

### Book of abstracts







#### **CONFERENCE INFORMATION**

#### How to get to the venue?

#### Scientific committee

Julian Aherne (Trent University, Canada) Arminda Alves (Universidade do Porto, Portugal) Linda Geiser (US Dept of Agriculture – Forest Service, Washington, District of Columbia, USA) Simonetta Giordano (University of Napoli Federico II, Italy) Manuela Giovanetti (CREA Research Centre for Agriculture and Environment, Bologna, Italy) Stefano Martellos (University of Trieste, Italy) Nathalie Sauret (Université Côte d'Azur, France)

#### **Conference Organizing Committee**

Sofia Augusto (Universidade de Lisboa) Ángel Fernández (Universidade de Santiago de Compostela) Paula Matos (Universidade de Lisboa) Silvana Munzi (Universidade de Lisboa) Pedro Pinho (Universidade de Lisboa) Nuno Ratola (Universidade do Porto) Bernardo Rocha (Universidade de Lisboa) Zulema Varela (Universidade de Santiago de Compostela)



#### PROGRAMME November 4, 2024 (Monday)

13:15-14:15		Registration of participants
14:15-14:30		Opening ceremony with Greetings from the Authorities
14:30- 17:30	Session	<b>"Lichen and Bryophytes Biomonitoring"</b> Moderators: Mira Aničić & Paolo Giordani
14:30-15:15	Ρ	Linda Geiser Making biomonitoring accessible to all. Creating digital infrastructure to facilitate the collection and application of biomonitoring information in environmental management and policymaking
15:15-15:30	R	<u>Hugo Counoy</u> Towards an interpretative framework for urban air quality biomonitoring using lichens: a meta-analysis of surveys following the European protocol
15:30-15:45	R	Luca Paoli Comparing the diversity of epiphytic lichens with a regional dataset of atmospheric emission sources: a long-term study around a landfill site (Tuscany, Central Italy)
15:45-16:00	R	Silvia Poponessi Bryophyte surveys within the long-term project biodiversity monitoring south Tyrol
16:00-16:30	<sup>\$\$5</sup>	Coffee break
16:30-16:45	R	Aldo Winkler Magnetochemical biomonitoring for the preventive conservation of the Palatine Hill archaeological site in Rome, Italy
16:45-17:00	R	Mira Aničić Urošević Can different moss species be interchangeably used in biomonitoring potentially toxic elements within the same survey?
17:00-17:15	R	Jana Borovská Biomonitoring of heavy metals in Slovakia



17:15-17:20	F	<u>Luca Paoli</u> Trace elements in the lichen <u>Lobaria pulmonaria</u> from remote areas: evidence from herbarium collections in Slovakia
17:20-17:25	F	<u>Paolo Giordani</u> A new tool for interpreting lichen biodiversity data: the Italian proposal
17:25-17:30	F	Sonia Ravera Assessing Lobaria pulmonaria (L.) Hoffm. as a proxy for minimal atmospheric pollution in mediterranean: insights from the BioConLobaria project on pollution impact and lichen viability

#### 17:30-18:30 IABEP Members meeting



#### November 5, 2024 (Tuesday)

09:00-09:45	Р	<u>Cristina Branquinho</u>
		Trends in pollution over time in the XXI century
09:45- 12:30	Session	"Plastics and Microplastics"
		Moderators: Merhiban Jafarova & Julian Aherne
09:45- 10:00	R	Zulema Varela
		Plastic debris in nests of white stork Ciconia ciconia
		population: characterisation and analysis of associated
		factors
10:00-10:15	R	<u>Lisa Grifoni</u>
		Biomonitoring of airborne microplastics: comparison
		between moss and lichen transplants
10:15-10:30	R	<u>Mehriban Jafarova</u>
		Does moss bag design influence the accumulation of
		atmospheric microplastics?
10:30-11:00	<sup>5<sup>1</sup>5</sup>	Coffee break
11:00-11:15	R	Fiore Capozzi
11.00-11.15	n	Sequential extraction of anthropogenic microfibers from
		leaves of <u>Pittosporum tobira</u>
11:15-11:30	R	Carla Gamelas
11.15-11.50	I.	Microplastic contamination in lettuces from Lisbon urban
		vegetable gardens
11:30-11:45	R	Julian Aherne
		Web of pollution: passive biomonitoring of atmospheric
		microplastics across Ontario
11:45-12:00	R	Maria Maisto
		The soil microbiota as a biomarker for the response to
		microplastics as an emerging pollutant. the case study of
		Volturno river sediments (Southern Italy)
12:00-12:15	R	Maria Antonietta Ranauda
		Molecular and microbial biomarkers in the soil-plant
		system response to emerging pollutants
12:15-12:30	<u>۲.</u> ۲	Discussion
12:30-12:35	•	Group photo
12:30-14:00	þ	Lunch



#### 14:00-15:50 Session "Plant-based biomonitoring"

		Moderators: Nathalie Sauret & Sergio Calabrese
14:00-14:15	R	<u>Jérome Ledauphin</u>
		Leaf absorption characteristics of a bioindicator plant
		<u>Elaeagnus ebbingei</u> towards PAHs
14:15-14:30	R	Zbigniew Ziembik
		Comparison of leaf elemental composition for
		biomonitoring purposes in urban environments
14:30-14:45	R	Antonello Prigioniero
		Leaf surface functional traits as driver to identify plants
		useful in PM and PTEs biomonitoring in urban environment
14:45-15:00	R	Sergio Calabrese
		Active and passive biomonitoring techniques in active
		volcanic environments
15:00:15:15	R	<u>Claudia Pisuttu</u>
		Suitability of <u>Tillandsia usneoides</u> for biomonitoring toxic
		elements in forest ecosystems
15:15-15:30	R	Lorenzo Pippi
		Towards ozone biomonitoring 3.0: Can vegetation
		spectroscopy help?
15:30-15:35	F	<u>Giulia Scimone</u>
		Sick building syndrome and ozone biomonitoring:
		Nicotiana tabacum cv. Bel-w3 seedlings exposure as a
		valid tool for the indoor air quality assessment
15:35-15:40	F	<u>Donatella Rosoni</u>
		Ozone biomonitoring is a versatile tool for science,
		environmental health and regulation: a case study from a
		regional agency for territory protection of central Italy
15:40-15:45	F	<u>Carla Gamelas</u>
		Identifying trace metal contamination in Lisbon urban
		vegetable gardens using lettuce as a biomonitor
15:45-15:50	F	<u>Mathilde Gaudin</u>
		Metabolites of phenanthrene as potential markers of
		polycyclic aromatic hydrocarbons in a bioindicating hedge
		plant <u>Elaeagnus ebbingei</u>
15:50-16:00	<b></b>	Discussion
16:00-16:30	5 <sup>5</sup> 5	Coffee break



#### 16:30-17:30 Session "Community Science"

		Moderator: Sonia Ravera & Stefano Loppi
16:30-16:45	R	Yannick Agnan
		Engaging citizens in an air quality assessment using lichen
		biomonitoring: a case study of the lichens GO project
16:45-16:50	F	<u>Silvana Munzi</u>
		How and why to become a soil ambassador in the Echo project?
16:50-16:55	F	<u>Samuele Risoli</u>
		Citizen science meets tropospheric ozone biomonitoring:
		insights from Italy
16:55-17:00	F	<u>Gemma Bianchi</u>
		How much do you know about ozone biomonitoring?
17:00-17:15	R	<u>Vlatka Filipović Marijić</u>
		Assessment of the spatial and temporal impact of
		wastewaters on biological responses in aquatic organism
		and water quality of the karst river water
17:15-17:30	<u> </u>	Discussion
19:30	ý	Dinner at "Restaurante Laurentina"
	/	https://www.restaurantelaurentina.com



#### November 6, 2024 (Wednesday)

09:30-12:30	Session	n "Ecosystem-based biomonitoring"
		Moderators: Martin Bačkor & Manuela Giovanetti
09:30-10-15	Р	Patricia Ventura Garcia
		Sleeping volcanoes, awaking health issues: lessons
		learned from wild mice
10:15-10:30	R	<u>Martin Bačkor</u>
		Lichens and mosses in remediation of metal polluted
		environments
10:30-10:35	F	Nuno Ratola
		Possible influence of forest fires on hg levels in soil and
		pine needles
10:35-10:40	F	<u>Gilda Perri</u>
		Toxic elements (PTEs) in serpentine-derived soils and
		bioaccumulation capacity of spontaneous vascular flora
		in the Calabria Region, Southern Italy
10:40-10:45	F	Paola Adamo
		Nature-based solutions and urban farming: the impact of
		green barriers on air quality, soil health, and food safety
10:45-11:15	<sup>555</sup>	Coffee break
11:15-11:30	R	Fabiana Cristofolini
		Pollen viability as bioindicator of air pollution: state of the
		art and future perspectives
11:30-11:45	R	Nenad Zarić
		Honeybees as effective biomonitors of spatial and
		temporal pollution
11:45:12:00	R	<u>Stefan Fraenzle</u>
		Biomonitoring using chitin: crayfish, grafted chitin and
		direct evaluation of adsorption thermodynamics
12:00-12:05	F	Daniela Baldantoni
		Multi-Element bioconcentration kinetics in <u>Dictyota</u>
		spiralis for active biomonitoring of coastal marine
		ecosystems



12:05-12:10	F	Maria Alexandra Oliveira Implementing a long-term network to monitor the effects of air pollution on terrestrial ecosystems in Portugal
12:10-12:15	F	Manuela Giovanetti Agrochemical residues in beebread as an indicator of landscape management
12:15-12:45	1988	Closure

12:45-14:00



ENGAGING CITIZENS IN SOIL SCIENCE: THE ROAD TO HEALTHIER SOILS

14:00-14:15		Opening: Short presentation of IABEP and ECHO project
14:15-15:00		Marcos Pedlowski (Universidade Estadual do Norte Fluminense - participation by Zoom)
15:00-15:30		Pesticides: who use them, where and when Isabel Brito (Universidade de Évora) When the use of pesticides support soil friendly
15:30-16:00		techniques Cristina Cruz (Universidade de Lisboa) How to monitor pesticide residues and their effects in soil
16:00-16:30	<sup>555</sup>	Coffee break
16:30-18:00		Roundtable with stakeholders – Moderator: Cristina Cruz - Paulo Carvalho (Vivifarms) - Diogo Pinho (Monte da Silveira Bio) - Francisco Lupi (Projeto Pronobis) - Teresa Dias (Universidade de Lisboa)
18:00	1353	Closure

### Session "Lichen and Bryophytes Biomonitoring"

Moderators: Mira Aničić & Paolo Giordani



# (P) Making biomonitoring accessible to all. Creating digital infrastructure to Making biomonitoring accessible to all. Creating digital infrastructure to facilitate the collection and application of biomonitoring information in environmental management and policymaking

Linda Geiser

Air Resource Management National Program Leader, US Dept of Agriculture – Forest Service, Washington, District of Columbia, USA

Humanity is facing multiple, existential environmental crises related to human alterations of the climate, biogeochemical cycles, and biosphere, and to the release of pollutants and novel entities into the air, soil, and water. To tackle these crises and achieve a sustainable future, we need effective, data-driven, environmental management and policies. By providing relevant data to evaluate environmental conditions and trends, biomonitoring can help decision-makers develop, and assess the efficacy of management plans, actions, and policies. Additionally, biomonitoring can be implemented at various geographical scales, at relatively low cost, and in areas without power. However, lack of access to biomonitoring. Web-accessible, standardized protocols, data archives, and automated quality assurance and interpretive tools can empower more people to collect and use high-quality biomonitoring data. Practical examples from the author's experience will be shared to jumpstart a conversation about how the IABEP might create a centralized repository and digital infrastructure to facilitate biomonitoring and a sustainable environment globally.



