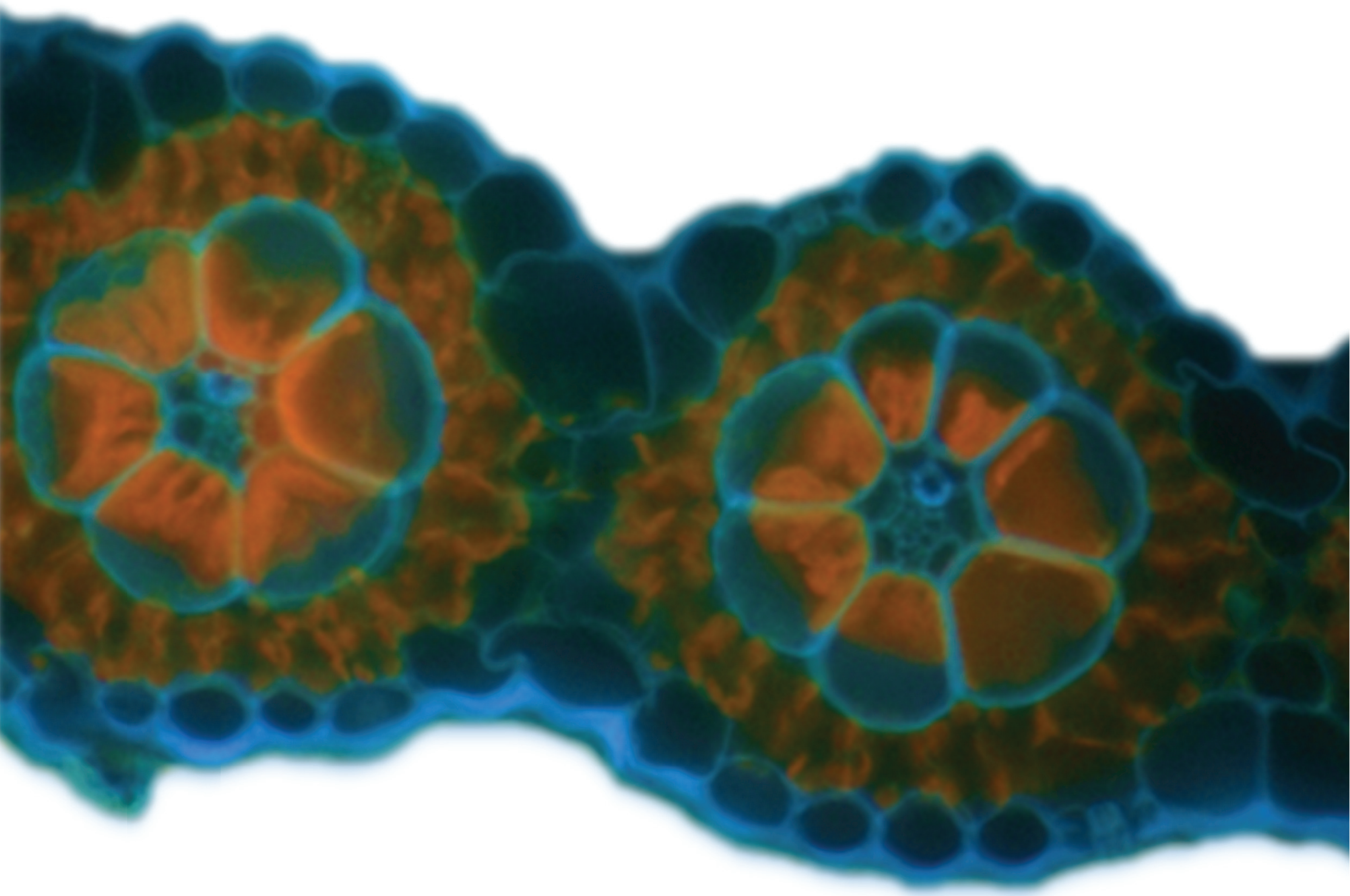

Book of Abstracts

PLANTMETALS

TRACE METAL METABOLISM IN PLANTS

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Annual Meeting 2024

Ljubljana, Slovenia,
September 17th - 20th, 2024

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Title page image (courtesy of Paula Pongrac):

Autofluorescence image of proso (*Panicum miliaceum* L.) leaf cross-section captured with Axioskop 2 MOT microscope equipped with an Axiocam MRc colour digital camera (Carl Zeiss AG, Göttingen, Germany) using UV excitation (365 nm). Length of the image is 275 μm.

Programme at a glance

Tuesday, 17th September 2024

Registration, poster mounting, opening session

Oral session 1

Get-togehter dinner

Wednesday, 18th September 2024

Oral sessions 2+3

MC-meeting

Conference dinner

Thursday, 19th September 2024

Oral sessions 4+5+STSM

Poster session

Friday, 20th September 2024

Oral session 6

Session “The future of PLANTMETALS”

Excursion

Programme

Tuesday, 17th September

15:00-16:30		**Registration, poster mounting, welcome coffee**	
16:30-16:45		**Opening address by PLANTMETALS Action Chair and the LOC**	
16:45-18:45	16:45-17:45	**Session 1 (WG 1 1/2)**	**Plenary lecture: Catherine Curie: A new role for the Mn transporter NRAMP2 in the seed including novel analytical and imaging tools to decipher metal homeostasis**
	17:45-18:00		Contributed talk 1: Seckin Eroglu: Iron accumulates in seed endosperm to trigger seed coat rupture
	18:00-18:15		Contributed talk 2: Sebastien Thomine: Regulation of intracellular free zinc concentration in *Arabidopsis thaliana*
	18:15-18:30		Contributed talk 3: Marie-Pierre Isaure: Various strategies developed by *Arabidopsis halleri* to cope with Cd toxicity
	18:30-18:45		Contributed talk 4: Sina Fischer: Ionomic phenotypes in neo-tetraploids
19:30-23:00		**Get-together dinner: Gostilna Čad (Cesta na Rožnik 18, 1000 Ljubljana), included in the registration payment.** **Location: https://hotel-cad.si/en/restaurant/)** 	

Wednesday, 18th September

08:30-10:00	08:45-09:15	**Session 2 (WG 2)**	**Invited lecture 1: Hendrik Küpper: Biochemical and physiological evidence for a beneficial role of chromium in plants**
	09:15-09:30		Contributed talk 5: Marie-Theres Hauser: Effects of nickel on pectinmethylesterase (PME)/ pectinmethylesterase inhibitor (PMEI) expression and activity in *Arabidopsis thaliana*
	09:30-09:45		Contributed talk 6: Fernando Antônio Gomes Brito: Cadmium (Cd) and zinc (Zn) isotope fractionation in a plant metallothionein (MT)
	09:45-10:00		Contributed talk 7: Jonathan Przybyla-Toscano: Adaptation of the membrane- and cell wall-associated proteome of *Arabidopsis thaliana* roots in response to uranium stress
10:00-10:30		**Coffee break**	
10:30-12:00	10:30-11:00	**Session 3 (WG 4)**	**Invited lecture 2: Matthias Wiggenhauser: Cadmium, zinc, and copper leaching rates determined in large monolith lysimeters**
	11:00-11:30		**Invited lecture 3: Hester Blommaert: The pathways of cadmium in cacao: in light of a synchrotron source**
	11:30-11:45		Contributed talk 8: Michel Mench: Phytomanagement of metal(loid)-contaminated soils with biomass sorghum: successes and failures
	11:45-12:00		Contributed talk 9: Gianluigi Giannelli: The multi-stress resistant PVr_9 bacterial strain mitigates Na^{+} ions toxicity in *Arabidopsis* and tomato and improves tomato fruit nutritional characteristics
	12:00-12:15		Contributed talk 10: Aida Bani: Agromining as an agricultural alternative in the serpentine soils of the Albania
	12:15-12:30		Contributed talk 11: Valerie Bert: Characterization of *Arabidopsis halleri* and *Urtica dioica* responses to Zn and Cd: Soil management practices to help biofortification?
12:30-14:00		**Lunch break**	
14:00-17:30		**Management committee meeting & Coffee break**	
19:00-23:00		**Conference dinner: Allegria (Nazorjeva ulica 8, 1000 Ljubljana) self-paid, 40 € without beverages, to be paid on-site at the registration desk.**	

Thursday, 19th September

08:30-10:00	08:30-09:00	**Session 4 (WG 3 1/2)**	**Invited lecture 4: Tomica Mišljenovič: Revealing Tl hyperaccumulation in the flora of the abandoned Allchar mine: New findings and future perspectives**
	09:00-09:15		Contributed talk 12: Stéphane Ravanel: Cellular and molecular responses of the metal-tolerant green microalga *Coelastrella* to uranium
	09:15-09:30		Contributed talk 13: Paco Romero: Physiological, biochemical and molecular approaches to understand the copper deficiency response in tomato
	09:30-09:45		Contributed talk 14: Petra Maskova: Horseradish, a panacea for arsenic contamination in temperate zone?
	09:45-10:00		Contributed talk 15: Emre Aksoy: Development of soybean lines with high iron and low phytate content via gene editing
10:00-10:30		**Coffee break**	
10:30-12:00	10:30-11:00	**STSM reporting**	**Overview of the Short-term Scientific Missions (STSMs)**
	11:00-11:10		STSM1: Valentina Bočaj: Species-specific and metal-induced changes in gene expression and metabolome of closely related *Noccaea* species under field conditions
	11:10-11:20		STSM2: Magdalena Pypka: How do plants find Zn source?
	11:20-11:30		STSM3: Noelia Jaime Pérez: Cadmium and zinc uptake in *Sedum alfredii*: Role of apoplastic and symplastic pathways
	11:30-11:40		STSM4: Kumbirai Deon Mandebere: Iron accumulates in seed endosperm to aid germination
	11:40-11:50		STSM5: Anna Kokavcová: The potential of *Pistia stratiotes* for phytoremediation of metal-polluted environment
12:00-13:30		**Lunch break**	
13:30-15:05	13:30-14:00	**Session 5 (WG 3 2/2)**	**Invited lecture 5: Marjana Regvar: Resolving structure-related trace element and molecular composition of Tartary buckwheat grain**
	14:15-14:30		Contributed talk 16: Josip Jurkovic: Norway spruce (*Picea abies*) needles as indicator for heavy metals pollution of Sarajevo
	14:30-14:45		Contributed talk 17: Pietro Peroni: Effect of root biostimulants on root growth and metal(loid)s uptake in *Miscanthus x giganteus*
	14:45-15:00		Contributed talk 18: Hagai Yasour: Involvement of trace metal nutrition in plant physiology process and abiotic stress responses
15:00-18:00		**Poster session & Coffee break**	

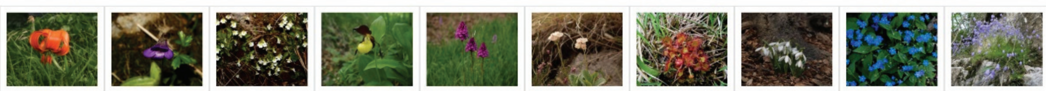
Friday, 20th September

<table>
<tr><td rowspan="5">08:30-10:00</td><td>08:30-09:00</td><td rowspan="5">Session 6 (WG 1 2/2)</td><td>Invited lecture 6: Oscar Siemianowski: How do plants manage their microelements? Untangling the Zn transport mechanisms between lateral roots</td></tr>
<tr><td>09:00-09:15</td><td>Contributed talk 19: Jessica Shadbolt: Good copper, bad copper: Characterising the physiological implications of contrasting alleles of HvHMA5</td></tr>
<tr><td>09:15-09:30</td><td>Contributed talk 20: Filis Morina: Micronutrients at the frontline of plant defence responses</td></tr>
<tr><td>09:30-09:45</td><td>Contributed talk 21: Filip Poščič: Iron-mediated mitigation of hexavalent chromium toxicity in Brassica juncea and Raphanus sativus</td></tr>
<tr><td>09:45-10:00</td><td>Contributed talk 22: Katarina Vogel-Mikuš: Mercury ligand environment in the food chain as affected by Se biofortification of plants</td></tr>
<tr><td>10:00-10:30</td><td></td><td colspan="2">Coffee break</td></tr>
<tr><td rowspan="2">10:30-12:30</td><td>10:30-11:30</td><td></td><td>Planning the future of the PLANTMETALS as a society</td></tr>
<tr><td>11:30-12:00</td><td></td><td>Poster removal, wrap-up</td></tr>
<tr><td>12:00-13:15</td><td></td><td colspan="2">Lunch break</td></tr>
<tr><td>13:30-20:00</td><td colspan="3">Botanical Excursion: Visit the Idrija valley and Hg mine
https://www.geopark-idrija.si/en/natural-heritage/plants/

The important European and international scientists who came to Idrija because of the mine did not only explore its geology, but also its botany. They laid the foundations of natural science in Slovenia. Knowledge of flora has a long tradition in the Idrija environment, which began with the arrival of the first botanist in Slovenia in the 16th century, P. A. Mattioli. He was followed by doctor and naturalist G. A. Scopoli, who discovered and described several previously unknown species, including the henbane bell, Scopolia carniolica, named after him. B. Hacquet worked in Idrija as the mine doctor. Two plants are named after him: Hacquetia epipactis and Pedicularis hacquetii. F. Hladnik, born in Idrija, is known as the founder of the Ljubljana Botanical Garden. Dedicating his life to researching the flora of the Carniolan region, he discovered a plant on the Trnovo Forest area that was later named Hladnikia pastinacifolia. In his memory, the left side of the Rake walking path was named Hladnik Botanical Ledge as many famous plants flower here in the spring. H. Freyer was born to a famous Idrija-based pharmacist Karl Freyer. He spent a lot of his time to studying botany in the Idrija region and was the first to describe Daphne blagayana, a flower even Frederick Augustus II of Saxony came to see.

Famous in Idrija and its vicinity is also Primula x venusta that is a hybrid between Primula auricula and Primula carniolica. In the Upper Idrijca area, there grow several species of orchids (Orchidaceae). Also special are Alpine plants such as the alpine snowbell, the flower of the sweet-lady, Rhodothamnus chamaecistus, and the Bertoloni columbine.

As a homage to the pioneering researchers of nature, Scopoli's Memorial Garden is planted in Idrija – at the entry point to the Rake Walking Path.


</td></tr>
</table>

Location:

Department of Biology, Biotechnical Faculty, University of Ljubljana
Večna pot 111, 1000 Ljubljana, Slovenia

Content

Understanding zinc redistribution mechanisms: insights from tobacco plants exposed to Zn-deficiency

Nina Adamek-Siwirykow[1], Anna Barabasz[1]

[1] *University of Warsaw, Poland*

The distribution of zinc (Zn) between plant organs depends on the metal concentration in the growth medium, showing a higher shoot/root Zn ratio under deficiency (Zn-def) compared to sufficiency. This study examines the kinetics of changes in shoot/root Zn ratio and Zn concentration in root parts after transplanting to Zn-def conditions. Tobacco plants, initially grown under optimal Zn conditions (1 μM Zn), were either transplanted to Zn-deficient medium or continued under optimal conditions. Samples from leaves, roots, or main root segments were collected at 0, 2, 4, and 6 days for Zn concentration (AAS) and gene expression (RNAseq, RT-qPCR) analysis.

Under optimal conditions, 5.5-week-old tobacco plants accumulated 40% of Zn in shoots. After 6 days in Zn-deficient conditions, the Zn distribution shifted to 50% in shoots. In contrast, plants continuing in optimal conditions showed increased Zn in roots, with only 35% in shoots. Analysis of the 7.5-cm middle main root segment revealed it as a Zn reservoir, holding 30% (Zn-def) and 63% (1 μM Zn) of initial Zn concentration. RNA sequencing identified genes involved in Zn redistribution, with higher expression in the middle root part under Zn-def conditions for 6 days. Notable genes, such as ZIP4A, ZIP4B, and ZIP5B, showed increased expression compared to time 0. Significant expression differences were observed for ZIP1-like and ZIP4B between day6def vs day6Zn and day6def vs day0 in the basal root part.

This study enhances the understanding of Zn redistribution, highlighting the middle root segment's importance. Exploring specific genes' roles in Zn root-to-shoot translocation is crucial for understanding the regulatory mechanisms influencing micronutrient supply in plants.

Development of soybean lines with high iron and low phytate content via gene editing

Damla Söbe[1], Kubilay Yıldırım[2], Sevgi Çalışkan[3], Emre Aksoy[1]

[1]*Department of Biological Sciences, Middle East Technical University, Ankara, Türkiye*
[2]*Department of Molecular Biology and Genetics, Ondokuz Mayıs University, Samsun, Türkiye*
[3]*Department of Plant Production and Technologies, Niğde Ömer Halisdemir University, Niğde, Türkiye*

Iron (Fe) deficiency, a prevalent form of hidden hunger, remains a significant global health concern, contributing to anemia incidence. Biofortification of staple crops offers promise in addressing this issue, yet natural anti-nutrients in Fe-fortified foods often hinder Fe absorption in the human body. To overcome this challenge, we propose harnessing cultivated plants with distinct forms of Fe storage, with soybean (*Glycine max* L. Merr.) being a primary candidate due to its significant Fe concentrations within ferritin in the seeds, facilitating enhanced Fe absorption upon consumption. However, soybean seeds also contain phytate, which inhibits Fe absorption. Previous mutation breeding strategies targeting phytate reduction encountered yield and germination problems. Conversely, mutating multidrug resistance proteins (MRPs) involved in phytate storage has shown promise without any compromise. Here we aim to develop soybean lines with high Fe and low phytate content through CRISPR-Cas9-based gene editing, utilizing the Turkish variety Arısoy. Departing from prior approaches, we target three GmMRP genes (GmMRP3, GmMRP13, and GmMRP19) simultaneously, aiming to reduce phytate levels without yield loss. We targeted conserved TMD1 and NBD1 domains of the GmMRP genes with multiple gRNAs, assessing efficiency in protoplasts and transgenic plants. T0 mutant soybean lines were generated, and after confirming mutations, homozygous lines with low MRP expression will be identified in the T1 generation. Greenhouse experiments will assess yield, phytic acid levels, and quality traits in T2 seeds from these lines. Our quest embodies the intricate balance between alleviating hidden hunger and fostering agricultural sustainability.

Effect of different olive mill waste biochar application rates with and without FYM on growth, yield and chemical composition of faba bean (*Vicia faba* L.)

Abdel Rahman Al Tawaha[1]

[1]*Al Hussein Bin Talal University, Jordan*

During the 2018/ 2019 growing season, a pot experiment was conducted in the Jordan University of Science and Technology (JUST) greenhouse in Irbid, north of Jordan, to investigate the effect of different rates of olive mill biochar with and without farm yard manure (FYM) on faba bean (*Vicia faba* L.) growth, nutritional value, and yield using two types of soil. The experiment was laid out in randomized complete block design (RCBD) with eight replications. Sixteen treatments in each replicate including four levels of biochar (0, 1, 3 and 5%) which is equal (0, 30, 90, 150 g/ pot respectively), two soil types (clay loam and sandy loam) and two levels of FYM (with or without). Plants treated with 3 percent biochar and grown in manured clay loam soil produced a higher grain yield of 1420.5 kg ha^{-1}. Also, plants treated with 3% biochar and grown in manured clay loam soil produced a substantially higher maximum shoot dry weight (5.87g). The results showed that faba bean plants grown in manured clay loam soil and treated with 3% and 5% biochar had the highest root dry weight (5.42 g and 4.71 g, respectively), and that faba bean plants grown in manured clay loam soil and treated with 5% biochar had significantly tallest plant (64.48 cm) compared to all other plants. The plants that showed the highest N uptake (58.11 and 57.86 kg ha^{-1}) were given 5% and 3% biochar, respectively, and grown in clay loam soil that had been treated with FYM. Plants treated with highest 5% biochar and grown in manure-treated clay loamy soil (5.67 kg ha^{-1}) had the highest P uptake, whereas plants treated with 3% biochar and grown in clay loam soil treated with manure had the highest K uptake (17.03 kg ha^{-1}). In clay loam soil with FYM, 5% biochar had a higher chlorophyll content (597.23 mg m-2) and a higher leaf relative water content at 5% biochar compared to other treatments (52.61%) and to the control in clay loam soil with FYM addition (52.35%). Overall, these studies showed that using 3% and 5% biochar can improve faba bean seed production, growth, and quality while also improving soil characteristics, especially clay loam soil.

Integrated monitoring of vegetation around mud volcanoes

Nihal Mustafayeva[1], Arif Huseynov[2], Ayten Huseynova[3], Esmira Alirzayeva[3]

[1]*Institute of Molecular Biology and Biotechnologies*
[2]*Institute of Oil and Gas*
[3]*Azerbaijan National Academy of Sciences, Azerbaijan*

Nowadays, natural phenomena such as volcanic eruptions, earthquakes, etc. are considered as strong sources of ecological disasters. Azerbaijan is known for the highest concentration of distribution of mud volcanoes (MVs) per unit area: 350 (both offshore and onshore) out of 2500 known MVs. Waste in all forms (gas, fluid and solid) are found in their emissions. Water enriched with micro and macroelements as well as toxic compounds (e.g. hydrocarbons and heavy metals) and key greenhouse gases (e.g. CH_4, CO_2) lead to the destruction and fragmentation of vegetation structure, loss or decline in some plant populations. The two-level monitoring approach (ground-based and remote sensing) was used to understand the threats to vegetation from MVs located in Dashgil and Lokbatan regions. Based on red and near-infrared reflectance measurements obtained from satellites (e.g., SENTINEL-2), NDVI (Normalized Difference Vegetation Index) was determined to range from -0.39 to +0.11 for Dashgil MV and from -0.021 to +0.049 for Lokbatan MV areas. Monitoring of plant cover was consistent with these data and four plant species were identified around Dashgil MV. While *Artemisia fragrans* was observed at a distance of 200-300 m from the crater of this MV, *Salsola dendroides* and *Halocnemum strobilaceum* were widespread, and *Limonium* spp. – less often at 50-100 m. However, only *S. nodulosa* was recorded at the Lokbatan mud flow, and plants were found to grow relatively vigorously and densely at 5-10 m from MV. In the leaves of all species studied, corresponding anatomical adaptation strategies to these strained conditions were revealed.

Role of OsbZIP48 transcriptionfactor in rice Zn deficiency response and Zn accumulation

Feiyang Jin[1], Grmay H. Lilay[1], André M. Cordeiro[2], Guilherme Leitão[2], Nelson J.M. Saibo[2], Ana G.L. Assunção[1,3]

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[2]*Plant Gene Regulation, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, Oeiras, 2780-157, Portugal*
[3]*BIOPOLIS-CIBIO, Research Centre in Biodiversity and Genetic Resources, University of Porto, Vairão, 4485-661, Portugal*

Zinc (Zn) is an essential micronutrient for all living organisms, and its deficiency in agricultural soils adversely affects the yield and nutritional quality of crops globally. While Zn uptake, transport, and storage is controlled by several factors, the response to zinc deficiency is regulated by F-bZIP transcription factors. The *Arabidopsis* group F bZIP (F-bZIP) transcription factors, bZIP19 and bZIP23, and their homologs from rice, wheat and barley have been identified as the key regulators of the zinc deficiency response, supporting the evolutionary conservation of this regulatory network across land plants. The *Arabidopsis* bZIP19 and bZIP23 also act as Zn sensors by directly binding Zn^{2+} ions to a Cys/His-rich Zn sensor motif (ZSM). Deletions or modification of the ZSM disrupts Zn binding, leading to a constitutive transcriptional activation of the Zn deficiency response. This, in turn, results in a significant increase in plant and seed Zn content. Previously, we identified the rice F-bZIP OsbZIP48 as the functional homolog of the *Arabidopsis* bZIP19 and bZIP23 transcription factors. Here, we will show results of genetically modified rice lines where CRISPR/Cas9-mediated knockout was employed targeting rice OsbZIP48. The response to Zn deficient supply in hydroponic nutrient solution and the effect on Zn uptake and accumulation in these lines was analyzed. Our results show the critical role of rice F-bZIPs in the Zn deficiency response and provide new promising avenue for the development of plant-based biofortification strategies to tackle the global Zn-deficiency health problem.

