava 1, 11030 Belgrade, Serbia
[3]Department for biology and ecology, Faculty of Science, University of Novi Sad, Dositeja Obradovića 1, 21000 Novi Sad, Serbia

E-mail of the presenting author: katarina.jovicic@ibiss.bg.ac.rs

Fish inhabiting contaminated aquatic environment tend to accumulate essential, but also toxic metals in their tissues. The extent of metal accumulation is influenced by several factors, including metal concentration in the environment, duration and frequency of exposure, mechanisms of metal uptake and intrinsic factors such as fish age, sex, or feeding habits. Various metals, depending on their type and role, have a different affinity to fish tissues. Fish muscles usually contain the lowest amounts of metals compared to other tissues, but are often used in studies because they are a part of the fish body used in the human nutrition [1, 2].

The northern pike is the top piscivore and a phytophilic species in a trophic pyramid of freshwater ecosystems that is highly dependent on backwaters and vegetated riverine areas for spawning and recruitment. In the autumn season 2024, 11 samples of northern pike were collected from the Tisza River using electrofishing (HONDA 1.2κW, 6 A). The content of metals (Ag, Al, As, Cd, Cr, Co, Cu, Fe, Li, Mn, Ni, Pb, Sr, Zn) was determined in muscle samples of fish of different ages using inductively coupled plasma optical spectrometry (ICP-OES). The age determination indicated that the sampled fish ranged from age classes 1+ to 5+. This study aimed to evaluate the potential human health risk associated with the consumption of fish muscle by calculating the estimated daily intake (EDI) of metals, following the methodology described by Javed and Usmani [3]. The risk assessment of metal exposure determined by parameters such as the EDI, involves not only the amount of contaminant ingested, but also factors such as the frequency and duration of exposure, the average body weight of the fish, and the oral reference dose.

Notably, nickel (Ni) and cobalt (Co) were below detection limits in the analyzed fish muscle samples. The concentrations of other detected metals were below the maximum concentrations set by the EU [4] and the Republic of Serbia [5]. The observed values for EDI were significantly lower than the corresponding reference doses, therefore all confirming safe human consumption.

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Microplastic occurrence in gastrointestinal tracts of freshwater fish: addressing data gaps in Serbia and adjacent countries

Djikanović, V.[1], Vranković, J.[1], Jovičić, K.[1]

[1]Department of Hydroecology and Water Protection, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, Despot Stefan Blvd 142, 11108 Belgrade, Serbia

E-mail of the presenting author: (djiki@ibiss.bg.ac.rs)

Among various environmental pollutants, microplastics (MPs) have become one of the most pressing environmental problems of the 21st century due to their ubiquitous distribution in global ecosystems. The widespread distribution of MP is due to their inherent chemical stability and very slow biodegradation, which allow them to persist in environmental systems over long periods of time with minimal structural changes.

Once dispersed in the environment, MPs are difficult to remove and can end up in drinking water and aquatic food sources, harming both the environment and human health. While the presence of MPs in the marine environment has been identified worldwide, the importance of MP pollution in the freshwater environment has also been emphasised in recent years. Several field studies have reported the presence of MPs in the gut contents of fish in freshwater habitats [1, 2]. The aim of our study was to highlight the need of investigation of the occurrence of MPs in the gut contents of freshwater fish from Serbia and its bordering countries (Slovenia, Croatia, Montenegro, Bosnia and Herzegovina, North Macedonia, Albania, Hungary, Bulgaria and Romania). The literature review was conducted as part of the Interreg Ipa-Adrion project, ADRIPLAST, which highlighted the general lack of data on MP in freshwater fish. In the current literature, there are only a few publications that deal specifically with MP in the gastrointestinal tract of freshwater fish, while most research has focussed on the presence of MPs in the aquatic environment (in water and sediment matrices). Although the results are relatively scarce, a few studies have been reported from the Eastern European countries, including Romania [3, 4], Slovenia [5], Croatia [6, 7], and Serbia [8]. These results emphasise the need for a systematic and comprehensive investigation of MP contamination of freshwater fish species in these regions. Edible fish species deserve special attention as they play a direct role in the human diet and are therefore a critical vector for the transfer of MPs) into the human food chain. Therefore, the ingestion and accumulation of MPs by these fish can lead to potential human exposure, raising concerns about the toxicological and health effects of microplastic pollution. The analysis of MP in freshwater fish provides important data for environmental monitoring, ecological risk assessment, fisheries management and ultimately contributes to the protection of public health.

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Erythron profile and liver enzymes as potential biomarkers of water and sediment pollution in vimba bream (*Vimba vimba*) from the Danube River: effects of varying pollution and sampling season

Subotić, S.[1], Jasnić, N.[1], Višnjić-Jeftić, Ž.[2], Kostić, J.[2], Sunjog, K.[2], Lenhardt, M.[3]

[1]*University of Belgrade – Faculty of Biology, Belgrade, Serbia*
[2]*University of Belgrade – Institute for Multidisciplinary Research, Belgrade, Serbia*
[3]*University of Belgrade – Institute for Biological Research „Siniša Stanković",National Institute of the Republic of Serbia, Belgrade, Serbia*