### (R) Towards an interpretative framework for urban air quality biomonitoring using lichens: a meta-analysis of surveys following the European protocol

<u>Hugo Counoy</u>(1); Laure Turcati(2); Patrick Bogaert(1); Yannick Agnan(1); and European lichen biomonitoring research groups

(1) Earth and Life Institute, Université catholique de Louvain, 1348 Louvain-la-Neuve, Belgium ; (2) Sorbonne Université, OSU Ecce Terra, 4 place Jussieu, 75252 Paris, France

Air pollution poses a significant threat to both human and ecosystem health, particularly in urban areas where over half of the global population resides. Accurately assessing air quality is essential for understanding population exposure and mitigating its harmful effects. Studying epiphytic lichen communities constitutes a complementary approach to physical-chemical sensors by enhancing spatial resolution at a lower cost. In addition, each lichen species has a specific sensitivity to atmospheric pollutants, making it possible to identify the pollutants involved. Although a standardized European protocol has been developed for lichen sampling in urban areas, there is to date no unified interpretative framework for bioindication data, which limits the potential for large-scale application in air quality assessment. Currently, national lichen databases are used to calculate ecological indices and functional diversity metrics. However, these indices are not directly related to pollutants, making it difficult to understand specific effects, such as nitrogen pollutants (NO<sub>x</sub> and NH<sub>3</sub>) with contrasted effects on eutrophic lichen species. We thus propose a meta-analysis of bioindication surveys conducted using the European protocol. Our goal is to identify species that are highly indicative across Europe and easy to identify. We have currently collected raw data from 47 studies across 14 European countries, representing over 7000 sampled trees and 375 lichen taxa. By combining these data with European environmental databases (including air pollutant concentrations, climatic variables, and land cover information) and applying generalized linear mixed models, we aim to understand the key drivers influencing the frequency of each individual species across Europe. Through this study, we seek to lay the foundation for a new interpretative framework for the bioindication community, enabling more robust and scalable applications in urban air quality assessment.



# (R) Comparing the diversity of epiphytic lichens with a regional dataset of atmospheric emission sources: a long-term study around a landfill site (Tuscany, Central Italy)

<u>Luca Paoli</u>(1); Stefano Loppi(2); Andrea Vannini(3); Zuzana Fačkovcová(4),(5); Elisa Bini(6); Chiara Collaveri(6); Camilla Grossi(6),(7)

(1) Department of Biology, University of Pisa, Italy; (2) Department of Life Sciences, University of Siena, Italy; (3) Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy; (4) Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia; (5) Department of Pharmacy, University of Genoa, Italy; (6) Centro Regionale per la Tutela della Qualitá dell'Aria, ARPAT Dipartimento di Livorno, Italy; (7) Department of Earth Sciences, University of Pisa, Italy

Landfilling is still the main waste disposal method in Europe and strict rules are prescribed by EU legislation for waste disposal in landfills. Biomonitoring of air pollution is suitable for the implementation of environmental policies on air quality and atmospheric pollution control, and it can be applied also for the implementation of waste management strategies. In our study, the biodiversity of epiphytic lichens has been used to assess the effects of air pollution around a landfill site in Central Italy, with regular monitoring campaigns carried out every two years. Focusing on the period 2010–2020, biomonitoring data have been combined with the available local emission data from the Regional Inventory of Atmospheric Emission Sources (I.R.S.E.) drafted by ARPAT (Regional Agency for Environmental Protection) for the Tuscany Region, which consists of a series of data (biannually or triennially updated) regarding airborne pollutants released into the atmosphere by anthropic activities and natural sources within a specific area, and aimed at providing an estimate of air pollution pressure within that area. The results indicated a decrease in lichen diversity near the landfill but no significant negative effect on lichen diversity in the surrounding area. Comparisons with I.R.S.E. emissions data revealed a relationship between the increase in nitrophilous lichens and ammonia  $(NH_3)$  emissions from the landfill. In addition, a BETA index, summarizing land use pressures related to NH<sub>3</sub> emissions, was applied and compared with the frequency of epiphytic lichens up to 2020 highlighting a relationship between lichen frequencies and the intensity of the pressure.



#### (R) Bryophyte surveys within the long-term project Biodiversity Monitoring South Tyrol

<u>Silvia Poponessi</u>(1); Roberto Dellavedova(1); Annalena Cogoni(3); Ulrike Tappeiner(1,2); Andreas Hilpold(1)

(1) Institute for Alpine Environment, Eurac Research, Bolzano, Italy; (2) Department of Ecology, University of Innsbruck, Innsbruck, Austria; 3: Department of Life and Environmental Sciences, University of Cagliari, Italy

Mountain regions, which are important global biodiversity hotspots, are increasingly being studied from a multidisciplinary perspective. The long-term project Biodiversity Monitoring South Tyrol examines 320 terrestrial study areas distributed throughout South Tyrol in a large number of different habitat types in regular monitoring cycles of five years. Since 2019, 64 sites have been monitored annually using standardized protocols with the aim of assessing plant and animal groups sensitive to climate and land use change, including vascular plants and bryophytes. Bryophytes play an important and often overlooked role in ecosystem functioning. Despite the high species diversity of bryophytes, a major obstacle to their use as study organisms has been the lack of basic floristic, ecological and alpha-taxonomic knowledge in many Italian regions. The distribution of these plants depends on several general climatic and ecological factors, as well as their interaction with other living organisms.

Sites were selected using a stratified sampling design to ensure coverage of the most representative habitat types, ranging from near natural to heavily anthropogenically modified. Data is also collected on abiotic factors, landscape structure and land use management. Another important aspect of the initiative is the ongoing exchange between experts and local authorities concerning pressing issues about the influence of agricultural and forestry practices on biodiversity and ecosystem services, as well as the current impacts of environmental change, such as extreme weather events or invasive species. The results of the project will also be used to evaluate or improve the effectiveness of species and habitat conservation strategies both within and outside of protected areas.



### (R) Magnetochemical biomonitoring for the preventive conservation of the Palatine Hill archaeological site in Rome, Italy

Lisa Grifoni(1,2); <u>Aldo Winkler(</u>1), Francesca Boldrighini(3), Luigi Antonello Di Lella(2), Alfonsina Russo(3), Antonio Sgamellotti(4), Lilla Spagnuolo(1), Gabriella Strano(3), Stefano Loppi(2)

(1) Istituto Nazionale di Geofisica e Vulcanologia, Rome, Italy; (2) Department of Life Sciences, University of Siena, Italy; (3) Parco Archeologico del Colosseo, Rome, Italy; (4) Accademia Nazionale dei Lincei, Rome, Italy

Magnetic and chemical biomonitoring methodologies were applied to the southern slopes of the Palatine Hill archaeological site in Rome, Italy. Four samplings of plant leaves and a three-months exposure of lichen transplants were carried out between July 2022 and June 2023 to assess the impact of vehicular particulate matter from Via dei Cerchi (VDC), a busy road coasting Circus Maximus, towards the archaeological area upon the Palatine Hill. The magnetic properties of leaves and lichens were compared to the concentration of trace elements. The bioaccumulation of magnetite-like particles, associated with tracers of vehicular emissions, decreased with longitudinal distance from the road, without any important influence of elevation from the ground. First Order Reversal Curves diagrams were useful to link the variations of the magnetic grain-size to the different traffic regime at VDC. In fact, in the first half of the road, brakes are used for slowing down the cars before reaching the queues due to parking and a traffic light, while, on the second half of the road, slow or start and stop traffic prevail, with emission of finer particles linked to fuel exhaust. Lichens demonstrated to be more efficient biomonitors of airborne fraction of PM than leaves, irrespective of the plant species. Conversely, leaves intercepted and accumulated all PM fractions, including road dust and resuspended soil particles. Thus, plant leaves are suitable for providing preventive conservation services that limit the overall impact of particulate pollution on cultural heritage sites within busy metropolitan contexts. Green barriers and plants should be respectively installed and planted as close as possible to the road for providing the most effective protection service.



### (R) Can different moss species be interchangeably used in biomonitoring potentially toxic elements within the same survey?