Autoinoculation: a novel experimental route in phytoremediation studies of aquatic systems

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This is the first report on the use of bacterial symbionts selected in order to autoinoculate a hyperaccumulating host plant. *Callitriche cophocarpa* is a macrophyte widely distributed in the temperate climate zone and a known aquatic hyperaccumulator of chromium. Ten pure symbiotic bacterial isolates of *C. cophocarpa* were obtained and identified. Three of the isolates showed the highest resistance to Cr(VI): *Microbacterium* sp. (Ct1), *Aeromonas* sp. (Ct3) and *Acinetobacter* sp. (Ct6). *Acinetobacter* sp. (Ct6). The isolates were also able to effectively detoxify Cr(VI) by reducing it to Cr(III). We tested whether inoculation of plants with a consortium consisting of Ct1, Ct3 and Ct6 affects: (1) the phytoextraction of chromium from leachates, (2) the physiological state of plants after Cr(VI) treatment. The experimental solutions contained 10.7 mg/L of Cr(VI) – an amount 530 times exceeding the legal limits. We influenced the plants with Cr in two 10-day steps, first using mature shoots and then apical ones. The highest Cr content were found in the autoinoculated young plants - 1274 mg/kg dry mass. The physiological status of the plants was assessed by biometric tests and chlorophyll fluorescence analyses. The photosynthetic activity of mature shoots was influenced by Cr(VI) more negatively than that of young apical shoots. The autoinoculation with the bacterial consortium significantly reduced the negative effect of Cr(VI) on mature organs. The results unequivocally show a beneficial effect of *C. cophocarpa* inoculation with the tested symbiotic isolates resulting in a significant improvement of the phytoremediation properties of this aquatic chromium hyperaccumulator.

Genomics of sesame for Se, Fe and Zn biofortification: A COST-PLANT METAL PROJECT

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[2]*Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana, Türkiye*

The world population is expected to increase to 9.8 billion in 2050. The increasing population brings with it hunger and malnutrition. Therefore, in order to combat nutrient deficiency, it is necessary to produce foods with high nutritional value that can be consumed by all segments of society. Therefore, the aim of COST project is to examine the Fe, Zn, and Se contents in a large sesame gene pool, determine the DNA markers associated with these contents. Through this COST funded project under Plant metal action, genome wide association mapping will be performed using highly diverse sesame panel and linked DNA markers will be identified. In addition, the discovery of new metal transporters responsible for Fe, Zn, and Se transport to sesame seeds/grains as a result of GWAS and/or the transcription factors controlling them. For this purpose, 289 sesame genotypes will be grown in Adana conditions for two years (2024, 2025) and the Fe, Zn and Se contents in their seeds will be determined. Simultaneously, thousands of SNP markers will be generated using DArTseq technology. DNA markers associated with Fe, Zn, and Se contents will be identified using various bioinformatics tools. As a result of gene ontology analyses of the loci associated with the micronutrients, the transporters responsible for trace element translocation into seeds/grains and/or the transcription factors controlling them will be determined, and their characterization will be carried out with bioinformatics tools. The core collection that will be developed in this project will be used in future projects. Identified linked DNA markers in this project will be further validated to develop KASP markers for marker assisted selection sesame breeding program focusing to increase the Fe, Zn and Se in sesame seed.

Agromining as an agricultural alternative in the serpentine soils of the Albania

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[2]Department of Botany, Faculty of Biology, University of Sofia, 1164 Sofia, Bulgaria

The ultramafic surface area in Albania covers 11.05% of the total surface of the country and contain elevated levels of metals such as Ni, Cr, Co and Fe. Surface horizons of the soils are characterized by extremely high Mg/Ca quotients of up to 30 and low nutrient status.

Most of Albania ultramafic agricultural area have abandoned by local farmers, so only small surface is currently being used for low-productivity agriculture, but they have the potential to provide multiple ecosystem services.

For years in those soils we have been applied phytomining, cultivating *Odontarrhena chalcidica* (syn. *Alyssum murale*) plants that are able to accumulate trace metals from metal-rich soils and transport them to the shoots (>1%), which can then be harvested as a bio-ore to recover highly valuable metals such as Ni. In the last years we have been applied phytomining in 5-10 ha cultivating *Odontarrhena decipiens*.

Our study showed us that *O. chalcidica* and *O. decipiens* are suitable for application of phytoextraction operations in mineralized and contaminated soils, since total biomass production and nickel concentration in shoots has been high.

After 2 years of successful practices in 5-10 ha we achieved a 12-ton biomass production, 193 kg ha^{-1} nickel yield of *O. chalcidica* and 14.6 -ton biomass production, 326.5 kg ha^{-1} nickel yield of *O. decipiens*.

We are applying agromining as an agricultural alternative in some mineralized soils of the country under different practices and to develop innovative agronomic practices using native Albanian Ni-hyperaccumulating species.

Characterization of *Arabidopsis halleri* and *Urtica dioica* responses to Zn and Cd: soil management practices to help biofortification?

Valérie Bert[1], Julien Goutant[1], Arnaud Pain[1], Arnaud Grignet[1]

[1] *Ineris, Parc Technologique Alata, BP2France60550VERNEUIL EN HALATTE France*

The (hyper)accumulators of Zn by their very high foliar concentration in Zn are relevant candidates for Zn-food supplement or Zn-biofortification. However, to verify the feasibility of such use, it is necessary to check that the leaf Zn is bioaccessible and in what proportion. In contrast, the Cd, which is usually accumulated in Zn (hyper)accumulators, should not be bioaccessible, as it is an undesirable element for this type of use. Other types of plants can be used as dietary supplement due to their nutritional content. Besides this advantage, they can contain Cd and other undesirable elements which can be bioaccessible. Characterization of gastric and intestinal bioaccessibilities of Zn and Cd by the Unified Barge Method (UBM; NF ISO 17924: 2019) validated for As, Cd, Pb and Sb in soils and applied to plants allow to assess the fraction of trace elements that have passed through biological barriers (bioavailable fraction). In this way, this method, more accurate than the measurement of the total TE concentrations in plants, can inform on the true nutritional quality of the plants ingested. Soil amendments (fertilizing products and arbuscular mycorrhizal fungi) can reduce Cd and other undesirable elements while increasing Zn and other essential TE in plants. The responses to TE of the Zn and Cd (hyper)accumulator *Arabidopsis halleri* and the plant species *Urtica dioica* collected on metal-polluted soils, where they are cultivated for phytomanagement purpose, will be presented focusing on the UBM results and soil management practices.

Phytomanagement of metal-contaminated soils by trees and herbaceous plants: associated microbial diversity and effect of fungal inoculation

Damien Blaudez[1], Lisa Ciadamidaro[2], Stéphane Pflender[2], Loïc Yung[1], Andy Laprie[1], Vincent Placet[3], Valérie Bert[4], Catherine Sirguey[5], Michel Chalot[2]

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Soil pollution by metals and land-use competition for production of food and non-food crops are pan-European concerns. The development of eco-innovative technologies capable of limiting the risks associated with contaminants while providing recoverable biomass is a priority. Phytomanagement combines a set of techniques using plants that contribute to rehabilitate and revalorize contaminated sites. However, the number of plant species tested in phytomanagement scenarios is still low. This is particularly true for tree species. Therefore, we screened, under large-scale experiments, the response of a set of tree species at three metal-contaminated sites. Growth data, metal concentrations in plants, as well as the effects on bacterial and microbial diversity, will be presented.

Characterization of plant microbiomes followed by the selection and inoculation of relevant plant-growth promoting (PGP) microorganisms such as mycorrhizal or endophytic fungi are recognized as key levers for improving plant growth and fitness. In this context, we also performed laboratory and in situ experiments based on the fungal inoculation on a variety of plants (poplar, nettle, *Noccaea caerulescens*) that can be used for bioenergy, fibre production or agromining perspectives. We showed that some strains are very effective at improving biomass production without affecting fibre quality or metal transfer in the harvested biomass.

Blommaert, Hester **Invited lecture 3**

The pathways of cadmium in cacao: in light of a synchrotron source

Hester Blommaert[1,9], Hiram Castillo-Michel[2], Giulia Veronesi[3], Rémi Tucoulou[2], Jacques Beauchêne[4], Gautier Landrot[5], Denis Testemale[6], Pathamanathan Umaharan[7], Erik Smolders[8], Géraldine Sarret[9]

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Synchrotron-radiation based methods such as X-Ray Absorption Spectroscopy and X-Ray Fluorescence imaging enable the measurement of the in situ chemical speciation of a metal in plant tissues, and the imaging of cellular and subcellular localization of metals. The potential applications for these techniques alongside the challenges in sample preparation will be presented. We will exemplify the use of these techniques in plant sciences with the study of cadmium (Cd) pathways in a high Cd accumulating cacao cultivar with background Cd concentrations (0.28 mg Cd kg^{-1}). We investigated Cd distribution and speciation within roots, branches, and leaves. Results revealed the incorporation of Cd into Ca-oxalate crystals with oxygen-ligand binding in roots and branches, potentially limiting its transfer to leaves and beans. Cd-sulfur species in root cells suggested a retention mechanism via thiol-containing ligands. Moreover, Cd accumulations in large, secreting channels of the branches suggested that mucilage may play a role in the transport of Cd within cacao trees. The leaves had local Cd enrichments in the parenchymatous tissues and displayed a higher Cd concentration in the mesophyll compared to the epidermis. These insights in Cd distribution and speciation underscore that *Theobroma cacao* L. develops original Cd detoxification strategies, strongly different from non-tolerant species such as cereals.

Species-specific and metal-induced changes in gene expression and metabolome of closely related *Noccaea* species under field conditions

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Understanding metal hyperaccumulation has yielded insight into important metal homeostasis mechanisms. Gene expression and metabolome of the hyperaccumulator Noccaea caerulescens and its close relative, *N. praecox* were studied. Leaf samples were collected at the flowering developmental stage from plants grown naturally at a metal-enriched location, Žerjav (Ze), and an unpolluted location, Lokovec (Lo). At the latter, both *N. praecox* (Np) and *N. caerulescens* (Nc) co-occur, allowing an in-field comparison. After extraction, total RNA was sequenced using TruSeq Stranded mRNA Sample Preparation Kit (Illumina, San Diego, USA) using Illumina NovaSeq 6000 platform (2 × 150 PE). Untargeted metabolomics was performed using Waters UPLC I-Class Plus (Waters, USA) tandem Q Exactive high-resolution mass spectrometer (Thermo Fisher Scientific, USA). Seventy differentially expressed genes (DEGs) and 11 differentially accumulated metabolites (DAMs) were detected between *N. praecox* growing at Ze and Lo. Furthermore, 126 DEGs and 25 DAMs were found between *N. praecox* from Ze and *N. caerulescens* from Lo. In addition, 111 DEGs and 19 DAMs were detected between *N. praecox* and *N. caerulescens* growing at the Lo. Among the DEGs, we found nine, 16 and 15 KEGG pathways to be enriched. Several of them have been frequently associated with metal stress, including glutathione metabolism, phenylpropanoid biosynthesis, and flavonoid biosynthesis, and will be presented in greater detail.

Modulation of metal content and antioxidant activity in wine by native non-*Saccharomyces* yeast

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Wine production involves alcoholic fermentation carried out by yeasts capable of converting sugars from grape must into ethanol and other compounds that influence the composition of the final product. Recent trends in winemaking have led to the use of commercial non-*Saccharomyces* yeasts in wine fermentation. Although microelements are present in the must in very small concentrations, they play a significant role for the normal development of alcoholic fermentation, as they are necessary for the metabolism of the yeast. Certain metals, such as Fe, Mn and Cu are important for regulating the cellular metabolism of yeasts, helping to maintain ionic balance, but very little is understood about how the use of non-*Saccharomyces* affects their concentration. In this study, electron spin resonance spectroscopy (EPR) was used to determine the individual effects of native non-*Saccharomyces* yeast on the utilization of metal ions in Maraština wine. Additionally, antioxidant activity was measured based on presence of reactive active species using EPR. The fermentation trails were set up with indigenous non-*Saccharomyces* yeast strains: *Metschnikowia pulcherrima* K-6, *Metschnikowia chyrsoperlae* K-11, *Metschnikowia sinensis/shanxiensis* P-7, *Lachancea thermotolerans* P-25, *Pichia kluyveri* Z-3, *Hanseniaspora uvarum* Z-7 and *Hanseniaspora guilliermondii* N-29, previously isolated from Croatian Maraština grapes, which are stored in the collection of native yeasts established at the Institute for Adriatic Crops and Karst Reclamation in 2021. Fourier transform infrared spectroscopy was used for monitor alcohol and sugar concentration during fermentation. This study provides insight into the mechanism of metal ion utilisation in alcoholic fermentation carried out by different native yeasts.

ESRF beamline ID21: trace metal mapping and speciation in plant science

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Trace metal mapping and speciation in plants is one of the core activities at beamline ID21 of the European Synchrotron. ID21 is a beamline dedicated to X-ray fluorescence (XRF) mapping and X-ray absorption spectroscopy (XAS) in the tender X-ray range (2-11keV), this energy range allows detecting important nutrient elements (P, S, K, Ca, Mn, Fe, Cu, Zn) as well as rare earths and pollutants (Cd, Ag, Ce, La, Gd). A brand-new X-ray nanoscope is now available at the beamline to complement the existing microscope. It offers enhanced capabilities for nano-XRF mapping, nano XAS and hyperspectral XRF mapping. This new state-of-the-art instrument offers higher lateral resolution (down to 150 nm) with better XRF detection capacities (sub-ppm), higher acquisition speed, an improved cryogenic sample environment. Cryo-fixed plant samples can better cope with intense X-ray beams and the elemental distributions, chemical states, and sample morphologies are close to the in-vivo state under frozen-hydrated conditions. This presentation will highlight the capabilities of ID21 and how they could be exploited to advance our knowledge about trace metals in plants. Some examples of research done at ID21 will be used to illustrate sample preparation protocols, data acquisition and data analysis strategies.

The role of RNA oxidation in plants response to metals

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Despite the important role of reactive oxygen species (ROS) in plants response to stress factors little is known about their impact on the process of RNA oxidation. The studies carried out in majority on animal models show that RNA oxidation is associated with the development of neurodegenerative diseases, cancer and with the process of aging. In addition, it has been shown that the process is selective and leads to inhibition of the translation. Interestingly, in seeds, RNA oxidation seems to be associated with the process of the breakage of dormancy. However, its occurrence and role in grown plants is still not clear.

Within our studies we have shown that exposure of soybean seedlings to short term metal stress, namely to cadmium (Cd), copper (Cu) and lead (Pb), results in accumulation of the most frequent oxidative modification of RNA, 8-hydroxyguanosine (8-OHG). The observed 8-OHG formation is an early response and precedes accumulation of the oxidative stress markers. The immunohistochemical staining revealed 8-OHG presence in inner layers of cortex pointing out to its tissue-specific formation. The sequencing of 8-OHG enriched transcript showed that RNA oxidation is a massive process occurring in thousands of transcripts. The obtained results indicate that RNA oxidation might be an important process in plants response to metals. However, elucidation of its exact role requires further studies.

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Curie, Catherine **Plenary lecture**

A new role for the Mn transporter NRAMP2 in the seed including novel analytical and imaging tools to decipher metal homeostasis

Alexandra Leskova[1], Marie-Pierre Isaure[1], Carine Alcon[1], Arnaud Comte[1], Javier Jimenez-Lamana[1], Katarzina Bierla[1], Joanna Szpunar[1], Tou Cheu Xiong[1], Stéphane Mari[1], Catherine Curie[1]

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Our lab studies iron (Fe) and manganese (Mn) homeostasis in the model plant *Arabidopsis thaliana*. Both metals are well-known to be scarcely available in the vast majority of the soils of the planet and hence to limit plant growth. In the past, we have identified and characterized the function of a number of transporters including IRT1 and NRAMP1 that carry out respectively, Fe and Mn high-affinity transport at the root surface and that are thus of paramount importance to cope with metal deficiency. Recently, we have reported that NRAMP2 mediates efflux of Mn from the trans-Golgi Network into the cytosol and acts as a central hub to distribute Mn to the main cell organelles (Alejandro, 2017). We are now focusing on the role of NRAMP2 in the seed. We will present unpublished data combining µXRF and laser ablation-ICP-MS (LaICP-MS) techniques applied on knock-out mutants of NRAMP2, that show the crucial role of NRAMP2 in partitioning Mn between seed coat and embryo.

The presentation will also describe new tools that we recently implemented in the lab, including (i) single particle-ICP-MS (SP-ICP-MS) analysis of metal content in *Arabidopsis* single pollen grains (Jimenez-Lamana et al., 2023), and (ii) new redox Fe fluorescent sensors that allow to selectively image either Fe^{2+} or Fe^{3+} species, separately or in combination, within living roots (Alcon et al., 2024). These redox Fe sensors helped reveal polar distribution of Fe^{2+} in root epidermis.

Alejandro, S., Cailliatte, C., Alcon, C., Dirick, L., Domergue, F., Correia, D., Castaings, L., Briat, J.F., Mari, S., Curie, C. (2017) Intracellular Mn distribution by the Trans-Golgi Network NRAMP2 transporter is critical for photosynthesis and cellular redox homeostasis. Plant Cell 29 : 3068-84. doi: 10.1105/tpc.17.00578.

Jimenez-Lamana, J., Bierla, K., Leskova,A., Curie, C., Szpunar, J., and Mari , S. (2023) Single cell ICP-MS as a powerful analytical tool to determine metal content in individual pollen grains. J. Anal. At. Spectrom. 38, 1560-1563. DOI: 10.1039/d3ja00140g

Alcon, C., Comte, A., Curie, C., Xiong, T.C. (2024) Imaging of Labile Fe2+ and Fe3+ in living Arabidopsis thaliana root. Plant Physiol., in press.

Forms of the metal transporters MTP1 in *N. caerulescens* Monte Prinzera

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The facultative metallophyte species *Noccaea caerulescens* is a notable hyperaccumulator, evolved in Europe across non-metalliferous, calamine (Zn, Pb, Cd enriched), and serpentine (Ni-rich) soils. Different ecotypes exhibit varied metal accumulation and tolerance. Specifically, the Italian ecotype Monte Prinzera (MP), growing on ultramafic soils with high Ni, Cr, and Fe levels, hyperaccumulates Ni. This study investigates the role of the vacuolar transporter gene MTP1 in Ni hyperaccumulation and hypertolerance in *N. caerulescens* MP. High constitutive MTP1 expression was confirmed, though the gene is downregulated upon Ni treatment in roots of this ecotype. Notably, two MTP1 sequences, differing in length, were identified in both *N. caerulescens* MP and GA ecotypes on gDNA and cDNA. The shorter form, MTP1-short, lacks the conserved His-loop region, a proposed Zn sensor. RealTime-PCR with Taq-Man probes differentiated the expression of MTP1-long and MTP1-short in leaves and roots of plants submitted to various metal treatments. Subcellular localization studies in tobacco cells confirmed that both MTP1 forms reside in the vacuolar compartment, suggesting a role in metal detoxification. Yeast complementation assays using the zrc1cot1 double mutant and WT strains of *Saccharomyces cerevisiae* revealed differences in Zn, Ni, and Co transport abilities between the two MTP1 forms. These findings indicate a potentially distinct role of MTP1 variants in metal detoxification, correlating with the diverse metal hyperaccumulation and tolerance properties of *N. caerulescens* ecotypes.

Role of KUP9 gene in adaptation of Arabidopsis arenosa to serpentine soils

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Serpentine soils represent powerful models to understand adaptation to local conditions because their extreme chemical and physical properties act as strong selective pressures. They are generally toxic for the plants due to high magnesium to calcium ratio and high concentration of nickel and other heavy metals. Despite these challenges, some plants, including several *Arabidopsis* species, adapted to this environment. Furthermore, the island-like distribution of serpentines across landscapes leads to repeated adaptation, making it a good model to study repeatability of evolution and various ways the plants can adapt to toxic soils.

Our genomic comparison of wild populations of *Arabidopsis arenosa* adapted and non-adapted to serpentine soils resulted in the list of candidate genes for adaptation. One of the main candidates with very frequent differences between adapted and non-adapted populations is KUP9 gene, belonging to KUP/HAK/KT family of K+ transporters. Here, we compare protein sequences of serpentine and non serpentine alleles of KUP9 gene in relation to the structure of the protein and the conserved regions of the protein. We show the potential functional implications of the differences, which will be tested by insertion of the respective alleles into *Arabidopsis thaliana* plants.

Iron accumulates in seed endosperm to trigger seed coat rupture

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Iron (Fe) is an essential element serving as a cofactor in many proteins including peroxidases. Fe has been thought to be accumulated almost solely in the embryo which develops to become the new plant. Surprisingly, we found Fe also accumulates in the endosperm of tomato and *Arabidopsis thaliana*. This internal Fe, as well as its supply from outside, increased germination speed specifically by facilitating seed coat rupture, the step preceding the endosperm rupture. Using histochemical stainings combined with synchrotron X-ray fluorescence mapping and XANES; we identified the localization and speciation of Fe in the wild type. This localization shifted in mutants that were previously shown to mediate embryo Fe localizations such as vit1 and mtp8. Fe accumulated as a ring through the chalazal part of the endosperm, which colocalized to the fluorescence of SUC2::GFP, indicating Fe localizes to the phloem remainings; a tissue which has been largely overlooked before in mature seeds. We further showed evidence that the Fe reserves in the mitigate seed coat rupture by fueling chalazal peroxidases. This study linked Fe to seed germination for the first time and explained the phenomenon mechanistically.

Phytoremediation strategies for soil metal cleanup and management

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Phytoremediation offers a promising strategy for mitigating trace metal contamination in soil and the environment, addressing the harmful effects on human health and ecosystems.

The recent proposal for a Directive of the European Parliament and of the Council on Soil Monitoring and Resilience (Soil Monitoring Law), in Article 15 (Risk assessment and management of contaminated sites) highlights the importance of reducing risks posed by contamination. It emphasizes considering factors such as cost, effectiveness, durability, and technical feasibility when implementing risk reduction measures. Among the remediation techniques outlined in Annex V of the Proposal, phytotechnologies emerge as notably cost-effective solutions. This directive underscores the necessity for sustainable soil management and promotes the adoption of eco-friendly remediation approaches, with phytoremediation taking center stage.

Plants release root exudates that influence the rhizosphere environment, fostering microbial activity. Soil microorganisms, such as mycorrhizal fungi and metal-resistant bacteria, enhance metal solubilization, sequestration, and detoxification, thus facilitating plant metal uptake and tolerance.