E-mail of the presenting author: ssubotic@bio.bg.ac.rs

Fish erythrocytes are ellipsoidal nucleated cells that are released from the erythropoietic sites, and become increasingly elliptical and less spherical in the course of their life. The relative abundance of the different developmental stages (immature, intermediary and mature) of erythrocytes represents an erythron profile that can be used as a sensitive indicator of contamination. Transaminase (aspartate aminotransferase, AST; alanine aminotransferase, ALT) activities, which are sensitive biochemical markers of liver function and the general physiological state of fish, could also be a good indicator of pollution. In this study, a benthopelagic fish vimba bream (*Vimba vimba*) was sampled at three locations characterised by different levels of pollution (Zemun - relatively clean, Višnjica - moderately polluted, and Banovci - heavily polluted) to assess the potential of the mentioned parameters as biomarkers of water and sediment pollution. In 2016-2017, a total of 156 vimba bream specimens were sampled and analysed in Zemun (92 specimens were collected in spring, 24 in summer, 30 in autumn, and 10 in winter) to determine the seasonal changes of investigated parameters. In Višnjica, 16 specimens were collected in autumn 2018 and in Banovci 5 specimens were collected in summer 2017. The total length and body mass of each fish were measured and used to calculate the Fulton's condition factor. Blood smears were prepared on microscopic slides and stained with the Bio-Diff kit. Random fields were photographed and approximately 50 erythrocytes, per individual, were analysed. Three morphometric parameters (area, perimeter, and length) of the erythrocytes were measured using the ImageJ programme. Serum was separated from blood and AST and ALT activities were measured using Randox assay. At each site, the overall condition of the analysed fish was good ($K > 1$). During the year the highest percentage of immature erythrocytes was observed in summer, when the temperature was highest and oxygen concentration and saturation were lowest. A higher percentage of mature erythocytes could indicate lower feeding effort or starvation, which was observed, and expected, in the winter season. The morphometric parameters of erythrocytes were significantly lower in autumn compared to the other seasons. Only in intermediary erythrocytes all three parameters correlated with temperature, oxygen concentration and oxygen saturation. Compared to Višnjica (autumn) and Banovci (summer), fish from Zemun had a higher proportion of immature erythrocytes. Fish from Višnjica had significantly higher morphometric parameters of erythrocytes for all developmental stages (except area in mature stage), while from Banovci had significantly lower parameters, compared to the individuals from Zemun. Transaminase activities showed no significant seasonal differences and there were no differences in their activities between fish from Zemun and Višnjica. Enzymes did not show significant correlation with temperature, oxygen concentration and oxygen saturation. The percentage of immature erythocytes could be an indication of environmental stress, and while the highest percentage in summer could be related to hypoxia during the summer months, the highest percentage found in fish from the Zemun sampling site, less polluted than Višnjica and Banovci, should be interpreted with caution since comparison involved different sampling seasons and years and various number of sampled fish.

Chub species as biomonitors of potentially toxic elements in river environments

Milićević T.[1], Miličić Lj.[2], Mijatović N.[2], Popović A.[3], Jakšić G.[4], Jovanović G.[1], Ilić B.[2] Mendaš G.[5], Herceg Romanić S[5].

[1] Environmental Physics Laboratory, Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia
[2] Institute for Materials Testing, Belgrade, Serbia
[3] University of Belgrade - Faculty of Chemistry, Belgrade, Serbia
[4] AQUATIKA-Freshwater Aquarium Karlovac, Karlovac, Croatia
[5] Institute for Medical Research and Occupational Health, Zagreb, Croatia

E-mail of the presenting author: tijana.milicevic@ipb.ac.rs

Aquatic ecosystems are highly vulnerable to contamination by potentially toxic elements (PTEs), with fish serving as biomonitors of environmental quality and sediments acting as long-term sinks of pollutants. In this study, we investigated PTE concentrations in three *Squalius* chub species (*S. cephalus*, *S. squalus*, *S. svallize*) and sediments from Croatian rivers during 2020–2021 (4 from Adriatic, 9 from the Danube basin and one from Plitvice Lake). Samples were prepared by acid microwave digestion, and chemical analyses were performed using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). For quality control and assurance, the blanks, replicates and certified reference material were analyzed. The spatial distribution patterns of 28 PTEs (Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mn, Mg, Mo, Na, Ni, P, S, Pb, Sb, Si, Sr, Ti, V, and Zn) in both filleted chub muscle tissue and sediment and their associations were investigated. The fish consumers' health risk assessment was determined by using models developed by the U.S. Department of Energy (DOE), Oak Ridge Operations Office (RAIS) [1]. In parallel, based on the PTE concentrations in the sediments collected from the same location where the chub was caught, the environmental implications were assessed through contamination factor (CF) and ecological risk indices (Eri, RI).

In the chub muscle tissue samples, elevated concentrations of Cr, Pb, and Zn were determined, with Cr and Pb concentrations surpassing EU regulatory limits, suggesting potential risks to consumers. Samples from the Neretva and Sava River basins were identified as having the highest total target hazard quotient (THQ), hazard index (HI), and carcinogenic risk (CR), with the most significant contribution to CR values arising from hexavalent chromium (Cr^{6+}). While overall CR and HI values for most samples remained within acceptable ranges ($CR < 1 \times 10^{-6}$; $HI < 1$), the occurrence of extreme cases among the investigated samples, with increased risks, highlights the need for targeted monitoring and restrictions or control on fish consumption from these locations (especially from specific samples from the Neretva River). Sediment results showed that most PTEs were below the international guideline values, except Cd, which frequently exceeded regulatory values and has the dominant ecological risk factor. Moderate environmental risk (RI>150) was obtained in sediments from Vidak-Stubičke toplice and Kupica-Brod na Kupi. Overall, the study highlights Cd as the primary sediment contaminant of concern, while Pb and Cr in fish represent significant food safety issues. The advanced Principal Component Analysis (PCA) biplot indicated the correlation of the fish and sediment samples from the same locations with PTEs that indicated the anthropogenic influence at these locations, while the ratio of concentrations in fish and sediment also indicated potential for bioaccumulation in the fish muscle tissues. The combined application of (bio)monitoring data with statistical tools and environmental and human health risk assessment provides a novel framework for river ecosystem monitoring, supporting the development of effective water management and public health protection strategies.

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AI-driven insights into PCB-138 dynamics in Mediterranean pelagic fish

Bezdan, T.[1], **Jovanović, G.[2]**, Herceg Romanić, S.[3], Mustać, B.[4], Perišić, M.[1,2]

[1]*Singidunum University, 32 Danijelova, Belgrade, Serbia*
[2]*Institute of Physics Belgrade, 118 Pregrevica, Belgrade, Serbia*
[3]*Institute for Medical Research and Occupational Health, Ksaverska Cesta 2, 10001, Zagreb, Croatia* [4]*Department of ecology, agronomy and aquaculture, University of Zadar, Trg Kneza Višeslava 9, 23000 Zadar, Croatia*

E-mail of the presenting author: gordana.jovanovic@ipb.ac.rs

Marine environments, particularly such as the semi-enclosed Mediterranean Sea, are long-term sinks for persistent organic pollutants (POPs), including organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). These compounds accumulate in small pelagic fish, essential for both marine ecosystems and human diets. In this study, seven OCPs and 17 PCB congeners were analyzed in sardine, anchovy, round sardinella, chub, and horse mackerel collected over three years in the eastern Mediterranean using high-resolution gas chromatography [1]. Focusing on PCB-138, a toxic, highly chlorinated congener, the study employed a modular artificial intelligence (AI)-based platform [2] identifying key factors influencing PCB-138 levels in fish.

