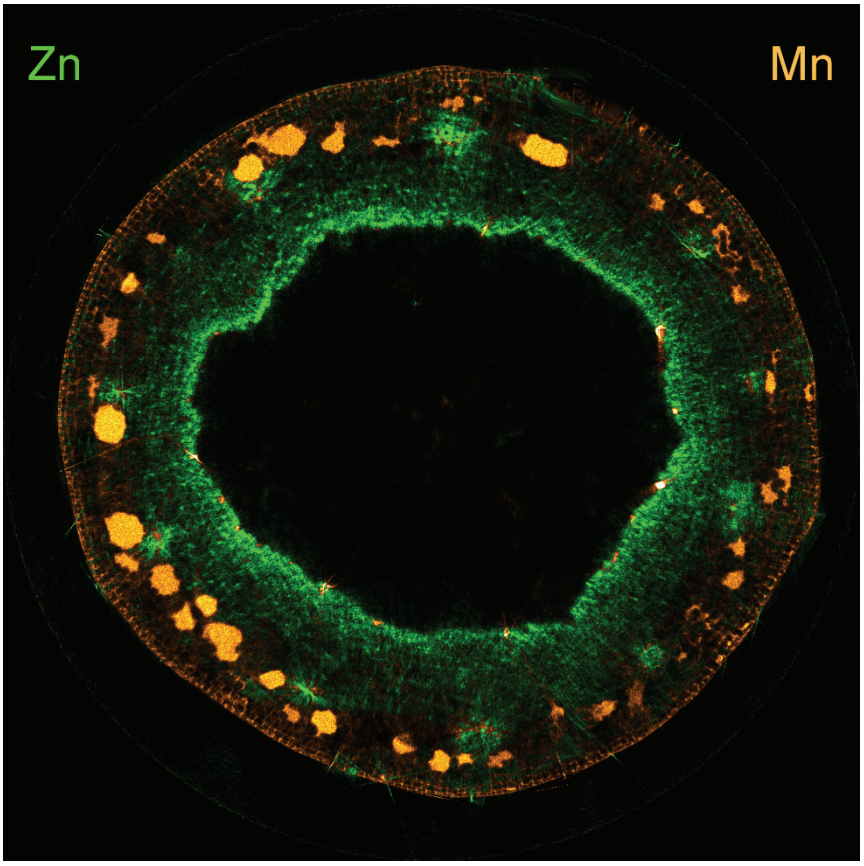

Book of Abstracts

PLANTMETALS

TRACE METAL METABOLISM IN PLANTS

Conference 2025

České Budějovice, Czechia
September 9th - 12th, 2025

Title: Book of Abstracts, PLANTMETALS
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Title page image (courtesy of Hendrik Küpper):

Micro-XRF map of a *Tilia cordata* leaf gall (caused by eriophyte mites) showing the distribution of Mn and Zn. The full data were published in Morina F*, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujić B, Falkenberg G, Küpper H* (2025) How eriophyid mites shape metal metabolism in leaf galls on Tilia cordata. New Phytologist 246, 2222-2242. https://doi.org/10.1111/nph.70103

Programme at a glance

Tuesday, 9th September

15:00 to 16:30: Registration, poster mounting, welcome coffee

16:30 to 19:00: Opening, Oral session "Pathways of TMs from the soil and through the plant"

19:30 to 23:00: Get-together dinner (included in the registration)

Wednesday, 10th September

09:00 to 13:00: Oral Session "Metalloproteins important for TM homeostasis, metalloenzymes" – with coffee break 10:20-11:00

13:00 to 14:30: Lunch break (funded from registration fee)

14:30 to 16:10: Oral Session "Responses of plant TM metabolism to the environment"

16:10 to 18:00: Poster session 1: discussions and networking over drinks and snacks

Thursday, 11th September

09:00 to 13:00: Oral Session "Agronomic aspects of TM homeostasis" – with coffee break 10:20-11:00

13:00 to 14:30: Lunch break (funded from registration fee)

14:30 to 16:00: Poster session 2, including coffee break 15:30 to 16:00

16:00 to 18:00: General Assembly Meeting of the Society

19:00 to 23:00: Conference dinner (self-supporting)

Friday, 12th September

09:00 to 12:00: Oral Session 5 (talks that belong to multiple topics) – with coffee break 10:30-11:00

12:00 to 12:30: Announcement of next conference, closing remarks

12.30 to 13:00: Poster removal, wrap up

13:00 to 14:00: Lunch break (funded from registration fee)

14:00 to 19:00: Excursion (Optional): Budvar brewery, České Budějovice

Detailed Programme

General remarks

- The registration desk will be open on Tuesday 9th of September 15:00 - 16:30 and Wednesday 10th September 8:30-9:00. If you need help for registration-related things later, please feel free to speak directly to members of the local team, marked with a red sign on their name tags

- The conference is open to all participants who have paid the registration fee

- Get-together dinner and lunches will be covered by the registration fee.

- Posters will be available for viewing during the whole conference

- In the BC/USB campus, there is public high-speed (10GBit/s) WiFi accessible via the international "eduroam" system. For those who don't have their own eduroam credentials, we will provide access to the Wifi during the conference days.

Tuesday, 9th September

<table>
<tr><td>15:00-16:30</td><td></td><td colspan="2">Registration, poster mounting, welcome coffee</td></tr>
<tr><td>16:30-16:40</td><td></td><td colspan="2">Opening address by PLANTMETALS Society Chair and the LOC</td></tr>
<tr><td rowspan="5">16:40-19:00</td><td>16:40-17:20</td><td rowspan="5">Oral session "Pathways of TMs from the soil and through the plant"</td><td>Ute Krämer: "Genome-enabled approaches in the study of metal hyperaccumulation and hypertolerance"</td></tr>
<tr><td>17:20-18:00</td><td>Olena Vatamaniuk: "Crosstalk Between Iron and Copper Homeostasis in Arabidopsis thaliana" - REMOTE talk</td></tr>
<tr><td>18:00-18:20</td><td>Yohan Pillon: "Global variation of the angiosperm ionome"</td></tr>
<tr><td>18:20-18:40</td><td>Ana Assuncao: "Expression patterns of Arabidopsis bZIP19 and bZIP23 elucidate their individual contribution to the regulation of the Zn deficiency response"</td></tr>
<tr><td>18:40-19:00</td><td>Marc Hanikenne: "Toward the identification of the genetic mechanisms governing zinc homeostasis in B. distachyon"</td></tr>
<tr><td>19:30-23:00</td><td></td><td colspan="2">Get-together party at the Pavilion ("Klub Kampa") of the South Bohemian University
   </td></tr>
</table>

Wednesday, 10th September

09:00-10:20	9:00-9:40	**Oral Session "Responses of plant TM metabolism to the environment" (I)**	**Jörg Rinklebe: "Trace elements in soils and plants"**
	09:40-10:00		Gabriel Sgarbiero Montanha: "Multi-scale approach shedding light on the ionome-protein interface in soybean seeds"
	10:00-10:20		Magdalena Pypka: "How do plants find and manage their zinc? "
10:20-11:00		**Coffee break**	
11:00-13:00	11:00-11:20	**Oral Session "Responses of plant TM metabolism to the environment" (II)**	Maria De Benedictis: "The endoplasmic reticulum quality control plays a role in cadmium stress response in *Arabidopsis thaliana*"
	11:20-11:40		Atta Mohi Ud Din: "Photosystems and antioxidative system of rye, wheat and triticale under Pb stress"
	11:40-12:00		Giovanni DalCorso: "Metal interactions in the Ni hyperaccumulating population of *Noccaea caerulescens* - Monte Prinzera"
	12:00-12:20		Elisa Fasani: "The different forms of metal transporters MTP1 modulate multi-metal transport in *N. caerulescens* Monte Prinzera"
	12:20-12:40		Hendrik Küpper: "Comparison of mechanisms leading to lethal vs. sublethal metal(loid) toxicity in plants"
	12:40-13:00		5-min poster talks: Anna Čepelová, Michel Francis, Tereza Koberová, Justyna Wronka
13:00-14:30		**Lunch break**	
14:30-16:10	**14:30-15:10**	**Oral Session "Metalloproteins important for TM homeostasis, metallo-enzymes"**	**Annette Rompel: "Functional Changes in the Family of Type 3 Copper Proteins."**
	15:10-15:30		Jonathan Przybyla-Toscano: "Identification of nickel-binding proteins in the nickel hyperaccumulator species *Noccaea caerulescens*"
	15:30-15:50		Ádám Solti: "Is the way of Fe removal from chloroplasts a form of ferritinophagy?"
	15:50-16:10		Marie-Theres Hauser: "Time-resolved responses of increased nickel concentration on root growth, cell wall modification and THESEUS1 dependent phosphorylation"
16:10-18:00		**Poster session 1: discussions and networking over drinks and snacks**	

Thursday, 11th September

09:00-10:20	**9:00-09:40**	**Oral session "Agronomic aspects of TM homeostasis" (I)**	**Marcel Bugter: "Regulatory and scientific challenges for micronutrient fertilizers"**
	09:40-10:00		Weronika Jozwiak: "Zinc uptake, distribution and biotransformation in soybean leaves after foliar fertilization"
	10:00-10:20		Matthias Wiggenhauser: "Exploring the role of thiols as trace metal filter in arable soils"
10:20-11:00		**Coffee break**	
11:00-13:00	11:00-11:20	**Oral session "Agronomic aspects of TM homeostasis" (II)**	Giancarlo Renella: "Potential of organic amendments to restore fertility and functionality of a contaminated soils at a former wood preservation site"
	11:20-11:40		Yingying Wang: "Understanding the relationships between the accumulation of biomass and that of contaminants and nutrients in wheat grains"
	11:40-12:00		Catherine Sirguey: "Co-remediation of multi-contaminated soils under multiple pressures: a long road ahead"
	12:00-12:20		Jean-Yves Cornu: "Copper dynamics in vineyard soils as affected by the supply of aerated compost tea"
	12:20-14:40		Mirko Salinitro: Treasure from trash: mining critical elements from secondary sources using plants
	12:40-13:00		5-min poster talks: Nadine Bouzari, Mihaela Gălean, Maria Kuzmik, Manon Quillerou Moreau
13:00-14:30		**Lunch break**	
14:30-16:00		**Poster session 2, including coffee break 15:30 to 16:00**	
16:00-18:00		**General Assembly Meeting of the Plantmetals Society** ***– only for registered members***	
19:00-23:00		**Conference dinner (self-supporting: 40 € incl. 2 drinks), restaurant & brewery "Solnice"**  	

Friday, 12th September

09:00-10:00	09:00-09:20	**Oral session for talks that belong to multiple topics (I)**	Damien Blaudez: "Plants and rare earth elements: from transfer and accumulation to cellular responses"
	09:20-09:40		Katarzyna Kinska: "Mobility of Te in soil and its bioavailability to selected plants - a model study"
	09:40-10:00		Yongchao Liang: "Mechanisms of silicon-mediated mitigation of heavy metals toxicity in plants"
10:00-11:00		**Coffee break**	
11:00-11:40	11:00-11:20	**Oral session for talks that belong to multiple topics (II)**	Robert Frost: "Assessing the radiological impact of major industrial facilities"
	11:20-11:40		Santiago Prochetto: "HY5 Regulates cadmium tolerance and accumulation in Arabidopsis roots"
11:40-12:00		**Preview to the next Plantmetals activities planned by the society members**	
12:00-12:30		**Closing of the conference**	
12:30-13:00		**Poster removal, wrap-up**	
13:00-14:00		**Lunch break**	
14:00-19:00	**Excursion (optional)** **Plan: Budvar Brewery followed by a guided tour through the historic town centre of České Budějovice.**		

Content

Disturbed electron transport beyond PSI changes metabolome and transcriptome in Zn-deficient soybean

Elisa Andresen[1], Filis Morina[1], Syed Nadeem Hussain Bokhari[1], Peter Koník[2], Hendrik Küpper[1,2]

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[2]University of South Bohemia, Faculty of Sciences, Branišovská 1645/31a, 11 370 05 České Budějovice, Czech Republic

Low Zn availability in soils is a problem in many parts of the world, with tremendous consequences for food and feed production because Zn deficiency affects the yield and quality of plants. In this study we investigated the consequences of Zn-limitation in hydroponically cultivated soybean (*Glycine max* L.) plants. Parameters of photosynthesis biophysics were determined by spatially and spectrally resolved Kautsky and OJIP fluorescence kinetics and oxygen production at two time points (V4 stage, after five weeks, and pod development stage, R5-R6, after 8-10 weeks). Lower NPQ at 730 nm and lower quantum yield of electron transport flux until PSI acceptors were observed, indicating an inhibition of the PSI acceptor side. Metalloproteomics showed that down-regulation of Cu/Zn-superoxide dismutase (CuZnSOD) and Zn-carbonic anhydrase (CA) were primary consequences of Zn-limitation. This explained the effects on photosynthesis in terms of decreased use of excitons, which consequently led to oxidative stress. Indeed, untargeted metabolomics revealed an accumulation of lipid oxidation products in the Zn-deficient leaves. Further response to Zn deficiency included up-regulation of gene expression of cell wall metabolism, response to (a)biotic stressors and antioxidant activity, which correlated with accumulation of antioxidants, Vit B6, (iso)flavonoids and phytoalexins.

Expression patterns of Arabidopsis bZIP19 and bZIP23 elucidate their individual contribution to the regulation of the Zn deficiency response

Ana Campilho[1], Grmay Hailu Lilay[2], Margarida Benvindo[1], Pedro Humberto Castro[1], Ana G. L. Assunção[1]

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Micronutrients are required by plants, at adequate concentrations, for optimal growth. In order to avoid deficiency or excess, plants rely on a tightly regulated micronutrient homeostasis network. The prevalence of micronutrient deficiencies in cultivated soils and plants are a global problem that negatively affect crop production and plant nutritional value, as well as human health and well-being (Assuncao et al., 2022).

In *Arabidopsis thaliana*, the transcription factors bZIP19 and bZIP23 regulate the homeostasis of the micronutrient Zn, acting as the key regulators of the Zn deficiency response and also as sensors of cellular Zn status (Lilay et al., 2021). Although there is a degree of functional redundancy between bZIP19 and bZIP23, their individual contributions in this regulatory process are not yet understood.

To gain insight on the individual contributions of bZIP19 and bZIP23, here we analysed the phenotypes of bzip19, bzip23 and bzip19bzip23 single and double mutants in response to Zn deficiency, and histochemical GUS staining in pbZIP19::GUS and pbZIP23::GUS lines.