Phytoremediation aligns with sustainable remediation principles by minimizing environmental disruption and maximizing resource utilization. Its scalability and adaptability make it suitable for diverse landscapes and ecosystems.

Through collaborative research and policy support, phytoremediation can significantly contribute to trace metal management and environmental sustainability goals outlined in the new European Soil Law. By embracing the interactions between plants and soil microorganisms, phytoremediation offers a promising pathway towards greener soil management practices, mitigating the detrimental effects of metal-polluted soils on human health and ecosystems.

Scanning X-ray microscopy at the hard X-ray micro/nano-probe beamline P06 (DESY) for bio and bio-medical applications

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The Microprobe experiment at the PETRA III beamline P06 is a versatile setup for scanning X-ray microscopy with X-ray fluorescence, X-ray absorption spectroscopy and X-ray diffraction / coherent scattering contrasts. It is used for studies in materials sciences, catalysis, cultural heritage, (asteroid-) mineralogy, etc., but the largest share of beamtime is given biological and bio-medical applications. The ability to collect megapixel images in less than an hour facilitates series of 2D images for full 3D fluo-tomography and spectro-microscopy. Samples can be chemically fixed, freeze dried or even fresh (unfixed). The golden standard, however, is shock-freezing and measurement in the frozen-hydrated state in order to avoid beam damage and artefacts of element re-distribution. A cryogenic sample transfer protocol is available both for measurements under a nitrogen cryo-stream or in a cryogenically cooled UHV chamber. Examples are presented for various sample types (tissues, bone, teeth, cells), scanning modes (fast 2D, tomographic 3D), and sample preparation techniques (frozen-hydrated, unfixed, chemically fixed).

The figure summarizes results of a study assessing cellular uptake of exogeneous coenzyme Q10 into human skin [1]. Zinc (cyan) and iodine (magenta) elemental maps for the full scan collected in high flux mode (a,b) and the fine scan of 3 selected cells (c,d), indicated by the blue rectangle in a), collected in high resolution mode. The direct comparison of iodine and zinc emphasizes the homogeneous distribution of iodine among the cytoplasm and agrees with the expected Q10 localization. The large number of individually resolved cells allowed to deduce the correlation between iodine mass per cell and projected cell area. A mean value of 46.5 fg iodine/cell was found with a cell-to-cell uptake variation of 10.7 fg/cell.

[1] T. Staufer, G. Falkenberg, D. Brueckner, et al., Antioxidants, 2022, 11, 1532.

Ionomic phenotypes in neo-tetraploids

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Whole genome duplication (WGD) in *A. thaliana* leads to increased shoot potassium (K)[1]. We observed ionomic phenotypes in neo-tetraploids under various conditions and observed specific changes in the ionome. Our results point to interesting cross talk between Fe and K homeostasis.

Through RNAseq and mutant studies[2] we were able to investigate the molecular mechanisms which underpins these ionomic changes. For example, neo-tetraploid mutants of K uptake (akt1 and hak5) still showed increased shoot K, revealing that these genes are not involved in higher K levels. Contrastingly, neo-tetraploid mutants of endodermal development (esb1), salt stress signalling (sos3) and Abscisic Acid (ABA) signalling (mbf1c) failed to accumulate higher shoot K levels. The gene expression in diploid and tetraploid plants was analysed. A network of genes was discovered, which changed expression in neo-tetraploid wild type plants but not in neo-tetraploid mutants. These mutants no longer displayed the increased shoot K. The gene network is enriched in functions like cell wall and Casparian strip development and ion-transport in the endodermis. We also show that WGD in the endodermis alone is sufficient to induce increased shoot K levels, underlining the importance of this root structure.

Future studies will now focus on identifying how K and Fe networks are connected. We will also study genes required for increased K in neo-tetraploids and create a plant with the benefits of neo-tetraploids, like higher survival under abiotic stress1, but without the loss of seed production that can often be seen in naturally occurring neo-tetraploids.

[1]: Chao et al. 2013, Science

[2]: Fischer et al., 2022, Plant Physiology

Studying the uptake of ESS-specific radionuclides by crops cultivated in Southern Sweden

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The ESS is a large neutron research facility under construction in Lund, Sweden [1], the activity of which will produce radioactive by-products that could be spread in the environment should an accident occur [2]. The Swedish Radiation Safety Authority has established a list of radionuclides that would contribute the most to the effective dose from exposure to ground deposition, following such an accident, and this list consists of radionuclides that do not result form more conventional nuclear activities. Examples of these radionuclides are isotopes of W, Hf, Ta, Gd and Lu, for which the migration in soil has rarely been studied, besides the case of tungsten [3], and similarly, experimental data on the accumulation in plants is very scarce [4]. As such, data on the transfer of these metals in the environment are currently lacking in the reference handbooks edited by the International Atomic Energy Agency [5], and considerable uncertainties exist regarding their fate once released into the environment [6]. To tackle this issue, a highly multidisciplinary project has been conceived, in which the migration rates in soil and the transfer from soil to edible plant species will be studied. An overview of the project will be presented, alongside preliminary results and a roadmap for the work which will follow in the coming years.

[1] R. Garoby et al., Phys. Scr. 93(1), 014001 (2017).

[2] K. Stenström et al., J. Haz. Mat., vol. 429, p. 128292 (2022).

[4] G. Pédehontaa-Hiaa et al., SSM2021-787 (2023).

[5] IAEA, Tech. Rep. 472 (2010).

[6] G. Pédehontaa-Hiaa et al., SSM2019-1010 (2019).

Stress induced by soil contamination with heavy metals and their effects on some biomarkers and DNA damage in maize plants from vicinity of Ferronikel smelter in Drenas, Kosovo

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The Ferronikel smelter in Drenas is one of the main industrial areas in the Kosovo and pollution by heavy metals caused serious threat for all living organisms on this area. The objective of this study was to determine the concentration of some heavy metals (Fe, Cu, Mn, Cr, Cd, Ni and Pb) in agricultural soils and in maize plants in this area, and their potential toxic effects on this plant through some very sensitive biochemical and molecular markers. Maize plant seedlings growth in nine soil samples from different sites of this area. The highest concentrations of heavy metals in soils and maize leaves were conducted close to the Ferronikel smelter, and in some sites, the nickel and chromium concertation in soils exceeded 800 mg kg^{-1}. A significant effects of heavy metals induced toxicity resulted in the, build-up aminolevulinic acid and reduced activity of δ-aminolevulinic acid dehydratase, and chlorophyll content in the maize leaves and increased the malondialdehyde and glutathione level. In general, maize seedlings growth in polluted sites presented an increase in nuclear DNA content and in G2M phase, and a decrease in G1 phase when compared to the control. FCM data indicates that no major DNA damage in maize leaves was induced on polluted sites. We concluded that sites close to the smelter are affected by soil heavy metals pollution and these biochemical and molecular analysis would a powerful ecotoxicological tool in biomonitoring of heavy metal pollution.

X-ray fluorescence and nuclear microprobe analysis for assessing the concentration of period-six transition metals in soil and seed crops

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The European Spallation Source (ESS) is a neutron research facility currently under construction in Lund, Sweden [1]. Upon its completion it will produce radioactive by-products that could spread in the event of an accident. The Swedish Radiation Safety Authority has listed unconventional radioisotopes like 187W, 172Hf, 182Ta, 148Gd, and 173Lu, highlighting gaps in the understanding of how these elements migrate in soil and are accumulated by plants [2]. To address this issue a highly multidisciplinary project was designed and as a part of the preliminary studies, data was collected from soil and seed samples taken from surrounding farmlands around the ESS site. The collected samples were analyzed using X-ray fluorescence and nuclear microprobe techniques. Baseline measurements on soil samples and seed crops, revealed the concentration and localization of the elements before contamination. Results obtained from this study will serve as a reference point for future measurements on artificially doped soil and plant samples, which will yield a better understanding of the dispersion of these period six transition metals in the environment surrounding the ESS site.

[1] Garoby, Roland, et al. ""The European spallation source design."" Physica Scripta 93.1 (2017): 014001.

[2] Pédehontaa-Hiaa, Guillaume, et al. ""Region-specific radioecological evaluation of accidental releases of radionuclides from ESS."" (2021).

The multi-stress resistant PVr_9 bacterial strain mitigates Na^+ ions toxicity in *Arabidopsis* and tomato and improves tomato fruit nutritional characteristics

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Soil salinity imposes ion toxicity, osmotic stress, nutrient deficiency, and oxidative stress on plants, resulting in detrimental effects on growth and development. In this study, the beneficial effects of the bacterial strain PVr_9 showing multi-stress resistance to As, Cd, NaCl and Pb were evaluated in *A. thaliana* and *S. lycopersicum* under 150 mM NaCl. In vitro analyses revealed a positive effect of PVr_9 on morphological parameters in both species. Under salinity, PVr_9-inoculated *A. thaliana* showed lower levels of ROS, 8-oxo-dG, osmolytes, and ABA content along with modulation of antioxidant enzyme activities. The SOS1 and NHX1 genes, involved in plant tolerance, were up regulated in both tomato and *A. thaliana* by PVr_9. Furthermore, 2D-PAGE analysis in PVr_9-inoculated salt stressed tomato plants showed an up regulation of enzymes involved in proline metabolism and a down regulation of proteins induced by stress. In greenhouse experiments, PVr_9-inoculated salt stressed tomato showed a significant increase in fresh biomass, chlorophyll content, antioxidant enzyme activity, and osmolytes. Finally, tomato fruits recovered from field-grown plants subjected to NaCl treatment during flowering, showed more lycopene, β-carotene, carotenoids, L-ascorbic acid, and an increased antioxidant capacity; furthermore, Pvr_9 inoculation limits the accumulation in fruits of heavy metals such as Cd and Pb. Overall, the results indicated a possible crosstalk between PVr_9 and plant roots to enhance salt tolerance and address this strain as a potential biostimulant to be applied in field trials of important agronomic crops, modulating nutritional molecules, and improving food safety by limiting the accumulation of dangerous contaminants.

Cadmium (Cd) and zinc (Zn) isotope fractionation in a plant metallothionein (MT)

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Metallothioneins (MTs) are proposed to play a role in the homeostasis of micronutrients such as Zn(II) and Cu(I), as well as in the detoxification of toxic metals like Cd(II). These ligands, rich in thiol groups (R-SH), could be used to investigate the functions of MTs using process tracing. Previous studies have shown that Cd and Zn isotope fractionation is systematically 'inverse' in various plant species. This contrasting isotope fractionation may be attributed to the processes that separate Cd from Zn, particularly the chelation of these metals by thiol groups. However, to date, there is no experimental data on isotope fractionation for Cd and Zn to thiols.

We aimed to determine the Cd and Zn isotope fractionation in a model MT, chickpea metallothionein (cicMT2). To this end, cicMT2 was recombinantly expressed in *E. coli* cells with a GST tag for purification purposes, which was subsequently cleaved to obtain the native protein sequence. Metal-free cicMT2 was incubated with Cd(II) and Zn(II) ions for different equilibration times. After separating the unbound metal ions, the protein samples were measured for Cd and Zn isotope composition.

Our results indicate that Cd isotopic equilibrium was achieved after 6 hours of incubation, while the Zn isotopic equilibrium may have been reached within the initial 10 minutes of incubation. This interpretation is based on a consistent trend in the mean values throughout the experiment and the decreasing standard deviations as the incubation time increases. The binding of cicMT2 to Cd and Zn caused a strong isotope fractionation between the complexed and free metals. Initially, the average isotope fractionation between the free and complexed metals was very strong and remained significant at isotopic equilibrium (Δ114/110Cdfree-MT = 0.53 to 1.23 ‰, Δ66/64Znfree-MT = 0.37 to 0.87 ‰). We will present preliminary data from *E. coli* experiments where we assess whether i) MTs (and thiols) are a major driver of Cd and Zn isotope fractionation in *E.coli* and ii) to what extent other processes such as membrane transport additionally fractionate Cd and Zn isotopes.

Assessment of indicators to qualify functionality and ecotoxicity of metal contaminated soil with plant-based solutions

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The failure to consider the environmental problems led to a progressive soil pollution, particularly in trace metal elements by atmospheric deposits contained in fumes and waste from factories on the soil.

Populations and ecosystems are faced with soil pollution situations that exposed them. They may then consider new uses for these soils in order to protect, restore, or even improve polluted soils through ecological rehabilitation approaches which take into account ecosystem degradation degree, future intended land uses, affordability of the solutions and impact of climate change.

Still underutilized, ecological rehabilitation techniques based on the use of plant species enable the management of polluted lands in situ, to preserve soil resources and provide other benefits related to the presence of vegetation cover on soil functions and Human health.

The poster will present the subject of my thesis, the methodologies, and the expected results as part of two ongoing projects (REVE, REECOL). Briefly, several plant covers that exist on two metal-contaminated sites will be monitored to assess the best strategies to reduce metal exposure pathways while providing other benefits.

Amongst indicators which will be studied are the soil-plant transfers (metal bioavailability in soil and plants, bioconcentration factor), the properties of the soils (pH, organic matter content, cation exchange capacity, etc.), the functionalities of soil (organic matter degradation, enzymatic activity) and the ecotoxicity of soil (viability, growth and reproduction of nematodes). Reduction of metal exposure (soil, plant) and soil ecotoxicity and the improvement of biodiversity are the main expected results of the study.

Effects of nickel on pectinmethylesterase (PME)/ pectinmethylesterase inhibitor (PMEI) expression and activity in *Arabidopsis thaliana*

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Nickel (Ni^{2+}) is an essential microelement for plants but due to human activities and agricultural practices, the amount of Ni^{2+} release rises imposing environmental concerns. We and others have recently shown that Ni^{2+} ions inhibit cell elongation in hypocotyl and root growth in *Arabidopsis thaliana*. To understand how Ni^{2+} inhibits growth, we focused to the effect of Ni^{2+} on modifications of cell wall component pectin. Pectins and in particular the homogalacturonans (HGs) are highly methylated and acetylated and the degree of these modifications is controlled by 67 pectin methylesterases (PMEs) and 76 PME inhibitors (PMEIs). To determine if Ni^{2+} changes the expression, RT-qPCR was conducted for the whole gene family of PMEs and PMEIs. The results indicate that the balance between PME and PMEI activity was shifted towards PME and thus demethylation of pectins. This result was corroborated by PME activity assays showing that Ni^{2+} exposure of seedlings induces PME activity. Changes in pectin methylation levels are a prerequisite for the production of pectin derived signaling molecules like pectates and oligogalacturonides (OGs). These signaling molecules are ligands of the *Catharanthus roseus* RECEPTOR LIKE KINASES 1 LIKE (CrRLK1L) family. We have previously shown that members of this cell wall integrity sensors mediate growth responses upon Ni^{2+} exposure. We will present data where PME activity in CrRLK1L mutants point to their role in suppressing demethylation of pectins. A model will be proposed and discussed how Ni^{2+} ion induce cell wall modifications leading to signaling events resulting in growth adjustments.

The role of metal accumulation in serpentine adaptation in the group *Alyssum montanum* s.l.

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Serpentine soil is one of the strongest challenges in nature for plants. It is naturally toxic, thanks to a high concentration of heavy metals, propensity to drought, and shortage of nutrients. In order to colonize serpentine soil, plants often have to evolve and (locally) adapt through special molecular and physiological traits. One such adaptation may be hyperaccumulation: the ability of a plant species to take up large amounts of heavy metals and storing them in its tissues. This ability is of particular interest for human purposes, as, if exploited, it can help restore damaged ecosystems through phytoremediation. Species encompassing populations inhabiting both non-toxic as well as toxic soils might provide ideal study systems for exploring the evolution of hyperaccumulation and adaptation towards toxic soils. In general, hyperaccumulators are mostly found in the Brassicaceae family and especially in the genus *Alyssum* s.l. We here tested two understudied species, *Alyssum gmelinii* and *Alyssum spruneri*, representatives of the typical hyperaccumulator tribe *Alyssae* (Brassicaceae), as promising new model species to untangle the role of metal (hyper)accumulation in adaptation to serpentine soil and establish their unknown phytoremediation potential. The species are distributed along Central Europe and the Balkans in both serpentine and non-serpentine environments. We performed a transplant experiment and tested the differences in fitness between the populations and metal accumulation in the plants' tissues. Moreover, we grew the same populations in anthropogenically polluted soil to assess the phytoremediation ability of the species. Our preliminary results show fitness advantages of plants originating from both types of soil in their substrate of origin. A subset of populations analyzed for Ni uptake shows *A. gmelinii* is likely not a hyperaccumulator, although there is considerable natural variation in the ability to accumulate Ni among populations.

Antioxidant properties of in vitro cultivated *Stevia rebaudiana* Bert. treated with valin Ag-nanofibers

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Stevia rebaudiana Bertoni is a plant of the Asteraceae family used in food and pharmaceutical industries as a source of non-calorie sweeteners due to the synthesized diterpene glycosides in its leaves. Conventional propagation methods are limited by poor seed viability, low germination rates, and weak rooting ability. To meet the increasing demand for this plant with a reduced population, the study focused on developing a suitable alternative biotechnological method for its propagation. The objective of the research was to explore the impact of two low molecular weight peptidomimetics (synthesized from the amino acid valine with pyridine residue) self-regulated at nanofibers on the plant growth parameters and the antioxidant activity of *S. rebaudiana* plantlets grown by direct organogenesis and their use as carriers of the biologically active agent silver ion (NM6-1% Ag and NM6-2% Ag). The supply of varied Ag nanofiber concentrations to the MS nutrient medium has led to increased growth of microplantlets at 10 and 50 mg L^{-1}, while the highest concentration of 100 mg L^{-1} inhibited growth. A significant increase in the percentage of root formation was also observed, which is almost zero in controls and plants grown in an MS with BAP adding. NM6-2% Ag application led to a higher activity of the enzymes with antioxidant activity (SOD, CAT, APX and GPX), than NM6-1% Ag adding. The enzyme activity levels rose with an increase in NM6-2% Ag concentration from 1 to 50 mg L^{-1}, while the addition of 100 mg L^{-1} led to a decrease in their activity.

Spatial distribution of heavy metals in soil and spruce seedling nutrients: case study in Bosnian forest nurseries

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The study aimed to identify spatial factors influencing heavy metal distribution among the three forest nurseries. It further explores correlations between soil heavy metal concentrations and seedling nutrient content. The concentrations of cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) were analyzed in both topsoil and subsoil layers using pseudo-total extraction methods. As control, corresponding unmanaged grassland soils were used to explain heavy metals origin. Soil type and it inherent properties such as soil texture, organic carbon (C) and nitrogen (N), pH values and cation exchange capacity were determined to understand their influence on heavy metal distribution. Simultaneously, concentrations of C, N, phosphorus (P), sulfur (S), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), and aluminum (Al) were assessed in the leaves, stems, and roots of spruce seedlings. Findings indicate significant variations in heavy metal levels across nursery locations, influenced by differing soil properties and anthropogenic factors. Correlations between heavy metal concentrations in soil and nutrient levels in seedlings provide insights into potential impacts on spruce growth and health. Research contributes with information for forest nursery management strategies.

Ilyas, Muhammad Faizan   Poster P22

Stability of chelated micronutrients (metals) in acidic and alkaline soils in relation to micronutrient availability to plants

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Most of the micronutrients (metals) become unavailable for plants at high soil pH which impedes the normal growth of plants, especially the crop plants that are sensitive to micronutrient deficiency like soybean. The chelated forms of micronutrients are more available in soil. However, the efficacy and overall fate of the EDTA-chelated micronutrient is unknown in the soil, which is also important from environmental aspects. Considering the aforesaid, a pot experiment is conducted to elucidate the stability of chelated Zn^-, Mn^- and Cu^- with EDTA and EDDS (biodegradable chelating agent) in acidic and alkaline soils. It was hypothesized that EDTA-chelated micronutrients are more suitable sources of micronutrients in high-pH soils compared to EDDS-chelated micronutrients. For comparison, a negative control with no micronutrients (metals) and a positive control with sulphated Zn^-, Mn^- and Cu^- were included. The Zn was added as 66Zn-stable isotope, whereas, Fe was added as FeHBED. 66Zn-stable isotopes containing -SO_4, -EDTA and EDDS were prepared. Soybean plants were grown and soil moisture contents were maintained at 60% water-holding capacity. There were seven soil sampling intervals viz. 0, 1, 7, 14, 28, 42, and 56 days after the start of the experiment. The measurement of nutrients concentration in plant and soil samples, and chelate concentration in soil samples is under process. The results obtained will be presented in the annual meeting of Cost Action PLANTMETALS.

Imran, Muhammad Poster P23

Sustainable use of chelated micronutreints

Various strategies developed by *Arabidopsis halleri* to cope with Cd toxicity

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Arabidopsis halleri is a Zn, Cd hyperaccumulating plant and a model to study adaptation to metal contaminations and metal homeostasis. Metal speciation and distribution in the plants are key information to untangle the mechanisms of metal hyperaccumulation. Various studies on Cd speciation and localization have been carried out on plants grown in Cd-spiked media. Here we will present the results obtained on these media as well as results on plants naturally grown in the contaminated site of Auby (France) and exposed to multiple contaminants. Cd speciation and distribution were studied by X-ray absorption spectroscopy and various imaging techniques including synchrotron X-ray fluorescence, laser ablation ICP-MS, and electron microscopy. We will show that, contrary to previous studies reporting that Cd was mainly coordinated by oxygen, sulfur ligands can predominate, particularly in plants from the field. Contrasted metal allocations were also evidenced. These results show that various Cd detoxification strategies occur depending on exposure conditions and age of the plants, and allow the plant to adapt to different environmental conditions. This adaptation is an advantage compared to less versatile plants.

Cadmium and zinc uptake in *Sedum alfredii*: Role of apoplastic and symplastic pathways

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This study explores the mechanisms underlying zinc (Zn) and cadmium (Cd) accumulation in *Sedum alfredii*, a Cd and Zn co-hyperaccumulator known for its potential in phytoremediation of contaminated soils. By comparing hyperaccumulating (HE) and non-hyperaccumulating (NHE) ecotypes, our research focused on the interactions between the apoplastic and symplastic (including transmembrane) pathways in Zn/Cd loading in the xylem and its subsequent distribution in the roots. While previous studies have predominantly emphasized the symplastic pathway in hyperaccumulators, recent findings suggest a significant contribution of the apoplastic pathway, particularly in regions with underdeveloped suberin lamellae. To elucidate the role of these pathways in Cd/Zn uptake in *S. alfredii*, we utilized advanced imaging techniques such as customized benchtop µXRF and synchrotron cryo-µXRF tomography. Our results show that Cd inhibits Zn accumulation in both ecotypes, with distinct impacts on transporter gene expression and suberin development. In HE, Cd primarily influenced symplasmic and transmembrane Zn transport, whereas in NHE, it modified both symplastic and apoplastic pathways. These effects can be attributed, to a significant extent, to alterations in transporter gene expression for the symplastic/transmembrane transport and changes in the Casparian strip (in particular, the suberin lamellae) for the apoplastic transport. 3D metalloproteomic analyses showed different profiles of high-affinity Cd-binding soluble proteins in HE and NHE roots. This indicates that further contributions to the symplastic pathway should be considered. Overall, this research sheds light on the intricate mechanisms governing Zn/Cd accumulation in *S. alfredii*, offering insights crucial for developing effective phytoremediation strategies for contaminated soils.