The framework integrates advanced machine learning, metaheuristics, and explainable AI leveraging seven ensemble regression algorithms (AdaBoost, CatBoost, ExtraTrees, Gradient Boosting, Histogram Gradient Boosting, LightGBM, XGBoost). Models were evaluated via five-fold cross-validation, and the top three were optimized using Sine Cosine Algorithm and Harris Hawks Optimization to improve predictive accuracy. For model interpretability, SHapley Additive exPlanations (SHAP) and Shapley Additive Global importancE (SAGE) were applied. The ExtraTrees model, optimized with SCA, showed excellent accuracy (mean absolute error=0.0697, mean squared error=0.1604, mean absolute percentage error=0.1394, R^2=0.9511).

The pollutant abundance order was PCB > DDT > HCH > HCB. Among PCBs, PCB-153 was the most dominant, while p,p'-DDE prevailed among OCPs. Global feature importance (SAGE) reveals that PCB-153 (48.4%), PCB-170 (19.6%), and PCB-180 (11.5%) together accounted for nearly 80%, predictive power, followed by PCB-118 (10.5%), PCB-123 (2.8%), and DDT metabolites (*p,p'*-DDD: 2.1%, *p,p'*-DDE: 1.8%). In contrast, HCB, HCH isomers, lower chlorinated PCBs, and biological variables (lipid content, fish size, sampling year) contribute minimally. The results indicate that pollutant load in marine fish is driven mainly by industrial and agricultural pollution, which remains detectable despite long-term bans, rather than biological variability. SHAP analysis reveals that PCB-153, PCB-170, and PCB-180 differ in their influence on PCB-138 concentrations. PCB-153 is the strongest and most variable predictor (SHAP values from –40% to +40%), becoming consistently positive above 1 ng g^{-1}. PCB-170 shows a threshold-dependent impact, negative below 0.1 ng g^{-1}, and strongly positive above while PCB-180 has a weaker, linear effect (SHAP values from –10% to +20%). Interaction analysis shows that PCB-153 and PCB-170 jointly reinforce PCB-138 predictions at higher concentrations, whereas the influence of PCB-153 and PCB-180 interaction remains minimal and stable. These findings suggest stronger co-accumulation between PCB-153 and PCB-170 in marine fish.

The findings enhance understanding of POP distribution in marine fish, highlighting the individual and combined effects of specific PCB congeners. They suggest a need to update monitoring strategies to include multiple pollutants and targeted congeners like PCB-170, beyond standard indicator PCBs. Despite data limitations, the scalable AI framework demonstrates strong potential for broader environmental modeling applications.

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Ecotoxicological Evaluation of Peloids from Serbian Spas

Roganović, J.[1], Veselinović, G.[2], Vukićević, E.[1], Mutić, T.[2], Burazer, N.[2], Milićević, T.[3], Gajica, G.[2]

[1] *Faculty of Chemistry, University of Belgrade, Studentski trg 12-14, 11000 Belgrade, Serbia*
[2] *Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia*
[3] *Institute of Physics Belgrade, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia,*

E-mail of the presenting author: jovanaorlic@chem.bg.ac.rs

A peloid, or therapeutic mud, is defined as a matured mud or muddy suspension with recognized therapeutic and cosmetic applications. It represents a complex mixture of fine-grained natural materials of geological and/or biological origin, combined with thermomineral water. Throughout the maturation process, interactions between the solid and liquid phases, supported by biological activity, induce modifications in both organic and inorganic components. These transformations improve the overall quality of the peloid, enhancing its physicochemical properties, including cooling behavior, plasticity, viscosity, and water retention capacity.

Serbia hosts several peloid-rich areas, many of which have developed into established spa centers. In this study, peloids from 15 spas across four regions of the Republic of Serbia were investigated: North (Kanjiža, Rusanda, Junaković), Central West (Koviljača, Vrujci, Selters, Ždrelo), South West (Lukovska, Kuršumlijska, Prolom, Jošanička), and South (Niška, Sijarinska, Vranjska, Bujanovačka).

To evaluate their potential environmental and therapeutic safety, the aquatic toxicity of water extracts of these peloids was assessed using the bioluminescent marine bacterium *Aliivibrio fischeri* (formerly *Vibrio fischeri*). This Gram-negative, free-living bacterium is widely applied as a sensitive bioindicator, as inhibition of its bioluminescence is considered a robust measure of nonspecific toxicity [1].

The toxicity assays followed ISO 11348 guidelines, employing freeze-dried *A. fischeri* (NRRL B-11177, Macherey-Nagel GmbH & Co. KG, Düren, Germany). Prior to testing, bacterial cultures were reconstituted with the supplied solution and incubated at 15 °C in 1 mL of 2% (w/v) NaCl containing varying concentrations of peloid water extracts. Bioluminescence was recorded after 15 min of exposure using the BioFix® Lumi-10 system (Macherey-Nagel GmbH & Co. KG, Düren, Germany).

Among all analyzed peloids, four exhibited measurable ecotoxicological activity, manifested as inhibition of *A. fischeri* luminescence. The strongest effect was observed for the Lukovska Spa peloid, with an EC_{50} of 0.69 μg/mL. Kanjiža Spa peloid also demonstrated marked toxicity (EC_{50} = 3.74 μg/mL). Peloids from Rusanda and Koviljača Spa were moderately toxic, with EC_{50} values of 32.66 μg/mL and 40.9 μg/mL, respectively [2]. Peloids from the remaining spas showed no detectable ecotoxic activity, as indicated by EC_{50} values exceeding 125 μg/mL.