<u>Mira Aničić Urošević(</u>1); Miloš Ilić(2); Tijana Milićević(1); Gordana Jovanović(1); Omari Chaligava(3); Inga Zinicovscaia(3,4); Aleksandar Popović(5)

(1) Institute of Physics Belgrade, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia; (2) University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, 21000 Novi Sad, Serbia; (3) Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Joliot Curie 6, 141980 Dubna, Russia; (4) Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, 30 Reactorului Str. MG-6, Bucharest Magurele, Romania; (5): Faculty of Chemistry, University of Belgrade, 11000 Belgrade, Serbia

The selection of appropriate biomonitor species is a crucial criterion for intercomparable biomonitoring over large spatial scales. It is important to select species that are ubiquitous and tolerant to pollution. However, different species prefer different habitats and can rarely be sampled at the same sites. Two moss species (Hypnum cupressiforme and Sphagnum girgensohnii) were exposed in bags, in parallel, searching for their comparative response to the presence of airborne potentially toxic elements (PTEs) in urban and agricultural areas of Serbia. In addition, in remote areas of Serbia passive moss monitoring involved the parallel collection of two mosses (H. cupressiforme and Brachythecium spp.) and a lichen (Evernia prunastri). PTEs were determined using destructive (ICP-OES and ICP-MS) and non-destructive (NAA) techniques. A type II linear regression model was applied to test the interchangeable use of the collocated biomonitor species and significant differences were detected. Regression analysis showed significant determination coefficients only for Cu. However, the spatial-temporal trends in the collocated biomonitors were similar. In Belgrade, both species recognized the city zones characterized by high, moderate, and low levels of air pollution. The same pattern of element distribution was found in vineyards experiments, but with the absolute values of element significantly higher in S. girgensohnii bags. Concerning passive biomonitoring, regression analysis showed significant determination coefficients only for Cd and S. To conclude, it should be careful with exchangeable using multiple biomonitor species, even from the same genus, within a single study.



#### (R) Biomonitoring of Heavy Metals in Slovakia

Jana Borovská; Tomáš Rusňák

Institute of Landscape Ecology, Slovak Academy of Sciences, Branch Nitra, Akademická 2, 949 01 Nitra, Slovakia

Environmental studies using bryophytes for biomonitoring of changes in atmospheric deposition of heavy metals in Slovakia started in 1990 in a 16x16 km grid. In the last ICP Vegetation Moss Survey 2020-23 new open and background sites were added. Collected mosses were analyzed by EA-TCD (N, C, S), AES-ICP (Al, P, Ca, Mg, K, Na, Fe, Mn, Zn, B, Cu), AES-ICP-U (As, Cr, Co, Cd, Ni, Pb) and by AAS-AMA (Hg). Correlation analysis and heat-maps were applied to identify relationships among different heavy metals and to provide insights into potential sources and interactions among contaminants. Hierarchical clustering analyses were used to identify similarities in contamination patterns among sampling sites and to reveal clusters with similar contamination profiles. Factor analysis was applied to uncover underlying factors influencing the observed contamination patterns. The scree plot indicated the optimal number of factors, while factor loadings provided insights into the contribution of each metal to the identified factors. Based on the Pollution load index the results showed that Slovakia has moderate to no pollution (PLI = 1.45), except a several hotspots with moderate to high pollution. These hotspots are situated in the eastern part of the country where is a center of metallurgy and steel production and in a region with long mining history with many pits for nonferrous ores, mainly magnesite, limestone and a large copper refined factory. In the western part of the country, the highest PLI is around a large and important fertilizer producer and in a region with dominant fiber glass and automotive industry. The highest mean values of contamination factors are Fe (3.16), Co (2.25) and Al (2.23), which are classified as slight contamination.

Acknowledgement: This work was supported by the project VEGA 2/0115/21 'Long-term changes of atmospheric pollution and their impact to ecosystems'.



### (F) Trace elements in the lichen *Lobaria pulmonaria* from remote areas: evidence from herbarium collections in Slovakia

Anna Guttová(1); Zuzana Fačkovcová (1,2); Luca Paoli(3)

(1) Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia; (2) Department of Pharmacy, University of Genoa, Italy; (3) Department of Biology, University of Pisa, Italy

Lichen collections can be used to evaluate past levels of chemical elements and assess their variation also in remote areas. In the present study, a revision of Lobaria pulmonaria collections from remote forests of the Western Carpathians (Slovakia) was carried out to characterize past and current profiles of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, S, Sb and Zn. Overall, twenty specimens from two collections (Plant Science and Biodiversity Centre, Slovak Academy of Science "Herb. SAV", and the Natural History Museum of Bratislava "Herb. BRA") were selected. Several peaks of concentrations, especially for Cd, Cu, Fe, Mn, Ni and Zn were observed in the period 1972-1989. A progressive decrease of concentrations was evident from the 1960s for As, Cr, Hg, S and Sb. The results also highlight a decrease in Pb concentrations after 1989 associated to the introduction of unleaded gasoline, whose positive effects could be observed also in remote areas. Based on the available specimens, background concentrations have been estimated for each period: the elemental content of L. pulmonaria between 1960 and 1989 in the Western Carpathians was at least four times higher than nowadays. On the whole, the study confirmed that botanical collections (in this case lichen collections) may provide valuable information to investigate air pollution, when other sources of data are not available.



#### (F) A new tool for interpreting lichen biodiversity data: the Italian proposal

<u>Paolo Giordani</u>; Renato Benesperi; Elisabetta Bianchi; Giorgio Brunialti; Giulia Canali; Fabio Candotto Carniel; Tania Contardo; Luca Di Nuzzo; Lisa Grifoni; Luana Francesconi; Luisa Frati; Stefano Loppi; Fabrizio Monaci; Silvana Munzi; Juri Nascimbene; Luca Paoli; Sonia Ravera; Carolina Stringa Basile; Mauro Tretiach; Andrea Vannini; Aldo Winkler

Working Group for Biomonitoring, Italian Lichen Society

Despite decades of work on the standardization of biomonitoring methods based on lichen diversity, some crucial points remain unresolved. This situation inevitably has an impact on the large-scale application of the methods, which in turn affects their reliability. The interpretation of lichen diversity results is undoubtedly one of the most critical aspects. As part of the activities of the Working Group for Biomonitoring of the Italian Lichenological Society, a methodological proposal has been developed which takes into account the various sources of variability in lichen biodiversity data, thus increasing their comparability. A database was established for the purpose of collating the data collected in recent years in Italy during various biomonitoring campaigns. Subsequently, the data were divided according to the main Italian bioclimatic regions and the types of bark on which they were collected. This division allows for the development of different interpretation tools. Each tool is represented by a two-dimensional plane, identified by the lichen diversity value on the ordinates and the proportion of oligotrophic species on the abscissas. Lines across the plane identify the percentile position of data, providing a comparable assessment of each subset of data in relation to the maximum values observed in homogeneous situations.



# (F) Assessing *Lobaria pulmonaria* (L.) Hoffm. as a proxy for minimal atmospheric pollution in Mediterranean: insights from the BioConLobaria Project on pollution impact and lichen viability

<u>Sonia Ravera(1);</u> Luca Paoli(2); Marta Agostini(2); Renato Benesperi(3); Juri Nascimbene(4); Elisabetta Bianchi(3); Patrizia Campisi(1); Silvia Del Vecchio(4); Zuzana Fačkovcová(5); Luana Francesconi(4); Gabriele Gheza(4); Giovanna Pezzi(2); Monica Ruffini Castiglione(2); Luigi Sanità di Toppi(2); Luca Di Nuzzo(4)

(1) Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STeBiCeF), University of Palermo, Italy; (2) Department of Biology, University of Pisa, Italy; (3): Department of Biology, University of Firenze, Italy; (4): Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy; (5): Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia

The lichen Lobaria pulmonaria is well-known for its sensitivity to airborne pollutants. Research consistently shows that pollutants like  $SO_2$ ,  $NO_x$  and acid rain have a dual negative impact: they cause direct phytotoxicity and indirectly acidify bark, which historically led to the decline or replacement of Lobarion communities with more acidtolerant species. Multiple studies have established L. pulmonaria as an effective indicator of lichen diversity and low environmental pollution. Its presence is often linked to these favorable conditions, making it a potential tool for large-scale surveys. This study aims to assess the species' potential as a proxy indicator for a rapid evaluation of minimal atmospheric pollution in the Mediterranean region. We present preliminary findings from the ongoing "BioConLobaria" project, which primarily focuses on understanding the response of L. pulmonaria transplants to various environmental factors within forest ecosystems. Our hypothesis is that atmospheric pollution impacts the growth of the transplants. The research is being conducted across three different forest habitats (mixed oak, chestnut, and beech forests) which are the primary substrates for L. pulmonaria in the Mediterranean. Study plots are situated at varying distances from pollution sources such as urban areas, roads, and industrial activities. This experimental setup has been replicated in three distinct regions of Italy: Emilia-Romagna, Tuscany, and Campania. Given that the viability of L. pulmonaria populations in response to environmental stressors often depends on the regenerative capacity of both young and adult thalli, we analyzed two types of transplants: those with meristematic (young) properties and those with non-meristematic (adult) properties. Growth rates and survival probabilities were assessed and modelled under each experimental condition to better understand how this species responds to environmental pollution.

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### Session "Plastics and microplastics"

Moderators: Merhiban Jafarova & Julian Aherne



#### (P) Trends in pollution over time in the XXI century

#### Cristina Branquinho

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Advances in technology have enabled us to better monitor and mitigate pollution, leading to decreased pollutant loads in various environmental compartments. Biomonitoring, a powerful tool for assessing environmental health, includes methods such analyzing pollutant accumulation in organisms or evaluating biodiversity patterns. While spatial patterns are relatively straightforward to assess through single-site sampling, temporal studies present significant challenges, including sampling design, methodological standardization, accounting for temporal fluctuations, data analysis, and resource allocation. This presentation will review several case studies that demonstrate the evaluation of long-term pollution trends through biomonitoring. Those case-studies will cover: i) different types of biomonitors from lichens to bryophytes; ii) different types of biomonitoring from pollutant accumulation to biodiversity patterns; iii) different types of pollution from macronutrients, through metals and Persistent organic compounds; iv) in different ecosystem matrixes such as air and water. A critical analysis of the results will assess the limitations of long-term biomonitoring, and the challenges posed by emergent pollutants.



### (R) Plastic debris in nests of white stork *Ciconia ciconia* population: characterisation and analysis of associated factors

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Most bird species are threatened by plastic pollution. The situation is particularly critical for the white stork (Ciconia ciconia). This emblematic migratory bird often nests near populated areas, where it obtains much of its building materials. Plastic is one of them, due to its abundance in landfills and surrounding agricultural fields. With this in mind, the aim of the study was to determine the types of plastics and microplastics present in nests, pellets and feathers of a white stork population and to check if there is any relationship with nest variables such as age or distance to potential sources of pollution. Samples were collected from 8 nests and chemically digested in order to isolate any plastics present and characterized using FTIR spectroscopy. Most of the plastics found are polyethylene or polypropylene. The predominant form are fibers, followed by fragments, ropes, cables, rubbers and sheets. There seems no relationship between the number of particles found and the age of the nests, as well as their proximity to different sources of contamination. Simultaneously, given that humans are the main cause of this situation, we wanted to know whether society perceives this environmental problem and what psychosocial variables (e.g. perception of environmental risk or attribution of responsibility) influence non-responsible behavior in the use of plastic.