Analysis of mutant phenotypes indicated unequal redundancy between bZIP19 and bZIP23. GUS expression in pbZIP19::GUS was observed throughout the plant, in differentiated cells and tissues, including those involved in transport pathways, whereas in pbZIP23::GUS was observed mostly in meristematic tissue. These results indicate that bZIP19 and bZIP23 have different expression patterns across plant tissues, which may explain the unequal redundancy between bZIP19 and bZIP23, and also their evolutionary retention as paralogs.

This knowledge has implications for the mechanistic understanding of the Zn sensing and deficiency response in Arabidopsis, and for improving Zn nutritional value and use-efficiency in crops.

Site-specific differences in the patterns of selected NtZIP genes promoter activity in tobacco roots under Zn- deficiency and sufficiency conditions

Anna Barabasz, Ewa Borzęcka, Justyna Walicka

University of Warsaw, Faculty of Biology

ZIP proteins are transmembrane zinc transporters. Some of them can also transport copper, iron or manganese. For most ZIPs (for which subcellular localization was studied) plasmalemma localization was demonstrated. The vast majority of ZIP genes are upregulated in the root under Zn-deficiency conditions. However, single ZIP gene mutants do not show a strong phenotype, indicating that the ZIP genes can function redundantly in maintaining Zn homeostasis.

Our studies have shown that site-specific ZIP expression in roots depends on: (a) concentration of individual microelements in the medium, (b) developmental stage of individual root parts. Transgenic plants harbouring promZIP::GUS construct were used to better understand the role of individual ZIP genes in the response to a 6-day Zn-deficiency / Zn-sufficiency condition. Seedlings of selected homozygous transgenic lines (T2 generation: promNtZIP1-like::GUS, promNtZIP4B::GUS, promNtZIP5B::GUS; T1 generation: promZIP8-GUS) after germination on solid medium were transferred to a liquid medium supplemented with Zn to 1 uM concentration. 17 days later, two plants for each line were subjected to the GUS reaction. Half of the remaining plants were transferred to a Zn-deficiency condition. The growth of the other half was continued in the presence of Zn. After 6-days, the GUS reactions were carried out on plants of all tested lines.

The obtained results will be presented during the conference and compared with the expression level (qPCR method) of the studied genes in the apical, middle and basal part of the main root.

Funding: project no. 2019/35/B/NZ9/04338 financially supported by the National Science Centre, Poland.

Beyond the vacuole: extracellular release of thiol-peptides in Populus alba under cadmium stress

Erika Bellini[1]*; Valentina Vitelli[2]*; Alessandro Saba[2,3], Andrea Andreucci[1], Luigi Sanità di Toppi[1]
*These authors equally contributed

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Cadmium (Cd) is a highly toxic heavy metal, whose widespread presence in the environment poses a serious threat to plant health. Plants counteract Cd toxicity through detoxification strategies involving thiol-containing ligands such as glutathione (GSH) and phytochelatins (PCn), which chelate Cd and mediate its vacuolar sequestration. Recent findings in the liverwort Marchantia polymorpha L. also revealed the extracellular release of thiol-peptides, suggesting a novel detoxification mechanism beyond intracellular ones.

In this study, we investigated whether a similar strategy is employed by *Populus alba* L. (Villafranca clone), a Cd-tolerant woody species with phytoremediation potential. Using a validated HPLC-ESI-MS-MS method, we quantified thiol-peptides in plants exposed to Cd. PCn levels, especially PC2, increased significantly under Cd stress, primarily in roots. GSH levels also showed an initial rise, but then decreased, likely due to oxidation or conversion into PCn. Notably, both GSH and PCn were also detected in the culture medium, as extracellular thiol-peptides. Inhibition experiments using sodium orthovanadate, a plasma membrane ATPase inhibitor, strongly suppressed GSH efflux and partially reduced PCn release, possibly suggesting distinct transport routes. These findings confirm that *P. alba* performs a dual detoxification strategy: intracellular chelation and vacuolar sequestration, together with extracellular export of thiol-peptides.

Taken together, our results suggest that the extracellular release of thiol peptides under Cd stress, previously demonstrated in *M. polymorpha*, is evolutionarily conserved in vascular plants. This highlights a possibly ancient and widespread mechanism in land plants to mitigate metal toxicity through both intracellular and extracellular thiol-peptide-based detoxification.

Plants and rare earth elements: from transfer and accumulation to cellular responses

Damien Blaudez[1], Nicolas Grosjean[1], Emmanuelle Montargès-Pelletier[1], Michel Chalot[2], Antony van der Ent[3], Marie Le Jean1[3]

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[2] *Université Marie et Louis Pasteur, CNRS, Chrono-Environnement, Montbéliard, France*
[3] *Wageningen University and Research, Laboratory of Genetics, Wageningen, The Netherlands*

Rare earth elements (REEs) gather 15 lanthanides plus yttrium and scandium. REEs can be divided into two subgroups: the light REEs (LREEs) versus heavy REEs (HREEs). REEs are increasingly crucial for modern technologies but their increasing demand raises concerns about their environmental impact.

The first objective was to discover new REE-accumulating plant species. We performed a screening on a large collection of fern species. New genera and species that preferentially accumulated LREEs were identified. A similar screening with Phytolacca species showed that the REE-hyperaccumulation trait was conserved within this genus, with a preferential transfer of HREEs to the shoots. We also performed an X-ray fluorescence survey at the Herbarium of the Paris Muséum on 2,000 collection specimens. New REE-accumulators within the Phytolaccaceae and Juglandaceae families and in ferns were identified. Consequently, the panel of plants harbouring high REE concentrations in their leaves is now extended.

However, little is known regarding (i) the accumulation sites of REEs in these plants, and (ii) the cellular and molecular effects of REEs in plants. The second objective was therefore to reveal REE accumulation sites in *Dryopteris erythrosora* by Synchrotron analyses. Unexpectedly, REEs accumulated mostly within the xylem, suggesting a poor discharge of the elements. Finally, we analysed the response of *Arabidopsis thaliana* exposed to REEs. At the transcriptome level, we observed the contribution of ROS and auxin redistribution to the modified root architecture following REE exposure. Fe homeostasis was perturbated by REEs in both roots and leaves, suggesting competition between REEs and Fe.

Comprehensive ultra-trace metal analysis and metalloproteomics

Syed Nadeem Hussain Bokhari[1], Hendrik Küpper[1,2]

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[2]*University of South Bohemia, Faculty of Sciences, Branišovská 1645/31a, 11 370 05 České Budějovice, Czech Republic*

Metalloproteins are vital for numerous biological functions across all life forms. Therefore, both metal deficiency leading to missing metal centres in proteins, and inhibitory binding of metals to proteins at toxic concentrations, cause severe disturbance of cellular functions. This presentation highlights our lab's advancements in ultra-trace metal speciation with a focus on metalloproteomics through the online coupling of ICP-MS (element-XR) with HPLC. We optimized key parameters including the sample introduction system in ICP-MS, chromatographic conditions (column selection, eluent composition, gradient design), and sample preparation and handling. Techniques such as anion exchange chromatography (AEC), chromatofocusing (CF), hydrophobic interaction chromatography (HIC), and size exclusion chromatography (SEC) were refined for high-resolution separation based on protein size, charge, and hydrophobicity. These methods offer precise analysis of metal-protein interactions at subnanomolar concentrations in complex biological samples. Recent applications include studies in soybean, *Sedum alfredii, Brassica napus*, *Capsicum annuum*, *Noccaea caerulescens*, *Medicago truncatula*, *Trichodesmium erythraeum,* and *Desmodesmus quadricauda* under metal stress.

Acknowledgements: This research has been supported by the Ministry of Education of the Czech Republic with co-financing from the European Union (grant KOROLID, CZ.02.1.01/0.0/0.0/15_003/0000336), the Czech Science Foundation (grant 17-14024S) the Deutsche Forschungsgemeinschaft (grants KU 1495/8-1 and KU 1495/8-2) and the Czech Academy of Sciences (RVO:60077344) and COST Action (CA 19116 Trace metal metabolism in plants-PLANTMETALS).

Crosstalk between iron homeostasis and immunity in *Arabidopsis thaliana*

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Iron (Fe) is an essential micronutrient for plant development, yet its bioavailability is often limited in soils. On the other hand, excess Fe is toxic. To cope with this, plants have evolved tightly regulated mechanisms to maintain iron homeostasis. Iron availability is often a critical determinant of the outcome of plant-pathogen interactions. Iron withdrawal during infection is a widespread defense response, known as nutritional immunity. In *Arabidopsis thaliana*, the vacuolar transporters AtNRAMP3 and AtNRAMP4 are crucial for remobilizing stored iron during germination. To identify new regulators of iron homeostasis, a genetic screen was performed and led to the isolation of a suppressor mutant that rescues the iron deficiency-sensitive phenotype of the nramp3 nramp4 double mutant. This suppressor, named isv8, carries a dominant mutation in NRG1a, a helper NLR known to play a central role in effector triggered immunity (ETI). Our work aims at understanding the functional link between NRG1 and iron deficiency responses, opening new avenues to explore the crosstalk between biotic stress responses and micronutrient homeostasis.

Regulatory and scientific challanges for micronutrient fertilizers

Marcel Bugter

Nouryon Micronutrients

Micronutrient fertilizer production is bound in Europe to both the Chemical Registration (REACH) as well as the Fertilizing Products Regulation (FPR; Regulation EU 2009_1009). This leads to different views from an ecological or an agronomic perspective. High minimum tonnage registrations linked to obligatory testing which are in place specifically for fertilizers, but not for general chemicals, are a barrier for new product introductions. In countries like Canada, Fertilizers are exempted from chemical registrations and are regulated within the Fertilizer Act, in Europe not.

Testing micronutrient product in scientific trails needs more attention to details. For iron, often chelates have to be used, but the actual Fe level will be compromised by exchange of Zn, Cu and Mn with Fe in the chelate. In high pH conditions Fe-EDDHA or Fe-HBED should be used, but only the Fe-ortho-ortho-isomer works. In many trial reports this information is not given, hence the dose rate or performance findings are not funded by good data.

Genetic basis of Ni hyperaccumulation in Balkan Noccaea and its role in serpentine adaptation

Anna Čepelová, Edita Tylová, Tereza Koberová, Filip Kolář

Faculty of Science, Charles University, Czechia

Hyperaccumulation is a fascinating trait found in only certain plant species. It presents an intriguing subject from both an evolutionary perspective and for its practical applications in environmental remediation and biomining. The genus Noccaea is widely distributed and includes species capable of simultaneously hyperaccumulating multiple metals. In addition to colonizing anthropogenically contaminated soils, Noccaea species also occur on ultramafic soils naturally metal-rich and toxic substrates with elevated levels of heavy metals, particularly nickel.

An open question remains: is the ability to hyperaccumulate nickel related to occurrence on ultramafic soils, and does this trait provide any adaptive advantage in such environments?

Populations of European *Noccaea caerulescens* are known to accumulate nickel, cadmium, zinc, and even lead, making this species a model for hyperaccumulation research. Closely related species, such as *Noccaea praecox* agg. from the Balkan Peninsula, also exhibit metal accumulation or hyperaccumulation, with populations found on both ultramafic and non-ultramafic soils.

This project investigates the genetic basis of nickel hyperaccumulation in Noccaea praecox agg. and explores the relationship between this trait and adaptation to ultramafic soils.

1. To examine the response of *Noccaea praecox* agg. to varying nickel concentrations under in vitro conditions using phenotypic characterization, elemental analysis of shoot tissues, and transcriptomic profiling.

2. To investigate the adaptive response to ultramafic substrates and nickel uptake in vivo through fitness predictors, tissue elemental analysis, and transcriptomic profiling of plants grown on ultramafic and non-ultramafic soils.

3.To identify candidate genes involved in both ultramafic soil adaptation and nickel hyperaccumulation.

Copper dynamics in vineyard soils as affected by the supply of aerated compost tea

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Aerated compost teas (ACT) are rich in soluble humic substances (SHS) that have high affinity for metals, notably Cu. Using a batch experiment, we measured the extent to which two ACTs altered Cu dynamics in vineyard topsoils. Total Cu concentration, free Cu ion fraction and size distribution of Cu ligands were measured in 0.01 M KCl extract to assess the impact of ACT on the mobility of Cu. DGT measurements were carried out to assess the effect of ACT on Cu bioavailability, and the dissociation rate of Cu-SHS complexes was measured. ACT increased the mobility of Cu from a factor 1.2 to 5.8 depending on the soil and on the ACT added. Cu mobilization was associated with an increase in absorbance at 254 nm and a decrease in the free Cu ion fraction in the KCl extract. Associated with the strong agreement between the size distribution of SHS and that of Cu ligands in the KCl extract of soils treated with ACT, these results showed that Cu was mobilized through complexation by the SHS present in ACTs. A fraction of the SHS supplied with ACTs sorbed onto the soil constituents, notably in calcareous soils where this fraction reached 86% for ACT B. This explains why the Cu mobilization efficiency of ACTs was on average lower in calcareous soils than in non-calcareous soils. Lastly, ACT increased the bioavailability of Cu in soils from a factor 1.3 to 4.2, due to the relatively high dissociation rate of Cu-SHS complexes.

Comparative genomics of *Noccaea caerulescens* reveals structural and regulatory drivers of metal hyperaccumulation

Damien M. Kuzmik [1]*, O. Courtin [1]*, AC. Cazale [1], C. Klopp[2], C. Deveaux[3], S. Merlot[1]

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The Brassicaceae species *Noccaea caerulescens* is a well-established model for studying metal hyperaccumulation, particularly zinc, cadmium, and nickel. Despite numerous molecular studies, the precise identification of the loci involved in the evolution of this trait remains limited because of the lack of high-quality genome assemblies across accessions with contrasting accumulation capacities.

Here, we performed long-read sequencing and genome assembly for five accessions of *Noccaea caerulescens*, selected to represent distinct genetic and edaphic groups, including ultramafic and calamine soils. For all accessions, we generated assembly of 7 chromosomes characteristic of the proto-Calepineae Karyotype. The comparison of these accessions did not reveal major chromosomal rearrangements, except for an inversion on chromosome 2 in the Cira accession. We then more specifically analyzed the structure of selected loci containing candidate genes involved in metal accumulation. Our analysis revealed a duplication of HMA4 in 4 accessions of N. caerulescens, with up to four copies in the accession Saint-Laurent-le-Minier. In contrast, the Firmiensis accession (aka Puy de Wolf) contains two functional copies of the metal transporter IRT1 that are expressed at a high level. Surprisingly, IREG2, involved in vacuolar storage of nickel, is not duplicated in the studied *N. caerulescens* accessions, suggesting that its high expression likely result from variation in cis elements present in its promoter.