The ecotoxic responses observed in certain peloids may also reflect antimicrobial potential, which could represent a beneficial therapeutic property [3]. These findings provide a valuable foundation for further research on the antibiotic activity of peloids against clinically relevant pathogens.

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Development and validation of methods for the determination of phthalates in milk and packaging materials by QuEChERS-GC-MS

Turgambek, A.[1], Baizhuma, Zh.[1,3], Chuvashova, E.[1], Dronova, X.[1], Akhmetsadykov, N.[1], Amutova, F.[1,2]

[1] *Antigen LLP, Scientific and Production Enterprise 040905, Almaty region, Kazakhstan*
[2] *Al-Farabi Kazakh National University, Faculty of Biology and Biotechnology, 050040 Almaty, Kazakhstan.*
[3] *Satbayev University, Department of Chemical and Biochemical Engineering, 050012, Almaty, Kazakhstan*

E-mail of the presenting author: turgambekk.a@gmail.com

Phthalate esters (PAEs) are organic compounds widely used as plasticizers [1], which can migrate into food products through supplementary materials and potentially endanger human health [2]. Food is one of the main exposures to PAEs, therefore requires regular monitoring and reliable detection of the presence and concentration of phthalate plasticizers for food safety assurance.

This study developed a GC-MS method for the determination of phthalate esters (DMP, DEP, DPrP, DIBP, DBP, BBP, and DEHP) in cow milk and milk packaging materials, based on liquid–liquid extraction combined with a QuEChERS approach. A hexane-dichloromethane mixture (1:1, v/v) was used as the extraction solvent for milk samples [3], while dichloromethane was applied for food packaging materials [4]. Pyrene was used as an internal standard. Due to the high lipophilicity of PAEs, an additional clean-up step was required. Therefore, a QuEChERS procedure with sorbent materials was applied, mainly silica-bonded octadecylsilane (C18), primary secondary amine (PSA), and anhydrous Na_2SO_4. Adsorbent C18 efficiently removed non-polar interferences such as lipids, while its combination with PSA improved purification from acidic matrix components. Anhydrous Na_2SO_4 was used to eliminate residual water.

The methods in milk and packaging matrices were validated by linearity, repeatability, accuracy, and limits of detection and quantification (LOD/LOQ) parameters. Calibration curves for all seven analytes showed good linearity (R^2=0.9871-0.9998). The method exhibited high sensitivity, with LODs of 2.9–24.7 µg/L and LOQs of 8.9–74.7 µg/L in cow milk products. For packaging materials, LODs ranged from 0.02 to 21.1 µg/L and LOQs from 0.1 to 64.0 µg/L, confirming the suitability of the methods for both food matrices and packaging. Recoveries rates (R) and precision (RSD) of the GC-MS-QuEChERS technique for blank milk samples spiked with PAEs were in the range of 84-109.4%, and for packaging spiked samples were 93.9-121.4%. The RSD range results were 1.4-19.5% for cow milk and 0.1-26.7% for milk packaging. This developed extraction method for determination of 7 PAEs in milk and packages demonstrates its high sensitivity and accuracy.

The established method was implemented to investigate 24 commercial cow milk (3,2% fat) samples and their packages. The research results showed that among 24 milk samples the concentrations of PAEs ranged between: DMP=2.1-55.4 µg/L, DEP=17.0-64.3 µg/L, DPrP=16.0-422.3 µg/L, DIBP=26.4-70.0 µg/L, DBP=130.9-1362.4 µg/L, BBP=108.3-641.6 µg/L, DEHP=100.5-368.6 µg/L. The corresponding food packaging materials demonstrated following results: DMP=3.4-91.8 µg/kg, DEP=4.8-144.7 µg/kg, DPrP=0.3-9.5 µg/kg, DIBP=36.3-535.4 µg/kg, DBP=205.3-9935 µg/kg, BBP=36.3-1611.5 µg/kg, DEHP=198.4-4941.3 µg/kg. Results showed that DBP, BBP and DEHP present at high concentrations in studied milk samples and food packaging compared with other PAEs. In conclusion, the developed analytical methods proved to be reliable for the detection of 7 PAEs in both milk and food packaging materials. These methods can be effectively applied in large-scale monitoring and serve as a valuable tool for comprehensive risk assessment across different age groups of consumers. Their implementation may also contribute to regulatory control, food safety assurance, and the establishment of preventive strategies to minimize human exposure to PAEs.