### (R) Biomonitoring of airborne microplastics: comparison between moss and lichen transplants

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There is growing interest in the use of moss and lichens to monitor airborne microplastics, yet few studies have compared the effectiveness of each biomonitor. Here we assess the ability of moss and lichen transplants collected from a remote area to accumulate microfibers (MFs) and Potentially Toxic Elements (PTEs) under the same deployment conditions, across a range of urban exposure sites. The results showed that both biomonitors accumulated similar amounts of MFs, both in terms of counts and on a mass basis, but when expressed on a surface area basis, lichens showed significantly higher values. Irrespective of the metric, moss and lichen data were strongly correlated. In contrast, there was no correlation between MFs and PTEs, suggesting that their sources were different. MFs accumulated by both moss and lichen transplants were dominated by polyethylene terephthalate (PET) and polypropylene polymers, suggesting that the main source of airborne MFs is synthetic textiles.



#### (R) Does moss bag design influence the accumulation of atmospheric microplastics?

<u>Mehriban Jafarova</u>; Carter Bertrim; Kelly Evans; Brandon Monteiro; Harriet Walker; Julian Aherne

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Active monitoring with "moss bags" has been widely used to evaluate air pollution in areas where naturally occurring species are unavailable. Moreover, studies have shown that mesh size and bag shape do not significantly influence the uptake capacity of transplanted moss for trace elements. However, few other atmospheric contaminants have been evaluated. Here, we assess the influence of moss bag design on its efficiency as an active biomonitoring technique for atmospheric microplastic deposition. Specifically, we evaluated the influence of mesh size and bag shape on the accumulation of atmospheric microplastics by the moss species *Pleurozium schreberi*. Further, we present observations of atmospheric microplastic using moss bags across 40 sites in the Greater Toronto Area, Ontario.



### (R) Sequential extraction of anthropogenic microfibers from leaves of *Pittosporum* tobira

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Anthropogenic microfibers (MFs) are human-produced fibers that can be categorized as natural, semisynthetic, or synthetic according to material of origin and production process; their widespread diffusion is becoming a global problem affecting both anthropized and natural environments. The distribution and transport of airborne MFs follow the paths of particulate matter. Recently, different screening methods to monitor the abundance of MFs fallout have been developed. Plants are known as good biomonitors of atmospheric deposition of several pollutants thanks to their intrinsic surface characteristics. Currently, several methods are used for the extraction of MFs from plants, essentially based on digestion and flotation of plant tissues, followed by visual counting of particles. The challenge for researchers is to find the best extraction technique, that should be reliable, quick, reproducible, and sustainable (i.e., with a reduced use of chemicals). The difficulty is to find a balance between the careful exclusion of all biological material and a good and constant recovery of the fibers, preserving their characteristics for chemical identification. So far, the most used extraction method is oxidative extraction, proposed for native mosses, although it is less useful for leaves of higher plants, due to the higher cellulose content and sometimes partial lignification of cell walls. Here we propose a four-step sequential extraction protocol for MFs from the leaves of Pittosporum tobira (Thunb.) W.T. Aiton. Assuming that the MFs identifiable by stereomicroscope are usually longer than 30 µm, and therefore they do not enter the leaf tissues, the method considers the MFs adhering to the leaf surface, and those possibly trapped in the cuticle. The results indicate that most MFs were accumulated outside the leaf cuticle (about 75%), while the removing of this latter enabled the recovery of the remaining 25%.



#### (R) Microplastic contamination in lettuces from Lisbon urban vegetable gardens

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Urban vegetable gardens (UVG) are increasingly common and provide numerous environmental and societal benefits, but vegetables grown in these sites may be affected by pollutants. Microplastics (MPs) are of growing concern due to their increasing presence in water, soil, air and even food. Considering that UVG are sometimes located in traffic areas, the accumulation of MPs by the vegetables grown in these settings should be evaluated. In this regard, lettuce (Lactuca sativa L.) is considered a good bioindicator of environmental quality, accumulating trace metals and MPs. The aim of the present study was to investigate the number of MPs accumulated by the leaves of lettuce plants grown in Lisbon UVG. For this purpose, MPs content was determined in the leaves of lettuces belonging to two cultivars collected in seven Lisbon UVG, in a rural area and in commercial samples, for comparison. Overall, 14 different lettuce samples (42 subsamples, considering triplicates) of washed outer leaves, were analyzed. The content of potentially toxic elements in lettuce leaves was also determined by PIXE and ICP-OES, for correlation with MPs content and identification of possible common sources. A total of 101 MPs were detected in the 42 sub-samples, and only 6 sub-samples were completely devoid of MPs. Mean MPs content in lettuce samples from rural  $(16.2 \pm 7.5)$ MPs/g) and urban (18.6 ± 7.4 MPs/g) environments were similar, and both were higher than those from commercial samples  $(10.7 \pm 0.2 \text{ MPs/g})$ . The results revealed significant differences in the MPs contents depending on the UVG location. This work provides, for the first time, insights into the MPs contamination of lettuces grown in Lisbon UVG and allows an estimation of the correspondent dietary intake.



### $(\ensuremath{\mathsf{R}})$ Web of pollution: Passive biomonitoring of atmospheric microplastics across Ontario

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Passive biomonitoring has been widely used to provide qualitative and quantitative spatial information on air pollution. Here we present a novel biomonitor of atmospheric microplastics in urban environments. Following a thorough search of the web, we asked three questions: (1) is the novel biomonitor widely distributed in urban environments? (2) does it accumulate microplastics above limits of detection? and (3) are microplastics easily extracted from the biomonitor? To answer these questions, we evaluated atmospheric microplastics across a web of sites in the Greater Toronto Area and Peterborough Ontario using the novel silken sensor.



### (R) The soil microbiota as a biomarker for the response to microplastics as an emerging pollutant. The case study of Volturno River sediments (Southern Italy)

<u>Maria Maisto</u>(1); Maria Antonietta Ranauda(1); Daniela (1); Maria Tartaglia(1); Alessia Postiglione(1); Antonello Prigioniero(1); Alessandra Falzarano(1); Pierpaolo Scarano(1); Valter Castelvetro(2); Andrea Corti(2); Francesca Modugno(2);, Jacopo La Nasa(2); Greta Biale (2); Rosaria Sciarrillo(1); Carmine Guarino(1)

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The microbiota of marine and riverine sediments is crucial for ecosystem services and stability and can be extremely sensitive to environmental disturbances. The rapid adaptive response of microorganisms to biotic and abiotic changes can lead to modifications in the structure and diversity of sediment microbial communities. This plasticity could make in situ microorganisms a useful marker for environmental approaches, such as the evaluation of the impact of anthropogenic activities on natural habitats. In this perspective, this study reports microplastics contamination (MPs) of Volturno River (Southern Italy) sediments and its possible ecological implications. A 16S metagenomics analysis was performed, using next-generation sequencing in Ion Torrent, to explore the bacterial taxonomy of sediments collected along the river watercourse and its possible modifications. MPs were detected in all samples, that were clustered into two distinct population data: high-MP contaminated and low-MP contaminated sediments. According to the Polymer Hazard Index (PHI), the risk of MP pollution of the analyzed sediments was categorized as Hazard level III/IV (corresponding to Danger category). Metagenomic data revealed that the presence of MPs significantly affects the abundance of microbial taxa, evidencing Flavobacteraceae and Nocardiaceae, known to degrade polymeric substances, in high-MP contaminated sediments. This study provides new insights of ecological relevance related to MP pollution and demonstrated the microbiological potential as bioindicator for environmental monitoring of plastic pollution.



### $(\ensuremath{\mathsf{R}})$ Molecular and microbial biomarkers in the soil-plant system response to emerging pollutants

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Emerging contaminants are extremely widespread in terrestrial environments, with potential impacts on the soil-plant system not yet well understood. In addition to heavy metals, polycyclic aromatic hydrocarbons and agrochemicals, macro- and microplastics (MPs) constitute a spreading contaminant in a variety of ecosystems. This study explores the effects of oxidized low-density polyethylene- microplastics (LDPE-MPs) on the rhizosphere ecology and plant fitness of *Fragaria x ananassa* (Duchesne ex Weston) Duchesne ex Rozier. The rhizospheric microbial community was investigated under the influence of 0,5% LDPE-MPs by internal transcribed spacer (ITS) and 16S rRNA metagenomic analysis; photosynthetic parameters, antioxidant enzyme activities, and nutrient accumulation were assessed to evaluate plant physiological and biochemical status. Genes related to jasmonic acid (JA), ethylene biosynthesis, and nitrate signaling pathways were analyzed to define the plant molecular response. Our results showed a shift in the rhizosphere microbial community. We identified MPs molecular biomarkers in the contaminated rhizosphere (Fusarium, Thanatephorus and Pseudallescheria) with potential pathogenic functions and two novel molecular biomarkers (Ohtaekwangia and Ascobolus). MPs pollution negatively impacts plant fitness, which showed decreased chlorophyll a and b (40 and 48 %, respectively), a change in macronutrient (N, Ca, S, P, K. and Mg), and micronutrient (Mn, Fe, Cu, and Zn) content (fluctuations between 14.42) and 26,7 %) at the leaf level and increased activity of antioxidant enzymes. Gene expression related to JA, ethylene biosynthesis, and nitrogen signaling pathways is enhanced in plants grown in contaminated soil, as well as the root endophytic and epiphytic microorganism interactions. Our results demonstrate that MPs pollution influences the rhizosphere microbial community and functions, and consequently, negatively impacts plant health.