Accumulation and distribution of Zn in *Cardamine waldsteinii*, a newly discovered hyperaccumulator species

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Hyperaccumulation of Zn is an extremely rare phenomenon, recognised at 3000 mg kg^{-1} and so far confirmed in 28 taxa. A systematic screening of the herbarium material of Brassicaceae in herbaria in Paris and Belgrade revealed Zn concentrations above the hyperaccumulation threshold in *Cardamine waldsteinii*, which was previously unknown in the field of metal accumulation. These concentrations were confirmed in material from the field, which led to an intensive study of accumulation in this species in the Balkans, the main distribution area of this species. Z-spec spectroscopy revealed concentrations of up to 7000 mg kg^{-1} in the leaves of *C. waldsteinii* from Mt. Ivanšćica in Croatia, with the hyperaccumulation threshold being exceeded in all samples from this site. The hyperaccumulators of Zn were also found at other sites but with mean concentrations slightly below the notional threshold. Distribution analysis using X-ray fluorescence microscopy showed that the leaf margins were the sites of highest accumulation in the leaves of *C. waldsteinii*, but the enrichment was also detected in roots, stems and petioles. Since hyperaccumulation of Zn was observed in the samples growing on soils relatively poor in this element, the accumulation potential and distribution in the plants experimentally grown on Zn-enriched soils will be the focus of the following investigations.

Effects of EM soil amendment and green manure on trace element content of oriental tobacco

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Green manure (GM) is commonly used in conjunction with organic fertilization, crop rotation, and mineral fertilization to improve the quality of agricultural soils. This study aimed to supplement the effects of GM with Effective Microorganisms (EM) soil amendment to improve the sustainability and trace element uptake of oriental tobacco (*Nicotiana tabacum* L.). The field experiment used a randomized complete block design with treatments including GM (seed material from fodder pea and barley), EM soil amendment (liquid bio fertilizer consisting of sugar cane molasses and lactic acid bacteria), a combination of both, and a control with no amendments. The results revealed that the combined application of GM and EM significantly enhanced the trace element concentrations in tobacco leaves. The average concentrations of trace elements in the treated plants ranged from 147 to 356 g/kg for iron (Fe), 68 to 78 mg/kg for manganese (Mn), 14 to 18 mg/kg for copper (Cu), and 20 to 40 mg/kg for zinc (Zn). The highest increases were observed in the combined GM and EM treatment, indicating a synergistic effect. The enhanced concentrations are attributed to improved microbial activity, nutrient cycling, and soil structure resulting from the amendments. These findings support the adoption of sustainable agricultural practices to improve the nutritional uptake of oriental tobacco while reducing reliance on chemical fertilizers. Further research is ongoing in order to optimize application rates and methods for different soil types and environmental conditions.

Jurković, Josip **Contributed talk 16**

Norway spruce (*Picea abies*) needles as indicator for heavy metals pollution of Sarajevo

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During winter, Sarajevo has one of the most contaminated air in the world. There are several reasons for that, but most important are position (basin surrounded by mountains, with low air flow), coal combustion and high traffic. Plant species can be utilized for bio monitoring the quality of the environment and reform the extent of pollution in both urbanized and industrial regions. In Sarajevo the Norway spruce is one of the most abundant evergreen specie. Needle samples of Norway spruce were collected in the winter 2024 (January to February), from 13 different locations in Sarajevo with high traffic pressure. Four locations, far away from traffic, were used as controls. From all of trees three different types of needles were taken: one, two and three years old (approximately 2021, 2022 and 2023). Samples were prepared by means of microwave digestion. The quantity of seven heavy metals were measured: Pb, Cd, Ni, Cu, Zn, Fe and Mn, by means of Atomic Absorption Spectroscopy with flame atomization (FAAS). For statistical evaluation, aside of basic statistical tools, correlations were used. The average values of metals found in urban area lie in the next sequence: Cd<Cu<Ni<Pb<Mn<Fe. Concentrations of metals found in controls had slightly different sequence: Cd<Ni<Cu<Pb<Fe<Zn<Mn. Higher average values in urban areas were found in case of Pb, Cu, Ni, Fe (Fe, ten times higher). Zn, Cd and Mn (Mn, eleven times higher) had higher average value in controls. Oldest needles had the highest amounts of heavy metals, suggesting their accumulation during years. We suggest that Norway spruce could be used for bio monitoring of heavy metals pollution in urban (industrial) areas, especially in case of Pb, Ni and Fe.

Solving small molecule and macromolecule structures with 3D Electron Diffraction

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Mobility of Te(IV)/Te(VI) in soil of different compositions: a step towards assessing metalloid uptake by plants

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Tellurium is a technology-critical element (TCE) with limited environmental data, particularly in the pedosphere. Its increased use, especially in the field of new energy technologies, will make tellurium one of the next-generation xenobiotics, similar to other TCEs. To evaluate metalloid uptake by plants, it is essential to understand the interaction between tellurium and soil, identify physicochemical transformations in the soil environment, and determine the mobility of different tellurium species.

This presentation will provide new information on the time-dependent sorption and desorption of Te(IV) and Te(VI) in the presence of additives that simulate different soil systems. This study obtained significant data on the retention of tellurium species through model studies on Te-spiked soils (varying levels of Mn/Fe (hydr-)oxides and organic matter). The study on fractionation involved single extractions of different fractions: the easily leachable/exchangeable and carbonate fraction (using 0.1 mol L^{-1} CH_3COOH), the reducible fraction (using 0.1 mol L^{-1} ascorbic acid in oxalate buffer), and the organic fraction (30% H_2O_2 at 85°C followed by 0.5 mol L^{-1} CH_3COONH_4).

The results showed that the efficiency of Te sorption depends on its oxidation state, soil composition, and the presence of compounds produced by rhizosphere activity, such as citrates. Tellurium retention is influenced by organic matter and the presence of Mn/Fe oxides. This, together with the fractionation study, revealed significant differences in the mobility of Te forms, which may also affect their uptake by plants.

Local adaptation to metal-rich soil in *Sabulina verna*

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Plants adapted to heavy metal-rich soils offer a compelling avenue for exploring local adaptation. Despite a long-standing interest in soils containing heavy metals and the plants that thrive in them, there remains a scarcity of research addressing the ecological and evolutionary aspects of pre-adaptation to these challenging conditions.

This study focuses on *Minuartia verna*, with populations found in mine dumps, serpentinite substrates, and non-toxic soils. Local adaptation and pre-adaptation were examined through a reciprocal experiment involving populations from each of these three groups. Multiple vegetative and generative plant traits were analyzed to calculate fitness. Comparing plant fitness across various soils revealed that non-metallicolous populations are not pre-adapted to heavy metal-contaminated soils. However, all populations exhibited great growth in serpentinite soil, suggesting a genetic adaptation to this specific substrate.

Analysis of metal content in the plants indicated their role as excluders of Ni and Pb, with root concentrations exceeding those in the leaves. Consistent trends in metal accumulation were observed across all populations. Furthermore, the roots of non-metallicolous plants absorbed more Zn in soils with elevated Zn levels compared to metallicolous populations, potentially signifying a lack of adaptation to high Zn concentrations.

These results suggest that the entire lineage of lowland Central European populations might have been pre-adapted to serpentinite substrates. Metallicolous populations in mine tailings likely adapted to heavy metal-rich soils at a later stage during colonization. This research provides valuable insights into the intricate relationship between plants and challenging environments, enhancing our understanding of plant adaptation.

Combined effect of antimony and low/high temperature on root system of *Zea mays*

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Unusual low and high temperatures during the spring and summer as a result of climate change, affect growth and development of agricultural crop production. Antimony (Sb) is a non-essential trace metalloid which is released to the environment due to human activities by mining, burning of fossil fuels and by industrial activities. Sb with increasing concentration in the soil negatively modifies plant growth and plant fitness.

We investigated how temperature (20 °C, 25 °C and 29 °C) in combination with Sb (10 mg Sb/L and 30 mg Sb/L) affected the root system of young maize plants cultivated hydroponically. Antimony with higher concentration reduced primary root length in combination with 20 °C or 25 °C temperature and reduced the number of lateral roots regardless of temperature.

In the case of exposure to Sb stress and low or high temperature, maize plants exhibited a decrease of antioxidant enzyme activities (SOD and G-POX) in roots in comparison to the control conditions. We also observed increasing synthesis of heat shock proteins at 29 °C in combination with higher Sb concentration.

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Kokavcová, Anna **STSM5**

The potential of ***Pistia stratiotes*** for phytoremediation of metal-polluted environment

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Collaborations between different laboratories are necessary to achieve important findings in the research. To enhance collaboration opportunities, COST Actions support participants in short-term scientific missions (STSMs), providing funding during their scientific stays. During this COST Action 19116, multiple STSMs were conducted. In this contribution, findings obtained during my scientific stay in the lab of Dr. Hendrik Küpper are going to be presented, where we explored the potential of the *Pistia stratiotes* (L.) for phytoremediation of metal polluted environments. Aquatic macrophyte *P. stratiotes* is an invasive species common in standing waters of Central Europe, effective in accumulation of toxic elements like Cd or Cr into its tissues. We treated the plants with environmentally relevant concentrations of Cu (redox active) and Zn (redox inert) to study their interaction and distribution at the root tissue level, as well as reveal the tolerance mechanisms against excess metals. The ionome of the roots and leaves was determined, the distribution of selected elements in the root tips using µXRF imaging was analysed, and chlorophyll fluorescence kinetics (OJIP) in leaves was assessed. These findings were supplemented with data on root lignification and suberisation from our lab. In conclusion, it was detected that Cu toxicity in used concentrations is more severe than Zn toxicity, suggesting that *P. stratiotes* is more suitable for phytoremediation of Zn-polluted waters.

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Mechanisms driving convergent plant adaptation to serpentine toxic soils

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Determining genetic underpinnings of adaptation is a major challenge of evolutionary biology. Repeated environmental adaptation offers powerful naturally replicated study systems, yet varying fraction of the genome exhibiting convergence precludes generalization. Multiple genomic and functional factors have been proposed to determine genomic convergence, but their relative importance is blurred by case-specific focus of the studies and varying evolutionary timescales. In our new project we are aiming to decipher convergent genetic basis of plant adaptation to serpentine substrates – soils that are naturally rich in heavy metals. We combine de novo assembly, ploidy-level screening (using flow cytometry), population-level resequencing and transcriptomics in ten Brassicaceae genera from serpentine-rich regions of Southeastern Europe to uncover genes that were repeatedly used in natural adaptation to challenging substrates. The project will identify general drivers of convergent genome evolution in nature and inform evolutionary predictions essential for efficient breeding and conservation.

Our ecological-genomics approach lacks the expertise in molecular genetic and ecophysiological aspects of heavy metal tolerance of plants, what is the primary motivation of our team to join the network. We are also seeking potential collaborators for possible follow-up studies on the genetic basis of metal adaptation in certain metal tolerant genera such as *Noccaea*, *Alyssum*, *Odontarrhena*, *Arabidopsis* and *Cardamine*.

Kováč, Ján Poster P31

The effect of potentially toxic elements (Zn, Cu) on anatomical and cytophysiological characteristics of *Noccaea caerulescens* species

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Alpine pennycress (*Noccaea caerulescens*) colonizes areas from non-contaminated meadows to soils or heaps enriched with heavy metals such as Zn, Cd, Pb or Ni. Plants tolerate a toxic environment and, in addition, accumulate contamination in huge concentrations in the above-ground parts. Tolerance and accumulation in this species are a common trait for plants living in both non-contaminated as well as in contaminated soils. Zinc and Cu in some concentrations are both essential for plants. Alpine pennycress, compared to many other species, is known as an extremely tolerant species to elevated Zn concentrations in soil. On the other hand, tolerance of this species for Cu is less known. In this work we compared anatomical and cytological characteristics of two populations from different localities. The first population comes from Zn-enriched heap, the second population from former Cu mining locality. The main goal of this work is to determine some possible predisposition of one population to Cu stress.

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Heavy metal content and non-enzymatic antioxidant capacity in the leaves of *Scilla lakusicii* and *Scilla litardierei*

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Scilla lakusicii and *S. litardierei* are plant species that inhabit the karst areas of the Dinarides. *S. lakusicii* occurs on rocky habitats while *S. litardierei* inhabits wet meadows and in challenging karst ecosystems they are exposed to different types of abiotic stress. The aim of our work was to determine the content of Fe, Mn, Cu, Zn and Ni in the leaf samples of *S. lakusicii* and *S. litardierei* as well as the content of phenolic compounds and non-enzymatic antioxidant capacity. The obtained results indicate that the leaves of *S. lakusicii* contain more Cu and Zn, while in the leaves of *S. litardierei* a higher content of Fe, Mn and Ni was measured. The higher content of phenolic compounds was measured in the ethanolic extract of *S. litardierei* leaves, as well as the antioxidant capacity measured by the ability to remove DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. On the other hand, *S. lakusicii* leaf extract showed a higher Cu reduction capacity. Differences in the metal content and antioxidant capacity of the two *Scilla* representatives may be a consequence of the specific micro-conditions of the habitats.

Key words: phenolic compounds, micronutrients, DPPH radical, abiotic stress, karst

Küpper, Hendrik **Invited lecture 1**

Biochemical and physiological evidence for a beneficial role of chromium in plants

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Chromium has never been proven to be a beneficial element for plants, and its effects on plants are mostly known from toxicity studies. We investigated the effects of Cr in the ultralow, environmentally relevant range of concentrations, on the submerged rootless plant *Ceratophyllum demersum* and soybean (*Glycine max*). We found that growth of the *C. demersum* decreased towards the lowest achievable Cr concentration (0.15 nM), with growth reaching zero when extrapolating towards zero Cr. Furthermore, Cr deficiency decreased soybean growth, especially the seed yield. Non-photochemical exciton quenching and photosynthetic oxygen release were affected by Cr deficiency in *C. demersum*. Metalloproteomics applying radioactive 51Cr, native gel electrophoresis and SEC HPLC-ICPMS of extracts from plants already showed Cr binding to several membrane proteins (about 50-60 kD) plus several <6 kDa soluble ligands. Identification of Cr-binding membrane proteins that we could purify by our self-developed AEC-HIC-SEC protocol showed that none of them binds the chemically similar Mo, and also element uptake on the whole-plant level did not suggest a replacement of Mo by Cr. One of these membrane proteins could be verified already in several purifications, and seems to be highly specific for Cr with possible binding also of Ni. According to the Uniprot database, this gene has previously only been known to be expressed according to transcriptomics (i.e. mRNA level). It belongs to a protein class that has been proposed to confer resistance against fungal pathogens. We discuss the function of potential newly discovered Cr-binding proteins for plant development and environment sensing.

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Cd accumulation in peanut seeds

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Peanut (*Arachis hypogaea* L.) (2n=4x=40) is an annual, tetraploid cultivar belonging to the legume family. Peanut ranks fifth among oilseed plants in terms of production in the world. Peanut is one of the important sources of vegetable protein in human daily nutrition and is considered a complementary food due to its sufficient nutritional value. Direct consumption of peanuts has increased in recent years. This has shown that, in addition to the oil quality of peanuts, it is also important to determine the beneficial and harmful components in the seeds for human health. The most important feature that distinguishes peanuts from other plants is that they form their flowers above the ground and their fruits underground, and this leads to a high probability of peanuts being contaminated with cadmium from the soil. Cd pollution is one of the heavy metal pollutions that has attracted great attention in recent years due to its wide mobility and high toxicity. Cadmium in soil has high chemical activities and can be easily absorbed by plants and included in the food chain; As a result, the element accumulates in the human body and is toxic to health. Previous studies have reported that the Cd accumulation capacity of peanuts is much higher than many other crops, especially in the acidic soils of Southern China. Therefore, determining the Cd contents accumulated in the seeds of peanut varieties/genotypes is of great importance for a healthy nutrition.

Identification of the essential steps of nickel hyperaccumulation in *Noccaea caerulescens*

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Metal hyperaccumulation is thought to result from changess in the expression or the activity of genes involved in metal homeostasis, particularly during root uptake, root-to-shoot translocation, and detoxification/sequestration in leaves. However, the precise molecular mechanisms underlying metal hyperaccumulation remain largely unknown.

The aim of this study is to identify candidate genes associated with different steps of nickel hyperaccumulation. To achieve this, existing and newly generated transcriptomic and genomic data from different *Noccaea caerulescens* accessions will be analyzed. This approach will allow us to cover a broad spectrum of genetic diversity and metal accumulation capacity. Comparative transcriptomics and genomics will allow the identification of differentially expressed (DE) genes associated with nickel hyperaccumulation, as well as genetic alterations such as single nucleotide polymorphisms (SNPs), insertions and deletions (INDELs), gene duplications, transposable element insertions (TE), affecting the coding sequence or the promoter region of candidate genes involved in metal homeostasis. The second phase of the project will focus on validating the role of candidate genes in nickel hyperaccumulation using *Arabidopsis thaliana* as a heterologous expression system. Candidate genes will be expressed under ubiquitous or tissue-specific promoters to mimic the expression patterns observed in *N. caerulescens*, ultimately allowing the construction of a synthetic nickel hyperaccumulator. Physiological assays and elemental analysis will be employed to characterize transgenic lines for nickel tolerance and accumulation.

This research aims to elucidate the molecular mechanisms underlying nickel hyperaccumulation in plants by identifying the minimum set of genes required for the trait, paving the way for sustainable solutions for metal extraction from contaminated soils.

Diversity of zinc homeostasis among accessions of the grass model *Brachypodium distachyon*

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Zinc (Zn) is an essential micronutrient for plants. In their environment, plants are often exposed to limited Zn supply in soils. Indeed, soils deficient in Zn cover a large surface of arable lands, including many densely populated and poor areas around the world. Zn deficiency lowers plant performance and crop yield, but also severely impacts human health. To a certain extent, Zn homeostasis mechanisms allow plants to maintain Zn concentrations in tissues within physiological limits in response to Zn deficiency. These mechanisms have been mostly examined in *Arabidopsis* and in rice, and translation to pooideae crops is lagging behind. Here, we exploit the natural diversity found in *Brachypodium distachyon* (*Brachypodium*) as a model to address this question. *Brachypodium* accessions mostly evolved around the Mediterranean region, which is characterized by highly variable Zn availability in soils.

First, we developed two growth systems enabling the manipulation of Zn supply in *Brachypodium*: (i) a circulating hydroponic system for seed-to-seed development, where Zn supply can be finely adjusted; and (ii) a soil with increased pH (using CaO), which decreases Zn and other nutrients availability, mimicking soil conditions encountered in the field. Plants of 15 *Brachypodium* accessions were grown in these conditions for 6 weeks. Germination rate, biomass production or leaf number were quantified, and root and shoot tissues were collected for ionome and gene expression profiling using ICP-AES and quantitative RT-PCR, respectively. This phenotyping revealed extensive variation among accessions for several traits. Leveraging this natural variation should enable a better understanding of Zn homeostasis in *Brachypodium*.

Rare earth elements perturb root architecture and ion homeostasis in *Arabidopsis thaliana*

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Rare earth elements (REEs) are crucial elements for current high-technology and renewable energy advances. In addition to their increasing usage and their low recyclability leading to their release into the environment, REEs are also used as crop fertilizers. However, little is known regarding the cellular and molecular effects of REEs in plants, which is crucial for better risk assessment, crop safety and phytoremediation. Here, we analysed the ionome and transcriptomic response of *Arabidopsis thaliana* exposed to a light (lanthanum, La) and a heavy (ytterbium, Yb) REE. At the transcriptome level, we observed the contribution of ROS and auxin redistribution to the modified root architecture following REE exposure. We found indications for the perturbation of Fe homeostasis by REEs in both roots and leaves of *Arabidopsis* suggesting competition between REEs and Fe. Furthermore, we propose putative ways of entry of REEs inside cells through transporters of microelements. Finally, similar to REE accumulating species, organic acid homeostasis (e.g. malate and citrate) appears critical as a tolerance mechanism in response to REEs. By combining ionomics and transcriptomics, we elucidated essential patterns of REE uptake and toxicity response of *Arabidopsis* and provide new hypotheses for a better evaluation of the impact of REEs on plant homeostasis.

Influence of mineral deficiency on cadmium accumulation in *Theobroma cacao*

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The new European regulation on the cadmium (Cd) limit of 0.6 mg $Cd.kg^{-1}$, in cacao products, affects the cacao market worldwide, especially in Latin America. Indeed, 30 to 50% of cacao bean production in this region exceeds this limit. Research on strategies to reduce Cd accumulation in cacao beans is currently limited by a lack of understanding of Cd transfer pathways from the soil to the bean. The purpose of this study was to test the influence of mineral deficiency on Cd uptake. Soil and cacao plant organs from representative agronomic cultivars (CCN-51 and Nacional) were sampled in cacao plantations and studied by a combination of chemical and imaging techniques. For the soils, trace and major element concentrations were measured on the total soil, and $Ca(NO_3)_2$, ammonium oxalate and DTPA extracts. These analyses revealed contrasted conditions in terms of micronutrient and Cd availabilities. For the plant organs, Cd accumulation was higher for the trees growing in soil with low availability in Fe, Mn, Zn and Ni. The distribution in the branches and roots, studied by LA-ICP-MS, was similar for the various conditions. The possible influence of mineral nutrition on Cd uptake and distribution will be discussed.

Database BIOREMET: On-line resource about the biodiversity of heavy metal rich habitats and aspects of bioremediation

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Heavy metal-enriched terrestric areas, both natural and man-made, are home to specialized metal tolerant organisms (MTOs). On the one hand, knowledge about the type, physiology and symbiosis of MTOs may contribute to reduction of the heavy metal load in the soil, i.e. bio-remediation. On the other hand, reduction of metals will lead to loss of specialized MTOs, a phenomenon that is observed frequently during the last years, at least in Europe.

With this database, we invite colleagues world-wide to contribute their experience of heavy metal rich sites. We aim to document the biodiversity of terrestrial heavy metal rich sites, mainly plants and microorganisms, as well as their symbiotic interactions. A comprehensive inventory of MTOs in relation to the environmental conditions together with research about the adaptations of MTOs shall lead to a holistic model about the functioning of heavy metal ecosystems, to an improved understanding of interactions and survival strategies of MTOs, and to the identification of plants and microorganisms suited for remediation actions where protection of the population and the environment are needed.

Data about heavy metal rich locations are connected with their physical and chemical characteristics, their geology and their mineralogy, where available. Data about vegetation, animals and microorganisms shall include all research about ecological and physiological traits. They will be linked with the publications and authors, relevant projects, finished and on-going, conferences, and excursions. We could also think about a topic of methods and protocols, as was given on the website of the recent project BIOREM.

Lux, Alexander Poster P39

Boarder cells and mucilage of the root cap protect plants against toxic nickel concentration

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Nickel plating is used extensively to provide excellent corrosion and wear resistance, and thus it is used for several decades without replacement. Nickel–cadmium battery is another example of use of this metal. Nickel deposits have been exploited in many areas of the world including Slovakia. The mines are closed, however large areas of Ni-rich substrates remain. Nickel in low concentrations is an essential element for plants, the excess of this element has negative impact on plant growth, crop yield and human health.