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Author ORCID

Family name	Given name	ORCID URL
Aarts	Mark	https://orcid.org/0000-0001-5257-0740
Aćimović	Jovana	https://orcid.org/0009-0000-4349-167X
Adamo	Paola	https://orcid.org/0000-0003-4054-4611
Agnan	Yannick	https://orcid.org/0000-0002-9349-6310
Agostini	Marta	https://orcid.org/0009-0005-7839-3160
Aherne	Julian	https://orcid.org/0000-0001-6974-1198
Akhatzhanova	Arailym	https://orcid.org/0000-0002-8400-1187
Akhmetalina	Nargiza	https://orcid.org/0009-0006-3301-8436
Akhmetsadykov	Nurlan	https://orcid.org/0000-0001-6076-7164
Aldreotti	Francesca	
Alves	Arminda	https://orcid.org/0000-0002-5090-621X
Amutova	Farida	https://orcid.org/0000-0003-3325-4018
Andrejić	Gordana	https://orcid.org/0000-0002-5515-9321
Angiolini	Claudia	https://orcid.org/0000-0002-9125-764X
Aničić Urošević	Mira	https://orcid.org/0000-0001-6917-5368
Antunes	Cristina	https://orcid.org/0000-0002-5934-388X
Arsenić	Ilija	https://orcid.org/0000-0002-5265-2890
Arsenijević	Sara	https://orcid.org/0009-0005-3775-2593
Arsenov	Danijela	https://orcid.org/0000-0002-1200-5023
Awada	Angela	
Bačeva Andonovska	Katerina	https://orcid.org/0000-0003-1277-1040
Baizhuma	Zhuldyz	https://orcid.org/0009-0003-5040-7132
Baratto	Aurora	-
Barrocas Dias	Cristina	https://orcid.org/0000-0002-1707-2209
Barrulas	Pedro	https://orcid.org/0000-0003-4075-3667
Bartonova	Alena	https://orcid.org/0000-0002-3350-1202
Batykova	Zhuldyz	https://orcid.org/0009-0000-2524-0607
Baubekova	Almagul	https://orcid.org/0000-0002-7838-1419
Bekteshi	Lirim	https://orcid.org/0009-0005-3280-1253
Belanović Simić	Snežana	https://orcid.org/0000-0003-0962-5595
Bell	Michael	https://orcid.org/0000-0003-3248-3265
Bellini	Erika	https://orcid.org/0000-0001-6542-3897
Beltrami	Sara	
Bernardo	Rocha	https://orcid.org/0000-0002-0904-1947
Bertoli	Marco	https://orcid.org/0000-0001-8345-6107
Beškoski	Vladimir	https://orcid.org/0000-0002-6372-4706
Bezdan	Timea	https://orcid.org/0000-0001-6938-6974
Bianchi	Gemma	https://orcid.org/0009-0007-5444-217X
Biljana	Ilić	https://orcid.org/0000-0003-0798-1748
Blagojević	Duško	https://orcid.org/0000-0001-6338-2833
Brdar	Jovana	/
Bronnikova	Polina	
Brueckner	Dennis	https://orcid.org/0000-0003-1714-5452

Brunetti	Cecilia	https://orcid.org/0000-0002-8531-6076
Buitrago	Daniela	https://orcid.org/0000-0003-3686-8242
Burazer	Nikola	https://orcid.org/0000-0002-5582-7393
Burazerović	Jelena	https://orcid.org/0000-0003-2772-9224
Caporale	Antonio Giandonato	https://orcid.org/0000-0002-7064-5600
Capozzi	Fiore	https://orcid.org/0000-0002-9076-7701
Carbognani	Michele	https://orcid.org/0000-0001-7701-9859
Carli	Marco	https://orcid.org/0000-0002-1808-1529
Caronni	Sarah	https://orcid.org/0000-0002-9614-2681
Casati	Enrico	
Caucheteux	Bastien	
Čelić	Tatjana	https://orcid.org/0000-0001-6332-8730
Chaligava	Omari	https://orcid.org/0000-0002-7231-9534
Chaparro	Marco A.E.	https://orcid.org/0000-0003-2832-2151
Chetyrkina	Valeriya	
Chuvashova	Elizaveta	https://orcid.org/0009-0000-0601-2211
Citterio	Sandra	https://orcid.org/0000-0001-5020-1095
Coelho	João Pedro	https://orcid.org/0000-0003-1975-8308
Collas	Claire	https://orcid.org/0000-0002-8291-1021
Ćosić	Nada	https://orcid.org/0000-0002-5852-1015
Costa	Mafalda	https://orcid.org/0000-0001-8764-2310
Cotrozzi	Lorenzo	https://orcid.org/0000-0002-4401-3896
Counoy	Hugo	https://orcid.org/0000-0003-4721-1115
Ćuk	Mirjana	https://orcid.org/0000-0002-8261-414X
Ćurčić	Milica	https://orcid.org/0000-0001-9239-0912
Ćurić	Valentina	https://orcid.org/0009-0003-1588-6748
D'Asaro	Lorenzo	
Davidović	Miloš	https://orcid.org/0000-0003-2438-0231
Dekan-Carreira	Vladimíra	https://orcid.org/0000-0001-9586-4124
Desandrè	Claudia	
Desideri	Sara	https://orcid.org/0009-0002-4474-9577
Di Lella	Luigi Antonello	https://orcid.org/0000-0002-5920-5073
Dimitrijević	Milena	https://orcid.org/0000-0003-1659-5945
Dojčinović	Biljana	https://orcid.org/0000-0003-1479-8060
Dolašević	Slobodan	https://orcid.org/0009-0003-6381-639X
Dolijanović	Željko	https://orcid.org/0000-0002-9224-3274
Đorđević	Vladan	https://orcid.org/0000-0002-3389-5176
Dronova	Xenia	https://orcid.org/0000-0003-3186-5157
Džoljić	Jovana	https://orcid.org/0009-0003-6103-890X
El Horri	Hafsa	
Fačkovcová	Zuzana	https://orcid.org/0000-0002-5760-5364
Falace	Annalisa	https://orcid.org/0000-0002-9671-5283
Faraguna	Fabio	https://orcid.org/0000-0003-4866-1038
Fedeli	Riccardo	https://orcid.org/0000-0002-8736-1313
Ferrarese	Roberta	
Ferreira	Sofia	N/A
Fiaschi	Tiberio	https://orcid.org/0000-0003-0403-2387