Altogether, our results indicate that both gene duplication and variation in cis-elements contribute to variation in metal hyperaccumulation in *N. caerulescens*. Future sequencing of additional N. caerulescens accessions and of related species will help us to understand the evolution of metal hyperaccumulation in this model species.

Thallium-induced metabolic adjustments in the facultative hyperaccumulator *Silene latifolia*

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Thallium (Tl) hyperaccumulation is a relatively rare and poorly studied phenomenon, with a few species identified as hyperaccumulators only recently. Among them, *Silene latifolia* has been shown to be a facultative Tl hyperaccumulator, with only the metallicolous populations capable of accumulating the metal and reaching up to 79,000 μg Tl g^{-1} d.w. in samples from a mine dump in Central Europe, thus representing the most toxic plant in the world.

By comparing a metallicolous and a non-metallicolous population of *S. latifolia*, the aim of the present study was to explore the metabolic adaptations underlying Tl hyperaccumulation. Plantlets were hydroponically grown, and untargeted metabolomics analysis was performed on the leaves and roots of samples exposed or not to 10 μM $TlNO_3$ for one week. Preliminary data revealed distinct metabolic profiles between the two populations, with a significant number of differentially accumulated metabolites (p-value < 0.05) observed in both leaves (394 out of 825) and roots (255 out of 872). These differences were particularly evident even in control conditions, indicating that the adaptations for hyperaccumulation are partly constitutively regulated rather than activated in response to stress. In addition, the two populations exhibited distinct modulation of the ascorbate-glutathione cycle, highlighting the fundamental role of this antioxidant machinery in maintaining redox homeostasis while facilitating Tl accumulation.

Metal interactions in the Ni hyperaccumulating population of *Noccaea caerulescens* - Monte Prinzera

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Hyperaccumulation is an intriguing characteristic found in certain plant species, allowing them to accumulate high levels of metal ions in their above-ground tissues without showing signs of toxicity. Noccaea caerulescens is a well-established model for studying metal hyperaccumulation and hypertolerance. In this study, a population of *N. caerulescens* from a serpentine soil site in the Italian Apennine Mountains (Monte Prinzera) was selected. The plants were grown hydroponically and exposed to varying concentrations of metals, both in excess and limiting amounts. The metals accumulated in the shoots and roots were measured using ICP-MS. Additionally, real-time PCR was used to assess the expression of metal transporters and genes regulated by iron deficiency in both the shoots and roots of the treated plants. The *N. caerulescens* population from Monte Prinzera demonstrated its ability to hypertolerate and hyperaccumulate nickel (Ni) but not zinc (Zn). Furthermore, excess Ni did not induce iron (Fe) deficiency, as seen in Ni-sensitive species. Instead, excess Ni competed with Fe translocation, rather than inhibiting its uptake.

The endoplasmic reticulum quality control plays a role in cadmium stress response in *Arabidopsis thaliana*

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Several types of stresses increase the accumulation of misfolded proteins in the plant Endoplasmic Reticulum (ER), resulting in ER stress. To restore ER homeostasis, cells activate the Unfolded Protein Response (UPR) pathway, which, together with the Endoplasmic Reticulum Quality Control (ERQC) and the Endoplasmic Reticulum Associated Degradation (ERAD) machineries, work to fasten glycoproteins folding and reduce the accumulation of the terminally misfolded ones in the ER.

Defects in the UPR pathway have been shown to reduce or increase plant tolerance depending on the type of stress, suggesting a complex regulation of this pathway. However, our knowledge about the role of the ERQC machinery, which assists protein folding, in mitigating the impact of Cd stress at ER level is so far limited. In our research, we took advantage of an *A. thaliana* mutant which has a reduced activity in one the main enzymes of the ERQC pathway and compared its response to Cd stress at phenotypic, biochemical and molecular level in comparison with wild-type (Wt) seedlings grown under the same conditions. Our data indicated that the ERQC mutant had constitutively high levels of ER stress already under standard growth conditions, but under Cd stress, it displayed a more tolerant phenotype compared to the Wt. Since the mutant accumulates Cd at comparable levels to the Wt, our results suggests that its greater ability to cope with the Cd stress leading to a more adapted and tolerant plant phenotype. Our results establish a role of the plant cell ERQC in the Cd sensing/response.

The different forms of metal transporters MTP1 modulate multi-metal transport in *N. caerulescens* Monte Prinzera

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The facultative metallophyte *Noccaea caerulescens* is a model species for the study of the evolution of metal tolerance and hyperaccumulation, due to its adaptation to very different soil types, both metalliferous and non-metalliferous. Such diverse edaphic conditions have led to the development of different adaptive strategies for metal homeostasis, and specific accumulation and tolerance properties towards Zn, Cd and Ni. Among them, *N. caerulescens* Monte Prinzera (MP, Parma - IT) is a serpentine ecotype growing on ultramafic soils (with high Ni, Co, and Fe levels) and characterised by superior Ni tolerance and hyperaccumulation. However, the mechanisms underlying these traits are still poorly understood.

In *N. caerulescens* MP, two different CDSs encoding for the vacuolar Zn transporter MTP1 were identified. The two different forms were characterised by different lengths, due to the absence, in the shorter sequence, of the His-rich loop, putatively involved in Zn sensing and selectivity in the plant. Both isoforms were confirmed to localise in the tonoplast. MTP1 expression was high and mostly constitutive; the long isoform was transcribed at high levels in all growth conditions, while the short form was induced in leaves upon Co supplementation. The two sequences were transformed in wild-type yeast and in the Zn- and Co-hypersensitive zrc1cot1 strain. Both isoforms restored Zn compartmentalisation in yeast, but produced different behaviours upon Ni and Co treatments. These findings suggest a role of MTP1 in modulating the tolerance of ions other than Zn and in the adaptation of N. caerulescens to different edaphic environments.

Unraveling the role nitric oxide in plastidial iron allocation using bioinfirmatics and multielement clustering

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Understanding essential transition metal allocation in plants cells is pivotal to decode metal homeostasis in organelles. In that revealing microscopy X-Ray Fluorescence (μXRF) is a powerful technique, yet indeed generates a massive amount of data, requiring appropriate bioinformatics in dataset handling. To understand intracellular Fe allocation in nitric oxide signaling and autophagy compromised *Arabidopsis thaliana* mesophyll cells, we applied 20 μm thick cryosection samples. Dataset on the K edge signal of transition metal distribution were gained at ID21, European Synchrotron Radiation Facility, and analyzed using two computational approaches. First, sample was analyzed as composed of foreground and background layers, differentiated by intensity thresholds computing, and morphological operations were applied to improve the clarity of segmented regions. Focusing on background layer, away from organelles overlapping or noise, provided detailed structural interpretation of Fe-rich zones. The second approach applied k-means clustering, treating each pixel as a point in multidimensional element space, where each organelle occupied a distinct region in this space region based on its characteristic elemental composition and corresponding to its specific functions. Both approaches independently indicated that compromised lines show altered plastidial Fe allocation at senescence initiation. This agreement increases the confidence in the biological findings and in the robustness of each method.

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Assessing the radiological impact of major industrial facilities

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Two Swedish industrial sites: The European Spallation Source (ESS) and the Ringhals Nuclear Power Plant (RNPP), are used as selected examples to demonstrate the research efforts being made to assess and predict the possible impact of both operational discharges and accidental releases from large industrial sites. The research projects discussed are largely funded by Swedish-state safety authorities, involve multiple academic institutions and strongly link academia, industry and public stakeholders. The ultimate goal of these collective efforts is to assess the radiological impact related to public exposure. With the ESS as example, the complexity of predicting the future impact on the environment and to the public, prior to the commissioning of a facility, will be illustrated. The ESS is situated in rich farmland and, as such, this example is primarily concerned with terrestrial pollution, with a key aspect being the potential release of foreign substances (in particular W, Hf, Ta, Gd and Lu) for which limited data on environmental transport is available. Details of a multi-year project to assess the pre-operational levels of contaminants in soil, water and biota around the ESS site will be presented, as well as current research on detection methodology and obtaining new environmental transport data. The example of the RNPP is used to illustrate how the continued monitoring of a long-standing site can be complicated by changing environmental conditions. RNPP, like all such power plants in Sweden, is constructed on the coast and this example is therefore focused on aquatic pollution.

Field-based insights from degraded sites: exploring a legume candidate species for soil remediation

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Our Heavy metal and organic pollutant contamination, along with soil salinity, are among the major abiotic stressors threatening environmental health, with tremendous impacts on plant growth and ecosystem functioning. Degraded sites provide valuable models for understanding contamination profiles, colonizing plant species, and their associated compartmentalized microbiomes. However, many such environments across Europe and globally remain insufficiently characterized, hindering the implementation of site-specific soil remediation strategies. In this study, we investigate and characterize four environmentally degraded sites in Romania, each affected by a distinct stressor: mercury (Hg), copper (Cu), hexachlorocyclohexane (HCH), and high salinity. A common legume species present across all sites serves as a model for examining plant-soil-microbe interactions under varying stress conditions. Ongoing analyses focus on soil parameters including pH, total organic carbon, organic matter content, available nitrogen (N), phosphorus (P), potassium (K), and concentrations of other different metals. In parallel, we assess metal accumulation in plant roots and shoots to trace element pathways from soil to plant tissues. Antioxidant enzyme activities (CAT, APX, SOD, GR) are measured to evaluate plant stress responses. To investigate microbial dynamics, we employ quantitative PCR targeting key functional genes (e.g., nifH, nirK, nirS, amoA, amoB) to assess how microbial gene abundance is influenced by different contaminants. Additionally, 16S rRNA gene amplicon sequencing is used to compare the rhizosphere microbiome with the nodule microbiome, contributing to a deeper understanding of legume-rhizobia symbioses under environmental stress. Correlation analyses reveal which soil parameters significantly shape the microbiome structure and function. Overall, this research explores the intercourse between legumes, soil types, and microbial communities in contaminated environments, contributing to the development of tailored and sustainable phytomanagement strategies.

Photosynthetic trait alterations in crops grown in metal nanoparticle-polluted soils of Mitrovica, Kosovo

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The increasing presence of metal nanoparticles (NPs) in agricultural soils, particularly due to industrial pollution and anthropogenic activities, raises concerns about their impact on plant physiological processes. This study examines the effects of metal nanoparticles derived from polluted soil on the photosynthetic performance of three major crop species: maize (*Zea mays*), tomato (*Solanum lycopersicum*), and pepper (*Capsicum annuum*). Plants grown in nanoparticle-contaminated soil were evaluated for key photosynthetic parameters, including chlorophyll content, gas exchange traits (net photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration), and chlorophyll fluorescence (Fv/Fm). The results revealed species-specific responses: while low to moderate nanoparticle levels in soil sometimes led to slight improvements in chlorophyll content potentially due to hormetic effects overall exposure to metal NPs such as zinc oxide (ZnO), titanium dioxide (TiO_2), and silver (Ag) from polluted soils resulted in reduced photosynthetic efficiency, increased oxidative stress, and impaired stomatal function. Tomato and pepper plants showed greater sensitivity to nanoparticle-induced stress compared to maize, which exhibited relatively higher tolerance. These findings underscore the risks posed by soil-borne metal nanoparticles to plant physiological health and productivity and highlight the importance of monitoring and mitigating soil pollution for sustainable crop production. This research is part of a project funded by the European Union under the NanoKos project.

Toward the identification of the genetic mechanisms governing zinc homeostasis in *B. distachyon*

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Understanding how plants adapt to variable soil environments is crucial for improving nutrient use efficiency and grain nutritional quality in crops. Although plants possess nutrient homeostasis mechanisms that help cope with variations in substrate composition, it is well established that soil mineral availability influences nutrient accumulation in grains. Zinc (Zn) deficiency, which is widespread in both soils and human diets, constitutes a global issue, especially in developing areas. Understanding how changes in Zn availability affect plant growth and grain quality, as well as elucidating the interplay between Zn homeostasis and other essential nutrients, would provide perspectives toward the production of more resilient and/or biofortified crops. Brachypodium distachyon, a model species for temperate cereals, offers a valuable system to investigate the regulation of metal homeostasis in response to variation in nutrient availabilities.

Using a custom-designed, and high plant density, circulating hydroponic system, and leveraging the natural genetic diversity among B. distachyon accessions, our research aims to elucidate the mechanisms underlying Zn homeostasis throughout the plant life cycle. To do so, we employ a combination of approaches : (i) to investigate how plants respond to changes in Zn availability during the vegetative stage; (ii) to evaluate the impact of Zn supply on reproductive development and grain production; and finally (iii) to evaluate the interplay between Zn and Nitrogen (N) nutrition.

The long-term goal of these studies is to establish the genetic mechanisms governing zinc homeostasis throughout the life cycle of temperate cereals, and how these mechanisms interact with nitrogen nutrition.

Time-resolved responses of increased nickel concentration on root growth, cell wall modification and THESEUS1 dependent phosphorylation

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The plant cell wall serves as a primary receiver of biotic and abiotic stress to initiate stress responses in plants. However, few studies have explored how fast the environment is sensed and responses are induced. We have shown that the cell wall integrity sensor THESEUS1 (THE1) is triggering growth responses upon increased nickel (Ni^{2+}) concentrations (1). To determine how fast Ni^{2+} is changing the plant physiology, changes of the root meristem activity were quantified and revealed a dynamic oscillating behavior. To address if Ni^{2+}stimulates modification of cell wall components involved in cell expansion, pectin methylesterase (PMEs) activities were quantified and revealed that PME activity is induced within 2 hours in a dose-dependent manner. Time-resolved phosphoproteomic experiments showed that Ni^{2+} changes the phosphorylation status of 61 phosphoproteins within 5 min. Among the Ni^{2+} dependent phosphoproteins are plasma membrane localized transporters but also nuclear proteins. To determine which of the Ni^{2+} dependent phosphoproteins depend on THE1, the data were combined with a phosphoproteomic analysis of the knock-out mutant the1-6. In conclusion, our research shows the fast and dynamic changes upon Ni^{2+} exposure and exemplify the importance of time-resolved analyses.

[1] Richter et al. 2017 Front Plant Sci. 8:1554. doi: 10.3389/fpls.2017.01554

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Deciphering the molecular mechanisms underlying the tolerance and bioaccumulation of uranium in a green microalga of the Coelastrella genus

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Pollution by heavy metals is harmful to the environment and poses significant health risks to humans. For effective phytoremediation of contaminated environments, it is crucial to identify and characterize organisms that can tolerate and accumulate metals. We have isolated a unicellular green microalga, *Coelastrella* sp. PCV, which thrives in uranium (U)-contaminated environments. To date, no genes specifically associated with U tolerance and accumulation have been identified in microalgae. Unraveling the molecular mechanisms underlying *Coelastrella*'s tolerance could provide valuable insights into its potential for U bioremediation.