In continuation of our previous study, we have separately analysed nickel concentration in boarder cells and mucilage released from the root cap, apical part of the root covered by the root cap and segment of root elongation zone. Sensitive and selective direct ultrasonic slurry sampling and electrothermal atomic absorption spectrometry method was used to determine the nickel concentration in the samples investigated. It was shown that majority of nickel is concentrated in the mucilage with the boarder cells. Maize (*Zea mays*) seedlings cultivated in aeroponics were used for experiments. The results indicate the specific role of boarder cells and mucilage as an important checkpoint in protection of plants against nickel toxicity reducing its translocation to the rest of plants. This study was supported by Slovak Grant Agency VEGA by grant VEGA 1/0472/22 and by the COST action CA19116 ´Trace Metal Metabolism in Plants – PLANTMETALS´.

The importance of trace elements follow-up in *Cannabis sativa* L. varieties

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The importance of trace elements analysis in cannabis flowers should be emphasized due to several reasons, encompassing health, quality and regulatory aspects. Although *Cannabis sativa* L. varieties have become largely included in medicinal, cosmetic or food industry they should be employed with caution not only because of the cannabinoids, (THC which is considered for psychoactive compound), but also for their quality and toxicological aspects. *Cannabis* plants can absorb trace elements such as lead, mercury, cadmium, and arsenic from the soil, water, or fertilizers. These elements can be harmful or even toxic and should be taken as a serious threat to consumers.

Some authors confirmed that a trace elements accumulation in *Cannabis inflorescence*, was mainly affected by the geographical origin but the botanical variety can also influence the potential for absorption of these elements in *Cannabis*. In this way we have investigated and compared the content of trace elements in six different varieties, grown in a green house in the same indoor conditions. The results have demonstrated that content of mercury and cadmium were highest in Jack Kush variety, and lead and arsenic concentration was most increased in Glueberry OG variety. Regular analysis ensures the maintaining of consistent quality across different varieties and/or batches of cannabis products. Therefore, identifying and mitigating contamination sources, such as soil, water, or agricultural inputs, from one side, and strict follow-up of trace elements content through in door production quality control will ensures the purity of the final cannabis products.

Potential of ***Bacillus mycoides*** to alleviate Cd toxicity in Swiss chard

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The main sink of trace metal pollution, including cadmium (Cd), is the soil. A lot of effort is made to minimize its impact on plants, especially on those grown for food and feed. Therefore, the aim of this experiment was to analyze the influence of Cd on Swiss chard grown on the soil and the potential of the inoculation with *Bacillus mycoides*, strain RB11, that exhibits a particularly high tolerance to Cd, to alleviate the toxic effects of Cd.

Before sowing, the seeds of Swiss chard were immersed for 24 h in Cd solutions (CdCl2 in deionized water) of the following concentrations: 0 (control), 1, 3 and 6 mmol Cd /l. The seeds were then sown in pots filled with a soil. Subsequent watering was as needed, and fifteen days after sowing (4 leaves stage), the plants were supplied with 200 ml of suspension of *B. mycoides*, strain RB11, or water (control). The plants were grown in a wired cage with a roof to prevent the inflow of precipitation, while the other conditions are the same as outside.

B. mycoides improved plant biomass production in Cd-treated plants. Cadmium increased Shoot/Root ratio. In addition, Cd increased variable chlorophyll fluorescence and *B. mycoides* reduced this increase. Cd and *B. mycoides* affected concentrations of vitamin C and H_2O_2, MDA, concentration of phenolic compounds, and the ability of cells to chelate Fe and reduce Cu in the leaves, even though the concentration of Cd in the leaves was below the detection limit.

Iron accumulates in seed endosperm to aid germination

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Iron (Fe) is an essential micronutrient for all living organisms. In plants, it functions as a cofactor in a range of metabolic processes towards growth and development. Although many processes in which Fe is involved are well-known, whether germination is one of them is yet to be explored. Endosperm weakening is a developmental process controlled by interactions between the endosperm cap and the radicle. Endosperm weakening can be facilitated by reactive oxygen species (ROS) through NADH oxidase-mediated enzymatic reactions. We observed iron(Fe) accumulation in tomato endosperm close to the radicle protrusion site. This Fe remained immobilized while the seedling is developing, implying unlike other nutrient stores in the endosperm, it does not fuel processes in the germinating embryo. Histochemical and fluorescent stainings showed Fe localization was conserved in *Arabidopsis thaliana*, albeit to a specific region in the chalazal part of the endosperm. Applying Fe exogenously increased germination speed, while this increase depended on the presence of an endosperm, indicating Fe prepones germination by acting on the endosperm. Screening of mutants unresponsive to Fe supplementation led to the identification of the genetic component of Fe-dependent regulation of germination speed. As far as we know, this study links Fe with germination for the first time. This information can contribute to optimizing seed germination and longevity.

Horseradish, a panacea for arsenic contamination in temperate zone?

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Arsenic (As), belongs to ten most dangerous chemicals that threaten public health and also plants including crops. Soil and water arsenic contamination is a serious problem particularly in Asia, but also in Europe. For As phytoremediation, an As-hyperaccumulating fern, *Pteris vittata*, is predominantly used. However, its distribution area is restricted to subtropics and thus we need to search for other phytoremediators. Our previous results showed that horseradish hairy roots are able to accumulate As effectively (Kofroňová et al., (DOI:10.1016/j.ecoenv.2019.02.028). The aim of this study was to test the viability and accumulation potential of whole horseradish soil-grown plants exposed to As. Young plants were treated twice with 6+6 or 25+25 mg of arsenic (AsV) per soil kg. Exposition to 25+25 mg/kg decreased biomass to about two thirds of control, lower treatment did not affect growth. Importantly, preliminary results showed that plants survived even 50+50 mg/kg As treatment. Arsenic exposure led to preferential biomass allocation to roots where arsenic is dominantly accumulated to high levels (up to 2 mg per g dw), almost exclusively in reduced (AsIII) form that can be detoxified. Net photosynthesis and transpiration rates were not impaired by arsenic stress, they even slightly increased. In summary, horseradish is able to effectively cope with arsenic stress. The growth limitation is minor. Although horseradish cannot be classified as hyperaccumulator (low As translocation to shoot) it forms easily harvested large storage-roots and belongs to cosmopolitan species growing in various habitats (preferentially temperate zone) thus might be a promising plant for As phytoremediation.

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Phytomanagement of metal(loid)-contaminated soils with biomass sorghum: successes and failures

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International energy policies, e.g. the EU Renewable Energy Directives and the US EPA Clean Energy Programs, have set objectives for using renewable sources of energy. Lignocellulosic energy crops matter due to their ability to grow on contaminated and degraded land, and thereby reducing the pressure on the limited arable land. *Sorghum bicolor* can produce a high biomass suitable for bioethanol, gasoline, diesel, pyrolysis oil, and sustainable aircraft fuel production on contaminated soils, while phytoextracting relevant Cd, Pb and Zn amounts without creating pollutant linkages. It is a C4 plant with a short growth cycle, high photosynthetic rates, water deficit tolerance, adapted to a wide range of soil conditions (e.g. salinity, excess metal(loid)s), and a fully mechanized crop. Shoot DW yield can reach up to 30 tons DW ha^{-1} $year^{-1}$ on low quality soils with limited water supply. Agronomic traits and shoot Cd uptake depend on varieties and N sources. In this decade, a series of field trials have been implemented in several countries, e.g. France, Poland, Italy, Greece, Brazil, and China. This communication gives an overview of the successes and failures out coming from this series. *Sorghum* biofuels reduced climate change impact, fossil fuel and ozone depletion, and eutrophication. The revenue generated from sorghum is figured to be 2.9 times its growing cost. *Sorghum* processing for biofuels may be limited by its high moisture content at harvest, and the use of pesticides to control weeds, pathogens, and insect attacks on seedlings. High shoot Cl concentration may promote boiler corrosion.

Bioaccumulation of metals from soil to grapevine

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The assessment of soil-plant interactions in grapevine varieties cultivated in Serbia unveils insights into potential metal accumulation tendencies. Investigating 10 grapevine varieties from three different growth vineyards, revealing varying biological accumulation concentrations (BAC) for different elements in different grapevine parts. Notably, leaves of selected varieties (Riesling rain, Riesling italian, Cabernet sauvignon and Cabernet franc) exhibited potential to be investigated as Zn accumulators, while others demonstrated potential for Cu (Riesling rain, Riesling italian) and Ni (Prokupac) accumulation capacities (Milićević et al., 2017). Differences in BAC between commercial and experimental conventional vineyards, influenced by soil pH and soil type, underscored the environmental complexity shaping metal uptake dynamics (Milićević et al., 2018a, b). Furthermore, organic cultivation displayed lower BACs, suggesting minimal element accumulation, with emphasis on B, K, Mg and P accumulation tendencies. Moreover, leaf samples highlighted the influence of airborne deposition and distant pollution sources on metal content, expanding the discourse on environmental metal influx (Milićević et al., 2021). Milićević et al., 2023 investigated the bioaccumulation of elements in grapevines by comparing their bioavailable soil fraction with concentrations measured in grapevine parts by describing pH and soil organic matter. The research on grapevine emphasizes the multifaceted interplay between grapevine physiology, soil composition, and anthropogenic factors in metal accumulation dynamics, underscoring the need for multidisciplinary approaches in environmental and agricultural management.

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Revealing Tl hyperaccumulation in the flora of the abandoned Allchar mine: New findings and future perspectives

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The abandoned Allchar mine in the Republic of North Macedonia is a globally unique deposit with the highest known content of Tl mineralisation. The aim of this study was to investigate the potential hyperaccumulation of Tl, the most abundant trace element in the Allchar mine area, in the leaves of over 30 species growing on the site. Extensive sampling was carried out over two years and the elementome of the plants was analysed using Z-Spec JP500 and E-Lite XRF instruments (Z-Spec Inc). Hyperaccumulation of Tl was detected in several taxa, with the highest concentration found in the samples of *Silene latifolia* (79,200 mg kg^{-1} Tl), making it the strongest Tl hyperaccumulator known to date. It is also worth noting that the Tl concentration in the rhizosphere of *S. latifolia* was only 862 mg kg^{-1} and the leaves were thoroughly washed with hexane to avoid the risk of soil particle contamination. Hyperaccumulation of Tl, recorded in two locally endemic *Viola* species – *V. allchariensis* and *V. arsenica*, as well as in the Balkan endemic *V. tricolor* subsp. *macedonica* was also confirmed through synchrotron µXRF analysis showing that Tl is endogenous in all *Viola* species, and not the result of the surficial contamination. In addition, concentrations of Tl above the hyperaccumulation threshold were measured in the leaves of *Anthyllis vulneraria*, *Clinopodium alpinum*, *Hesperis matronalis*, *Linum hirsutum*, *Minuartia verna* and *Plantago lanceolata*. Experiments under controlled conditions and dosing trials will provide further insights into the mechanisms of Tl hyperaccumulation and tolerance.

Functional insights and emerging roles of plant metallothioneins

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Metallothioneins (MTs) form a superfamily of proteins characterised by a large cysteine content and a small molecular mass (<10 kDa), that are present in all life domains. Their most outstanding characteristic is their ability to coordinate d10 metal ions, such as essential Zn(II), Cu(I), and toxic Cd(II), Hg(II), etc. via the thiol groups of the cysteines. Moreover, the thiol groups of MTs can act as powerful antioxidants. Hence, MTs are associated with roles in metal ion homeostasis, as well as protection against metal toxicity and oxidative stress.

In plants, MTs are classified in 4 groups based on the cysteine distribution patterns in the amino acid sequences. *Arabidopsis thaliana* has 7 known functional MTs, and the expression of its different MT genes is tissue-specific and under developmental control. It is also induced by different abiotic and biotic stresses. This suggests that plant MTs have a role that goes beyond the maintenance of micronutrient homeostasis and toxic metal detoxication. Moreover, plant MTs have been reported to be involved in physiological events such as germination and embryogenesis, root development, suberization and senescence. However, the specific role of MTs in these molecular mechanisms is not understood. In this context, here we present the recent findings in our group to functionally characterise plant MTs.

Micronutrients at the frontline of plant defence responses

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The focus of our research is to reveal the role of micronutrients (Fe, Cu, Mn and Zn) in metal non-hyperaccumulating plants in defence responses against biotic stress. We investigated whether in non-hyperaccumulators (including crops and economically important species) metals act by binding to proteins and low molecular weight compounds in response to biotic stress, rather than by direct toxicity. Recently, we showed that surplus, non-growth limiting Zn nutrition (Zn priming) provided efficient defence against *Botrytis cinerea* infection in *Capsicum annuum* plants (Kuvelja et al. 2024). This was demonstrated by an increased abundance of immunity-related Zn-binding proteins by SEC-HPLC/ICP-MS, identified as NEDD8-activating enzyme E1 regulatory subunit, phenylalanine-tRNA ligase, polyribonucleotide nucleotidyltransferase, subtilisin-like proteases, and others. In addition, Zn complexation with *C. annuum* the low molecular weight ligand, acetophenone, increased its antimicrobial activity in vitro. Further, our latest results point at the involvement of several metals in response to gall-inducing mites in *Tilia cordata* leaves. This was evident by increased (sub)cellular metal localization in idioblasts (Mn, Fe) and nutritive tissue (Cu, Zn) of galls; metal-biding ligands were characterised by XANES and EXAFS. Genes involved in regulation of metal metabolism, including plant development, defence responses and signalling, were upregulated in the galls. Our ongoing work contributes to understanding the importance of metal mobilization in plant immunity against various biotic factors.

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Accumulation of Heavy Metals in Vegetable Species Planted in Serpentine Soils of RrajcaVillage in Albania

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The object of this contribution is to investigate heavy metals accumulation in vegetables species and to assess the human health risks of vegetable consumption. The vegetable types were cultivated on serpentine soils, in Prrenjas and Rrajca village. These soils are rich with heavy metals (Co, Cr, Ni, Fe). Vegetables are an essential part of the human diet. Different concentrations of heavy metals were found in edible parts of the different vegetables. The concentrations of heavy metals decreased in the sequence as leafy vegetables, root vegetables, legume vegetables. The ability of leafy vegetables to uptake and accumulate heavy metals was the highest. From the results obtained, it was found that the amount of total metals Co, Cr, Ni, Fe in the plants was at high levels, showing an accumulation ability of the different agricultural plants analyzed. From the total values were respectively higher and through the risk of the residents may face health risks from thevegetables consumption. Is suggested the use of phytoremediation technologies for the remediation of the Rrajca Village in Albania.

Keywords: heavy metals, Rrajca, phytoremediation, vegetables, serpentine soils.

Nadeem, Muhammad Azhar

Genome-wide association study unveiled genetic basis associated with seed manganese content in Turkish common bean (*Phaseolus vulgaris*) germplasm

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Lack of micronutrients, such as manganese (Mn), exerts a profound impact on billions of individuals globally, and poses major health risks. Biofortifying food crops, particularly common beans, offer a promising solution. We investigated genetic variations among 183 common bean accessions to identify genomic regions linked to Mn concentration in seeds. Field experiments were conducted according to augmented block design under two locations (Bolu and Sivas) for three consecutive years (2016,17 and 2018). The analysis of variance (ANOVA) revealed that genotype by environment interaction was a statistically highly significant ($p < 0.05$) influence on Mn contents. The mean Mn content across the germplasm was found to be 31.69 mg kg^{-1}. Notably, Bingol-16 exhibited the lowest Mn concentration at 11.75 mg kg^{-1}, while Malatya-59 showcased the highest concentration at 56.54 mg kg^{-1}. A total of 10 most stable accessions were evaluated from stability analysis. A total of 7900 DArTseq markers were used for association analysis, identifying 16 markers across four chromosomes (Pv02, Pv05, Pv07, and Pv11). Notably, markers DArT-3374915 & 3375187 exhibited consistent associations across different environments, making them promising candidates for Mn-focused breeding programs. Our findings lay a foundation for marker-assisted breeding efforts in common bean for seed Mn content improvement.

Keywords: Common bean, GWAS, Manganese, Micronutrient deficiency, Biofortification, DArTseq

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Heavy metals bioaccumulation and biochemical profiles in some species of the genus *Tulipa*

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The purpose of this study was to determine the concentration of some metals (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn Ca and Mg) in soil of serpentine and limestone sites, their bioaccumulation and impact on some biochemical parameters in *T. luanica*, *T. kosovarica* and *T. albanica* plants. The research showed that concentrations of Cd, Co, Cr, Fe, Mn and Ni were significantly higher at serpentine soil sites in comparison with limestone sites, while concentrations of Pb, Cd, Co and Cr in bulbs, leaves and seeds were under the limit of detection. The concentration of Ni in plant samples of *T. kosovarica* was significantly higher in comparison with its concentration in *T. albanica*, but in *T. luanica* was under the limit of detection. Moreover, concentrations of Al and Fe in leaves of *T. kosovarica* and *T. albanica* were higher in comparison with *T. luanica*. The concentration of Mg was significantly higher in *T. kosovarica* and *T. albanica* than in *T. luanica*. The δ-aminolevulinic acid dehydratase (ALA-D) activity, malondialdehyde (MDA) and glutathione (GSH) contents in leaves of *T. luanica* were higher in comparison with *T. kosovarica* and *T. albanica*. In addition, the amounts of total chlorophyll and δ-aminolevulinic acid (ALA) in leaves of *T. albanica* were higher in comparison with *T. kosovarica* and *T. luanica*. Our findings show that target organs of metal accumulation in three *Tulip* species appears to be leaves>seeds>bulbs, while the biochemical parameters show that limestone sites represent a less stressful habitat for growing these plant species in comparison with serpentine sites.

Identification of nickel-binding proteins in plants

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Nickel (Ni) is an essential micronutrient for most organisms. This cofactor plays a key role in maintaining protein structure, electron transfer functions, and catalytic reactions[1]. While several Ni-containing proteins have been reported in prokaryotes[1] only a few proteins have been associated with Ni homeostasis in plants. The best characterised example is for the Ni metalloenzyme urease and its maturation factors[2]. Given that Ni is an essential micronutrient for plant and that some plant species can accumulate high concentrations of the metal when grown in Ni-rich soils, we hypothesize that additional Ni-binding proteins may exist. These proteins remain to be identified in plants, as well as most of molecular actors involved in the uptake, transport, and storage of this metallic element. Here, we developed a metalloproteomic approach aiming at identifying target proteins of Ni using Arabidopsis thaliana and the Ni-hyperaccumulator Noccaea caerulescens. First, the root and shoot soluble proteomes of plants grown hydroponically in the presence of Ni was fractionated using ion-exchange chromatography. Then, potential Ni-binding proteins were retained by affinity capture and the Ni content was determined by ICP-MS. My preliminary results demonstrate that Ni was associated with several chromatographic fractions, suggesting that several pools of proteins may bind Ni in vivo. The next step in this work will be to identify these potential Ni-binding proteins by tandem mass spectrometry. This identification process is an essential step towards a better understanding of the role of Ni in essential cellular processes and deciphering the molecular basis of Ni accumulation in plants.

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Effect of root biostimulants on root growth and metal(loid)s uptake in *Miscanthus x giganteus*

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Miscanthus x giganteus is one of the most promising industrial crops for biomass production. Its cultivation on contaminated land would allow the implementation of efficient phytomanagement strategies, avoiding competition with food crops. However, *Miscanthus* is known to be an excluder species with limited potential for metal(loid) phytoextraction, but with possible positive effects on their phytostabilisation by reducing their bioavailability due to its deep and extensive rhizomatous root system. To implement these ecological services in marginal areas, research is now interested in studying the effect of biostimulants. The aim of this study was to investigate the effect of a combination of root biostimulants (i.e. mycorrhizae and humic and fulvic acids) on the growth and development of miscanthus roots and the related dynamics of uptake and reduction of metal bioavailability through the soil profile. The study was conducted in a greenhouse for 7 months in cylindrical rizhotrons approximately 1 m deep. The rizhotrons were filled with soil taken from a former illegal landfill contaminated with Ni, Pb, Cu and Zn. At the end of the experiment, the effect of the applied treatment resulted in an increase in root growth especially in the deepest layer (approximately four times more than the control), due mainly to an WUE increase (+ 2.5 g L^{-1}). In relation to the different depths investigated, a positive effect of the treatment also emerged on the metal roots concentrations (Ni, Zn), their increased accumulation (Pb, Cu, Zn) and a reduction in their bioavailable fraction (Cu, -18% in Soil Pore Water).

Community ionomics of a hyperdiverse shrubland on metalliferous soil in New Caledonia

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The island of New Caledonia in the southwest Pacific has particularly large surfaces of ultramafic substrates which have low concentration of macronutrients (N, P, K) and excess concentration of metals (Cr, Mn, Ni). It has nevertheless a rich and unique flora with a unique shrubby vegetation, maquis, and is considered as a biodiversity hotspot. The flora encompasses a large number of metal hyperaccumulating plant species, which are able to accumulate in their leaves metals at concentrations 100 to 1000 times higher than normal plants. We established a plot 20 × 20 m plot on Ferritic Ferrasols where we sampled all individual plants exceeding 1 m height. We measured concentrations of 20 elements on 474 individuals representing 37 species and 22 families. The plot included a large diversity of root symbioses and both Ni and Mn hyperaccumulators. We observed a large diversity of mineral nutrition strategies of plants, and this is in line with the Old Climatically Buffered and Infertile Landscape (OCBIL) theory which predicts nutritional specialization on infertile substrates. Nickel and manganese hyperaccumulation can be two different responses to the same soil conditions. Underground niche partitioning for mineral resources could explain the high alpha diversity observed on adverse geological substrates.

Exploring phytoremediation potential of native plants and rhizosphere microorganisms in metal-contaminated sites

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Soil supports 25% of Earth's biodiversity and plays a crucial role in carbon and water retention, but over 60% of EU soil ecosystems are degraded due to various factors. Mercury pollution is a global threat due to its toxicity, mobility, and persistence, stemming from abandoned mines and chlor-alkali plants despite reduced usage. Expensive engineering solutions are impractical for large affected areas, making phytomanagement a viable alternative.

Our research focuses on assessing metal resistance strategies of native plants at a polymetallic Hg-contaminated site and identifying rhizosphere microorganisms aiding plant growth under Hg contamination. Conducted at an urban site formerly housing a chlor-alkali plant, field studies using portable X-ray fluorescence spectrometry revealed soil Hg concentrations 96 times higher than industrial thresholds, with a potential ecological risk index (ERI) of 769600 and elevated Pb ERI. Despite severe contamination, pioneer plants like *Diplotaxis muralis* and *Lotus tenuis* show promise for cultivation on highly Hg-contaminated soil. Microbial communities associated with *D. muralis* utilize Hg detoxification mechanisms, enhancing plant resilience. Actinomycetota were prevalent in merA-positive rhizosphere communities, showing increased ABC transporters.

Assessment of cultivable rhizosphere microorganisms revealed 32 heavy metal-resistant fungi, with Ascomycota showing diverse resistance spectra, particularly to ecologically risky elements compared to Mucoromycota. Species like *Cladosporium* sp., *Didymella glomerata*, *Fusarium oxysporum*, *Phoma costaricensis*, and *Sarocladium kiliense* demonstrated high Hg biosorption capacities (33.8-54.9 mg/g dw) and removal efficiencies (47% - 97%).

Integrating native plant species and their associated soil microbiota could facilitate selection of plants and microbial inoculants resilient to high Hg levels for sustainable phytomanagement strategies.