Filipović Marijić	Vlatka	https://orcid.org/0000-0003-0581-6511
Fontasyeva	Marina	https://orcid.org/0000-0003-2366-4873
Gabetti	Alice	https://orcid.org/0009-0003-3525-3710
Gaglione	Anna	https://orcid.org/0009-0002-1578-0559
Gajica	Gordana	https://orcid.org/0000-0002-7875-8984
Gatina	Evgeniya	https://orcid.org/0000-0002-4684-0465
Geiser	Linda	https://orcid.org/0000-0003-3343-8925
Giordani	Paolo	https://orcid.org/0000-0003-0087-7315
Giordano	Simonetta	https://orcid.org/0000-0002-0705-8550
Glarey	L	
Goessler	Walter	https://orcid.org/0000-0002-0142-9373
Gojgić Cvijović	Gordana	https://orcid.org/0000-0001-5598-0585
Goran	Jakšić	
Gordana	Jovanović	https://orcid.org/0000-0001-8657-423X
Gordana	Mendaš	https://orcid.org/0000-0002-2661-2465
Gorelova	Svetlana	https://orcid.org/0000-0003-4922-5684
Gori	Antonella	https://orcid.org/0000-0002-7304-7526
Granata	Angelo	https://orcid.org/0000-0001-9236-9830
Grifoni	Lisa	https://orcid.org/0009-0007-4793-2701
Grujić	Tara	https://orcid.org/0000-0002-0122-6402
Guarnieri	Chiara	
Hayes	Felicity	https://orcid.org/0000-0002-1037-5725
Herceg Romanić	Snježana	https://orcid.org/0000-0003-2382-4734
Homem	Vera	https://orcid.org/0000-0002-3955-9042
Huguet-Cizo	Marion	https://orcid.org/0009-0007-4653-3135
Ilić	Anđelija	https://orcid.org/0000-0003-0859-6358
Ilić	Miloš	https://orcid.org/0000-0002-7613-7762
Ilijević	Konstantin	https://orcid.org/0000-0002-3905-0019
Ilijin	Larisa	https://orcid.org/0000-0003-1728-6140
Izrael Živković	Lidija	https://orcid.org/0000-0002-0385-4373
J. Ángel Fernández	Ángel	https://orcid.org/0000-0002-7629-6106
Jafarova	Mehriban	https://orcid.org/0000-0001-5394-7589
Jakovljević	Ksenija	https://orcid.org/0000-0002-1457-6807
Jakovljević	Olga	https://orcid.org/0000-0002-3079-8602
Jasnić	Nebojša	https://orcid.org/0000-0003-0333-333X
Joana	Martelo	https://orcid.org/0000-0002-0660-8944
Joksimović	Kristina	https://orcid.org/0000-0002-9371-4381
Jordanović	Jelena	https://orcid.org/0000-0002-8145-640X
Jovančević	Nikola	https://orcid.org/0000-0001-5385-5297
Jovanović	Gordana	https://orcid.org/0000-0001-8657-423X
Jovanović	Maja	https://orcid.org/0000-0002-0780-9801
Jovašević - Stojanović	Milena	https://orcid.org/0000-0003-0765-6603
Jurjanz	Stefan	https://orcid.org/0000-0001-8223-6612
Kalinović	Tanja	https://orcid.org/0000-0003-0161-0065
Kalinović	Jelena	https://orcid.org/0000-0003-0088-2683
Kane	Sonila	https://orcid.org/0000-0002-0863-2331
Karadžić	Ivanka	https://orcid.org/0000-0003-4694-7972

Karadžić	Vesna	https://orcid.org/0000-0001-8647-9136
Kasalica	Kristina	https://orcid.org/0000-0001-7731-2043
Kasiotis	Konstantinos	https://orcid.org/0000-0001-6631-0667
Keškić	Tanja	https://orcid.org/0000-0003-3289-1605
Kirshina	Alina	
Knežević	Jelena	https://orcid.org/0000-0002-8114-3630
Knežević	Goran	https://orcid.org/0000-0002-0344-1704
Kojić	Danijela	https://orcid.org/0000-0002-6422-9790
Kojić	Ivan	https://orcid.org/0000-0001-7737-1844
Konuspayeva	Gaukhar	https://orcid.org/0000-0003-0171-3582
Kosior	Grzegorz	https://orcid.org/0000-0002-8610-9271
Kostić	Jovana	https://orcid.org/0000-0003-2474-3223
Kostić	Aleksandar Ž.	https://orcid.org/0000-0002-1012-4029
Kovačević	Snežana	https://orcid.org/0000-0002-0970-1511
Kovačević Filipović	Milica	https://orcid.org/0000-0002-3776-8764
Kralj	Tomislav	https://orcid.org/0000-0003-0729-5078
Krivokuća	Nikola	
Krmar	Miodrag	https://orcid.org/0000-0002-0337-2411
Krmpot	Aleksandar	https://orcid.org/0000-0003-2751-7395
Krnjajić	Slobodan	https://orcid.org/0000-0001-5278-4589
Kučić Grgić	Dajana	https://orcid.org/0000-0001-8427-6482
Kuštro	Marija	https://orcid.org/0009-0003-7098-2919
Landi	Marco	https://orcid.org/0000-0003-0121-0715
Lapenta	Virginia	
Lauria	Giulia	https://orcid.org/0000-0001-8401-2073
Lazo	Pranvera	https://orcid.org/0000-0002-7202-4058
Lazović	Vojislav	https://orcid.org/0000-0003-3926-118X
Legnaro Diamanti	Mirko	https://orcid.org/0009-0007-6119-213X
Lenhardt	Mirjana	https://orcid.org/0000-0003-3390-3292
Lješević	Marija	https://orcid.org/0000-0002-8164-6043
Lo Piccolo	Ermes	https://orcid.org/0000-0001-9789-2524
Lončarević	Branka	https://orcid.org/0000-0002-2226-511X
Loppi	Stefano	https://orcid.org/0000-0002-3404-1017
Lorenzini	Giacomo	https://orcid.org/0000-0002-6371-5105
Louzon	Maxime	https://orcid.org/0000-0003-0079-5766
Lugonja	Nikoleta	https://orcid.org/0000-0002-5093-4448
Machera	Kyriaki	https://orcid.org/0000-0001-6499-3468
Maganza	Alessandra	https://orcid.org/0009-0002-4083-1725
Mailfert	Noémie	https://orcid.org/0009-0007-8257-118X
Mamirova	Aigerim	https://orcid.org/0000-0002-4274-5081
Manea-Karga	Electra	https://orcid.org/0000-0002-0241-611X
María Bouso	María	
Marié	Debora C.	https://orcid.org/0000-0002-5505-2559
Marjanović	Žaklina	https://orcid.org/0000-0001-5254-178X
Marković	Ivana	https://orcid.org/0009-0001-9498-8325
Marković	Dragan	https://orcid.org/0009-0005-0064-4759
Marte	Fernando	https://orcid.org/0009-0009-2192-1304