A transcriptomic analysis was conducted to identify the key genes involved in U tolerance and accumulation in *Coelastrella*. Gene ontology analysis of differentially expressed genes revealed that the cell cycle, translation, photosynthesis, and biosynthesis of amino acids and nucleotides were significantly down-regulated under U stress. In contrast, up-regulated genes were enriched in the categories catabolism, lipid metabolism, and transport. Furthermore, several genes encoding proteins potentially involved in U detoxification were upregulated, including ion transporters, ABC, and MATE transporters. Additionally, genes coding putative metalloproteins were upregulated and may contribute to U sequestration.

So far, no genetic transformation method has been reported for any *Coelastrella* species, which hinders the functional characterization of candidate genes in this organism. Therefore, we selected knockout mutants in orthologous genes from the model green microalga *Chlamydomonas reinhardtii*. Also, *C. reinhardtii* strains expressing *Coelastrella* genes are currently being constructed. A phenotypic and molecular characterization of null mutants and overexpressing strains has been conducted to assess their implication in U tolerance and accumulation.

Cd and Zn uptake in *Sedum alfredii*: purifying and characterizing Cd-binding proteins

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This study focuses on how zinc (Zn) and cadmium (Cd) are distributed and accumulated in *Sedum alfredii*, which is known for its ability to hyperaccumulate both metals and its potential use in phytoremediation. We compared hyperaccumulating (HE) and non-hyperaccumulating (NHE) ecotypes to understand how these metals move through the two main pathways: the apoplastic (outside the cells) and symplastic (inside the cells, including across membranes), as they enter the xylem and move through the root. To study these processes, we used advanced imaging techniques, such as customized benchtop μXRF imaging and synchrotron cryo- μXRF tomography, as well as chlorophyll fluorescence kinetic imaging. Furthermore, Cd-binding proteins were purified from both ecotypes by a combination of anion exchange (AEC1), chromatofocusing (CF), and size-exclusion chromatography (SEC) with online coupling to ICP-sfMS and subsequently identified by Tims-TOF. Integrating protein identification with transcriptomics led to detailed expression profiling in response to Cd and Zn in the roots of HE and NHE. After visualizing the proteomics results using heatmaps with hierarchical clustering we observed distinct patterns between ecotypes, with several candidate Cd-binding proteins showing significantly higher or lower abundance in HE compared to NHE. Whether these differences are ecotype-specific adaptations to metal stress or have a direct role in Cd accumulation is a topic of current research, with the aim to better understanding how *S. alfredii* responds to metal exposure.

Zinc uptake, distribution and biotransformation in soybean leaves after foliar fertilization

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Many studies reported that the application of foliar sprays can help to preserve crop yields and quality by effectively correcting microelements deficiencies. The main aim of this study was to investigate, using micro-XRF techniques, the absorption, distribution and biotransformation of chelated zinc - ZnIDHA, applied in soybean leaves. Metal distribution was analysed on intact, shock-frozen hydrated samples by micro-XRF using a benchtop machine and by μXRF tomography at the beamline P06 (PETRA III DESY, Hamburg, Germany). Zn speciation was studied by μXANES tomography at the same beamline. The OJIP induction kinetics were measured in a direct imaging method on darkadapted leaves. Zinc distribution on tissue-level was examined by μXRF. Higher accumulation was observed in the veins of positive control (optimal nutrition) and Zn-deficient leaves 18 days after treatment compared to negative control. Micro-XRF tomography revealed on cellular level that upon fertilization the incoming Zn in newly grown leaves arrived via the phloem and accumulated in the epidermis in addition to photosynthetic mesophyll. Zn distribution in the epidermis was mostly symplastic in the ZnlDHA treatment and similar to positive control. In Zn-deficient leaves, Zn was preferentially accumulated in the mesophyll. Micro-XANES demonstrated different ligands in the tissues. We demonstrated the delivery of Zn via phloem to Zn-deficient leaves that grew after ZnIDHA application (not treated with fertilizer), and it showed how Zn speciation and distribution differs between Zn-deficient, Zn-replete and Zn-fertilized plants. Our study confirmed that biodegradable ZnIDHA is a sustainable foliar fertilizer that improves Zn nutritional status of plants.

Mobility of Te in soil and its bioavailability to selected plants - a model study

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Tellurium is a critical element for the development of innovative technologies. Its wider use, e.g. in the production of solar panels, is expected to significantly increase Te emissions into the environment over the next 10-15 years. Data on this topic is very limited, so the aim of this study is to investigate the mobility and bioavailability of different chemical forms of Te, and to follow their transformations in soil and plant tissues. Due to the possible simultaneous ingress and interaction of Te and Cd with the environment (photovoltaics), their mutual influence on plants is investigated. Selected plant species were grown under conditions of increased stress associated with the presence of Te and/or Cd. The research on model systems, soils of a specific composition and hydroponic crops, enabled distinction of Te mobility and toxicity depending on its chemical form. Solid-liquid extraction with selective extractants was used to study soil systems in the context of elements' affinity to particular soil phases. Fractionation of tellurium-enriched soils distinguished exchangeable/carbonate, reducible and organic fractions. The fractionation study showed that Te(VI) is in general more mobile than Te(IV), and associated with different soil phases. Of the plants tested, bear garlic showed higher resistance to Te stress, regardless of its chemical form. White mustard, on the other hand, started showing poisoning symptoms in the presence of 5 mg/L Te, more severe for Te(IV). Accumulation of Cd and Te also varies, depending on the form of the latter.

Life on the edge: polyploidy and adaptation to ultramafic soils in Noccaea

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Polyploidization, or whole-genome duplication, is a key evolutionary process that significantly enhances plant adaptability. This study focuses on the impact of polyploidization on plant adaptation to extreme environments, specifically to toxic ultramafic substrates rich in metals such as nickel, chromium, and cobalt. We investigate whether certain ploidy levels show a preference for ultramafic soils and whether polyploidization influences metal accumulation in plants. Furthermore, we aim to uncover links between polyploidization, adaptation to ultramafic conditions, and metal hyperaccumulation. The genus Noccaea, known for its capacity to hyperaccumulate metals and adapt to ultramafic soils, was selected as a model system. By combining ploidy analysis, genomic approaches, reciprocal transplant experiments, and metal accumulation assessments, this study seeks to uncover the genetic basis of ultramafic adaptation and the role of polyploidization in this process. The findings will offer new insights into the mechanisms of adaptation and evolution in plants inhabiting extreme environments.

Assessment of silicon and copper exposure in the roots of *Triticum aestivum* L.: a comparative study between soil and hydroponic cultivation

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Silicon (Si), the second most abundant element in the lithosphere, is widely recognized as a beneficial element for plant growth and copper (Cu) is an essential micronutrient required for various vital physiological processes. However, when its concentration exceeds a certain threshold, it can become toxic. The interaction between Si and Cu has been previously studied. This study presents, for the first time, a comparative analysis of field and hydroponic data on the dynamics of silicon and copper supplementation, evaluating their effects on the growth and development of wheat (*Triticum aestivum* L.). The results indicate that although hydroponic systems allow for clearer observation of treatment effects, the physiological responses observed under real field conditions are more intricate and complex compared to the ones obtained in the laboratory. Root lengths results revealed statistically significant differences between two cultivation systems. Results of stem length from hydroponics have shown statistically significant differences in comparison to soil cultivation. Leaf lengths average has been the same in both systems; however, Cu-treated plants grown hydroponically were more adversely affected than those cultivated in the field. Regarding dry biomass distribution, root dry weights have been markedly higher, and differences have been statistically significant in field conditions compared to hydroponics. Conversely, stem dry weight followed an inverse trend.

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Genome-enabled approaches in the study of metal hyperaccumulation and hypertolerance

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The extremophile metal hyperaccumulator *Arabidopsis halleri* is a diploid outcrossing stoloniferous perennial among the closest relatives of *Arabidopsis thaliana*. *A. halleri* is known for a vast phenotypic range of its leaf ionome, with leaf Zn concentrations generally far above critical toxicity thresholds of ordinary plants, and for its exceptionally broad edaphic range that includes soils containing toxic levels of heavy metals as well as non-contaminated soils. Our objective is to unravel the genetic and physiological basis of phenotypic divergence both within *A. halleri* and by comparison to closely related species, in an ecological context. I will briefly outline earlier insights related to metal homeostasis from cross-species comparative approaches and then sketch some recent developments building on this work. We established a biodiversity resource of ca. 1,000 accessions of European *A. halleri* collected in the field, which are edaphically and ionomically indexed, that is, leaf and soil elemental composition at the site of origin are known for each genotype (Stein et al., 2017). I will present results from QTL mapping using the F2 progeny of crosses of phenotypically contrasting parents, and also from environmental association studies. Then I will show how haplotype-phased genome assemblies can help to identify critical genetic variation at candidate loci. A subset of the identified genomic regions are under study towards the identification of causal genes and polymorphisms. Our results provide examples of the genetic architectures, genomic alterations, and gene functions underlying naturally selected extreme physiological traits in plants. At least in part, the exceptionally large phenotypic range of *A. halleri* may reflect how plants utilize metals in mineral co-option for functions outside the nutritional requirements of the metalloproteome that are largely shared among plants (Krämer, 2024). Our work provides insights into how plants cope with environmental change, and can inform crop improvement and the development of plant-based technologies such as phytomining and phytoremediation.

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Comparison of mechanisms leading to lethal vs. sublethal metal(loid) toxicity in plants

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Many trace metals are essential for plant growth, but at higher concentrations they become toxic. Many potential mechanisms of metal toxicity-induced damage in plants have been proposed in the past years. However, to induce fast effects, metal concentrations applied were often far above those commonly found in polluted environments. For these reasons, for many years, research in our lab has focused on evaluating the relevance and specificity of different proposed mechanisms of metal-induced toxicity. In this work, mechanisms occurring under conditions leading to chronic toxicity at typically nanomolar concentrations were compared with acute toxicity within a short-term exposure, typically at 10x to 1000x higher concentrations. The results that will be presented as examples were obtained with toxicity by Cd as a redox-inert non-essential metal, Cu as a redox-active essential metal, and As as a redox-active metalloid. Results from the shoot model plant *Ceratophyllum demersum* and the crop model *Glycine max* (soybean) will be mainly compared, others will be shown for specific cases. Generally, in all studies, low-sublethal metal(loid) concentrations led to specific inhibition of a few processes, while acute toxicity at higher concentrations led to less specific inhibition of many processes. At chronic sublethal toxicity, the light-harvesting antenna LHCII and the PSII reaction centres were key primary targets in shoots, while few enzymes were key targets in roots. The differences between low-level specific vs. lethal non-specific inhibition were also related to drastic changes in metal(loid) distribution inside the tissues, with apoplastic binding becoming more important at higher concentrations.

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Comparative genomics of *Noccaea caerulescens* reveals structural and regulatory drivers of metal hyperaccumulation

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The Brassicaceae species *Noccaea caerulescens* is a well-established model for studying metal hyperaccumulation, particularly zinc, cadmium, and nickel. Despite numerous molecular studies, the precise identification of the loci involved in the evolution of this trait remains limited because of the lack of high-quality genome assemblies across accessions with contrasting accumulation capacities.

Here, we performed long-read sequencing and genome assembly for five accessions of *Noccaea caerulescens*, selected to represent distinct genetic and edaphic groups, including ultramafic and calamine soils. For all accessions, we generated assembly of 7 chromosomes characteristic of the proto-Calepineae Karyotype. The comparison of these accessions did not reveal major chromosomal rearrangements, except for an inversion on chromosome 2 in the Cira accession. We then more specifically analyzed the structure of selected loci containing candidate genes involved in metal accumulation. Our analysis revealed a duplication of HMA4 in 4 accessions of *N. caerulescens*, with up to four copies in the accession Saint-Laurent-le-Minier. In contrast, the Firmiensis accession (aka Puy de Wolf) contains two functional copies of the metal transporter IRT1 that are expressed at a high level. Surprisingly, IREG2, involved in vacuolar storage of nickel, is not duplicated in the studied *N. caerulescens* accessions, suggesting that its high expression likely result from variation in cis elements present in its promoter.

Altogether, our results indicate that both gene duplication and variation in cis-elements contribute to variation in metal hyperaccumulation in *N. caerulescens*. Future sequencing of additional *N. caerulescens* accessions and of related species will help us to understand the evolution of metal hyperaccumulation in this model species.

How does the fern *Dryopteris erythrosora* accumulate Rare Earth Elements ?

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Rare earth elements (REEs) are strategic metals strongly involved in low-carbon energy conversion. However, these emerging contaminants are increasingly disseminated into ecosystems, raising concern regarding their toxicity. Evidences for REE transfer and impact to living organisms such as toxicity or hormesis have been reported, without identifying the molecular effectors involved in REE uptake and transport. REE-accumulating plants are promising phytoremediation tools but are also crucial subjects to better understand REE uptake and transfer to the trophic chain.

This led us to search for REE accumulating ferns that could reveal the specific mechanisms allowing for REE uptake and transfer to the plant. To that end, the screening of a rustic fern collection, including the REE accumulating species *Dryopteris erythrosora* and some of its relatives highlighted contrasted behaviors between genera but also, between species, regarding REEs accumulation in fronds. The common point for all the ferns, was a preferential uptake and transfer of the light vs. the heavy REEs (1).

We selected the two most contrasted members of the Dryopteris genera, and implemented a comparative de novo transcriptomic approach. The combination of the Oxford Nanopore technology and Illumina NextSeq500 allowed us to (i) assemble a reference transcriptome and (ii) compare the DEGs between the two species. This dataset is a goldmine to find determining factors of REE accumulation in ferns.

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Letort, Fabien Poster

Exploring the capabilities of HERFD-XAS to resolve Cd speciation in environmental samples

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Cadmium (Cd) is a non-essential metal classified as class 1 carcinogen[1], which can induce detrimental effects for humans. For the general population (excluding smokers), dietary intake is the primary route of Cd exposure. Understanding Cd speciation in crops is essential to unravel its transport and accumulation pathways, particularly to develop effective mitigation strategies aimed at reducing Cd levels in edible parts. The Cd concentration in crops exposed to low Cd contamination is below the detection limit of X-ray Absorption Spectroscopy (XAS). High Energy Resolution Fluorescence Detected X-ray Absorption Spectroscopy (HERFD-XAS) offers enhanced sensitivity a spectral resolution. At the BM20 beamline of the European Synchrotron, we recorded a database of HERFD-XAS spectra of Cd compounds. We analyzed both organic (Cd-phytate, -malate, -tannate, -acetate, -cell wall-bound, and -calcium oxalate at varying Ca/Cd ratios) and inorganic (Cd-O, -S, -CO_3, -$(PO_4)_2$, -Cl_2, -SO_4, -$H_2(PO_4)_4$, and -Fh) forms of Cd. These spectra were compared with previous XAS data acquired at the ID21 beamline[2] of the European Synchrotron. Our results demonstrate clear spectral distinctions between different Cd compounds, offering improved spectral resolution, especially on the Cd-O binding at ~3540 eV. This work offers promising perspectives to identify Cd speciation in complex environmental samples. The new reference database will (i) support future Cd speciation analyses in crops and (ii) be made publicly available to the scientific community.