### Session "Plant-based Biomonitoring"

Moderators: Nathalie Sauret & Sergio Calabrese



### $({\sf R})$ Leaf absorption characteristics of a bioindicator plant Elaeagnus ebbingei towards PAHs

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We previously highlighted that the hedge plant *Elaeagnus ebbingei* could be used as a bioindicator of the atmospheric concentrations of Polycyclic Aromatic Hydrocarbons (PAHs). Scanning Electron Microscopy (SEM), Optical Microscopy and Infra-Red Fourier Transform Spectroscopy (IRTF) analyses revealed two different structures of adaxial and abaxial surfaces of E. ebbingei leaves. The amounts of PAHs were evaluated in leaves for which the abaxial surface was removed by abrasion. Compared to representative initial leaves, abraded ones contained lower levels of these atmospheric pollutants reflecting an heterogeneous repartition of PAHs in E. ebbingei leaves. Moreover, this indicates that PAHs are mostly localized in the layers of trichomes. E. ebbingei trees were exposed, over different periods of time, in a chamber to an air flux contaminated with phenanthrene. Experiments showed the great capacity of the leaves to absorb this PAH (>  $3 \mu g g^{-1}$  versus around 10 ng g<sup>-1</sup> in trees grown in an urban area). Moreover, a rather linear accumulation along time was recorded confirming the bioindicator characteristics of this plant. After contamination with phenanthrene, plants were placed outdoors in the open air. Phenanthrene disappeared of the leaves following a first order kinetic process. A half-life around 100 hours was calculated. The concentrations of phenanthrene in contaminated leaves converge endless to that of leaves of non-contaminated plants. In conclusion, the analyses of the leaves of E. ebbingei allowed to inform on the absorption pathway of PAHs and their localization in that hedge plant. Chamber experiments enable us to assume that the species presents a great absorption potential for high PAH levels. Further studies are conducted to understand the physical-chemical behavior (absorption/desorption, metabolization, photodegradation) of PAHs between the air and this bioindicator hedge plant.



### (R) Comparison of leaf elemental composition for biomonitoring purposes in urban environments

Zbigniew Ziembik; Agnieszka Dołhałczuk-Śródka; Andrzej Kłos; Grzegorz Kosior

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In urban environments, air contamination is related to the transport of particulate matter over short and long distances. Due to the abundance and variety of pollutant sources, the distribution of hazardous substances can be significantly non-uniform. As a result, pollutant distribution maps constructed using classical methods of pollution determination are coarse and require extensive support with mathematical modeling. The chemical composition of plant parts growing in urban regions can provide information regarding pollution in the surrounding area. Cities usually have many green spots, such as single trees or groups of trees. Tree leaves collected from these green spots can provide information about local contamination. The application of plants for biomonitoring purposes in urban environments faces several obstacles. Urban green areas often contain different plant species. The chemical substance content in tree leaves may depend not only on local contamination but also on the tree species, local growth conditions, season, and other factors. To estimate the influence of tree species on the chemical element content in their leaves, samples from willow (Salix alba L.), spruce (*Picea abies* (L.) Karst), and linden (*Tilia x europaea*) trees were collected. These trees grow in the same green spot. Starting in the middle of April 2024, leaf samples were collected approximately every two weeks. The influence of water concentration in the leaves on element content was also considered. To calculate water content, the weight of fresh and dried material was determined. The concentration of chemical elements in the dried material was determined using the XRF method. Concentrations of Ag, Ca, Cd, Cl, Co, Cu, Fe, K, Mn, Mo, Nb, Ni, P, Pd, Rb, S, Si, Sr, Th, Ti, U, W, Y, Zn, and Zr were determined. The studies have shown that one of the factors affecting heavy metal concentrations may be the water content in the leaves.



### $({\rm R})$ Leaf surface functional traits as driver to identify plants useful in PM and PTEs biomonitoring in urban environment

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In this study, we explored the relationships between the accumulation rates of particulate matter (PM) and potentially toxic elements (PTEs) occurred over 30 days of exposure to urban pollution, and the functional traits (trichome morphology and density, trichome surface per leaf area, stomata density and surface per leaf area, cuticle thickness and chemical features) of the leaf surface of twenty-eight trees and shrubs occurring in a Mediterranean urban environment. Following double sampling (August-September) in the city of Naples (Southern Italy), we quantified coarse and fine PM (PM<sub>10</sub> and  $PM_{2.5}$ ) deposited or adsorbed on leaves surface by successive extraction with distilled water and chloroform respectively, and gravimetric analysis using paper filters at different porosity. PTEs were extracted from paper filters and from leaves resulting from water and chloroform extraction. Then PTEs concentration was defined by ICP-MS. For each species, leaf surface functional traits were determined by SEM microscopy. Significant differences were found between species in the arrangement of functional traits, concentrations and daily uptake of pollutants. Correlations between variables were studied by means of principal component analysis. The trichomes-related traits scaled positively with PM concentration daily uptake, whereas for PTEs, the trichomes and cuticle features are relevant. Trichomes increase the leaf area several folds providing extra surfaces for PM retention, while cuticle thickness and esterification regulate the PTEs retention. Results provide novelty on the relationships between leaf surface functional traits and plant species potentially useful as biomonitor for urban air pollution, outlining an innovative way of measuring leaf surface functional traits. In addition to broadening the economic spectrum of functional traits, it emphasizes how important leaf surface functional traits are in the nature-based solutions definition and planning.



#### (R) Active and passive biomonitoring techniques in active volcanic environments

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Explosive eruptions and volcanic passive degassing inject large quantities of gas and particles into the atmosphere that are ultimately deposited at the Earth's surface through wet or dry deposition processes, affecting the atmosphere, the hydrosphere and the biosphere. The use of active (moss-bags) and passive (leaves of endemic plants) biomonitoring techniques in areas affected by volcanic emissions has proved particularly effective for studying volcanic aerosol and atmospheric deposition. In this contribution, we present the main results obtained in the last twenty years of research in active volcanoes (Etna, Stromboli and Vulcano, Italy; Nisyros, Greece; Nyiragongo, D.R. Congo; Gorely, Kamchatka). Moss bags (Sphagnum species) were exposed around Etna, Vulcano and Nyiragongo at different distances from the active vents to evaluate the impact of volcanic emissions into the atmosphere and in the local surroundings. Interesting results were also obtained through biomonitoring surveys using leaves of trees (Betula aetnensis, Castanea sativa, Pinus nigra and Populus tremula) and endemic species (Senecio aethnensis, Rumex aetnensis, Cistus salviifolius and creticus, Erica manipuliflora and arborea). The results confirmed the huge amount of silicate, sulphate and halide compounds emitted by volcanoes. The contents of major and trace elements significantly increased in mosses after their exposure to volcanic emissions, confirming them as efficient accumulators. Metals uptake rate by leaves of endemic plants rapidly decreases with the distance from the volcanic emission vents; the elements that showed the greatest accumulation include S, Na, K, Ca, Fe, Al, Cu, V, As, Cd, Li, Se, Sc, Th, Bi, Te and Tl. In general, both active and passive biomonitoring have yielded some very useful results in assessing the impact of volcanic emissions and mapping their areal extent.



### (R) Suitability of *Tillandsia usneoides* for biomonitoring toxic elements in forest ecosystems

<u>Claudia Pisuttu</u>(1); Clara Merlini(2); Alessandra Lepidio(1); Simone Bernardi(1); Lorenzo Cotrozzi(1); Giacomo Lorenzini(1); Cristina Nali(1); Elisa Pellegrini(1); Zuzana Fačkovcová(3); Luca Paoli(2)

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A rootless Bromeliad species (Tillandsia usneoides L.) was exposed for 90 consecutive days (May-August 2023) in nine sites of the Pollino National Park (Southern Italy), and used as biomonitor of airborne trace elements, with a focus on fluoride and sulphur, identified as the most emitted pollutants in the area. Unwashed and washed samples were analyzed by ICP-MS. Fluoride concentrations resulted higher in urban/traffic and/or suburban/traffic sites, whereas sulfur levels were much higher in the industrial site than in rural/remote ones (+70%). These results highlighted the occurrence of a clear locationspecific pattern of pollutant levels, strictly related to the potential emission sources. Following these promising outcomes, a second campaign was performed in 2024, surveying six sites located in Southern Tuscany (Central Italy), and characterized by different vegetation (i.e., oak, beech or chestnut forests) and forest management practices. Two remote sites were also set in San Piero a Grado (Central Italy), as controls. In each site, four plants of *T. usneoides* were collocated on the north side of different trees. Samples were collected after 90 and 180 consecutive days of exposure (May-August-October) and processed as reported above to quantify fluoride, sulfur, and mercury. Being high resistant/tolerant to heavy metal toxicity, as well as suitable to quantitatively characterize the pollution level of sampling sites, *T. usneoides* was shown to be a reliable biomonitor to reflect the intrinsic characteristics of each sampling area. and detect differences related to forest management practices. Overall, this study proposes the use of rootless Bromeliad species as an opportunity to easily characterize different morphological and climatic areas, especially those of the Mediterranean Basin.


#### (R) Towards ozone biomonitoring 3.0: Can vegetation spectroscopy help?

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Plants signal their health in a broader spectrum than we can see with our eyes and vegetation spectroscopy (VS) is a mature tool to monitor their responses to the environment, being non-destructive, rapid, and relatively low-cost. Reflection of light in the VIS-SWIR range (350-2500 nm) can assess macroscopic features and the underlying responses of plants to environmental constraints. This approach is scalable from leaf to remote sensing level, using airborne and space platforms. However, the use of this technique for environmental biomonitoring is underdeveloped. The present work aims to elucidate the potential of VS for the biomonitoring of tropospheric ozone  $(O_3)$ , one of the most toxic and abundant air pollutants. After a brief report of basic concepts of VS, including the approaches for exploiting information from multi- and hyperspectral data, collected with imaging/non-imaging sensors (spectral indexes, trait retrieval, spectral classification and mapping), some case studies are presented. Comparing the hyperspectral profiles (400-2400 nm) collected on leaves of different species (sage, grapevine, pomegranate, date palm), it was possible to discriminate with high accuracy (>80%) plants exposed to increasing O<sub>3</sub> (even in the absence of visible injuries). A number of hyperspectral models were developed to accurately predict an array of leaf functional traits (validation R<sup>2</sup>: 0.53-0.92). Furthermore, the collection of multispectral images of the  $O_3$ -supersensitive Bel-W3 tobacco, both as mature plants in pots and as seedlings in miniaturized kits, was shown to strongly help this well-known approach for the detection of  $O_3$  distribution in large scale surveys (spectral indexes were strongly related with the observed injury index). These outcomes may encourage further research to highlight the potential of VS to support the biomonitoring of air pollution in terms of processing speed, minimization of human errors and easy data management due to digitalization.