Martellos	Stefano	https://orcid.org/0000-0001-5201-8948
Matek Sarić	Marijana	https://orcid.org/0000-0003-2734-7955
Medić	Ana	https://orcid.org/0000-0001-9919-2281
Merlini	Clara	
Metalli	Alessandra	
Mijatović	Nevenka	https://orcid.org/0000-0002-1751-6498
Milićević	Tijana	https://orcid.org/0000-0003-1258-910X
Miličić	Ljiljana	https://orcid.org/0000-0002-3002-7058
Milošević	Ivana	https://orcid.org/0000-0002-4463-5986
Minić	Zoran	https://orcid.org/0000-0003-2520-0239
Mišljenović	Tomica	https://orcid.org/0000-0001-7583-0891
Mitić	Jelena	https://orcid.org/0000-0003-0349-829X
Momčilović	Miloš	https://orcid.org/0000-0001-9117-5903
Morais	João	
Moses	Jesse	https://orcid.org/0000-0002-3793-7994
Mossotto	Camilla	https://orcid.org/0009-0001-1337-0591
Mrdaković	Marija	https://orcid.org/0000-0003-3946-5828
Mustać	Bosiljka	https://orcid.org/0000-0002-1374-5883
Mutić	Tijana	https://orcid.org/0009-0009-5461-2970
Mutić	Jelena	https://orcid.org/0000-0002-0545-1578
Nali	Cristina	https://orcid.org/0000-0002-8228-0258
Nikolić	Maria Vesna	https://orcid.org/0000-0001-5035-0170
Nikolić	Dušan	https://orcid.org/0000-0003-2004-1662
Obradovic	Darija	https://orcid.org/0000-0001-5678-034X
Orčić	Snežana	https://orcid.org/0000-0002-0020-8763
Pajić	Tanja	https://orcid.org/0000-0003-3794-7655
Panont	Davis	
Paoli	Luca	https://orcid.org/0000-0001-5479-6428
Pastorino	Paolo	https://orcid.org/0000-0002-0585-1168
Pavlović	Ratko	https://orcid.org/0000-0001-6170-3621
374Pećić	Marija	https://orcid.org/0000-0003-4477-8976
Pedrelli	Athos	https://orcid.org/0000-0002-6935-2886
Pelfrêne	Aurélie	https://orcid.org/0000-0001-5339-7361
Pellegrini	Elisa	https://orcid.org/0000-0001-9237-5341
Pergal	Miodrag	https://orcid.org/0000-0001-8722-5840
Perić-Mataruga	Vesna	https://orcid.org/0000-0002-2899-7418
Perišić	Mirjana	https://orcid.org/0000-0002-8287-4136
Petraglia	Alessandro	https://orcid.org/0000-0003-4632-2251
Petrović	Bojana	https://orcid.org/0009-0004-4089-4683
Pikelj	Kristina	https://orcid.org/0000-0001-8789-8068
Pinho	Pedro	https://orcid.org/0000-0001-5571-9619
Pippi	Lorenzo	
Pisinov	Boris	https://orcid.org/0000-0002-4304-0462
Pisuttu	Claudia	https://orcid.org/0000-0002-7411-6394
Pizzul	Elisabetta	https://orcid.org/0000-0001-9916-5005
Platt	Stephen Mathew	https://orcid.org/0000-0002-8439-6633
Pontiroli	Daniele	https://orcid.org/0000-0002-9990-539X

Popović	Milica	https://orcid.org/0009-0003-3781-4083
Popović	Aleksandar	https://orcid.org/0000-0003-0162-9612
Popović	Slaviša	https://orcid.org/0000-0002-6656-009X
Popović	Dušanka	https://orcid.org/0000-0002-0762-2465
Popović	Milica	https://orcid.org/0009-0003-3781-4083
Popović-Djordjević	Jelena	https://orcid.org/0000-0003-4057-3826
Potter	Mark	
Prearo	Marino	https://orcid.org/0000-0002-2847-6006
Purać	Jelena	https://orcid.org/0000-0003-0028-9847
Qarri	Flora	https://orcid.org/0009-0000-2763-401X
Rabasović	Mihailo	https://orcid.org/0000-0002-9235-4388
Radenković	Milica	https://orcid.org/0009-0002-2979-3991
Radnović	Dragan	https://orcid.org/0000-0003-3753-1887
Radojević	Ana	https://orcid.org/0000-0003-3138-154X
Ranđelović	Dragana	https://orcid.org/0000-0003-4976-7766
Rašković	Božidar	https://orcid.org/0000-0001-7190-5833
Ratola	Nuno	https://orcid.org/0000-0002-4102-9606
Rebelo	Rui	https://orcid.org/0000-0003-2544-1470
Relić	Dubravka	https://orcid.org/0000-0002-0876-4561
Rezapour	Salar	https://orcid.org/0000-0002-7267-443X
Ribeiro	Ana Rita	https://orcid.org/0000-0002-4445-5379
Ricci	Gian Piero	
Risoli	Samuele	https://orcid.org/0000-0002-6232-3775
Rocha	Filipe	https://orcid.org/0000-0003-1166-1990
Roganović	Jovana	https://orcid.org/0000-0002-0338-2441
Roje	Vibor	https://orcid.org/0000-0001-9142-4607
Roljević Nikolić	Svetlana	https://orcid.org/0000-0002-3139-0289
Rondeau-Gagné	Simon	
Rosoni	Donatella	https://orcid.org/0009-0005-0635-6045
Ruffini Castiglione	Monica	https://orcid.org/0000-0002-3777-6596
Ruso	David	n/a
Russo	Claudia	https://orcid.org/0009-0003-5522-6197
Sabovljevic	Marko	https://orcid.org/0000-0001-5809-0406
Sala	Andreah	
Salinitro	Mirko	https://orcid.org/0000-0001-7829-4350
Saljnjikov	Elmira	https://orcid.org/0000-0002-6497-2066
Salvi	Gianguido	https://orcid.org/0000-0002-9394-1138
Samson	Roeland	https://orcid.org/0000-0001-7582-1450
Sanità di Toppi	Luigi	https://orcid.org/0000-0002-8731-4904
Sannikov	Pavel	https://orcid.org/0000-0001-7973-301X
Santrač	Isidora	https://orcid.org/0000-0001-8533-4250
Savić-Sević	Svetlana	https://orcid.org/0000-0002-6406-0745
Šariri	Sara	https://orcid.org/0009-0004-3397-5127
Schuhmacher	Rainer	https://orcid.org/0000-0002-7520-4943
Scimone	Giulia	
Scoccimarro	Martina	https://orcid.org/0009-0003-3604-0549
Seggi	Linda	https://orcid.org/0009-0008-4632-8972