Transcriptome response in flag leaves of proso millet to agronomic biofortification

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Biofortification aims to enhance crop micronutrient content through plant breeding and agronomy. Agronomic biofortification is based on optimal fertilisation techniques with which it has the potential to increase crop biomass, yield, and stress tolerance, while increasing concentration of essential elements. Biofortified crops have been shown to be a cost-effective way to reduce micronutrient deficiency and improve human health. An essential micronutrient often lacking in our diets is zinc (Zn). The aim of this study was to assess agronomic biofortification with Zn in two populations of proso millet (*Panicum miliaceum* L.) from Slovenia, i.e. in Sonček and Odranci populations. Plants were grown in a pot experiment in a glasshouse and were fertilised with 3 mM or 15 mM concentrations of $ZnSO_4$ twice: first, at flowering stage and again at grain filling stage. Biomass and concentration of Zn in leaves, flag leaves and grain were determined: there was no effect on the biomass and yield but an increase in leaf Zn concentration of both populations and increase in grain Zn concentration of Sonček population already at the 3 mM $ZnSO_4$. RNAseq of flag leaves uncovered only small transcriptome response with the largest number of differentially expressed genes between the two $ZnSO_4$ concentrations, many of which were involved in the toxicity response. Results indicate the need to examine relationships between achievable increase in grain Zn concentration without yield and metabolic penalties. Experiments in the field should be performed to meaningfully confirm our findings of increased grain Zn concentrations in proso millet.

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The impact of applying mycorrhizal fungi on the mineral profile of tomato seedlings

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Phosphorus (P) is crucial for root development, but other macro or micronutrients may also impact vegetable growth and fruit quality. Arbuscular mycorrhizal fungi (AMF) can improve P uptake and are recommended for soils with low biologically available phosphorus as well as other micronutrients such as zinc (Zn), manganese (Mn) or copper (Cu). This study aimed to assess the impact of AMF application on the root mycorrhization percentage, shoot and root morphology, and the mineral status of tomato seedlings. The experiment used a randomised design with Red Valley F1 tomato seeds. The treatments included an untreated control and seeds inoculated with AMF. Forty-five days after sowing, the height of the plants above the ground was higher for those inoculated with AMF compared to the non-inoculated ones. The content of P, Ca, Mn, and Cu in the stems and leaves of tomato seedlings was higher in seeds inoculated with AMF. There was no significant difference between treatments for Mg or Zn content. As can be expected, root mycorrhizal colonization occurred only in seeds inoculated with AMF, compared to the untreated control. However, the treatments did not significantly affect root length, surface area, average diameter, volume, and dry weight.

Iron-mediated mitigation of hexavalent chromium toxicity in *Brassica juncea* and *Raphanus sativus*

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Hexavalent chromium [Cr(VI)] is one of the most widespread toxic trace elements found in agricultural soils, with potential negative impacts on crop growth and yield. The role of iron (Fe) in mitigating Cr(VI) toxicity in plants remains poorly understood. Understanding plant Cr(VI) tolerance, accumulation, and its interaction with Fe is crucial for evaluating the feasibility of managing Fe application to mitigate Cr(VI) toxicity.

Cr(VI) tolerance was evaluated in *Brassica juncea* and *Raphanus sativus* exposed to a series of Cr(VI) concentrations (0-512 µM) supplied as $K_2Cr_2O_7$ in nutrient solution. Additionally, plants were exposed to a factorial combination of Cr(VI) at the maximum non-effect concentration (NEC = 8 µM) and the effective concentration inhibiting root elongation by 50% (EC50 = 64 µM), along with three Fe-EDDHA concentrations (10, 20, and 40 µM).

Significant differences were observed between species in Cr uptake efficiency and nutrient status. Under NEC, Fe treatments did not significantly alter plant growth and Cr concentrations in leaves (0.23 and 0.15 µmol g^{-1} DW in *B. juncea* and *R. sativus*, respectively). However, under EC50 at 40 µM Fe, plants showed an increased leaf area and significantly higher leaf Cr concentrations (0.83 and 0.58 µmol g^{-1} DW in *B. juncea* and *R. sativus*, respectively) compared to the 10 and 20 µM Fe treatments, not significantly different among themselves (0.50 and 0.25 µmol g^{-1} DW in *B. juncea* and *R. sativus*, respectively).

Exogenous Fe application could alleviate Cr(VI) phytotoxicity by increasing leaf Fe content and enhancing photosynthesis.

Adaptation of the membrane- and cell wall-associated proteome of *Arabidopsis thaliana* roots in response to uranium stress

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Uranium (U) is a non-essential and toxic metal for plants, which have the ability to take up uranyl ions from the soil and preferentially accumulate them in the roots. We showed that the bulk of the radionuclide accumulates in the root insoluble proteome of *Arabidopsis* plants challenged with U. Therefore, to elucidate new molecular mechanisms related to U stress response and tolerance, we used label-free quantitative proteomics to analyze the dynamics of the root membrane- and cell wall-enriched proteome under U stress. Of the 2,802 proteins identified, 458 showed differential accumulation in response to U. Biological processes affected by U include response to stress, amino acid metabolism, and previously unexplored functions associated with membranes and the cell wall. Indeed, our analysis supports a dynamic and complex reorganization of the cell wall in response to U stress, including lignin and suberin synthesis, pectin modifications, polysaccharide hydrolysis, and Casparian strips formation. Water flux through aquaporins and vesicular trafficking were also significantly perturbed by U stress. Finally, the abundance of metal transporters and iron, calcium, and other metal-binding proteins was affected by U. These proteins may play a role in controlling the fate and toxicity of U in plants.

Action of peroxidase Class III and lignin synthesis in *Halacsya sendtneri* (Boiss.) Dörfl. (Boraginaceae) rhizome as possible response to challenging serpentine habitat. Histochemical localization

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Halacsya sendtneri (Boiss.) Dörfl. (Boraginaceae) is a Balkan endemic species from a group of obligate serpentine plants. Serpentine soils often display copious heavy metal (HM) presence. Plants found in these environments indicate strong development of below-ground mass. Heavy metal tolerance mechanisms detected in serpentine-tolerant plant species include metal exclusion at the root level, sequestration to various plant organs or toxicity tolerance. The cell wall can be an effective barrier against HMs from the external environment by increasing lignification and higher activity of class III peroxidases (POX). Our previous work showed high POX activity as well as high phenolics concentrations in rhizome, which upholds its excluder strategies. The aim of our research was histochemical localization of lignin, phenolics and POX in rhizome of *H. sendtneri*, collected in Pribinić (B&H). The content of heavy metals (HM) in soil samples from Pribinić locality was in following order: Fe>Ni>Cr>Cu. Histochemical detection of lignin in rhizome showed that xylem elements in pith was a dominant place of polymer lignin deposition, and phenolic compounds in the cortex. In addition, POX activity was shown in both cortex and pith with higher POX activity in cortex. Our observations imply that rhizome might be a first step to dealing with heavy metal stress.

Keywords: serpentinophyte, heavy metals, antioxidative metabolism, lignification.

The impact of heavy metal stress on the growth and chemical composition of *Camelina*

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The primary purpose of the research on camelina (*Camelina sativa* L.) is to determine whether it can compete with oilseed rape in intensive agricultural production. Oil crops such as sunflower, soybeans, canola, castor bean, and others are good sources of oil with about 40% of the oil in the seeds. Camelina oil is rich in essential omega-3 fatty acids, and the seeds have a relatively high protein level. It is a short-season crop which has a high level of resilience to pathogens. Because of these characteristics, camelina can serve both as feed and food. It is utilized in the production of cold-pressed oils, biodiesel, sustainable farming, and the cultivation of so-called marginal land. The goal of this study was to find out how heavy metals (HMs) affect the absorption and accumulation of certain essential macro- and micronutrients as well as undesirable HMs, which may be important if camelina is used in phytoremediation. The experiment was done on camelina, variety Stepski 1. The seedlings were exposed to 1 μM Cd or Cu and 10 μM Ni or Zn from the moment of germination. Hoagland solution at ½ strength was used for the growth of plants in a greenhouse under semi-controlled conditions. The same HM concentrations as during germination were added to Hoagland solution. The contents and distribution of Ca, Mg, P, K, Fe, and Mn in roots and shoots were altered by higher quantities of Cd, Cu, Ni, and Zn.

How do plants find Zn source?

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Zinc deficits globally underscore the urgency to explore diverse approaches for Zn biofortification in plants, the primary source of this essential nutrient. Zn in the soil is distributed heterogeneously, making Zn uptake and distribution a complex process. Although many studies highlight the phenomenon of Zn-tropism in plants, the mechanisms responsible for this remain unclear. Our project aims to uncover how plants determine the direction of growth in soil with an uneven distribution of Zn.

Our research focuses on *Nicotiana tabacum* L. as a model plant. We cultivate plants in a Zn-deficient condition in agar medium (¼ Knop with no added Zn). Once the plants' root systems reach the desired size, we add the point of Zn source (¼ Knop agar medium with added 40 μM Zn) and documenting it growth. We showed that approximately 60% of plants in Zn-deficient conditions change root growth towards a zinc source.

We hypothesize that roots growing near a Zn source will have relatively higher Zn concentrations on the side closest to the Zn source compared to the other side. This asymmetry could lead to differences in cell growth, which may be an effect of either hormonal regulation or, more interestingly, the mechanical properties of cell walls, with pectins playing a crucial role.

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Geobotanical and biogeochemical prospecting– potential application in phytoremediation of mine sites

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Geobotanical and biogeochemical characterization was applied in the area of Bor mining site, indicating the degree of correlation between the underlying processes in rocks, mine soils and present plant species. Mine waste sites were formed by non-selective disposal of extracted rock material, where different lithological groups colonized by plants could be distinguished: hornblende andesites, altered andesites, melange, conglomerates and loose sediments. Four initial vegetation development stages have been determined and associated with the established lithological groups and mine soil characteristics that incurred during disintegration and weathering of rock material. This relation was especially pronounced among the lithological groups whose mineralogy generates acid reaction, and lithological groups that generate neutral to basic reaction of mine soil solution. Pseudo-total and EDTA-available contents of investigated trace elements are decreasing in order: Cu>Zn>Pb>As>Cr. Overburden mine soils are characterized by high contents of copper and arsenic, that are exceeding the prescribed limits and remediation values, and in respect of which it is necessary to remediate the area. Plant species with the highest degree of frequency were selected and analyzed for element content in roots and shoots. The contents of trace elements in the roots decrease in order: Cu>Zn>As> Pb> Cr, while the contents of trace elements accumulated in the shoots decrease in order: Cu>Zn>As>Cr> Pb. With decreasing of pH value, which affects the mobility of trace elements in mine soils, and increasing of the available sulfur concentration and electrical conductivity, species with higher bioconcentration factors begin to dominate the habitat.

Cellular and molecular responses of the metal-tolerant green microalga *Coelastrella* to uranium

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Uranium (U) contamination of terrestrial and aquatic ecosystems poses a significant threat to the environment and human health due to its chemotoxicity to all living organisms. The characterization of organisms that tolerate and accumulate U is crucial to decipher the mechanisms evolved to cope with the radionuclide and to propose new effective strategies for the bioremediation of contaminated environments. We isolated a unicellular green microalga of the genus *Coelastrella* that is hypertolerant to U and accumulate remarkably high amount of the metal. *Coelastrella* can also remediate U and lead from natural waters contaminated with metals. In this work, we used a combination of (sub)cellular imaging and molecular approaches to gain insight into the mechanisms responsible for U tolerance and accumulation in *Coelastrella*.

Resolving structure-related trace element and molecular composition of Tartary buckwheat grain

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Edible grains serve as the primary carbohydrate resource in our diets. However, before their consumption, grains undergo several processing steps that include removing grain outer coats and milling, thus reducing their nutritional value. The spatial distribution of essential trace elements and beneficial biomolecules is, therefore, a primary concern of grain biology and food science.

Micro-particle-induced X-ray emission (micro-PIXE) enables quantitative tissue-specific spatial analysis of nutritionally important elements in grain with an imaging resolution below 1 μm. The secondary Ion Mass Spectrometry (SIMS) imaging method, referred to as MeV-SIMS, is a complementary technique used for molecular imaging of grain tissues. As such, both methods have an excellent potential to help resolve fundamental questions in food quality and safety. We selected the Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) grain as a suitable model due to its distinct elemental spatial resolution demonstrated previously using micro-PIXE. It contains large concentrations of antioxidants rutin and quercetin however, their exact localization remains elusive. Both analyses can be performed subsequently at the accelerator facility of Jožef Stefan Institute, Slovenia. After micro-PIXE, the morphology of the sample was captured using scanning electron microscopy followed by laser-ablation-inductively-coupled plasma MS to determine the localisation of Al and Si with better sensitivity. Furthermore, prior to the MeV-SIMS, bright light and UV-excitation microscopies were used to capture the morphology of the sample. With careful consideration of grain tissues with the highest concentrations of grain nutrients, losses in grain nutritional value can be substantially reduced.

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Thallium uptake by tomato plants grown on a thallium polluted soil in relation to grafting combination and plant vigor

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The release of trace metals into the environment caused by productive activities, pose threats to ecosystems and human health. In the agricultural environment uptake of heavy metals by cultivated plants is controlled by soil properties and metal chemical forms, whereas the effects of selection of varieties and other biotechnological options such as grating on uptake, translocation and accumulation into edible parts have been poorly investigated. We present the results of a greenhouse trial on the absorption of thallium (Tl) in tomato plants grafted in different combinations grown on a Tl polluted soil taken from from Baccatoio Valley in the municipality of Valdicastello Carducci, an area heavily contaminated by Tl and several other trace elements from historical mining activities. The soil was also polluted by barium (Ba) due to the presence of barite ores in the area. The trial involved the grafted plants on 3 different rootstocks, self-grafted plants and non-grafted plants to mimic the current domestic and professional horticulture practiced in the polluted area. Main results were that grafted tomato plants showed higher growth and productivity than non grafted ones, and in general more vigorous plants absorbed more Tl and trace elements. Surprisingly, despite the high levels of pollution Tl concentrations were below the limit of quantification, and concentrations of other potentially toxic elements in tomato fruits were not at dangerous levels, whereas Ba concentrations were of some concern. Dynamics and danger of Tl in agricultural systems and effects of grafting biotechnology on trace metal uptake is still little characterized.

Physiological, biochemical and molecular approaches to understand the copper deficiency response in tomato

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Copper (Cu) deficiency (CuD) is a stress facing European agriculture that needs Cu-based fertilizers to be solved, hence posing high environmental and human risks. However, little is known about the effects of CuD on the quality of fruit horticultural crops. We performed a holistic approach to understand Cu homeostasis in fruits by selecting tomato (*Solanum lycopersicum*) as a model. We identified and characterized the high affinity Cu transporters (COPTs) into the tomato genome and revealed the molecular mechanisms occurring in plant organs and red ripe fruit in response to CuD growing conditions. Functional analyses highlighted the induction of 'metal ion transport' and 'transmembrane transport' biological processes in all tissues; while 'defense response' was induced specifically in the root and 'lipid biosynthesis', 'cell redox homeostasis' and the 'response to phosphate starvation' were repressed only in stem, leaf and fruit, respectively. SlCOPT2, Cu chaperones, ferric reductases and divalent cation transporters were among the most induced genes in response to CuD in all tissues, while Cu superoxide dismutases, plastocyanin and laccases were repressed. In addition, CuD increased fruit cracking incidence and the susceptibility to pathogen infection in red ripe fruit while reduced the Cu content and altered the accumulation of other micronutrients (Fe, Zn, Mn) in the fruit peel along fruit ripening. We will follow this research by understanding the hormonal regulation of Cu homeostasis in fruits, aiming to provide biotechnological tools to reduce food waste and to improve the sustainability of this crop.

Phytochelatin synthase in land plants: new perspectives for an ancient enzyme

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Important evidence points to the existence of well-known and emerging functions of the phytochelatin synthase (PCS) enzyme. Based on a careful review of the literature, 5 key-features of PCS can be delineated:

1. PCS acts as a "papain-like" cysteine peptidase. The conserved N-terminal catalytic domain of eukaryotic PCS displays a □-glutamylcysteine-dipeptidyl-(trans)peptidase activity, while the variable C-terminal domain stabilizes and modulates the enzyme activity.

2. PCS is constitutively expressed in a range of eukaryotes, encompassing plants, algae, lichen photobionts, fungi, some Animalia and Amoebozoa, SAR, and, more rarely, Excavata. The shorter PCSs from prokaryotes have been identified in cyanobacteria and in some proteobacteria.

3. Through its transpeptidasic products – i.e., the metal(loid)-binding oligopeptides named phytochelatins (PCn) – PCS plays a pivotal role in the detoxification of harmful metal(loid)s (e.g., cadmium, lead, arsenic, etc.) and plays a part in the homeostatic regulation of metal micronutrients such as zinc, copper, and iron.

4. PCS can hydrolyze GSH and GS-conjugated xenobiotics through the cleavage of glycine from GSH. As it is also present in prokaryotic PCSs, the breakdown of GS-conjugates may be considered a primigenial function of PCS, not related to the capacity to synthesize PCn.

5. Novel evidence implicates PCS in plant defense against pathogens, essentially in terms of Microbe-Triggered Immunity.

In conclusion, PCS is receiving ever-increasing attention in plant biology, due to its involvement in several other important processes. Advances in this area of knowledge may well help clarify the evolutionary history and roles of this quite mysterious "multi-tasking" enzyme for plant organisms.

Calcium oxalate crystals in cacao trees and their interactions with cadmium

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The 2019 European regulation on the cadmium (Cd) limit of 0.6 mg Cd.kg^{-1}, in cacao products, affects the cacao market worldwide, especially in Latin America. Indeed, 30 to 50% of cacao bean production in this region exceeds this limit. Research on strategies to reduce Cd accumulation in cacao beans is currently limited by a lack of understanding of Cd transfer pathways from the soil to the cacao beans. Recent studies on native cacao trees revealed that calcium oxalate was involved in Cd storage in branches. The purpose of this study was to further explore the relationship between calcium oxalate and Cd, on representative agronomic cultivars (CCN-51 and Nacional). Cacao roots, branches, leaves, and nibs sampled in the field were studied by a combination of chemical and imaging techniques. Calcium oxalate was present in all organs of the two cultivars. In the branches, the compartment with the highest concentrations, Ca oxalate accounted for 5 to 15% of the biomass. Ca oxalate was present as isolated crystals of various sizes (from a < 1 µm to a few tens of µm). Ca oxalate and Cd concentrations were positively in each organ, with highest correlations found in the branches, which represent the major Cd storage compartment in the tree. Ca oxalate is constitutively present in cacao plant, but its synthesis could be enhanced in the presence of Cd, and it could participate to Cd storage and detoxification.

Zinc excess: how *Arabidopsis* responds in the root tip compared to the remaining root system

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Zinc (Zn) is an essential micronutrient, but toxic when present in excess in the environment. Notably, Zn excess negatively impacts primary root growth and induces secondary iron (Fe) deficiency in *Arabidopsis*. Root growth processes (meristematic divisions, cell elongation, differentiation) take place in the apical part of the root and are driven by many actors, several of them requiring Zn. The effects of Zn excess on these processes are unclear. Here, we examined the specific impact of Zn excess on the root tip (RT, i.e. the apical ~2-3 mm) in comparison to the remaining root system (RR). We show that, after a 24-48h exposure to Zn excess, the Root Apical Meristem size, as well as the elongation zone, were shortened with differentiation initiating closer to the tip of root. These morphological changes were associated to a specific transcriptomic response in RT. Upon Zn excess, Zn accumulated at a lower concentration and with a distinct distribution in RT than in RR, and this pattern was associated to distinct expression of Zn transporter and Fe deficiency response genes. Zn and Fe transporter genes were overall less expressed in control conditions, and less regulated upon Zn excess in the RT, indicating a distinct response compared to RR. Moreover, several genes contributing to specialized metabolism pathways involved in antioxidant and biotic stress response were specifically up-regulated in the RT upon Zn excess. This suggests that a metabolic response may at least partly contribute to Zn tolerance and compensate for the lower expression of transporter genes in the less differentiated RT cells.

Shadbolt, Jessica **Contributed talk 19**

Good copper, bad copper: Characterising the physiological implications of contrasting alleles of HvHMA5

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Copper (Cu) is an essential co-factor for metalloproteins and participates in key oxidation reactions in both plants and humans. However, in excess this micronutrient has severe physiological implications that result in Cu toxicity. Understanding the genetics of grain Cu accumulation in barley (H. vulgare) is important due to the underpinning nutritional role barley plays in the staple diets of the poorest populations, in addition to the increasing deposition of Cu into arable soils. In this presentation, I will identify Heavy Metal ATPase-5 (HvHMA5) as the likely gene underlying a quantitative trait locus contributing to variation in barley grain Cu accumulation in a population of contemporary European 2-rowed barley cultivars. Resequencing experiments defined two distinct haplotypes of HvHMA5 in cultivated barley associated with contrasting levels of Cu and other grain micronutrients. Through protein modelling I will demonstrate that the difference in nutrient accumulation of these alleles can likely be attributed to a single amino acid substitution resulting in differing Cu transport capabilities. An analysis of georeferenced genotypic data from more diverse germplasm led to the tentative speculation that the HvHMA5 allele associated with increased grain Cu is under positive selection in cultivated germplasm. Through breeding approaches, the identified HvHMA5 alleles may facilitate the development of biofortified barley or varieties with increased heavy metal stress tolerance.

How do plants manage their microelements? Untangling the Zn transport mechanisms between lateral roots

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Zinc is an essential element for plant development. However, Zn distribution in soil is heterogeneous, impacting e.g. root growth efficiency in Zn-deficient regions. While control over Zn distribution within and between plant organs exists, the underlying mechanisms remain elusive.

In our research, we utilize tools such as transparent soil, a hydrogel-based medium that mimics soil properties, to heterogeneously distribute Zn (both Zn-sufficient and Zn-deficient regions within a single pot) and monitor root growth. Our findings demonstrate that the expression of tobacco ZIPs (Zn transporters) depends on the overall accessibility of the root system to Zn, rather than local Zn levels. Moreover, utilizing µXRF (X-Ray Fluorescence) at the first Polish Synchrotron SOLARIS (beamline POLYX), we have confirmed for the first time that under conditions of partial Zn deficiency in the medium (half with Zn, half without), Zn is transferred between Zn-sufficient and Zn-deficient lateral roots of the same plant. We have also identified Zn relocation sites, where Zn is potentially transferred from the xylem to the phloem to be delivered to lateral roots growing in Zn-deficient regions of the medium. Our current focus is on the molecular level to identify Zn transporters facilitating Zn relocation within these sites. Ultimately, we aim to uncover the mechanisms underlying Zn distribution within the root system, particularly to lateral roots in conditions mirroring natural soil Zn heterogeneity.

The successful unraveling of these mechanisms may open avenues for new Zn biofortification strategies, including enhancing the efficiency of Zn fertilizer utilization.