# (F) Sick building syndrome and ozone biomonitoring: *Nicotiana tabacum* cv. Bel-W3 seedlings exposure as a valid tool for the indoor air quality assessment

<u>Giulia Scimone(</u>1); Gemma Bianchi(1); Clara Merlini(1,2); Elena Alberti(2); Giorgia Semeraro(2); Sofia Trevisan(2); Mariagrazia Tonelli(1); Elisa Pellegrini(1); Claudia Pisuttu(1)

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Biological monitoring of ozone  $(O_3)$  pollution with the supersensitive tobacco cv. Bel-W3 has been successfully performed so far. It consists in the exposure of plants/seedlings to ambient air and in the quantitative evaluation of the intensity of O<sub>3</sub>-induced foliar symptoms (i.e., bifacial necrotic spots). However, any work has never focused on the potential of this methodology to assess the impact of  $O_3$  levels that occur in buildings, which are partially protective against the high outdoor concentrations of this pollutant, by playing a key role in the "sick building syndrome" (SBS). In this study, a group of four inexperienced scorers were involved in a two-week biomonitoring campaign aimed to generally stimulate awareness about the SBS due to air pollution and evaluate the efficacy of indoor tobacco exposure technique. Volunteers were firstly trained on symptoms assessment by performing four tests with 63 color photographs in full-scale of mature tobacco leaves (3 replicates of 21 photos of 21 different leaves) showing visible injuries. Subsequently, the above cited miniaturized kits were displayed inside and outside selected monitored sites. At the end of every exposure, volunteers were asked to estimate  $O_3$  foliar injury. During the training, the average accuracy of scorers resulted to be 60% and the average repeatability was 69%. The extreme classes were easily scored, while central classes proved to be more difficult to evaluate. Outdoor expositions revealed higher averages of damage in comparison to the indoor ones (2-fold), even if in few cases symptoms in indoor exposed tobacco plants were not negligible, confirming the potential of this approach to evaluate  $O_3$  inside buildings.



# (F) Ozone biomonitoring is a versatile tool for science, environmental health and regulation: A case study from a regional agency for territory protection of Central Italy

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The wide diffusion of tropospheric ozone ( $O_3$ ) is a major environmental problem in urban, rural and remote localities. Its increasing man-related levels are connected with severe impacts on human life and welfare, in terms of adverse health effects and plant injury. Biological monitoring is a powerful tool for filling the gap between the causes and the impact of environmental toxic compounds, as bioindication agents may assess in an easy-to-detect process the effects of pollution on biota. The present work aims to elucidate the potential of biomonitoring as a versatile tool for regulatory authorities to assess exposure levels and safety of  $O_3$ . Miniaturized kits based on the use of 2-week-old O<sub>3</sub>-hypersensitive tobacco germlings (*N tabacum* Bel-W3) were utilized in conjunction with four calibrated automatic analyzers (belonging to the air quality monitoring network managed by ARTA Abruzzo in compliance with the Directive 2008/50/CE) to monitor the distribution of ground-level  $O_3$  during the summer of 2023. Ozone was a relevant presence in the troposphere of the area investigated. The critical level for protecting agricultural crop species from 5% yield losses (indicated as an accumulated exposure over a threshold of 40 ppb of 3000 ppb h during daylight, which not to be exceeded over a 3-month period), has been approached after a couple of weeks in Cepagatti (Chieti; suburban-traffic monitoring station). Ozone concentrations were well above background levels and able to induce typical bifacial necrotic symptoms on the leaves of sensitive biological targets after a few days of exposure. Injury Index was read on more than 800 cotyledons and processed by statistical analyses. The correspondence between data from the automatic analyzers and those from the bioindicators was fair ( $R^2 = 0.91$ ), confirming that the proposed biomonitoring approach could be used by regulatory agencies to provide a health perspective.



### (F) Identifying trace metal contamination in Lisbon urban vegetable garden using lettuces as biomonitor

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Urban vegetable gardens (UVG) are gaining attention in the context of sustainable food supply, but vegetables grown in these sites are exposed to pollution, namely from traffic. The uptake of trace metals (TM) may lead to their accumulation in the edible parts of vegetables and introduction in the food chain, with the associated health risks. Lettuce (Lactuca sativa L.) is considered a good bioindicator of environmental quality and food safety. The aims of the present study were: 1) to assess the accumulation of TM in lettuces grown in Lisbon UVG and respective soils; 2) to estimate the potential exposure of local citizens to TM through dietary intake of these lettuces. TM were determined in lettuces belonging to 2 cultivars collected in 7 Lisbon UVG, in a rural area and in commercial samples, for comparison. Leaves (washed and unwashed), roots, and the respective soils were analyzed, to evaluate bioaccumulation and translocation from roots to aerial parts. The concentrations of Al, Br, Ca, Cl, Cu, Fe, K, Mn, Ni, P, Rb, S, Si, Sr, Ti and Zn were determined by PIXE and those of B, Cd, Cr, Mg, Mo, Na, Ni, Pb by ICP-OES. The contamination was assessed by comparison with the EU food quality and soil legislation. TM concentrations in leaves were used to assess the potential health risk through the estimation of the daily intake and hazard index. The correlation between TMs in leaves was used to ascertain possible common sources. TM soil contamination was assessed relative to the geochemical background through the geo-accumulation index and the enrichment factor. The results revealed significant differences in the accumulation of TM depending on the location. The implications of the study conclusions will be presented from the perspective of the environmental management of UVGs, to protect human health.



## (F) Metabolites of phenanthrene as potential markers of Polycyclic Aromatic Hydrocarbons in a bioindicating hedge plant *Elaeagnus ebbingei*

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Strong correlations were previously determined between Polycyclic Aromatic Hydrocarbons (PAHs) atmospheric concentrations and their amount in *Elaeagnus* ebbingei plant leaves. As a consequence, this hedge plant can be considered as a bioindicator of the presence of these air pollutants. Elaeagnus ebbingei leaves are absorbing high concentrations of phenanthrene which is then disappearing following a first order kinetic process. Thus, we developed a new method to monitor its presence using Solid-Phase Microextraction followed by Gas Chromatography-Mass Spectrometry (SPME-GC-MS). This method enables also to detect light PAHs (<188 g/mol) and offered adequate linearities for amounts in leaves lesser than 5  $\mu/kg$ . Our objective is to determine the physical-chemical behavior (absorption/desorption, metabolization, photodegradation) of PAHs between air and this bioindicator plant. To achieve this goal, an experiment chamber is used where *E. ebbingei* plants are exposed to a phenanthrene flow during a short period of time (10 h). After absorption of phenanthrene by the plant, the amounts of phenanthrene are monitored in the leaves and in the headspace of the plant using polyurethane foam (PUF). The presence of phenanthrene in PUF indicates that leaves are thus releasing this PAH. Moreover, after exposition to phenanthrene, the presence of potential metabolites is tentatively monitored in leaves to explain a possible metabolization correlated to the disappearance of this PAH. Indeed, possible metabolites of phenanthrene like 2-biphenylcarboxaldehyde were already identified in plants like Silene acaulis; others potential metabolites such as 6H-dibenzo[bd]pyran-6one and 9,10-phenanthraquinone are likely to be formed during photochemical processes. The influence of light, temperature and moisture will be investigated to characterize the behavior of phenanthrene and metabolites between air and the E. *ebbingei* plant

### Session "Community Science"

Moderators: Sonia Ravera & Stefano Loppi



### (R) Engaging citizens in an air quality assessment using lichen biomonitoring: a case study of the Lichens GO project

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Air pollution has a long-term impact on human and ecosystem health. In urban environments, air quality depends on both sources and microclimatic conditions, leading to considerable heterogeneity between neighborhoods and even between streets. However, high-resolution data are relatively scarce due to the limited number of measurement points in urban environments. The use of biomonitoring data derived from citizen science can overcome this resolution challenge by filing spatial gaps. This participatory approach also raises public awareness of current environmental concerns, while introducing them to the scientific method. An example is the French Lichens GO project (www.lichensgo.eu) that aims to assess air quality through lichen biomonitoring using a simplified version of the standardized European protocol to allow comparisons with expert data. To limit the sampling effort, while improving the data quality, we made a series of adjustments during co-construction phases. For instance, we evaluated the performance of several protocol steps (including site selection or potential identification observer bias) and the protocol applicability (e.g., time required, list of lichen species considered, etc.). Since the program was launched in 2018, we collected more than 2800 observations from 338 sampling sites. Some locations (e.g., Paris, Clermont-Ferrand, Limoges) presented an extensive sampling effort, enabling us to better understand the spatial heterogeneity of urban air quality data. Through this project, we seek to empower citizens to actively take part in environmental monitoring, while generating valuable data for air quality assessment at the national level.



#### (F) How and why to become a soil ambassador in the ECHO project?

#### Silvana Munzi

Centro Interuniversitário das Ciências e da Tecnologia, Universidade de Lisboa, Portugal

The ECHO Project is a citizen science initiative aimed at engaging communities in soil monitoring to enhance environmental awareness and improve data collection. Over the past year, since the first IABEP meeting, we have made significant progress in expanding our network of soil ambassadors, establishing assessment frameworks for citizen science protocols and dataset for use in data gathering. We have also actively raised awareness of the ECHO Project through participation in conferences and other events. In this presentation, we will outline how individuals can get involved by introducing the call for ambassador individuals dedicated to promoting and participating in the project within their local communities.



#### (F) Citizen Science meets tropospheric ozone biomonitoring: insights from Italy

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The rising levels of tropospheric ozone  $(O_3)$ , primarily caused by human activities, pose significant environmental challenges in urban, rural, and remote areas, greatly impacting on ecosystems health. In this scenario, biomonitoring provides valuable insights into the effects of  $O_3$  pollution on (selected) biota. Citizen science (CS) plays a crucial role in scientific research, enhancing the scope and scale of research-based studies and fostering public engagement in scientific processes of public interest. In 2024, a CS initiative aimed at validating the possibility of employing citizen scientists for the biomonitoring of O<sub>3</sub>, was launched with the help of the association Bioblitz Lombardia, involving volunteers and experts in exploring and cataloguing biodiversity in protected areas of Lombardy (Northern Italy). More than 120 citizens and 20 expert naturalists, affiliated with seven different protected areas, were involved in the campaign (i.e., held during Bioblitz 2024). The activity consisted of one round of exposure of miniaturized kits of O<sub>3</sub>-supersensitive Bel-W3 tobacco (*Nicotiana tabacum* L.) plantlets, from May 12 to 19, 2024. More than 1000 biological data (i.e.,  $O_3$  injury on cotyledons) were recorded, and statistically compared with chemical data collected by the automatic analyzers located near the monitored areas. Overall, the area monitored was ca. 3000 km<sup>2</sup>. Under the supervision of researchers and expert naturalists, citizens gained hands-on experience across various study areas and disciplines and were introduced into the scientific process in a clear and engaging manner. Beyond educational objectives, the initiative returned substantial research findings, enhancing understanding and awareness of local air quality issues, highlighting the effectiveness of biomonitoring in promoting public and youth engagement in environmental stewardship.