Keywords: Cadmium – Speciation – HERFD-XAS – Database.

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Mechanisms of silicon-mediated mitigation of heavy metals toxicity in plants

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Heavy metals pollution in soil poses severe threats to eco-environment, plant growth, food security and human health. It has been well-documented that silicon (Si) is extremely effective in mitigating heavy metals toxicity. However, the mechanisms behind remain poorly understood. We have recently put forward some new insights. First, studies using protoplasts and suspension cells have revealed that the cell wall is crucial for the function of Si. By using apoplastic pathway tracing techniques, we have found that Si acts as a barrier in the apoplast, significantly reducing the proportion of heavy metals absorbed through the apoplastic pathway. Second, Si can reduce the acidification process of roots by increasing the soluble phosphorus content in plants. This helps to reduce the bioavailability of heavy metals. Third, our multi-season in-situ field remediation studies have shown that Si-based fertilizers can reduce the concentrations of arsenic and cadmium in rice grains by affecting the microbial community in the roots of rice. More importantly, Si can increase the abundance of root-surface reducing microorganisms, thereby reducing the uptake of arsenite by rice. Finally, we have proposed a novel method that utilizes biosafety cationic macromolecules to help plant roots quickly establish a Si defense barrier, which is also effective on many low-Si plants. These findings provide unique insights into the role of Si in the plant-soil system and, more importantly, offer references for the rational application of Si-based fertilizers to address heavy metals pollution, improve phosphorus use efficiency, balance soil acidity and maintain sustainable agricultural development.

Application of 1,3-dioxolane in phytoremediation of cadmium: a case study of two populations of *Silene vulgaris*

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Silene vulgaris (Salisb.) Sm. belongs to the Caryophyllaceae family. It is commonly found across Europe. It has a large number of ecotypes resistant to heavy metals such as Cd, Cu, Ni, Pb, and Zn, occurring separately or in combinations.

For the study, seeds of inflated campion were used from two different populations. One population was exposed to cadmium, while the other developed without the presence of cadmium in the substrate.

1,3-Dioxolane is a compound that could potentially be used in agriculture as a new adjuvant in herbicides and in foliar fertilization. It is a compound that does not belong to microplastics and exhibits both hydrophilic and hydrophobic properties.

The aim of the study was to determine whether 1,3-dioxolane affects the physiological state of Silene vulgaris regardless of its origin when in contact with heavy metal contamination in the form of cadmium.

The experiment was conducted based on hydroponic cultivation, where cadmium and 1,3-dioxolane were supplied at a concentration of 10 μM. The nutrient solution was replaced twice a week, and the entire experiment lasted for two weeks. Subsequently, the physiological state of the plants was assessed based on measurements of fresh and dry mass, pigment content, chlorophyll a fluorescence, cadmium content in the plants, levels of H_2O_2, MDA, proline, and redox activity.

As a result of the experiments conducted, it can be concluded that 1,3-dioxolane has a mitigating effect on the action of cadmium, which is reflected in greater growth and mass of plants growing in the presence of 1,3-dioxolane compared to those without it. Plants growing in the presence of 1,3-dioxolane contained less cadmium than those growing in the presence of cadmium alone.

The role of root cap in protection against antimony toxicity in aquatic plant *Pistia stratiotes*

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Pistia stratiotes L. is a floating aquatic species with roots immersed in water and leaf rosette exposed to the air. It is distributed worldwide and is considered an invasive species in many parts of the world, recently including Slovakia. In some areas, it was recommended as a suitable species for phytoremediation of polluted waters.

We have previously investigated the role of the root cap in *Pistia* plants exposed to low concentrations of copper and zinc. The results have shown that some elements, including other metals, were specifically bound to the root cap. This influenced their distribution within the plant body. Due to our present focus on some metalloids released from the former mines, we investigated the effect of antimony (Sb) on *Pistia* plants. In addition, we have used this species as a suitable model for a study of the root cap removal effect on the uptake and distribution of this element in plants.

Results of our study have shown the inhibitory effect of Sb on the growth and morphology of the root system. Antimony also negatively affected the growth and pigmentation of roots and leaves. The study was accompanied by physiological and biochemical analysis of treated plants. The results of this study will be combined with our investigation of other plants and their potential for metal accumulation.

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Photosystems and antioxidative system of rye, wheat and triticale under Pb stress

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Lead (Pb^{2+}) pollution in the soil sub-ecosystem has been a continuously growing problem due to economic development and ever-increasing anthropogenic activities across the world. In this study, the photosynthetic performance and antioxidant capacity of Triticeae cereals (rye, wheat and triticale) were compared to assess the activities of antioxidants, the degree of oxidative damage, photochemical efficiency and the levels of photosynthetic proteins under Pb stress (0.5 mM, 1 mM and 2 mM Pb (NO3)2). Compared with triticale, Pb treatments imposed severe oxidative damage in rye and wheat. In addition, the highest activity of major antioxidant enzymes (SOD, POD, CAT, and GPX) was also found to be elevated. Triticale accumulated the highest Pb contents in roots. The concentration of mineral ions (Mg, Ca, and K) was also high in its leaves, compared with rye and wheat. Consistently, triticale showed higher photosynthetic activity under Pb stress. Immunoblotting of proteins revealed that rye and wheat have significantly lower levels of D1 (photosystem II subunit A, PsbA) and D2 (photosystem II subunit D, PsbD) proteins, while no obvious decrease was noticed in triticale. The amount of light-harvesting complex II b6 (Lhcb6; CP24) and light-harvesting complex II b5 (Lhcb5; CP26) was significantly increased in rye and wheat. However, the increase in PsbS (photosystem II subunit S) protein only occurred in wheat and triticale exposed to Pb treatment. Taken together, these findings demonstrate that triticale shows higher antioxidant capacity and photosynthetic efficiency than wheat and rye under Pb stress, suggesting it as a potential Pb tolerant plant.

The role of micronutrients in plant responses to biotic stress

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Emerging evidence points to the vital role of micronutrients such as Zn in the immunity of metal non-hyperaccumulating plants. In contrast to hyperaccumulators, crop plants do not accumulate the metals to the level that can exert direct toxicity towards the pathogen.

Our work aims to reveal the molecular mechanisms by which Zn and Cu mediate enhanced induced resistance in crops. Using the *Capsicum annuum* - *Botrytis cinerea* pathosystem, we investigated the photosynthetic performance, metal localisation and metalloproteomic profile of plants exposed to a range of concentrations, from Zn or Cu-deficiency (0.08 μM Zn, 0.02 μM Cu), control (1 μM Zn, 0.3 μM Cu) and surplus, not-growth-limiting metal concentrations, 5 μM Zn and 1 μM Cu. In our recent work (Kuvelja et al., 2024), we showed that Zn priming maintained the photosynthetic performance of infected plants and decreased the pathogen amount compared to control plants. Increased Zn localisation at the infection site was in part related to increased abundance of immunity-related Zn-binding proteins revealed by SEC-HPLC/ICP-MS. Additionally, Cu accumulated around the infection site in all treatments, and Cu-binding proteins were upregulated in infected Zn-primed plants. Our work opens promising new opportunities for agricultural practices towards using low, environmentally sustainable metal fertilisation instead of pesticides to enhance plant resilience.

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Do root architecture and biomass partitioning affect the uptake and the allocation to shoots of metallic contaminants and nutrients in wheat at vegetative stage?

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The root uptake of trace contaminants like cadmium (Cd), nickel (Ni), arsenic (As) and lead (Pb) by wheat is a serious concern for food safety because these elements are transferred aboveground and in the end, to the grains. On the other hand, wheat is also an important source of micronutrients like iron (Fe) and zinc (Zn). Therefore, wheat breeding should aim at selecting genotypes with a low potential of accumulation of toxic metals while preserving the content in micronutrients. The objective of this study was to investigate if the variability of the root architecture and of the biomass partitioning between roots and shoots between wheat genotypes were correlated with the root uptake of trace metals (contaminants and micro-nutrients) and with their allocation to shoots. For this, 10 genotypes of bread wheat and 7 of durum wheat were grown in greenhouse in tubes filled with glass beads and nutrient solution. At the stem elongation stage (6 leaves), the 4 replicates of each genotypes were sampled to determine the root architecture by root image analysis, the root cation exchange capacity, and the biomass and element contents (As, Cd, Ni, Pb, Fe, Zn , Ca, P, S, Ca, K, Mg, Cu, Mn, Mo) of both roots and shoots. The results are discussed in the perspective of identifying which of the measured plant traits can be associated to safe wheat genotype regarding the accumulation of toxic metals.

Assessing the effects of polluted soils containing metal nanoparticles on different traits of crops in the Mitrovica region, Kosovo

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Industrial and mining activities in northern Kosovo, especially near Mitrovica and the Trepça Industrial Park, have led to significant environmental pollution. Heavy metal contamination in soil, water, and plants exceeds EU standards. Despite long-term industrial activity, no studies have examined the presence of nanoparticles, particularly metallic ones, in the region's environment. This research aims to identify and track metal nanoparticles and assess their movement from water to soil and into food plants, evaluating potential risks to humans via direct plant consumption or indirectly through the food chain. To achieve this, ten agricultural sites near the Trepça battery factory will be selected, along with water sampling at three points along the Sitnica and Iber rivers. Focus crops include pepper, tomato, and maize, chosen for their local relevance and bioindicator potential. Advanced analytical techniques SEM associated EDX, XRD, XRF and biochemical and physiological measurements will assess nanoparticle identification and toxicity and its stress effects on plants. Expected outcomes include detailed data on nanoparticle distribution and associated risks in the Mitrovica region, insights into nanotoxicity and ecosystem impacts, and proposals for mitigation strategies such as phytoremediation and filtration. The results will contribute to policy recommendations for environmental and public health protection. These results provide crucial information for understanding the local impact of nanoparticles in this region and guide future policies for environmental management and public health protection. To ensure the sustainability of the project, we also plan to pursue further research through larger, EU-funded projects.

Biostimulant-based molecular priming enhances the yield and improves the quality of raspberry and strawberry fruits

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The *Ascophyllum nodosum*-based biostimulant SuperFifty can improve the yield and quality, and mitigate stress symptoms in model and crop plants such as *Arabidopsis thaliana*, pepper, and tomato. However, the effect of SuperFifty on raspberries and strawberries has not been previously investigated in detail. The aim of this study was to assess the influence of SuperFifty on the quality and quantity of raspberry and strawberry fruits, with a focus on metabolic composition and essential metalloid elements. For the purpose, metabolome analysis by liquid chromatography -mass spectrometry (LC-MS), and essential elements analysis by inductively coupled plasma-mass spectrometry (ICP-MS) were performed on plants pretreated or not with the biostimulant. We demonstrate that SuperFifty increases the size of both raspberry and strawberry fruits, thus enhancing the yield by 42.1% and 33.9%, respectively. The metabolome analysis revealed that most antioxidants, essential amino acids, organic acids, sugars, and vitamins as well as secondary metabolites, such as caffeic acid, p-coumaric acid, kaempferol, and quercetin, maintain their levels in SuperFifty-primed fruits. The results from the ICP-MS showed that elements important for human health, such as Zn, Mn, Fe, B, Cu, K, and Ca, also do not have their concentration significantly changed. Importantly, however, SuperFifty induced accumulation of magnesium, a crucial element known as a co-factor in many enzymatic reactions related to both plant and human physiology. Altogether, this biostimulant-induced molecular priming approach offers an environmentally friendly, efficient, and sustainable way to enhance the yield and quality of berry crops, with clear benefits to both producers and consumers.

The alleviating role of iron homeostasis in cadmium-induced photosynthesis disruption in maize (*Zea mays*)

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Introduction:

Cadmium (Cd) is a heavy metal that lacks any known biological function and is considered one of the most toxic pollutants in the biosphere. Its exposure in plants often results in severe damage to cellular components and critical metabolic processes, impacting photosynthesis, respiration, and nutrient uptake. It has been demonstrated that Cd disrupts chloroplast structure, degrades chlorophyll, and impairs photosystem activity, thereby leading to a reduction in carbon fixation. Increased iron availability mitigates the detrimental impact of Cd on photosynthetic apparatus, highlighting iron's essential role in chlorophyll synthesis and the proper functioning of electron transport chains. Since Cd enters the roots through iron (Fe) transporters, it seems reasonable to investigate whether the presence of elevated iron in the growth environment reduces the toxicity of Cd on plants. As photosynthesis disruption is one of the most sensitive factors indicating stress in plants, the main goal was to assess the Cd-Fe interactions in processes connected with photosynthesis.

Methods:

The experiments were conducted on Zea mays seedlings grown hydroponically. Chlorophylls' level was determined using a Dualex Scientific+ (Force-A, France) sensor. Photosynthetic efficiency was analysed by measuring Chlorophyll a fluorescence using a Fluorimeter (PocketPEA, Hansatech Instruments Ltd., England). Gas exchange analysis was performed with an infra-red gas analyzer (LCpro SD, ADC Bioscientific UK). The measurements of saturated photosynthesis rate (A), transpiration rate (E), and stomatal conductance (gs) were done.

Results and Conclusion:

Fe supplementation mitigates the negative effects of Cd stress in maize, supporting plant physiology and increasing the effectiveness of phytoextraction in soil remediation.

Global variation of the angiosperm ionome

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Most plants require 17 essential elements to complete their life cycle: nine macronutrients (H, C, O, N, K, Ca, Mg, P and S) and eight micronutrients (Cl, B, Fe, Mn, Zn, Cu, Ni and Mo). Some plants require additional nutrients, known as beneficial elements such as Si, Al, V, Na, etc. The diversity of mineral nutrition in vascular plants has received relatively little attention. One representation of plant strategies in mineral nutrition is the ionome (or elementome), consisting of all the mineral nutrients and other trace elements present in an organism. In order to capture global variation in seed plant ionomes, 463 wild and herbarium were sampled to represent almost all angiosperm families and all gymnosperm families. 50 elements were measured by ICP-MS. Variations in the concentrations of individual elements are discussed, particular with regard to hyperaccumulation. Plants are considered to hyperaccumulate a given metal when its concentration is 100-1,000 times higher than the normal level found in plants. Is this threshold valid? Do hyperaccumulating plants represent distinct categories? What about elements that have received little investigation? Preliminary answers will be drawn from this pilot dataset.