Segura Roux	Marta	https://orcid.org/0009-0003-7771-6322
Sekulić	Zoran	https://orcid.org/0000-0002-6858-9304
Šerbula	Snežana	https://orcid.org/0000-0001-7560-4130
Sharps	Katrina	https://orcid.org/0000-0003-3265-1505
Shirzad	Hossein	
Siavash Moghaddam	Sina	https://orcid.org/0000-0001-8643-8991
Sidoli	Michele	
Signorini	Linda	https://orcid.org/0009-0009-2149-5958
Šikuljak	Tijana	https://orcid.org/0000-0003-4153-4896
Šinžar-Sekulić	Jasmina	https://orcid.org/0000-0002-8181-7164
Skendžić	Tea	https://orcid.org/0000-0003-1969-4359
Skorić	Stefan	https://orcid.org/0000-0002-3577-2735
Smederevac-Lalić	Marija	https://orcid.org/0000-0003-4346-1432
Soligot-Hognon	Claire	https://orcid.org/0009-0002-2767-6492
Sorrentino	Maria Cristina	https://orcid.org/0000-0001-5150-0409
Spagnuolo	Lilla	https://orcid.org/0009-0006-7849-3476
Spagnuolo	Valeria	https://orcid.org/0000-0002-5021-6817
Spanò	Carmelina	https://orcid.org/0000-0002-6980-5021
Spariosu	Kristina	https://orcid.org/0000-0003-4943-2907
Spasojević	Pavle	https://orcid.org/0000-0001-8903-2102
Spasojević	Ivan	https://orcid.org/0000-0002-3150-3087
Špoljarević	Marija	https://orcid.org/0000-0002-9052-5307
Stanić	Marina	https://orcid.org/0000-0002-2779-7932
Stanisavljević	Ljubiša	https://orcid.org/0000-0002-6229-6535
Stanković	Slađan	https://orcid.org/0000-0001-7002-3601
Stefanović	Slobodan	https://orcid.org/0009-0007-7386-6365
Stevanović	Katarina	https://orcid.org/0000-0001-9733-2367
Stojiljković	Danica	https://orcid.org/0000-0002-9585-7298
Stringa Basile	Carolina	https://orcid.org/0009-0008-6541-4200
Subotić	Srđan	https://orcid.org/0000-0002-9597-2288
Sunjog	Karolina	https://orcid.org/0000-0002-7533-1256
Tasić	Aleksandra	https://orcid.org/0000-0002-8361-5697
Tijana	Milićević	https://orcid.org/0000-0003-1258-910X
Todorović	Dajana	https://orcid.org/0000-0003-1796-9312
Tonelli	Mariagrazia	
Toscano	Domenico	https://orcid.org/0000-0002-5000-4128
Todorović	Nataša	https://orcid.org/0000-0003-2626-0794
Trabucco	Raffaella	
Turgambek	Arailym	https://orcid.org/0009-0004-0583-3367
Tzanetou	Evangelia	https://orcid.org/0000-0001-9568-2283
Ugrinović	Martina	https://orcid.org/0009-0002-1237-2584
Valić	Damir	https://orcid.org/0000-0003-0497-5457
Varela	Zulema	https://orcid.org/0000-0002-4751-7686
Van der Stenn	Josef	https://orcid.org/0000-0002-4742-6637
van den Heuvel	Joost	https://orcid.org/0000-0002-8678-1527
Vannini	Andrea	https://orcid.org/0000-0003-3789-6691
Vasiljević	Zorka	https://orcid.org/0000-0002-7817-8648

Vegini	Emanuele	
Velázquez-Román	Luis	https://orcid.org/0009-0003-9751-2749
Vergel	Konstantin	https://orcid.org/0000-0001-8158-5589
Veselinović	Milorad	https://orcid.org/0000-0002-2134-7631
Veselinović	Gorica	https://orcid.org/0000-0001-6939-451X
Vesović	Marija	https://orcid.org/0000-0002-3506-8705
Višnjić-Jeftić	Željka	https://orcid.org/0000-0002-8206-2138
Vizi	Aleksa	https://orcid.org/0009-0006-5253-7530
Vlahović	Milena	https://orcid.org/0000-0002-7815-091X
Vlku	Ankica	https://orcid.org/0000-0002-5999-4973
Vujanović	Dragana	https://orcid.org/0000-0001-8376-728X
Vujić	Ante	https://orcid.org/0000-0002-8819-8079
Vujičić	Milorad	https://orcid.org/0000-0002-2152-9005
Vukajlović	Filip	https://orcid.org/0000-0003-4879-0171
Vukićević	Emilija	https://orcid.org/0009-0008-6859-6484
Vukov	Dragana	https://orcid.org/0000-0001-9737-6538
Winkler	Aldo	https://orcid.org/0000-0002-0653-0059
Wyonch	Jacob	https://orcid.org/0009-0001-0669-0650
Xiao	Jianbo	https://orcid.org/0000-0003-3311-770X
Yamjabok	Jitpanu	https://orcid.org/0009-0006-6897-2756
Yttri	Karl Espen	https://orcid.org/0000-0001-9904-5716
Zarić	Nenad	https://orcid.org/0000-0001-5419-8460
Žerađanin	Aleksandra	https://orcid.org/0000-0001-5896-6025
Zinicovscaia	Inga	https://orcid.org/0000-0003-0820-887X
Živković	Marija	https://orcid.org/0000-0002-3415-5145
Živković	Milica	https://orcid.org/0000-0003-1022-5767
Živković	Sanja	https://orcid.org/0000-0003-1499-1645
Živić	Miroslav	https://orcid.org/0000-0002-0314-5032

Authors are responsible

for the content of their papers.

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