#### (F) How much do you know about ozone biomonitoring?

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Citizen science can be a helpful tool in the tropospheric ozone  $(O_3)$  biomonitoring. Usually, this approach consists in five different steps: (i) recruitment, (ii) training, (iii) involvement of volunteers in specific biomonitoring campaigns, (iv) data collection, and (v) data validation. The necessary condition to the first point thus, to involve as much as possible stakeholders, are the dissemination and the divulgation of specific topics, using methods citizen-oriented. This study aimed to understand how ordinary citizens are informed about the role of  $O_3$  in air pollution and how they are effectively interested in the topic, trying to provide potential solutions to increase their consciousness. A survey was distributed to 114 volunteers of different ages (60 people under 30 years old, 38 over 30 and under 60 years old and 16 over 60 years) and instruction grade (8 people with lower secondary school diploma, 46 with high school diploma, 54 graduates and 6 PhD). Obtained outcomes highlighted that only 3% of the people interviewed were not interested in air pollution. Of these, 33% were represented by secondary school candidates. The 27% of the volunteers appeared to be well-informed about biomonitoring and the percentage grew as the instruction level increased. However, only 33% of the people consider O<sub>3</sub> among the most dangerous pollutants, but 58% associate it to the O<sub>3</sub> hole (a little bit of confusion!). The 43% of interviewed would like to be involved actively (according to the instruction level), while 45% of the people did not trust with this kind of activities, being more sceptic as the age increases. Overall, it seems fundamental to launch informative campaigns at different scales: training courses, specific projects to sensibilize scholars and low-time consuming initiatives BioBlitz-model based are just a few of the several activities, which may be organized to make informed, responsible and active the citizens.



# (R) Assessment of the spatial and temporal impact of wastewaters on biological responses in aquatic organism and water quality of the karst river water

<u>Vlatka Filipović Marijić(</u>1); Sara Šariri(1); Želimira Cvetković(2); Damir Valić(1); Tomislav Kralj(1); Tatjana Mijošek Pavin(1); Zuzana Redžović(3)

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Comprehensive understanding of risk assessment of dynamic river medium under the influence of multiple anthropogenic stressors should involve chemical but also biological responses, that reflect long-term changes in the environment. Water quality and toxic effects on organisms in the wastewater impacted karst Krka River (Croatia) was assessed by the spatial and temporal (long-term and seasonal) variability of the physical-chemical water parameters, acute toxicity testing with microalgae (Pseudokirchneriella subcapitata (Korshikov) Hindák, 1990) and crustaceans (Daphnia magna Straus, 1820) and by biomarker responses in fish brown trout (Salmo trutta L., 1758). Results indicated high toxicity of the industrial wastewater (toxicity tests with algae and daphnids), and degraded water quality and disturbed seasonal fluctuations at wastewater-influenced sites, primarily due to high levels of nutrients and organic matter. However, improvement was observed downstream in the Krka National Park (KNP), confirming the selfpurification and underground flows as important processes in dynamic and complex karst ecosystems. Natural seasonality, observed at sites without wastewater influence, was mainly driven by fluctuations in water levels and primary production during the year. Significantly higher malondialdehyde concentrations in brown trout from the sites impacted by wastewaters and in the Krka National Park compared to the river source pointed to oxidative stress, which was also confirmed by the need of enhanced levels of both antioxidants (glutathione and catalase) at these two sites. Our results indicated negative influence of wastewater on the river water quality and freshwater organisms of different trophic levels. Evident effects on the biota, even in KNP, presented indication of potential risks to the protected area and emphasized the need for continuous and rigorous monitoring to preserve the KNP and other sensitive karst ecosystems.

### Session "Ecosystem-based Biomonitoring"

Moderators: Martin Bačkor & Manuela Giovanetti



#### (P) Sleeping volcanoes, awaking health issues: lessons learned from wild mice

Patrícia Ventura Garcia

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It is estimated that 10% of the worldwide population lives in the vicinity of an active volcano. However, volcanogenic air pollution studies are still outnumbered when compared with anthropogenic air pollution studies, representing an unknown risk to human populations inhabiting volcanic areas. Present-day volcanic activity in the Azores archipelago is marked by several hydrothermal manifestations consisting of active fumarolic fields, thermal and cold  $CO_2$  springs and soil diffuse degassing areas. This type of "silent" volcanic activity is responsible for the emission into the environment of several noxious compounds, such as gases (some radioactive, such as radon, other neurotoxic, like gaseous elemental mercury) and heavy metals, which can cause adverse health effects in organisms that live in these areas, including humans. In this presentation we will unveil some of the silent risks of living in these environments, by presenting and discussing our most recent studies using wild mice as bioindicators to assess the health effects of vulcanogenic pollutants on the nervous and respiratory systems.



#### (R) Lichens and mosses in remediation of metal polluted environments

<u>Martin Bačko</u>r(1,2); Blažena Drábová(1); Marián Tomka(1); Dana Rajnincová(1); Dana Urminská(1); Michal Goga(2)

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In this presentation we critically evaluate the possibility of using lichens and mosses, frequently occurring cryptogams, in phytoremediation of metal(loid)s from the soil and water. We discuss various sources and harmful effects of metal(loid)s on lichens and mosses, factors affecting metal(loid)s bioavailability, advantages and disadvantages of use of lichen and mosses in remediation at different levels, from populations to cells and from ecology to molecular biology. Mechanisms of metal(loid)s accumulation and detoxification in lichens and mosses are discussed. Special emphasis is placed on extracellular deposition of metal(loid)s, ultrastructural changes and different physiological parameters which are frequently employed in phytoremediation studies using lichens and mosses, e.g. membrane integrity, assimilation pigment composition, chlorophyll a fluorescence, photosynthesis and respiration parameters. Composition of amino acids, ergosterol content in lichens, non-protein thiols synthesis, activity of antioxidant enzymes or expression of stress proteins are recently also utilized in studies focused on responses of lichens and mosses to metal(loid)s excess in the environment. Acknowledgement: this work was financially supported by the Slovak Research and Development Agency under contract No. APVV-21-0289, Slovak Grant Agency KEGA under contracts No. 008SPU-4/2023 and 009UPJŠ-4/2023, and Slovak Grant Agency VEGA (VEGA 1/0252/24).



#### (F) Possible influence of forest fires on Hg levels In soil and pine needles

#### Zulema Varela(1); María Bouso(1); J. Ángel Fernández(1); Nuno Ratola(2,3)

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Forest fires are guite common in the Iberian Peninsula and are a source of environmental, social and economic disruption in many communities. Lately, extreme wildfires have been increasing in frequency and severity with an enhanced negative impact on directly but also indirectly impacted areas. Mercury is a very toxic yet common element on our planet and forest fires reportedly may have an influence in its local and planetary distribution. By analyzing the Hg content in pine needles and soils collected (in November 2017 and November 2018) after the terrible 2017 forest fires in Portugal in impacted and non-impacted areas, this work intends to be a first attempt to assess this effect by using biomonitors with a recognized ability to uptake both elements and organic pollutants. Acknowledgement: this work was financially supported by: LA/P/0045/2020 (AliCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE; DOI: 10.54499/UIDB/00511/2020), funded by Portuguese national funds through FCT/MCTES (PIDDAC). Z. Varela thanks María Zambrano Grants Programme for the attraction of international talent in Spain (Ministry of Science, Innovation and Universities, Spain) and the Programme IACOBUS Estadias 2023. N. Ratola was supported by FCT under the Scientific Employment Stimulus Institutional Call CEECINST/00049/2018 (DOI: 10.54499/CEECINST/00049/2018/CP1524/CT0007).



# (F) Potential toxic elements (PTEs) in serpentine-derived soils and bioaccumulation capacity of spontaneous vascular flora in the Calabria region, southern Italy

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The presence of serpentinite rocks in Calabria region (Southern Italy), has been the focus of numerous studies due to environmental and human health concerns. Indeed, in serpentinite rocks asbestos frequently occur. Potentially Toxic Elements (PTEs) hosted in asbestos is one of the factors that determines their toxic/pathogenic effects. Furthermore, the potential leaching of PTEs, released by asbestos-containing rocks, into the air, water and soils can increase environmental pollution. On such substrates, vegetation is sporadic and limited to a few species, typical of recolonization phenomena. The aims of this work were to determine the PTEs concentrations, in soils and, consequently, to establish the bioavailable portion to plants. In this context, we sampled and analyzed several serpentinite-derived soils outcropping in the Mount Reventino (Calabria, Southern Italy) along with the spontaneously growing taxa. The studied species included Dittrichia viscosa, common to all investigated sites, Asparagus acutifolius and Erica arborea. Additionally, we sampled the same plant species from a noncontaminated site in the Calabria region to use as control. To determine the elemental composition of soils and plant tissues, we performed analyses using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In soils, the concentrations of some PTEs (e.g., Cr and Ni) exceeded the thresholds established by Italian law for sites intended for commercial and industrial use. Moreover, a comparison between the same species revealed elevated levels of several elements in plants on serpentinites. These data provide insights into the capacity of PTEs found in serpentinites-derived soils to penetrate biological systems. This understanding is crucial for assessing the potential risks to human health and ecosystems associated with exposure to asbestos-contaminated environments.



# (F) Nature-Based Solutions and Urban farming: the impact of Green Barriers on Air Quality, Soil Health, and Food Safety

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Urban agriculture offers social, economic, and environmental benefits. However, the potential risks associated with cultivating edible crops in urban areas, notorious for their poor air quality and pollution, have been overlooked in many studies. This study aims to assess air quality near urban gardens, evaluating the effectiveness of green barriers (Platanus occidentalis; Nerium oleander) in containing the spread of particulate matter within the green zones designated for urban gardening. The investigation focuses on barriers' ability to reduce the deposition of airborne particles on soil and plants, mitigating the potential risks of assimilation and accumulation. Additionally, the feasibility of leaves as biomonitors to intercept PM and associated pollutants is explored. Soil sampling was conducted both outside and inside the green barrier, and soils were analyzed to determine their geochemical profiles by acid mineralization and ICP-MS. Air quality in terms of number of particle concentrations, both outside and inside the green barrier, was monitored by a condensation particle counter for fine particles (20 to 1000 nm) and by a charger dosimeter for ultrafine particles (10 to 300 nm). The leaves forming the green barrier were collected outer and inner sides of the canopies and categorized into washed/unwashed samples, which were analyzed for the presence of potentially toxic metals and polycyclic aromatic hydrocarbons. This study provides insights into the efficacy of green barriers in mitigating urban air pollution. The findings shed light on the risks associated with urban agriculture, emphasizing the importance of considering both the benefits and challenges of urban agriculture.