Functional resilience of rhizosphere microbiota in a legume species under long-term mercury exposure

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Mercury (Hg) is a globally pervasive toxicant and persistent environmental pollutant that disrupts key biological processes, reduces soil biodiversity, and threatens food safety through trophic transfer and bioaccumulation. This study examined the structure and functional adaptation of bacterial and fungal communities in the rhizosphere of *Lotus tenuis*, a Hg-tolerant legume, along an in situ gradient of Hg contamination (40 - 1964 mg kg^{-1}). Microbial communities were profiled in both rhizosphere and bulk soils from Hg-impacted and uncontaminated sites. While taxonomic composition varied significantly between contaminated and control soils, alpha diversity remained stable. This pattern supports the stress gradient hypothesis, whereby facilitative microbial interactions under toxic conditions maintain community richness despite environmental filtering. The *L. tenuis* rhizosphere under Hg stress was enriched in functionally relevant taxa. *Pseudomonas* spp. and *Streptomyces* spp., known for metal resistance and metabolic plasticity, were markedly more abundant. Rhizobial plant-growth promoters such as *Mesorhizobium* and *Shinella* were prevalent, alongside drought- and metal-tolerant taxa including *Nocardioides* and *Skermanella*. Fungal communities showed increased abundance of arbuscular mycorrhizal fungi (Glomerales) and dark septate endophytes (*Darksidea*, *Acrocalymma*), both of which may enhance host plant tolerance by improving nutrient and water acquisition. Functional gene analysis revealed the presence of the mercury reductase gene merA in 85% of rhizospheric samples from contaminated soils, but not in controls, suggesting active microbial detoxification via enzymatic reduction of Hg^{2+} to $Hg(Hg^0)$. Furthermore, specific fungal taxa such as *Septoglomus*, *Dioszegia*, and *Articulospora* were positively correlated with elevated Hg accumulation in plant shoots, implying roles in Hg mobilization or uptake. This study demonstrates the compositional resilience and functional versatility of rhizospheric microbiota under Hg contamination and identifies microbial taxa with potential utility in microbially-assisted phytoremediation strategies.

HY5 regulates cadmium tolerance and accumulation in Arabidopsis roots

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Cadmium (Cd) is a highly toxic environmental pollutant that severely impairs plant growth, particularly affecting root development. While Cd exposure reduces root growth, the molecular mechanisms underlying these responses remain poorly understood. This study investigates the role of ELONGATED HYPOCOTYL 5 (HY5), a bZIP transcription factor, in regulating Cd tolerance and accumulation in *Arabidopsis thaliana* roots.

Transcriptomic analysis of root tips revealed enrichment of bZIP transcription factor binding sites in Cd-responsive genes, with HY5 target genes consistently enriched across conditions. Functional validation using *hy5* mutants demonstrated enhanced Cd sensitivity compared to wild-type plants, with mutants showing greater root growth inhibition under Cd stress. Importantly, *hy5* mutants accumulated higher Cd concentrations in roots and shoots, accompanied by increased phytochelatin levels, indicating elevated cytosolic Cd concentrations. RNA-Seq analysis of *hy5* mutants further revealed dysregulation of genes involved in cell wall organization, cell differentiation, water and xenobiotic transport, flavonoid metabolism, and oxidative stress responses, underscoring HY5's role in maintaining root growth under metal stress.

This study uncovers a novel role for HY5 in limiting Cd accumulation and promoting tolerance, emphasizing HY5 as a central regulator in plant responses to environmental stress.

Keywords**:** metal homeostasis, cadmium, RNA sequencing, ELONGATED HYPOCOTYL 5.

Identification of nickel-binding proteins in the nickel hyperaccumulator species *Noccaea caerulescens*

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Nickel (Ni) is a micronutrient essential for plant metabolism, notably as a cofactor for urease, an enzyme involved in nitrogen metabolism, and for certain Ni-dependent glyoxalases participating in cellular detoxification pathways. Trace amounts of Ni are sufficient to support plant growth and development, whereas moderate concentrations of Ni in the soil can be toxic to most plant species. Remarkably, a limited number of plant species, referred to as Ni-hyperaccumulators, have evolved mechanisms to tolerate and accumulate substantial amounts of Ni in their foliar tissues, enabling them to thrive in Ni-rich soils. To gain deeper insight into the molecular basis of Ni tolerance, accumulation, and its essential cellular functions, we explored the hypothesis that previously uncharacterized Ni-binding proteins (NiBPs) may contribute to these processes. To identify novel plant NiBPs, we developed a metalloproteomic approach using the Ni-hyperaccumulator species *Noccaea caerulescen* Cira accession). Plants were exposed to high Ni levels, and the leaf soluble proteome was sequentially fractionated by ion exchange and metal-based affinity chromatography. The Ni content of each protein fraction was quantified by inductively coupled plasma mass spectrometry, allowing the identification of several pools of NiBPs. Then, Ni enriched protein fractions were sequenced by tandem mass spectrometry to identify the associated proteins. The application of stringent selection criteria (i.e. intrinsic MS/MS criteria combined with specific criteria) led to the identification of novel candidate NiBPs. Ongoing biochemical characterization of identified proteins is currently underway to confirm their Ni-binding capacity. Further *in planta* genetic analysis will elucidate their potential role in Ni homeostasis.

How do plants find and manage their zinc?

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Zinc (Zn) deficiency is a global issue, highlighting the urgent need to explore diverse approaches for Zn biofortification in plants, which serve as the primary source of this essential nutrient. The heterogeneous distribution of Zn in soil complicates its uptake and distribution by plants. Although many studies have highlighted the phenomenon of Zn-tropism in plants, the mechanisms underlying this process remain unclear. Our project aims to uncover how plants determine the direction of growth in soils with uneven Zn distribution and how they subsequently distribute Zn within their organs.

Our research focuses on *Nicotiana tabacum* L. as a model plant. We cultivate the plants under Zn-deficient conditions using agar medium (¼ Knop solution with no added Zn). Once the root systems reach the desired size, we introduce a localized Zn source (¼ Knop agar medium supplemented with 20 μM Zn) and document the resulting growth patterns. Moreover, we employed X-ray spectroscopy to monitor the kinetics of Zn localization and quantify its concentration within the root system.

Our results showed that approximately 60% of plants grown under Zn-deficient conditions alter root growth direction toward the Zn source. These results suggest that specific root zones are responsible for sensing and uptaking Zn, distinct from root regions that do not contact the Zn source. This is a highly dynamic process, and a deeper understanding could enhance Zn uptake efficiency in plants. Identifying the transporters involved in Zn distribution throughout the root system and into the shoots could enable the development of plants with increased Zn content in edible parts.

Potential of organic amendments to restore fertility and functionality of a contaminated soils at a former wood preservation site

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Organic amendments can mitigate the effects of metal(oid) contamination, allow the cultivation of biomass plant species, and restore key soil ecological functions. However, long-term effectiveness of this practice remains an open question. This study reports the effects of five organic amendments, namely fresh pig manure (PM), biodigested pig manure (PD), compost (C), compost pellets (P), and green waste compost (G), five years after the amendment of a Cu-contaminated soil at a former wood preservation site. Impacts on physico-chemical soil properties, metal bioavailability, biochemical activity and microbial community structure, plant growth and soil fertility were evaluated.

Main results were that all amendments improved physical and chemical properties, increased enzyme activities involved in C, N, P and S decomposition and mineralization, and increased the bacterial diversity, and increased the yield of winter barley as compared to the non amended soils. The observed effects could be ascribed to the significant reduction of the bioavailability of viarious metals such as Cd, Ni, Pb, and Zn, compared to untreated control plots, whereas the observed decrease in Cu availability was not significant due to the high spatial variability in the Unt plots. The compost (C) and compost pellets (P) amendments were enriched with microbial species often associated with soil fertility and good plant yields, indicating an overall significant improvement of soil health.

Results support the use of organic amendments as a sustainable approach for the phytomanagement of metal contaminated soils, capable of reducing the metal availability while enhancing microbial activity and soil fertility.

Trace elements in soils and plants

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Many soils around the world are contaminated with numerous metals. Those metals are deposited to soils mainly due to anthropogenic activities and pose a significant threat to human health via the mobilization of metals from the soil solid phase to the soil liquid phase (e.g., pore water and groundwater), leading to the soil-plant transfer of metals.

This presentation is aiming at (a) discussing the phytoavailability of metals as affected by various soil parameters, and by plant defense mechanisms related to uptake and translocation; (b) examining soil and plant indices related to TE phytoavailability; (c) clarifying the challenges and problems related to phytoremediation; and (d) exploring the often encountered discrepancies of lower-than-expected metal toxicity.

As for soil, the effect of pH, redox potential, clay and organic matter contents, as well as aging of elements will be discussed. The latter is a key factor in interpreting the observed lower than expected toxicity to plants in real field conditions. This is because the discrepancy is very often generated by growth experiments that expose plants to metals directly from metal-laden solutions or by studies that spike soils with metals without proper aging before planting. Also, the behavior of metals depends on their nature and quantity. As for plant, metal absorption or exclusion is highly related to species-specific defense mechanisms developed by plants so that they are exposed to metal-induced stress. These mechanisms address metal exposure by operating both outside and inside the plant body; outside with the assistance of root exudates, and the rhizosphere microflora, and inside with selective translocation and storage processes.

Functional Changes in the Family of Type 3 Copper Proteins

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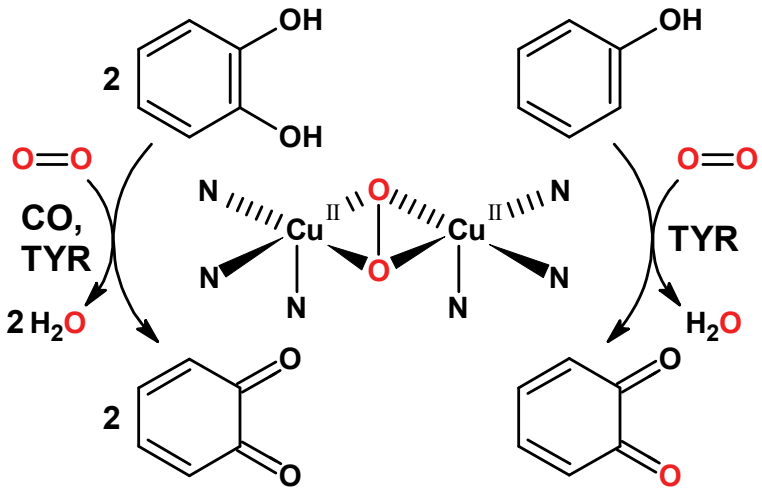
Tyrosinases (TYRs) are a family of copper-containing metalloenzymes that are present in all domains of life. TYRs catalyze the reactions that start the biosynthesis of melanin, the main pigment of the animal kingdom, and are also involved in the formation of the bright colors seen on the caps of mushrooms and in the petals of flowers. TYRs catalyze the *ortho*-hydroxylation and oxidation of phenols and the oxidation of catechols to the respective *o*-quinones. They only need molecular oxygen to do that, and the products of TYRs—*o*-quinones—are highly reactive and will usually react with the next available nucleophile. This reactivity can be harnessed for pharmaceutical applications as well as in environmental and food biotechnology. The majority of both basic and applied research on TYRs utilizes "mushroom tyrosinase", a crude enzyme preparation derived from button mushroom (*Agaricus bisporus*) fruiting bodies. Access to pure TYR preparations comes almost exclusively from the production of recombinant TYRs as the purification of these enzymes from the natural source is usually very laborious and plagued by low yields. Here an introduction into the biochemistry of the enzyme TYR will be given, followed by an overview of available structural data of TYRs, the current model for the catalytic mechanism, and a review of the applications of TYRs.



Scheme 1: Reactions catalyzed by TYR and catechol oxidases (CO). For each oxygen molecule consumed, one molecule of a phenolic substrate or two molecules of a catecholic substrate are converted to the respective *o*-benzoquinone.

TYRs have recently gained increasing attention in an environmental context due to their ecosystem-shaping effects. They are ubiquitously encountered in soils and have been proposed to be involved in plant symbiosis, the protection against competing microbes and secondary plant metabolites, and the synthesis of melanins, which act as protectants against oxidants, heat, enzymatic hydrolysis, and phagocytosis.

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Comparative analysis of role of iron in cadmium induced stress responses in *Zea mays*, *Solanum lycopersicum*, and *Cannabis sativa*

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Introduction:

Cadmium (Cd) contamination of agricultural soil poses a significant threat to the functioning of plants, particularly crops such as maize (*Zea mays*), tomatoes (*Solanum lycopersicum*), and hemp (*Cannabis sativa*). Despite species-specific differences, all three exhibit similar impairments in photosynthesis and redox homeostasis when exposed to Cd. It has been demonstrated that Cd competes with iron (Fe) at the transporter level, thereby reducing its availability for critical processes such as chlorophyll biosynthesis, electron transport, and antioxidant defence. As Fe is essential for enzymes such as Fe-superoxide dismutase and catalase, a deficiency in Fe has been shown to amplify oxidative stress. Understanding the molecular basis of Cd-Fe interactions reveals how plants maintain metal homeostasis under stress. This knowledge could help identify regulatory nodes and enhance the resilience of crops in cultivated soils.

Methods:

The experiments were conducted on hydroponically cultivated seedlings of maize, tomato, and hemp. The chlorophyll, flavonol, and anthocyanin content was measured using the Dualex Scientific+ pparatus (Force-A, Orsay, France). Leaf area analysis was performed using a laser leaf area meter (model CL-202; Bio-Science, USA). The activity of antioxidant enzymes (CAT and SOD) and the level of stress markers (H_2O_2 and MDA) were measured spectrophotometrically.

Results and Conclusion:

The results obtained allow to conclude that higher iron concentrations have a mitigating effect on the oxidative stress level associated with the presence of cadmium. In the case of hemp and maize, this effect was observed for both increasing CAT and SOD enzyme activities and decreasing levels of oxidative stress markers.