Assessment of the possibility of using several iodoquinolines for biofortification of the potato *Solanum tuberosum* L. with iodine - process optimization in a pot experiment

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There are various results of potato biofortification studies documenting very poor to very high iodine accumulation allowing the Recommended Daily Allowance for iodine (RDA-I) to be covered. The aim of the study was to determine the efficiency of iodine biofortification of potato using six iodoquinolines compared to KIO_3. Better tuber biofortification efficiency was obtained with a dose of 50 µM than 10 µM of the iodine compounds used. A range of iodine enrichment of tubers for both doses, respectively: KIO_3 (1.44-28.53% RDA-I) > 5,7-diiodo-8-quinolinol (1.53-9.80% RDA-I) > 5-chloro-7-iodo-8-quinoline (1.24-8.81% RDA-I) > 8-hydroxy-7-iodo-5-quinolinic acid (1.06-7.35% RDA-I) > 7-iodo-4-hydroxy-3-quinoline carbocyclic acid (0.94-6.53% RDA-I) > 6-iodo-4-hydroxy-3-quinoline carbocyclic acid (1.03-3.10% RDA-I) > 8-iodo-4-hydroxy-3-quinoline carbocyclic acid (1.08-1.18% RDA-I) versus control (0.42-0.94% RDA-I). Iodoquinolines occur naturally in control plants. The iodine metabolite profile (including content of: iodotyrosine, iodosalicylates and iodobenzoates) after application of iodoquinolines was similar to endogenous proportions of iodine compounds in potato. The tested compounds had no negative effect on yield. The mineral economy of potato plants and the nutritional and health-promoting value of tubers were also studied.

Nutrient survey of nickel concentrations in fruits of apple, sweet cherry, sour cherry and kiwifruit cultivars grown in the Department of Decidouous Fruit Trees in Naoussa, Greece

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Nickel (Ni) plays an important role in biological systems, and the bioavailability for plants is dependent on its origin and the soil characteristics, in which the pH has a crucial role. The scope of this research was to make a first attempt to make a screening of various commercial apple, sweet cherry, sour cherry and kiwifruit cultivars regarding their Ni concentration of fruits. The research was carried out in the experimental orchard of the Department of Decidouous Fruit Trees in Naoussa, of the Institute of Plant Breeding and Genetic Resources, Greece.

The higher Ni concentration of fruits among 17 sweet cherry cultivars grafted on mazzard (*Prunus avium* L. seedlings) was measured for the cultivar Blaze Star, whereas the lowest for the cultivar Grace Star. Ni concentration was diminished in the following order: Blaze Star> Negre di Bistrita> Tsolakeiko> Regina> Sabrina> Satin> Lory Bloom> Ferrovia> Cristalina> Sweetheart> BlackStar> Kordia> Canada Giant> Samba> Big Star> Skeena> Grace Star. Sour cherry 'Oblacinska' had higher Ni concentration in fruits than 'Konstantinoupoleos'. The higher Ni concentration of fruits among 18 apple cultivars grafted on M9 rootstock was measured for the cultivar Gala Buckeye, whereas the lowest for the cultivars Gala Shniga and Gala Brookfield. Ni concentration was diminished in the following order: Gala Buckeye> Super Chief> Golden Reinders> Golden Delicious> Gold Chief> Jeromine> Ozark Gold> Red Chief> Fuji Aztec> Scarlet Spur> Forlady> Mutsu> Fuji Kiku> Red Jonaprince> Jonagored> Granny Smith> Gala Brookfield=Gala Schniga. Among the 3 common kiwifruit cultivars grown in Greece, the cultivar 'Tsechelidis' had the highest Ni concentration of fruits, followed by 'Hayward clone 8', whereas 'Hayward' had the lowest.

From the above results it appears that the studied cultivars showed a different capacity of Ni absorption from the soil.

Effect of thorium uptake on plant metabolism

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Phytoremediation is the use of plants to extract, sequester, and/or detoxify pollutants. It is widely viewed as the ecologically responsible alternative to the environmentally destructive physical remediation methods currently practiced. Plants have many endogenous genetic, biochemical, and physiological properties that make them ideal agents for soil and water remediation. Significant progress has been made in recent years in developing native or genetically modified plants for remediation of environmental contaminants. Because elements are immutable, phytoremediation strategies for radionuclide and heavy metal pollutants focus on hyperaccumulation above-ground.

Soil contaminated with radionuclides pose a long-term radiation hazard to human health through exposure via the food chain and other pathways. Remediation of radionuclide-contaminated soils has become increasingly important.

Thorium is a radionuclide that occurs naturally all around the world. It is commonly found in rock, soil, water, and in very low concentrations also in air and organisms. The thorium contamination of surface soils and waters has resulted from the development of nuclear industry, which involves mining, milling and fabrication of various thorium products. Selection of appropriate techniques for the remediation of soils and waters contaminated with thorium and other xenobiotics belong to main goal of some research laboratories worldwide. Therefore, better understanding of plant responses to stress induced by thorium exposure is necessary prerequisite for phytoremediation.

The aim of our study has been contribution to understanding of the mechanisms of thorium uptake and its distribution in plants which can enhance effectiveness of phytoremediation in cleaning-up sites polluted by thorium.

Bacterial endophytes: ecological and biotechnological agents

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Bacterial endophytes isolated from plant hyperaccumulators are a source of extensive research with fundamental and applied implications. This study reports on a bacterial endophyte isolated from the roots of a selenium (Se) hyperaccumulator, *Stanleya pinnata* (Fam. Brassicaceae), growing on seleniferous soils in Colorado, USA (Staicu et al. 2015). The isolated was classified as *Pseudomonas moraviensis stanleyae*, based on fatty acid methyl ester (FAME) and multi-locus sequence analysis (MLSA) using 16S rRNA, gyrB, rpoB and rpoD genes. The isolate exhibited extreme tolerance to selenite (up to 120 mmol/L) and selenate (>150 mmol/L). In addition, in an inoculation experiment, it stimulated the growth of crop species *Brassica juncea* by 70% with no significant effect on Se accumulation. Interestingly, only selenite was reduced to red Se(0), via a biomineralization process, whereas no selenate removal was measured by capillary electrophoresis. Se(0) accumulation occurs intracellularly, indicative of a detoxification process (Ni et al. 2015). This is in contrast with the formation of extracellular Se(0) as a by-product of anaerobic respiration (e.g. *Shewanella*) (Staicu et al. 2022). In conclusion, selenium-rich plants may be used to provide dietary Se to humans and livestock, and also to clean up Se-polluted soils or industrial effluents (Sura-de Jong et al. 2015). Furthermore, the ecological relation between the isolate and the host plant deserves an in-depth exploration since the high Se resistance exhibited by the bacterium might act as a line of defense against the toxic Se naturally present in abundance in these soils.

Ni et al. (2015) Nanoscale 7(41):17320-7; Staicu et al. (2015) J Appl Microbiol 4119(2):400-10; Staicu et al. (2022) Environ Pollut 306:119451; Sura-de Jong et al. (2015) Front Plant Sci 6:113.

Accumulation of some heavy metals of the essential nutrients group in agricultural plants

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Biological selectivity of chemical elements allows plants to control, within certain limits, their chemical composition, specifically microelements, including heavy metals (HM). Concentrations of these microelements in plants often have a positive correlation with their content in soils and even in underlying rocks. Herein, the content of microelements and ultra-microelements was studied in vegetable crops: tubers (potatoes), root vegetables (carrots, beets), onions (onions). Vegetable samples were prepared as follows: dried in an oven at 105 °C until constant weight; 1 g of dried sample was digested with 8 ml HNO_3 65% and 3 ml H_2O_2 20% in a closed microwave digestion system (Marinussen and Van der Zee, 1997); the following elements were determined: Cd, Ni, Cr and Co - by graphite furnace atomic absorption spectrometry (GF-AAS), LOQ = 0.04 mg/kg; Fe (LOQ = 1.00 mg/kg), Cu (LOQ = 0.80 mg/kg), Zn (LOQ = 0.40 mg/kg) and Mn (LOQ = 0.40 mg/kg) – by inductively coupled plasma optical emission spectrometry (ICP-OES). The found Fe content was from 10.4 (potatoes) to 66.7 mg/kg (beets), Zn from 10.9 (carrots) to 26.5 mg/kg (carrots); Cu from 3.5 (carrots) to 9.7 mg/kg (potatoes); Mn from 2.37 (carrots) to 46.8 mg/kg (beets); Co 0.026 (onion) to 0.23 mg/kg (carrots). These amounts were found as optimal for plants but exceed the maximum residue levels (MRLs) established for humans. Based on the obtained results it can be assumed that the content of HMs in quantities exceeding the MRL is a consequence of their high content in soils of agricultural land.

Modelling the spatio-temporal migration of some heavy metals in the water-soil-plant chain

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The toxicity of heavy metals (HMs) is expressed in their ability to accumulate and then actively react with organic matter during metabolism, forming new compounds that are dangerous to the organism. In this context, the problems of environmental pollution assessment require the application of methods of mathematical modelling of pollutant distribution, aimed at predicting the development of the environmental situation. In the future, the results of HMs migration modelling can be used to predict areas with expected increased pollution due to emissions of various hazardous pollutants. However, research is complicated by the fact that metal ions rarely occur in isolation in the natural environment. Their various combinations lead to changes in the properties of individual elements as a result of their effects on living organisms. The kinetic characteristics of the growth of maize B73 (as a control sample) and an adapted local variety were studied under field conditions. According to the results obtained, the background (natural) adaptation of the plant is quite consistent with the results of drought modelling. Furthermore, the concentrations of changes of a number of HMs in soil and plant samples before and after irrigation were studied, taking into account the climatic conditions and the dynamics of accumulation of these HMs during the vegetative period of maize growth at the time of the experimental field. The prevalence of Zn, Cu and Mn in the water-soil-plant system was studied during the plant maturation period. The sampling points for water, soil and plant samples were located at fixed distances from each other in accordance with the objective of the HMs migration study. The results were used to develop a possible scenario of spatial and temporal concentration changes of HMs. The following comparative series were identified as a result of the identification of migration peculiarities of HMs series in the water-soil-plant system under field conditions: Zn < Cu < Mn - for water samples; Zn < Cu < Mn - for soil samples; Zn > Cu > Mn - for maize grain samples.

The effect of agricultural practices on of apple orchard soils' total and available copper and zinc content and geochemical fractions of these elements

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Apple orchard soils are enriched in terms of copper (Cu) and zinc (Zn) elements due to anthropogenic activities such as applying fungicides and fertilization. Result of the successive and continuous application of these fungicides for fighting against apple scab disease, washing off fungucides from the surface of the leaves by rainfall, senescing of fungicide-applied leaves and accumulation of pruned shoots on the soil surface and use of phosphorus fertilizers produced by phosphate rock containing high amounts of Cu and Zn, these elements accumulate in the soil. In order to determine Cu and Zn pollution in apple orchards, soil samples were taken from depth of 0-20 cm of apple orchard soils which are of different ages as 0-5, 5-10, 10-15, 15-20 and >20 years and from pasture and non-cultivated field as a control samples. In order to evaluate the behavior, mobility, bioavailability and phytotoxicity of Cu and Zn elements in the soil, total and available Cu and Zn and 7 different Cu and Zn geochemical fractions (water soluble and exchangeable, bound to carbonates, bound to Mn oxides, bound to amorphous Fe/Al oxides), bound to crystalline Fe oxides, bound to organic and sulfides, residual fractions) were determined. As the orchard age increased, there was an increase in the total Cu, available Cu and Zn content, and the proportional distribution of the some fractions of Cu and Zn. Especially in apple orchards which are older, higher values were found in terms of total and available Cu amount compared to the control samples.

Distribution of selected obligate serpentine Balkan endemics in Bosnia and Herzegovina

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The largest serpentine areas in Europe are located on the Balkan Peninsula. Serpentine habitats of the Balkans are well known as centres of floristic differentiation, speciation, and refugia. The flora of Bosnia and Herzegovina is characterised by significant diversity. Obligate serpentine Balkan endemics form a special and noteworthy group. The aim of this work is to show the distribution of selected obligate serpentine Balkan endemics in Bosnia and Herzegovina: *Euphorbia glabriflora* Vis. (Euphorbiaceae), *Euphorbia gregersenii* K. Malý ex Beck (Euphorbiaceae), *Fumana bonapartei* Maire & Petitm. (Cistaceae) and *Potentilla visianii* Pančić (Rosaceae). The listed species are legally protected in the Republic of Srpska, and are also on the Red List of the Flora of the Federation of Bosnia and Herzegovina. This work contributes to a better knowledge of the distribution of selected obligate serpentine Balkan endemics, and the monitoring of the state of their populations and the preservation of the habitats they inhabit is particularly noteworthy.

Key words: *Euphorbia glabriflora*, *Euphorbia gregersenii*, *Fumana bonapartei*, *Potentilla visianii*, flora.

The influence of the soil properties on the restoration of the pure oak forest in the urban environment

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The main objective of this research is to determine the influence of soil properties on the regeneration of oak forests in urban areas. Three sites AT1, AT2, and AT3 were selected at Avala Mountain (Mt.) (Serbia) for sampling soil, herbaceous vegetation, and sessile oak leaves. Two sites AT1 and AT2 are located at the top of Avala Mt., and in 2016 were exposed to strong ice breaks. The third site AT3, is at a lower altitude and consists of a good quality sessile oak forest. At the site, AT2 soil moisture has the lowest values. The specific mass density for all tested soils is in the range of 24.2 - 25.6 kN/m3. Observing values of shear resistance, surface samples have higher values. Clay and silt particles are moving from the surface to deeper layers. The concentration of macroelements in soils are in accordance with the geological composition of the bedrock. Concentrations of almost all analyzed microelements for sites AT1 and AT2 are higher than in site AT3. Concentrations of Cr, Zn, As, Zr, Cd, W, Pb, and Bi are statistically significantly higher in herbaceous vegetation at sites AT1 and AT2 compared to the concentrations in sessile oak leaves. Concentrations of Sr and Ba in all three studied localities are higher in herbaceous vegetation with extremely high values of BCF. Statistically significant higher concentrations of Sb, Te, I, and Cs were measured in sessile oak leaves compared to the concentrations in herbaceous vegetation. No increase in leachate is expected due to climate change. Based on the results the proposed method of restoration of the pure oak forest in urban areas would include the installation of dry stone walls.

Regulation of intracellular free zinc concentration in *Arabidopsis thaliana* roots

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Zinc (Zn) is an essential micronutrient for all living organisms. It acts as a structural or catalytic cofactor in proteins, with Zn-binding proteins representing nearly 10% of the proteome in eukaryotes. The risk of Zn malnutrition affects one third of the global human population and plants represent the main entry point for Zn into the food chain (Assunção et al., 2022/ 10.1093/jxb/erac014; Stanton et al., 2022/ 10.1016/j.molp.2021.12.008). Enhancing Zn accumulation and Zn-use-efficiency in crops is required to improve plant nutritional value and production in nutrient-deficient soils. Recent findings indicate that Zn uptake in plant root is regulated by F-bZIP transcription factors that directly perceive intracellular free Zn concentrations (Lilay et al., 2021/ 10.1038/s41477-021-00856-7). Here, we use eCALWY, a genetically encoded fluorescent Zn sensor, expressed in the cytosol of *Arabidopsis* cells to investigate the regulation of intracellular Zn concentration in roots (Vinkenborg et al., 2009/ 10.1038/nmeth.1368, Lanquar et al. 2014/ 10.1111/nph.12652). We find that cytosolic free zinc concentration lies in the 100 pM range and increases transiently in response to an elevation of Zn concentration in the medium. We analyze the regulation of cytosolic Zn concentration in mutant backgrounds lacking MTP1 or MTP3, the transporters involved in Zn storage inside the vacuole, or HMA4, the plasma membrane Zn efflux pump. This research will highlight the mechanisms regulating intracellular Zn concentration and in turn the activation of Zn deficiency responses or toxicity due to Zn excess.

Light stress triggers accumulation of manganese and zinc in leaf tips and outer tipburn in lettuce

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Tipburn (TB) is an injury occurring on tips of young inner leaves (inner TB) and on edges/tips of outer leaves (outer TB/outer marginal necrosis) of lettuce and leafy vegetables. In this study, the aim was to investigate changes in accumulation of inorganic ions in different leaf types and segments of lettuce (*Lactuca sativa* 'Frillice') in response to increased irradiance (150→300 μmol m-2s-1) for shorter (8 days) and longer (25 days) time with the main focus on outer TB. Short term high irradiance (HI) did not change the nutrient distribution between young and mature leaves. Long-term HI induced an extreme heterogeneous distribution of Ca and Mn, causing high accumulation in leaf edges of mature leaves, but Ca and Mn deficiency in inner young unexpanded leaves. Anatomical studies with scanning electron microscopy (SEM) revealed a high density of stomata on tips of outer mature leaves but very few stomata on tips of inner leaves. Laboratory based micro-X-ray fluorescence (μ-XRF) elemental mapping was used to image the composition and spatial distribution of metal ions in healthy and injured tips of outer leaves. High accumulation of Mn, Zn, and Ca along with depletion of K and Cl was observed in injured parts of the tips. Very early (small necrotic spots), corresponding to the area with high stomatal density, showed high accumulation of Mn and Zn. The striking changes in accumulation and distribution of Mn and Zn between healthy and injured parts suggest a role for local metal stress in light stress induced outer TB.

Sound of photosynthesis

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Effect of silicon on growth of crops exposed to antimony

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Both antimony (Sb) and silicon (Si) belong to the group of metalloids. Antimony is considered as soil pollutant which enters the environment especially due to mining activities and related ore processing, traffic and various kinds of industrial activities. It is dangerous element for plants and causes various disorders in their growth and development. On the other hand, silicon is the 2nd most abundant element in the Earth crust and is considered as beneficial element in plant nutrition. Si is taken up in a large quantities by certain groups of plants, among them also by Graminae. Moreover, Si was found to ameliorate various forms of abiotic and biotic stresses, and Si-based mechanisms allowing plants to cope with stress have been developed at multiple levels. In our experiments have found that Si is affecting the growth of young maize plants exposed to Sb by modifying the uptake of Sb, affecting the activity of various enzymatic and non-enzymatic antioxidants, and by affecting the stability of cell membranes. Additionally, another experiments on sorghum revealed that Sb and Si probably share the same uptake pathways.

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Assisted *Pteris vittata* growth and As phytoextraction by enrichment of rhizosphere bacterial strains isolated from a naturally As-rich soil

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This greenhouse study evaluated the effects of *Pteris vittata* rhizosphere bacteria on the growth and accumulation of arsenic (As) in *Pteris vittata* grown on a naturally As-rich soil. Inoculations were performed with a consortium of six bacteria resistant to > 100 mM arsenate. Selected bacteria from the consortium were also utilized individually: PVr_9 homologous to *Agrobacterium radiobacter* that produces indole acetic acid and siderophores, and shows ACC deaminase activity, PVr_15 homologus to *Acinetobacter schindleri* that contains the arsenate reductase gene and PVr_5 homologous to *Paenarthrobacter ureafaciens* that possesses all traits from both PVr_9 and PVr_15. Frond and root biomass significantly increased in ferns inoculated with the consortium or only with PVr_9, (by ~61% and ~65% and ~124% and ~172% respectively), while only an increased root length was found in those inoculated with either PVr_5 or PVr_15. Arsenic content significantly decreased in ferns inoculated with PVr_9 by 50%, while increased in those inoculated with PVr_5 and PVr_15 (77.6% and 83.7%, respectively). These results indicate that PVr_9 promotes plant growth and decreases As phytoextraction efficiency, while PVr_5 and PVr_15 promote root elongation and increase As phytoextraction efficiency. Inoculations with these bacteria could be used to increase As tolerance and reduce its accumulation in crops or in remediating contaminated soils.

Accumulation of rare earth elements by macroalgae collected in post-mining lakes

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Post-mining lakes of northern Czechia represent an exceptional aquatic ecosystems. They are yearlong habitats of underwater macroalgal meadows. To evaluate their bioremediation potential, two genera of macroalgae *Vaucheria* sp. (Xanthophyceae) and *Chara* spp. (Charophyceae) were collected by scuba diving technique from these meadows. Sampling was conducted in the post-mining lakes Most, Medard and Milada in different seasons to follow concurrently seasonality of the species. The individual algae samples were incubated under controlled laboratory conditions in photobioreactors in the lake water of their origin, at natural temperature (ranging 4 °C – 23 °C), and natural light intensity (ranging 5 - 180 µmol $m^{-2}s^{-1}$) for 96 h. To determine their capacity for metal accumulation a mixture of rare earth elements (REEs) was added to the cultures in final concentration 40 µM ($LaCl_3$, $CeCl_3$, $NdCl_3$, $GdCl_3$). The algae biomass was harvested after 4 days of treatment, washed thoroughly with distilled water, frozen at -60 °C, and freeze-dried. Quantitative analysis of REEs in the algae biomass was done by inductively coupled plasma-mass spectrometry (ICP-MS). Both macroalgae accumulated REEs from aquatic environment, Vaucheria sp. being a more efficient accumulator than *Chara* spp. Variability in the REE content in algae biomass was found, depending on location and season.

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Mercury ligand environment in the food chain as affected by Se biofortification of plants

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Although selenium (Se) is not essential for plants, it may counteract various abiotic stresses e.g. mercury (Hg) toxicity, when applied in trace amounts. This work aimed to study physiological responses and Hg speciation in plant/ fungi-animal food chain. Lettuce (*Lactuca sativa*), porcini (*Boletus edulis*) and Spanish slug (*Arion vulgaris*) were taken as model organisms. The plants, fed to the slugs, were grown in $HgCl_2$ contaminated soil or soil from the vicinity of Hg mine in Idrija with traces of HgS and methyl Hg, and foliarly sprayed with Se. Hg L3-edge (12284 eV) XANES and EXAFS spectra of plant, fungal and animal material were measured at the BM30B beamline of the ESRF, Grenoble. The results showed that the addition of Se alleviated Hg toxic effects in the food chain fom $HgCl_2$-contaminated environment, while for Idrija environment, containing low amounts of highly toxic methyl-Hg, the beneficial effect was less prominent. No Hg-Se complexes were detected in plants, while the complexation was confirmed in mushrooms and slugs. The addition of Se to the plants, however, changed Hg ligand environment in plant tissues from sulphur to nitrogen ligands. Hg and Se both target the -SH functional groups in the plant tissues, so the toxic effects of Hg were rather enhanced than alleviated by the addition of Se. Nevertheless, the addition of Se to the plants decreased Hg toxicity for the primary consumers, the slugs.

Isotope source tracing and mapping to investigate the fate of foliar applied zinc under drought stress in wheat

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Droughts can reduce the soil zinc (Zn) bioavailability and its translocation from the root to the shoot. Hence, droughts complicate agronomic Zn biofortification approaches that seek to increase grain Zn concentration with the addition of Zn fertilizer. In this regard, foliar Zn application can by-pass these adverse soil conditions and markedly increase the cereal grains with Zn to improve its nutritional value. Previous studies have shown that foliar Zn application is a promising approach to improve the grain Zn content in wheat under drought stress. However, the effects of droughts on foliar Zn uptake and translocation have been rarely investigated. Improving this mechanistic understanding will potentially help to improve foliar Zn application strategies and thereby improve crop quality under drought stress.