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### (R) Pollen viability as bioindicator of air pollution: state of the art and future perspectives

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To understand the potential of using pollen viability as a bioindicator of air pollution, the current state of research is analyzed, and future perspectives are proposed. A bibliographic search was conducted on July 3, 2024, using the Web of Science database with the following criteria: pollen AND viability AND pollut\* AND air NOT allergy. The search yielded 41 records, of which 12 were excluded due to irrelevance to pollen viability analysis in relation to air pollution. The content of the remaining 29 papers was evaluated. All articles were in English, with only five published before 2000. Italy (9) and India (7) were the leading countries in this research area. Most studies (21 out of 29) were carried out in field analyzing the response of wild plants. Tree species were considered in 19 studies, and 16 studies examined a single taxon. Regarding pollutants, 8 articles were related to different levels of air pollution, while 12 assessed the impact of specific air pollutants such as  $O_3$ ,  $PM_{10}$ ,  $SO_2$ ,  $NO_x$ , and  $NO_2$ ; heavy metals, acid rain, and formaldehyde were less frequently studied. Methods to analyze pollen viability primarily included colorimetric tests (mainly TTC test), and germination test with pollen tube elongation. Some studies also evaluated pollen-stigma interactions and alterations in pollen grain shape and size. The results generally indicated a reduction in pollen viability in response to pollutants, with a certain variability within and between species. More sensitive species, such as *Pinus nigra*, *Prunus avium* and *Rosa rugosa*, were suggested as potential bioindicators. The recent development of automated systems and analyze pollen viability, Amphasys instruments to such as the devices (www.amphasys.com), which can rapidly and automatically assess pollen number, shape, size, and viability (e.g., over 10,000 grains in a few minutes), offers promising applications for future research using pollen viability for air pollution biomonitoring.



#### (R) Honeybees as effective biomonitors of spatial and temporal pollution

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Many living organisms have been proposed as potential biomonitors. In terrestrial ecosystems biomonitors are mostly plants, which are stationary organisms that represent pollution only in a small area in their immediate vicinity. Therefore, we propose honeybees as excellent terrestrial biomonitors. They reflect pollution in all aspects of the environment: in air by collecting PM on their hairy bodies, in the water by drinking it, and in the soil by collecting pollen and nectar from plants that take it up from the soil. Honeybees were used to compare spatial and temporal differences in metal pollution at different locations in Serbia and Austria. Differences in element concentrations were found depending on the sampling location. Bees from urban areas showed higher concentrations of Cu, Zn and Pb; from rural areas of Ba, Cd and Ni; from industrial areas of Cr, Fe, As and Al. In addition, metal pollution has been tracked over the years. In South Banat district, Serbia it was observed that the concentration of elements in bees has decreased over the years (from 2013 to 2015). The results prove that bees are good biomonitors of spatial and temporal patterns of metal pollution. The toxicity of some elements depends not only on their concentration, but on their chemical form (e.g. As and Se). The HPLC-ICPMS method has been used to study the different species of these elements in the environment. Inorganic As was the predominant As species, while the majority of Se was determined to be protein-bound selenomethionine. In addition to metals and metalloids, pesticides are also monitored in honeybees. Neonicotinoids are a new class of insecticides that have a negative impact on honeybees. Not only the parent compound, but also its metabolites can be toxic. We therefore monitor the commercially available neonicotinoids and all their known metabolites in honeybees.



## (R) Biomonitoring using chitin: crayfish, grafted chitin and direct evaluation of adsorption thermodynamics

#### Stefan Fränzle; Felix Blind

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Some favorite items of biomonitoring, like arthropods and lichens, are covered with chitin. While this biopolymer is a chemically robust and potent sorbent (if pH > 3), and times (10 min) and protocols for adsorption for biomonitoring purposes had been optimized before in my team, we now tried to understand metal ion-or metal complex-chitin interactions by a more direct electrochemical approach. Up to now, this covered both classical environmental pollutants Pb, Cd, Ni, Cu, and a couple of other metals fairly stable in water (In, Sn, Bi, Co), and also addressed additional effects caused by natural (particularly in eutrophic waters) or anthropogenic ligands. The setup is very simple, essentially comprising a concentration-difference electrochemical cell. Now, establishment of constant voltage takes ca. 24 h on average, and ligands surprisingly enhance electrochemistry. While these observations do not cast doubt on the application of chitin for biomonitoring, both ancient (Precambrian) functions of chitin and its contribution to shaping bioinorganic chemistry in limnetic biotopes and underneath sediments must be reconsidered. There are additional applications of the chitin-/metal cells studied in my lab.



### (F) Multi-element bioconcentration kinetics in *Dictyota spiralis* for active biomonitoring of coastal marine ecosystems

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Environmental alterations of chemical element abundances may affect marine ecosystems either directly, due to toxicity, persistence and biomagnification, or indirectly by altering ecosystem dynamics. In this context, investigating element accumulation in organisms lay the foundation for their use in biomonitoring and bioremediation applications. In the present research, the bioconcentration kinetics of 15 elements were investigated in a brown seaweed, Dictyota spiralis Montagne, recently described as an effective passive biomonitor, using both devitalized (by boiling in a 35% NaCl solution) and non-devitalized samples, washed in a 100 µM EDTA solution to reduce the initial element concentrations. The alga, collected in a marine protected area, was characterized for lipid (1.0±0.4 % d.w.), glucose (6.7±1.7 mg/g d.w.), fructose (3.9±1.4 mg/g d.w.), galactose (3.3±1.3 mg/g d.w.), starch (20.1±2.2 mg/g d.w.) and polyphenol (0.04±0.02 mg GAE/g d.w.) concentrations before processing and exposure. Algae were placed in 2 mm mesh polyethylene/polypropylene bags and exposed in 4 sites characterized by different anthropogenic pressures. Bags were collected in triplicate at 2, 4, 8, 16 and 24 days, and analyzed for element concentrations. In terms of preprocessing, Student paired t-tests and ANOVAs demonstrated the uselessness of, respectively, EDTA treatment in reducing the initial concentrations and devitalization in affecting element bioconcentrations (apart from S showing reduced concentrations). Kinetics had either peaked responses, with the reaching of the highest concentrations in a few days (on average 4 days for As, Cd, Mg, Ni, P and S), or monotonic increases in concentration (Co, Cr, Cu, Fe, Mn, Pb, Si, V and Zn), suggesting that saturation may occur after the 24 days of exposure - an important finding in active biomonitoring applications.



### (F) Implementing a long-term network to monitor the effects of air pollution on terrestrial ecosystems in Portugal

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Monitoring the effects of air pollution on terrestrial ecosystems in Europe, and related research, has been mostly focused on forests through the International Co-operative Programme (ICP) on Assessment and Monitoring of Air Pollution Effects on Forests (ICPForests, ICPF). The ICP, launched in 1985 under the Air Convention (former CLRTAP), following discussions on strategies and policies to reduce air pollution, focused at the time on sulfur compounds. Nowadays, the National Emissions reduction Commitments (NEC) Directive requires that Member States establish monitoring networks to assess the effects of sulfur, nitrogen, and ozone on ecosystems. Selection of sites to be included in the networks should be based on risk and cost-benefit strategy, through a set of criteria that consider spatial and ecosystem representativeness (percentage of land occupation), and inclusion of highly sensitive ecosystems. At the European scale, many countries will base their networks on pre-existing ICPF sites. However, some countries, such as Portugal, lack active ICPF monitoring, and must establish new networks. This presents a challenge and an opportunity to create a long-term monitoring network that maximizes the number of parameters to be measured and establishes clear doseresponse relationships at both the individual and ecosystem scales. In Portugal, representative ecosystems include woodland and forests, grasslands, heathland and scrub, and croplands. Parameters in each monitoring site include air, soil, plant, and soil pore water chemistry, as well as biodiversity metrics. Site selection was based on a framework that overlaps existing air quality stations, representative ecosystems, Natura 2000 sites or other protection status, sensitive habitats, and public ownership. Here, we describe the steps taken in the selection of 24 new monitoring plots, highlighting limiting factors, strengths, and weaknesses of the new Portuguese long-term monitoring network.



#### (F) Agrochemical residues in beebread as an indicator of landscape management

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Pesticides are recognized as one of the leading drivers of the current pollinator decline, alongside habitat loss and degradation, both of which are closely linked to agricultural practices and landscape management. In our study, we assessed the health of agroecosystems in the Emilia-Romagna region (Northern Italy) as part of the BeeNet national monitoring project. We analyzed pesticide residues in 100 beebread samples collected from 25 BeeNet stations during March and June of 2021 and 2022. Our investigation focused on evaluating the diversity and concentrations of pesticide residues, their toxicity-weighted concentrations (TWC) for honeybees, and their relationship with land use patterns. Of the samples analyzed, 84% tested positive for at least one pesticide residue, with 63 different active ingredients detected out of 373 screened. The active ingredients belonged mainly to fungicides and insecticides, accounting for 46% and 44% of the total residues, respectively. Notably, 15 compounds were not approved as plant protection products (PPPs) in the EU, raising concerns about illegal use or contamination through beeswax recycling. In seven samples, the TWC exceeded the risk threshold, mainly due to one or two active ingredients. Additionally, TWC for four insecticides (Carbaryl, Fipronil, Imidacloprid, and Thiamethoxam) surpassed the safety threshold. We observed moderate to strong positive correlations between both TWC and the number of active ingredients, and the percentage of land used for orchards. Given that TWC does not account for possible synergistic effects among different chemicals, and with an average of five active ingredients found per sample, our results point to a significant toxicological risk to bees associated with current agricultural practices. This underscores the need for urgent adoption of eco-sustainable and pollinator-friendly strategies.

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