Treasure from trash: mining critical elements from secondary sources using plants

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The rising global demand for critical elements such as nickel (Ni), cobalt (Co), and selenium (Se) necessitates sustainable recovery strategies. Agromining, which uses hyperaccumulator plants to extract metals from enriched substrates, offers a promising solution. This study tested the recovery of Ni, Co, and Se from mine wastes amended with sewage sludge (SS) using *Odontarrhena chalcidica*, *Berkheya coddii*, and *Astragalus bisulcatus*, respectively. Mine wastes from three Italian sites (identified as PRIN, CAMP, NORD) were treated with 0, 15, and 30 kg/m^2 SS, the three hyperaccumulators were then cultivated on these substrates under field conditions. Subtrates metal concentrations reached up to 1500 mg/kg for Ni, 200 mg/kg for Co, and 25 mg/kg for Se, under SS30 treatments. SS amendments significantly boosted biomass (2-fold for *O. chalcidica* and *B. coddii*; 9-fold for *A. bisulcatus*) but reduced hyperaccumulation efficiencies by 33 - 74%. Annual Co and Se yields improved with SS application (Co: 11.5 to 30.6 mg/m^2/year; Se: 4.5 to 14.5 mg/m^2/year), while Ni yield decreased (1140 to 670 mg/m^2/year). SS addition increased Ni and Co bioavailability, while *A. bisulcatus* reduced Se availability. Results demonstrate that hyperaccumulator cultivation on SS-amended mine wastes can enhance critical element recovery, particularly for Co and Se. However, optimizing the SS/mine waste ratio is essential to balance biomass production with efficient metal accumulation. These findings advance the development of agromining as a tecnology for critical element recovery from waste materials.

Beyond the vacuole: extracellular release of thiol-peptides in Populus alba under cadmium stress

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Cadmium (Cd) is a highly toxic heavy metal, whose widespread presence in the environment poses a serious threat to plant health. Plants counteract Cd toxicity through detoxification strategies involving thiol-containing ligands such as glutathione (GSH) and phytochelatins (PCn), which chelate Cd and mediate its vacuolar sequestration. Recent findings in the liverwort *Marchantia polymorpha* L. also revealed the extracellular release of thiol-peptides, suggesting a novel detoxification mechanism beyond intracellular ones. In this study, we investigated whether a similar strategy is employed by *Populus alba* L. (Villafranca clone), a Cd-tolerant woody species with phytoremediation potential. Using a validated HPLC-ESI-MS-MS method, we quantified thiol-peptides in plants exposed to Cd. PCn levels, especially PC2, increased significantly under Cd stress, primarily in roots. GSH levels also showed an initial rise, but then decreased, likely due to oxidation or conversion into PCn. Notably, both GSH and PCn were also detected in the culture medium, as extracellular thiol-peptides. Inhibition experiments using sodium orthovanadate, a plasma membrane ATPase inhibitor, strongly suppressed GSH efflux and partially reduced PCn release, possibly suggesting distinct transport routes. These findings confirm that *P. alba* performs a dual detoxification strategy: intracellular chelation and vacuolar sequestration, together with extracellular export of thiol-peptides. Taken together, our results suggest that the extracellular release of thiol peptides under Cd stress, previously demonstrated in *M. polymorpha*, is evolutionarily conserved in vascular plants. This highlights a possibly ancient and widespread mechanism in land plants to mitigate metal toxicity through both intracellular and extracellular thiol-peptide-based detoxification.

Does metal deficiency affect Cd accumulation in cacao?

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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. In particular the relationship between nutrition in essential metals and Cd accumulation is not clear. In this work we studied cacao samples from trees growing on contrasted soils. Total elemental concentrations were determined in soils, soil chemical extracts and cacao plant organs, and elemental distributions in roots and branches were imaged by LA ICP MS. Results suggest an impact of mineral deficiency and Cd accumulation and distribution. Further studies in controlled conditions should be conducted to clarify the mechanisms.

Multi-scale approach shedding light on the ionome-protein interface in soybean seeds

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Soybean seeds are a major source of protein in both human and animal diets. Recent evidence has shown that protein biosynthesis and accumulation within seed tissues are intricately dependent on the availability of mineral nutrients, such as sulphur (S) and zinc (Zn). Nevertheless, the spatial dynamics of these protein-elemental interactions remain poorly understood. In this scenario, we combined the unique beamline capabilities of the Brazilian and Canadian Light Sources and employed synchrotron-based Fourier-transform infrared spectroscopy (FTIR), X-ray fluorescence (XRF), X-ray absorption near edge structure (XANES), and micro-computed tomography (μ-CT) to characterise the distribution of proteins, Zn, S, as well as the sub-cellular structure and the S speciation in soybean seed genotypes exhibiting contrasting total protein content, either subjected or not to a time-specific exogenous S and Zn top-up supply. Our results revealed a spatially and quantitatively conserved association between the protein-characteristic amide-I FTIR band and Zn intensity in the cotyledonary cells, i.e., the primary sites of protein storage within specialised vesicles. It also demonstrated the efficiency of top-up supply on the increase of S and Zn content in these cells. Furthermore, it showed that S-rich regions in the cotyledon were primarily bound to methionine and cysteine sulphur-amino acids. Altogether, our findings highlight the feasibility of coupling multiple synchrotron-based techniques and shed light on the functional role of S and Zn localisation in protein biosynthesis in soybean seeds, suggesting the potential of mineral-based approaches for generating more nutritious food.

Cadmium-induced impairment of photosynthesis in tomato (*Solanum lycopersicum*): the regulatory role of iron homeostasis

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Introduction:

Cadmium (Cd) is a non-essential toxic metal with well-documented phytotoxicity. The effect of Cd is manifested through the disruption of the structure of chloroplasts, the inhibition of the biosynthesis of chlorophyll, and the interference with photosynthetic electron transfer. Iron (Fe) is an essential micronutrient for plant growth, actively participating in vital metabolic processes. Fe is an essential component in the biosynthesis of photosynthetic pigments and numerous proteins that are closely associated with both photosystems. Consequently, Fe-nutritional status has been implicated in influencing photosynthetic reactions and electron transfer. The close association of Fe and Cd in plants is attributable to the similarity of their chemical properties and the shared use of transporters. This prompts the hypothesis that Fe has the capacity to mitigate the toxic effects of Cd on tomato plants, with particular reference to processes associated with photosynthesis.

Methods:

All the experiments were conducted on tomato (*Solanum lycopersicum* L.) seedlings cultivated hydroponically. Chlorophylls' level was determined using a Dualex Scientific+ (Force-A, France). Photosynthetic efficiency was analysed using the FMS-300 chlorophyll fluorimeter (Hansatech Instruments Ltd., England). Gas exchange analysis was performed with an infra-red gas analyzer (LCpro SD, ADC Bioscientific, UK) through measurements of saturated photosynthesis rate, transpiration rate, and stomatal conductance.

Results and conclusion:

In the presence of Fe in elevated concentrations, a decrease in the toxic effect of cadmium on the efficiency of the photosynthesis process in the tested plants was observed. This may indicate the mitigating effect of iron on cadmium-induced stress in tomatoes.

Co-remediation of multi-contaminated soils under multiple pressures: a long road ahead

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The success of phytoremediation of multi-contaminated soils is linked to the ability of the plant species used to establish, develop and rapidly produce significant plant biomass, i.e. the physiological response of the species to abiotic stress. This requires both the ability of the plant to tolerate persistent organic pollutants and the ability to phytoextract trace metal elements. In this context, metal-hyperaccumulating plants appear as potential candidates. However, in the context of large-scale development of phytoextraction, the plant may be exposed to stresses other than the presence of metals, such as the presence of organic co-pollutants, high salinity, or extreme soil pH. Our work in recent years has therefore focused on the response of the Cd, Ni and Zn hyperaccumulator *Noccaea caerulescens* to a multistressful environment. Through a series of experiments under field and controlled conditions, we evaluated the effects of different abiotic stresses on the development and growth of two contrasting *N. caerulescens* accessions (one non-metallicolous and one metallicolous), focusing on root development and antioxidant response. We also investigated whether the application of phytohormones could alleviate stress and protect plants during phytoextraction. Our results indicate that, in addition to contaminants, other abiotic stresses such as salinity and drought may limit the implementation of hyperaccumulator plants on multicontaminated sites. It is therefore necessary to consider these stresses in the development of phytoremediation strategies. The application of phytohormones seems to be an effective way to improve plant establishment and recover phytoextraction potential, although we observed an interaction between plant accession, type of stress and type of hormone.

Is the way of Fe removal from chloroplasts a form of ferritinophagy?

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Autophagy is essential in the turnover of cell components. In animals, ferritinophagy, targeting ferritins an important way for intracellular iron mobilization. In plants, ferritins are targeting chloroplasts and mitochondria, whereas the majority of cellular iron is found in chloroplasts. Little is known, however, on the iron recycling from chloroplasts. Accumulation of ferritins under developmental senescence has been long described, the connection between ferritins and iron remobilization has not been fully revealed. In *Arabidopsis thaliana* Col-0 leaves iron content of chloroplasts, and the iron peak at plastidial loci gained by low energy X-ray fluorescence microscopy (LEXRF) indicated strong coincidences: a peak accumulation occurred at early senescence that disappeared in later stages. Appearance of ferritins is, however, a rare event found exclusively at early senescence. Expression and protein amount of of FERs also indicated the same pattern. In autophagosome defective (atg5.2, atg7.2, ati1) lines, iron accumulation was revealed at plastidial locations associated increased plastidial Fe content, FER expression and protein amount. Although we have no data on any co-localization of ferritins to autophagosomes, data at the current stage suggest that ferritinophagy might be the way of iron recycling from plastids during developmental senescence.

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How do the rare earth elements effect plant development?

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This study focuses on the effects of rare earth elements (REEs) on maize (*Zea mays*) growth under two contrasting water regimes: standard irrigation and drought conditions. Rare earth elements, though traditionally considered non-essential for plant development, have recently gained attention due to their potential to enhance crop productivity and stress tolerance. In this experiment, selected REEs were applied to maize plants to evaluate their influence on physiological and morphological parameters, including plant height, biomass accumulation, chlorophyll content, and root development. Under standard irrigation, tested REEs (cerium, erbium and lanthanum) were observed to promote moderate decreases in plant growth and vigor, suggesting an inhibitory effect on metabolic processes. Under drought stress, the impact of REEs became more pronounced. Treated plants exhibited lower drought tolerance, reflected in lower water retention, formerly wilting, and lower biomass compared to untreated controls. These findings indicate that testes REEs probably don't play a role in enhancing plant resilience to water scarcity by modulating stress response pathways. However, further research is needed to understand the long-term effects of REE application, optimal dosages, and their environmental safety. This study provides a foundation for future investigations into the practical use of REEs in improving crop performance under variable water availability.

Control of zinc availability in plant cells

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Zinc (Zn) is an essential micronutrient for all living organisms. 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. Zinc acts as a structural or catalytic cofactor in nearly 10% of the proteins in eukaryotes. The cytosolic buffered available zinc concentration, $[Zn^{2+}]$cyt, is a critical parameter that must be maintained within a narrow range in the cytoplasm to ensure proper protein metalation and avoid toxicity. Recent findings indicate that Zn uptake in plant roots is regulated by F-bZIP transcription factors that directly perceive $[Zn^{2+}]$cyt. In plant cells, the vacuole and the cell wall serve as high capacity buffers, allowing metal storage and reuse. We have used eCALWY fluorescent nanosensors to monitor dynamically $[Zn^{2+}]$cyt in roots of the model plant Arabidopsis thaliana. We grew plant roots in microfluidic devices allowing long-term microscopic observation and a tight control of $[Zn^{2+}]$ in the perfusion medium. Using this experimental set up, we measured the resting $[Zn^{2+}]$cyt and its changes upon increase in changes in $[Zn^{2+}]$ext in root cells. We compared wild type Arabidopsis with mutants impaired in the plasma membrane Zn efflux pump, HMA4, or in MTP1, the vacuolar transporter responsible for Zn sequestration in the vacuole. We will discuss the network controlling Zn homeostasis in plant cells.

Variability in response of selected willow species and ecotypes to cadmium, antimony and nickel

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Heavy metal and metalloid pollution is one of the crucial factors that negatively affect the environment and quality of our life nowadays. Proper and environmentally suitable land use is often not possible without costly remediation issues. Therefore, there is an urgent need to find out various strategies and solutions for sustainable soil decontamination or appropriate land use. One of the strategy for decontamination of land using plants is processes of phytoextraction. However, recent decades showed that not all contaminates sites might be easily decontaminated due to extreme high doses of contaminants presented on sites or due to unfavourable conditions of local environment that does not allow to survive metal accumulating plants on sites. Therefore, alternative strategy might be phytostabilisation of contaminated sites using tolerant species and varieties of plants. In the recent study we investigated several variable species and ecotypes of willows (*Salix* sp.) and compared their response to elevated concentration of cadmium, antimony and nickel in hydroponics. We evaluated the biomass production, quantum yield of photosystem II and chlorophyll content as well as uptake of these three contaminants in aboveground tissues to identify the proper isolates that might be used for extraction of metals, or alternatively that are able to cope with contaminants by their restricted uptake, and, might be therefore rather use for phytostabilisation of metal(loid) contaminated soils.

Acknowledgements: This work was supported by the Slovak Research and Development Agency (grant numbers APVV-23-0318, and APVV SK-TW-24-0008), and by Slovak Grant Agency VEGA (grant number VEGA 2/0047/25).

Screening of fast-growing tree species for tolerance and accumulation of metal(loid)s in abandoned mining sites

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Slovakia has a long and rich history of mining and was once among the most important mining regions in Europe. Although mining activity has significantly declined in recent decades, environmental pollution in the form of waste rock dumps and tailings still persists. Toxic elements such as arsenic, antimony, lead, copper, and mercury continue to contaminate soil and water in affected areas. One possible way to reduce the risk of further contamination is through the stabilization of these elements in the environment and reforestation of degraded mining sites. Fast-growing tree species may assist in this process, as they have the potential to remediate contaminated soils through phytoextraction or phytostabilization.

We conducted a screening of several fast-growing woody species in former mining areas of eastern Slovakia, where elevated concentrations of toxic elements are continuously present. Soil and above-ground plant tissue samples were collected in the vicinity of Rudňany, Poráč, Poproč, Gelnica, and Slovinky. The samples were analyzed to determine concentrations of various elements, including heavy metals and metalloids. The levels of metal(loid)s in soil correlated with historical records of ore mining, reflecting the composition of the extracted mineral deposits. In several cases, elevated concentrations of specific elements were also detected in the above-ground parts of the plants. Selected plant species and ecotypes will be used in further experiments to evaluate their accumulation capacity and tolerance to metal(loid) stress. Acknowledgement: This work was supported by Slovak Grant Agency VEGA, grant 2/0047/25 and Slovak Research and Development Agency, grant APVV-23-0318. We thank Irena Mravíková for the technical support.