The objective of this study is to trace the foliar Zn uptake and translocation in wheat (*Triticum aestivum*) under drought stress. In the ongoing experiment, two wheat cultivars that differ in drought tolerance will be grown in the greenhouse. The enriched stable isotope 66Zn will be applied to the flag leaves at flowering. By measuring 66Zn: 64Zn isotope ratios in wheat, we will quantify how much of the foliar applied Zn will be translocated from the leaves to the grains and other plant parts. Furthermore, we will seek to map for the first time the 66Zn: 64Zn isotope ratios using laser ablation ICP-MS to decipher the Zn uptake pathways in wheat leaves on a microscale. At the conference, first results will be presented and discussed in the context of droughts and Zn biofortification.

Cadmium, zinc, and copper leaching rates determined in large monolith lysimeters

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Soil mass balances are used to assess the risk of trace metals that are inadvertently applied with fertilizers into agroecosystems. The accuracy of such balances is limited by leaching rates, as they are difficult to measure. Here, we used monolith lysimeters to precisely determine Cd, Cu, and Zn leaching rates in 2021 and 2022. The large lysimeters (n = 12, 1m diameter, 1.35m depth) included one soil type (cambisol, weakly acidic) and distinct cropping systems with three experimental replicates. Stable isotope tracers were applied to determine the direct transfer of these trace metals from the soil surface into the seepage water. The annual leaching rates ranged from 0.04 to 0.30 for Cd, 2.65 to 11.7 for Cu, and 7.27 to 39.0 g (ha a)$^{-1}$ for Zn. These leaching rates were up to four times higher in the year with several heavy rain periods compared to the dry year. Monthly resolved data revealed that distinct climatic conditions in combination with crop development can have a strong impact on trace metal leaching rates. In contrast, fertilization strategy (e.g., conventional vs. organic) had a minor effect on leaching rates. Trace metal leaching rates were up to 10 times smaller than fertilizer inputs and had therefore a minor impact on soil mass balances. This was further confirmed with isotope source tracing that showed that only small fractions of Cd, Cu, and Zn were directly transferred from the soil surface to the leached seepage water within two years (< 0.07%). A comparison with models that predict Cd leaching rates in the EU suggests that the models overestimate the Cd soil output with seepage water. Hence, monolith lysimeters can help to refine leaching models and thereby also soil mass balances that are used to assess the risk of trace metals inputs with fertilizers.

Defense strategies of halotolerant sea aster conditioning its tolerance to cadmium, copper and zinc

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In numerous halophytes the mechanisms determining tolerance to excessive salinity are crucial for maintaining undisturbed growth in the presence of toxic concentrations of metals. The sea aster (*Aster tripolium* L., syn. *Tripolium pannonicum* (Jacq) Dobrocz.; *Tripolium pannonicum* subsp. *tripolium* (L.) Greuter), a short-lived perennial halophyte growing in the coastal salt marsh areas, has lately gained interest due to its high degree of adaptation to saline habitats. Its anatomical and physiological features make the species prospective for utilization in land desalination and phytoremediation.

In this study the responses of sea aster to suboptimal doses of Cd^{2+}, Cu^{2+} and Zn^{2+} were evaluated at the level of growth performance and a range of physiological parameters related to defense strategies: scavenging of reactive oxygen species, metal detoxification machinery (metal-binding compounds), and DNA protection. In the case of Cd-treated plants also carbohydrate profiling and anatomical examination of developed organs was conducted.

Aster tripolium had an efficient system protecting DNA from damage, since none of the tested metals induced DNA fragmentation. Additionally, no significant level of oxidased base excision was detected. Also the level of lipid peroxidation was stable, as revealed by lipoxygenase activity and MDA accumulation. Synthesis of thiol compounds was stimulated by metal treatments, however there was no increase in the pool of free glutathione. In turn, in Cu-treated plants higher level of protein glutathionylation occurred.

Involvement of trace metal nutrition in plant physiology process and abiotic stress responses

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Trace metals have a crucial role in different plant physiological processes. Low concentrations of these elements are needed for normal plant growth and development. It also plays a role in plant responses to biotic and abiotic stresses. Our research focuses on the effect of metal nutrition on reproductive physiology (flower and fruit organ development and function) and fruit quality, mainly understanding the role in mitigating abiotic stress adverse effects. For example, zinc imbalances affect maize male inflorescence development, manganese enrichment effects on pepper heat damage, and the possible role of calcium in these physiological disorders. Also, we are interested in understanding sulfur deficiencies in iron-sulfur complexes' activity in nitrogen fixation processes in legume crops (peanuts). The sulfur deficiencies might be relevant due to future sulfur deficiencies in agricultural areas worldwide.

Production of porous carbon from pistachio shell and its Cu^{2+} adsorption performance

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Thanks to its benefits, including high specific capacitance value, environmental friendliness, low cost, and easily available natural materials, biomass-derived carbons have been thoroughly studied for wastewater treatment applications. In this study, Pistachio Shell derived biochar, was created from Pistachio Shell by wet impregnation with $ZnCl_2$ and subsequent heating to 500, 700, and 900 °C in a CO_2 atmosphere. Investigated how the newly acquired biochar affected the capacity to extract the Cu^{2+} heavy metals from the aqueous solution. Through the use of FT-IR, XRD, SEM, BJH, BET, DSC, EDX, and TGA studies, the prepared Pistachio Shell derived biochar were identified. The ideal pH for the adsorption of Cu^{2+} heavy metals was found to be 2. Using an initial concentration of 100 ppm of Cu^{2+} heavy metals and a dose of 1.5 g L-1 PS-B-700, the maximum percentage clearance of Cu^{2+} ions was 99.10%. The highest adsorption capacity (Qm) of the PS-B-700 was 78.46 mg g^{-1}. Langmuir, Freundlich, and Tempkin isotherm models were used to examine the data. The experimental data of PS-B-700 were best suited by the Freundlich isotherm model. Pseudo-first-order (PFO), Pseudo-second-order (PSO), Intraparticle diffusion, Film diffusion and Elovich models were used to analyze kinetic data. The PSO rate model had a strong correlation (R2 > 0.985), and it was principally responsible for controlling the adsorption rate. The outcomes show that PS-B-700 may be used repeatedly without noticeably diminishing adsorption effectiveness and that it is efficient at eliminating the Cu^{2+} ions from water.

Keywords: Biochar, $ZnCl_2$ Activation, Pistachio Shell, Cu^{2+} ions, Adsorption

Phytoremediation of multielement -contaminated dredged sediment with *Brassica napus* – field trial experiance

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This work was conducted within the H2020 Phy2Climate project which aims to develop phytoremediation solutions for contaminated lands worldwide and couple it with clean biofuel production. Our work's main objective was to assess *Brassica napus*'s potential for phytoextractions of multi-element contaminated dredged sediment in a real environment and authentic natural conditions.

Field trials were performed during 2022. and 2023. at the dredged sediment landfill located near the Serbian-Romanian border. The total landfill area was approximately 2000 m^2. Deposited sediment was highly heterogeneous with heavy metals varying in a wide range of concentrations not only in the surface layer (0-20 cm) but in deeper layers.

Metal concentrations in the surface layer were the following: Cr: 87-150 mg/kg; Cu: 57 – 168 mg/kg; Zn: 154 – 219 mg/kg; Cd: 1,5 – 4,8 mg/kg and Pb: 66 – 163 mg/kg). For the field test, the winter variety of *Brassica napus* was selected and sown in September 2022. and harvested at full maturity at the beginning of July 2023. The estimated seed yield was 380 kg (1,9 t/ha) and approximately 8.000 kg of dry harvest residues was produced (estimation is based on the number of planted seeds per m2, emergence rate, and its average mass (dry) at the moment of harvest). The average metal concentrations in seeds were Cr: 0,97 mg/kg; Cu: 6,78 mg/kg; Zn: 50,25 mg/kg; Cd: 0,19 mg/kg and Pb: 0,30 mg/kg and in aboveground biomass: Cr: 1,1 mg/kg; Cu: 10,12 mg/kg; Zn: 47,56 mg/kg; Cd: 1,61 mg/kg and Pb: 0,71 mg/kg.

Lead and zinc content in the *Achillea millefolium* plant in Mitrovica, Kosovo

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This study observes the content of lead and zinc in the plant *Achillea millefolium*, collected from the area of Mitrovica, Kosovo. Mitrovica is known for high environmental pollution, especially from heavy metals, due to the intensive mining and industrial activity in the region. To assess the impact of these pollutants on plants, analyzes of soil samples and *Achillea millefolium*, a widely used medicinal plant, were carried out. Samples were collected from different locations in Mitrovica, and were analyzed for lead and zinc content by digestation with microwaves and reading in ICP OES. The content of Pb and Zn in the soil has ranged from 69.– 685.4 7 mg kg^{-1} (average 351.7), respectively 77.2 – 2104.3 mg kg^{-1} Zn (1019.2). The results show different levels of accumulation of these metals in different parts of the plant, reflecting the degree of pollution in the respective locations. Higher accumulation of Pb was shown in roots up to 65.65 mg kg^{-1}, respectively 30.71 in stems and 17.34 in leaves. While Zn is accumulated more in leaves up to 178.04, roots 69.1 and stems up to 48.5 mg kg^{-1}. This study sheds light on the potential of *Achillea millefolium* as a bioindicator for monitoring environmental pollution and for potential health risks associated with the use of medicinal plants collected from polluted areas.

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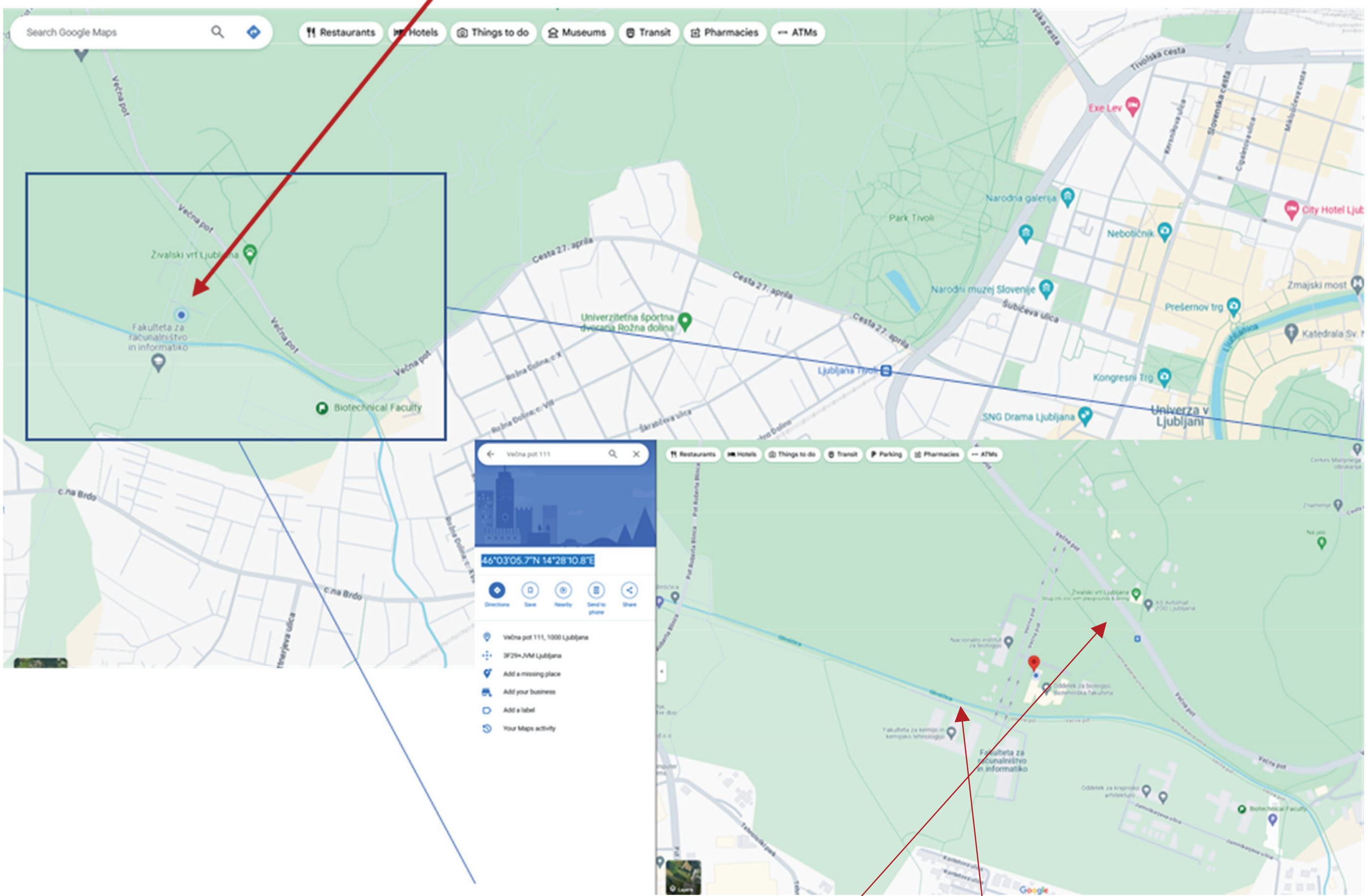
Getting from and to the Ljubljana airport:

Please consult the airport page: https://www.lju-airport.si/en/transport/. Note, that there is no train from the airport to the city centre. Most frequently we use shuttles, also when flying to neighbouring (Zagreb, Trieste, Venice, etc) airports, for example https://www.goopti.com/en/.

Conference venue:

Department of Biology, Biotechnical Faculty, University of Ljubljana

Address: Večna pot 101, Ljubljana (46°03'05.7"N 14°28'10.8"E), a location opposite to the Ljubljana ZOO



Access:

1. Ljubljana Bus no. 18

For timetable and payment methods: https://www.lpp.si/en

2. Rent-a-bike (Bicikelj)

More details: https://www.bicikelj.si/en/home

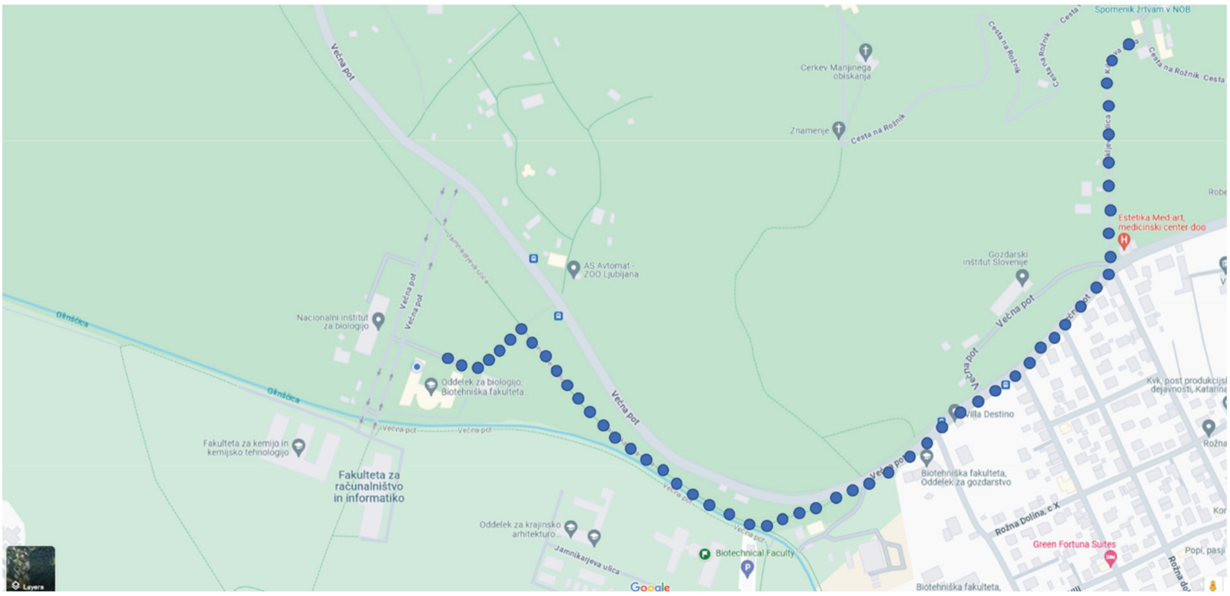
3. Car

There is free parking (gravel parking lot) opposite the ZOO

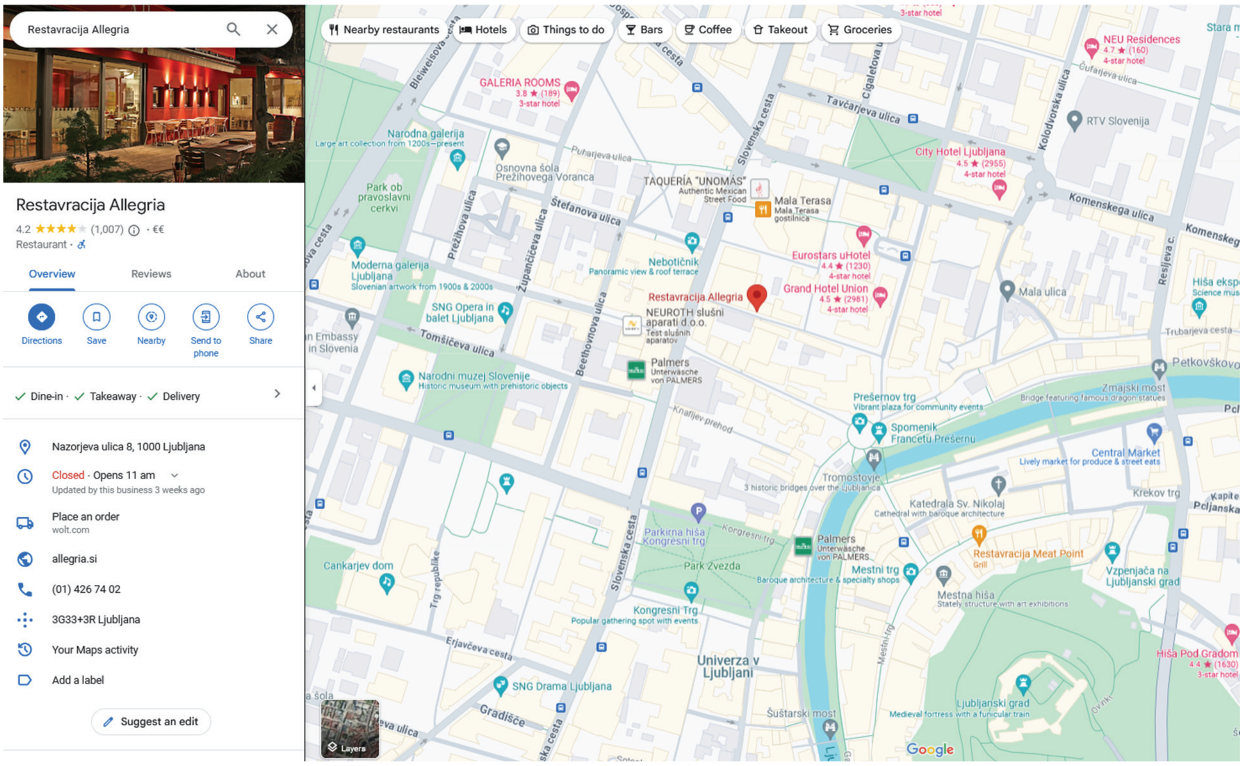
Lunches: 3-minute walk to the cafeteria of the Faculty of Chemistry and Chemical Technology (follow signs)

Coffee breaks: at the venue

Get-together dinner (Tuesday, 17.9.2024, starting at 19.30): Čad Bistro (https://hotel-cad.si/): Cesta na Rožnik 18, 1000 Ljubljana; a 20 min walk from the conference venue as shown on the map below.



Conference dinner (Wednesday, 18.9.2024, starting at 19.00): Allegria restaurant: Nazorjeva ulica 8.

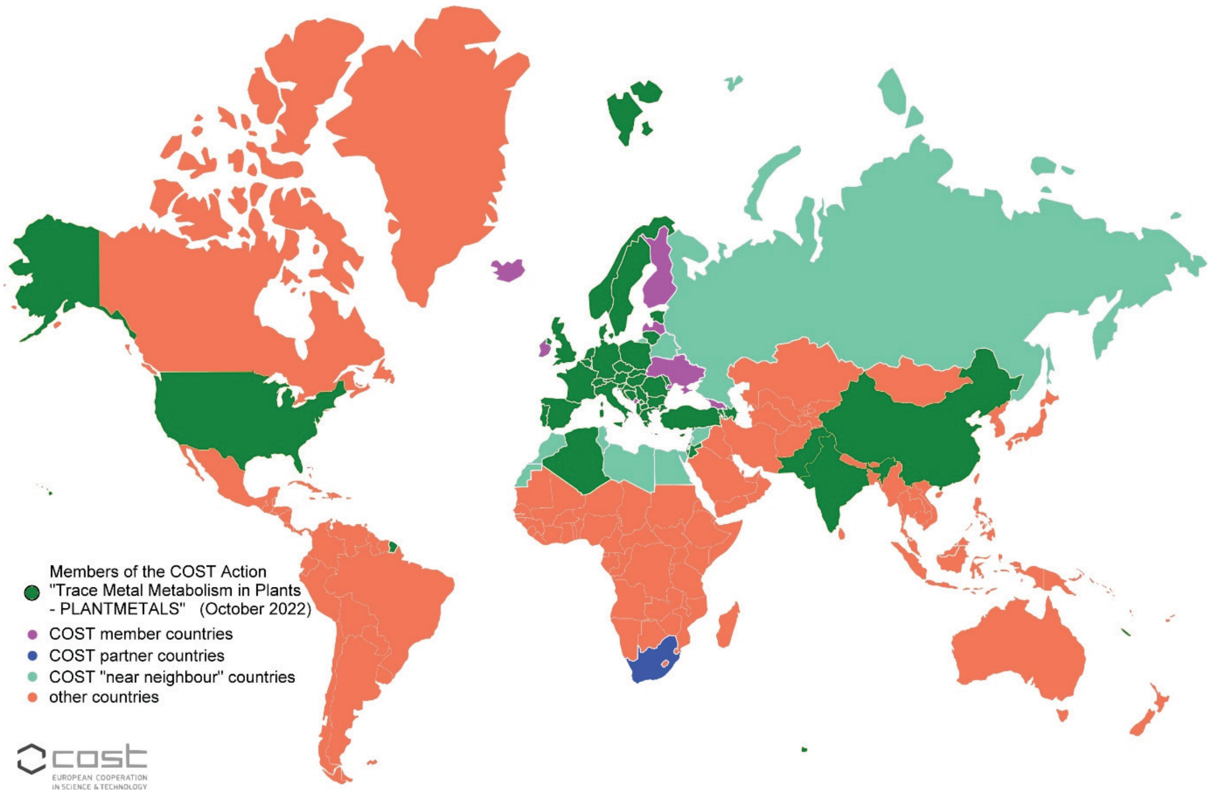


Excursion to Idrija:

Bus will depart from the conference venue on Friday after lunch and should return to Ljubljana around 20.00 and will comprise botanical part at the Wild Lake (https://en.wikipedia.org/wiki/Wild_Lake) and the neighbouring Idrijca river, followed by a visit to the Mercury Mine (https://www.cudhg-idrija.si/en/anthonys-main-road, 1h 30min) or the Mercury Smelting Plant (https://www.cudhg-idrija.si/en/idrija-hg-smelting-plant-project, a 1h 15min).

Organised in liaison with the Slovenia Society of Plant Biology

Slovensko društvo za biologijo rastlin
Slovenian Society of Plant Biology



Metals, Plants and People