Crosstalk between iron and copper homeostasis in *Arabidopsis thaliana*

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Iron (Fe) and copper (Cu) are essential yet potentially toxic redox-active transition metals with interconnected metabolic pathways. Crosstalk between their homeostatic networks is therefore expected, but the underlying molecular mechanisms remain poorly defined. We will report our recent findings that CITF1 (COPPER DEFICIENCY INDUCED TRANSCRIPTION FACTOR 1), an established regulator of Cu homeostasis, physically interacts with FIT (FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR), the central regulator of Fe homeostasis in *Arabidopsis thaliana*. The CITF1-FIT complex promotes the expression of Cu uptake genes under Cu deficiency, correlating with the increased abundance of CITF1-FIT complex in these conditions. Proteasome-dependent degradation regulates the stability of CITF1 and FIT, and Cu deficiency delays CITF1 turnover, stabilizing FIT in a CITF1-dependent manner. Conversely, under Fe deficiency, the abundance of CITF1-FIT complex decreases, and CITF1 negatively affects Fe homeostasis by disrupting FIT interactions with bHLH38/39, thereby reducing the expression of the Fe uptake genes. Consistently, the citf1 mutant shows attenuated sensitivity to Fe deficiency and increased seed Fe accumulation. Finally, our data point to the existence of an unidentified CITF1-regulated Fe transporter(s) active under Cu deficiency. This research highlights the complexity of Cu/Fe transcriptional networks and opens new avenues for decoding Cu/Fe crosstalk mechanisms. We will also present findings showing that Cu can partially substitute for Fe in long-distance shoot-to-root signaling and that this event is orchestrated by the Fe/Cu transporter OPT3. Together, these findings bring us closer to untangling the complexity of Cu/Fe crosstalk used by plants to ensure balanced Cu and Fe nutrition.

Xenobiotics impact on cyanobacterial biomass viability and further usability as bioaccumulators of rare earth elements

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The growing environmental and industrial demand for rare earth elements (REEs) necessitates innovative and effective recovery methods, particularly the use of biological systems. In this study, we systematically evaluate the dual role of cyanobacterial cells in both biotransformation of xenobiotics and bioaccumulation of REEs, specifically gadolinium (Gd) and europium (Eu). Four cyanobacterial strains: *Limnospira indica*, *Limnospira maxima*, *Synechococcus bigranulatus* and *Leptolyngbya foveolarum* were employed for this purpose. Two xenobiotics: epoxymethyl dimethyl phosphonate and 1-phenylethyl acetate were selected to induce bioconversion in these strains. Ecotoxicity assays were performed to assess the impact of Gd and Eu on both pre- and post-bioconversion biomasses. Sub-lethal concentrations of REEs were determined using 96-well plate growth inhibition assays. After ecotoxicity tests, cyanobacterial biomasses were exposed to selected concentrations of Gd and Eu for 3 days. The experiments were conducted under controlled conditions in photobioreactors, monitored biomass growth (OD720) and dry weight over 72 hours. Inductively coupled plasma mass spectrometry analysis was used to quantify the accumulated REEs. The ecotoxicity test results showed strain-specific variations in REE tolerance, with all strains demonstrating higher resilience. Dry weight analysis showed increased biomass in the REE-treated cultures, suggesting that REEs can enhance cyanobacteria growth and subsequently increase resource recovery. Microscopic analyses confirmed no significant morphological changes between pre- and post-bioconversion biomasses. These findings highlight the potential of cyanobacteria to not only thrive under xenobiotic exposure but also to sequester REEs, benefiting applications such as the synthesis of high-value bioproducts (e.g., dimethyl (1E)-3-hydroxyprop-1-enylphosphonate and enantiomerically pure phenyl ethanol), resource recovery, and biofertilizer production.

Development and optimization of adapter for guided cryostream to be mounted on Oxford cryostream

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Work in cryogenic conditions is essential for obtaining valid data on metal speciation and distribution in biological tissues. The environment for the sample measuring in μXRF/μXANES cryotomography requires specific conditions that will prevent sample melting. However, existing commercial cryostreams have a general problem of producing vibrations, caused by turbulent gas flow, icing caused by air humidity and suboptimal temperature stability of the cooled object. Therefore, we developed a guided cryostream to prevent icing and vibrations, optimised for long measurements of μXRF/μXANES cryo-tomography. This was named "guided Cryostream" and is attached to the end of the commercial Cryostream. The main advantage of this cryostream guide is that it separates the sample from the environment by a design that keeps the sample in a very smooth laminar flow of pure cold nitrogen. This became possible by shielding the inner nitrogen stream with the temperature below -120°C from the environment by two layers of X-ray transparent windows. In between these, the outer N_2 stream flows at room temperature. In this way, the outer stream is prevented from mixing with air and in a way that prevents icing of the samples and the windows. The vibrations problem was solved by optimisations of the tube walls shape to achieve laminar flow. The problem of icing of the outer window was solved by optimisations of the walls thickness and interdistance. It has been used successfully for all μXRF and μXANES tomography beamtimes of the team since summer 2022. It was published as part of the first study where it was used, and where it was an essential prerequisite for achieving the high data quality (Morina et al. 2025).

Morina F, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujić B, Falkenberg G, Küpper H (2025) How eriophyid mites shape metal metabolism in leaf galls on Tilia cordata. New Phytologist 246, 2222-2242. https://doi.org/10.1111/nph.70103

Understanding the relationships between the accumulation of biomass and that of contaminants and nutrients in wheat grains

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Wheat is a major staple crop and an essential source of calories, proteins and inorganic nutrients but it also accumulates trace contaminants like cadmium (Cd), nickel (Ni), arsenic (As) and lead (Pb). The objective of this study was to gain a better understanding of the relationships between the concentration of contaminants and nutrients in wheat grains and the grain biomass. We report a set of two experiments on one genotype of each bread and durum wheat for which the trace elements and the biomass accumulation in grains were modified by the following individual treatments. The phloem pathway was stopped by girdling the spike peduncle with hot air at mid grain filling stage. The flag leaf blade was cut at anthesis to reduce the phloem pathway and also to alter the xylem to phloem exchanges at the first node level connecting the stem to the rachis. The xylem pathway was restricted at anthesis by cutting awns and for durum wheat an additional treatment consisted in covering the spike to reduce its transpiration. Grains dry matter and As, Cd, Ni, Pb, Fe, Zn , Ca, P, S, Ca, K, Mg, Cu, Mn, Mo contents were determined according the grain positions within each spikelet (lateral variability) and between spikelets (longitudinal variability). Results are discussed in the perspective of the trade-off required regarding the grain biomass and their micronutrient contents if the goal is to decrease the grain contents in contaminants.

Exploring the role of thiols as trace metal filter in arable soils

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While the role of thiols (R-SH) on detoxification and sequestration of trace metals in plants has been extensively researched, their function in soils in controlling trace metal phytoavailability remains to be elucidated. X-ray absorption spectroscopy data (XANES) indicates the presence of thiols in soils, likely derived from decomposed plant material. Furthermore, a few studies indicate that a considerable proportion of soft metals such as the contaminant cadmium can be complexed by soil thiols. However, the precise quantification of soil thiols and the binding of trace metals to soil thiols remains largely unexplored. Such data would be particularly relevant for aerated soils that are pertinent to crop production. The objective of this ongoing project is to determine the role of soil thiols on trace metal phytoavailability and crop trace metal uptake. It is hypothesized that soils with high concentrations of thiols may possess a significant capacity to filter cadmium from the micronutrient zinc, thereby increasing the zinc to cadmium ratio in the phytoavailable soil pool and consequently in crops. In order to test this hypothesis, we have started to develop methods that aim to quantify soil thiols, including synchrotron XANES and a wet-chemistry method (qBBr). These methods will be applied in soil incubation and plant growth trials to quantify the role of soil thiols on the phytoavailability and plant uptake of cadmium and zinc. The fundamental knowledge obtained will be used to develop soil management methods that aim to produce healthy crops that are rich in zinc and depleted in cadmium.

Cell wall as a multistress sensor in halotolerant and metallotolerant species treated with cadmium and salinity

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Plant cell wall is a dynamic structure that promptly reacts to environmental challenges. Significant rearrangements of its components and the changes in activity of cell wall-bound enzymes allow rapid response to external stimuli, conditioning the survival of the cell itself, as well as the entire plant organism. In natural conditions, plants are subjected to a wide range of stresses that often act simultaneously, influencing the growth and survival. As a defence strategy, numerous species develop structural and metabolic adaptations to specific ecological features of the habitats, enabling them to tolerate suboptimal doses of stress factors. We have focused our interest on cell wall as a multistress sensor in representatives of extremophytes, plants naturally adapted to inhabit extreme environments. This study aimed at comparing structural and metabolic rearrangements of the cell walls in Brassicaceae extremophytes: halotolerant *Lobularia maritima* and metal tolerant ecotype of *Alyssum montanum*. Plants cultivated in in vitro conditions were subjected to 5 μM Cd^{2+}, 50 mM NaCl and the combination of both. The responses on the level of cell walls were evaluated in the roots and shoots on the basis of changes in the activity of cell wall-bound enzymes, microscopic evaluation of cell wall structure and deposition of polymers, with the view of distinguishing phenomena specific for metal exposure, salinity and combined multistress. Detailed results are to be presented and discussed on the poster.

NtZIP5B and NtZIP5C genes: two co-participants in the orchestra playing under the direction of "The Zinc Deficiency"

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ZIP proteins are transmembrane transporters primarily of zinc, but in some cases also iron, manganese and copper ions. Plant genomes usually contain over a dozen ZIP genes. Since Nicotiana tabacum emerged from hybridization of two other tabacco species genomes (*N. sylvestris* and *N. tomentosiformis*), most of its genes are present in two copies, known as homeologs. While analyzing the reference mRNA tobacco sequences to identify its ZIP genes (NCBI database), we found not two, but four distinct sequences of ZIP5 gene. One of them (provisionally named ZIP5C), compared to the other three, has ORF shorter on the 5' end. That suggests the full gene sequence was yet to be properly identified. Importantly, our qPCR studies showed that NtZIP5C expression profile differs from that of NtZIP5A and NtZIP5. Based on sequences present in the parental genomes, we identified a putative full-length version of the ZIP5C gene, as well as its promoter. Both elements were subsequently cloned. Finally, we generated a tobacco line containing promZIP5C::GUS cassette. These plants were used to investigate promoter expression pattern in Zn-deficiency/Zn-sufficiency conditions.

At the conference, we will present the similarities and differences between the NtZIP5B and NtZIP5C genes, including nucleotide and amino acid sequences, expression levels (qPCR), and site-specific promoter activity patterns.

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Venue

On site

The meeting will be hosted at the University of South Bohemia and the Biology Centre (BC) of the Czech Academy of Sciences (CAS) in České Budějovice, Czech Republic. Both are located together at on a joined campus. **The conference will be in building C of the Faculty of Science of South Bohemian University, with the lectures taking place in hall C1 and the poster sessions and coffee breaks in the hall in front of this hall.**

For the welcome party and the daily lunches we will use the BC/USB campus facilities as shown on the map provided in the abstract book.

GPS

DD – decimal degrees: 48.9783207 N, 14.4477556 E

DMS – degrees, minutes, seconds: N 48° 58' 41.955' E 14° 26' 51.92''

International travel

České Budějovice is a large regional town (about 95 000 inhabitants) and the capital of the Region of South Bohemia (Jihocesky kraj). It is easy to reach by ground transportation as described in several variants below.

Train

From most neighbour countries, the most convenient and cheapest way of reaching České Budějovice is by train, using the European high-speed railway system until Prague main station (Praha hlavní nádraží = Praha hl. n.) or Linz and then a direct train from these stations to České Budějovice (which is in the middle of the train line connecting Prague to Linz). Travel times vary from the Express trains that take around 1.5 hours to the regular trains that take about 3 hours. Furthermore, the Express trains have onboard highspeed WiFi and offer some snacks and drinks while they cost the same as the regular trains.

Flying

Prague Airport Vaclav Havel (PRG).
Most airlines fly to Prague, although not always are direct flights available at suitable times.
From the airport, there are regular Airport Express Bus connections to Prague main railway station (60 CZK per person, please have cash ready!). Make sure you go to the Prague main train station (Hlavní nádraží); Prague has four larger railway stations and several smaller stations. Travelling time by bus is about 35-40 minutes; a taxi is usually not much quicker but much more expensive. Trains for České Budějovice leave quite regularly. Beware, the last express train to České Budějovice leaves at 21:58. Travel times vary from the Express trains that take around 2 hours to the regular trains that take about 3 hours. Furthermore, the Express trains have onboard highspeed WiFi and offer some snacks and drinks while they cost the same as the regular trains.
Alternatively, the RegioJet busses also stop regularly at the airport, and they connect directly to RegioJet busses to České Budějovice railroad station. It takes 45 minutes to the Prague RegioJet hub, then a 45 min wait, followed by a 2:15 journey to České Budějovice. Most of these busses have on-board WiFi, WC, and individual screens for films etc. The last RegioJet bus leaves the airport at 17:30. You have to reserve tickets in advance.

Although Prague Airport (PRG) seems the most logical choice, nearby airports include the Austrian airports of Linz (LNZ, 90 km) and Vienna (VIE, 215 km). Both have regular rail connections to České Budějovice. We would not recommend Brno Turany Airport (BRQ), because connections to České Budějovice are slow.

Private car

When travelling with several people and not too far a distance, time- and money-wise a shared car can be an interesting alternative. České Budějovice is easy to reach, although coming from Austria or Bavaria the last section in the Czech Republic will be mainly good quality national roads (“highways”) with only short sections of motorways. Coming from the north, there are mostly motorways, but beware of traffic jams in particular around Prague.

Visa

If you are from within the EU, no visa is needed. If you are from outside the EU, you might need a visa. Please check this with the Czech Embassy in your country. You have to do this early; this process normally cost time and you might need original confirmation of participation from us. In addition, still ongoing COVID restrictions might make this process even longer.

Accommodation

Neither the University nor the Academy has suitable on-campus accommodation. However, there are numerous possibilities nearby and in the town. The best is to check with booking.com or similar online booking websites. If you prefer smaller "bed and breakfast" pensions, which are usually not listed in booking.com and similar websites, you can find them on www.mapy.cz by typing in Ceske Budejovice and then zooming in using the "+" button on the upper right corner until close to the maximal zoom level. You will see that with each zoom level more of the accommodation symbols appearing – there are very many of those in town, usually they are good (sometimes excellent) quality and inexpensive. www.mapy.cz always displays the webpages or phone number of these pensions when you click on them - not all but many of the owners speak English or German! We recommend to book as soon as possible, because on Thursday August 26 the very popular national agricultural exhibition "The providing Earth" starts, and popular accommodation will be booked out soon.

Local travel·

Walking

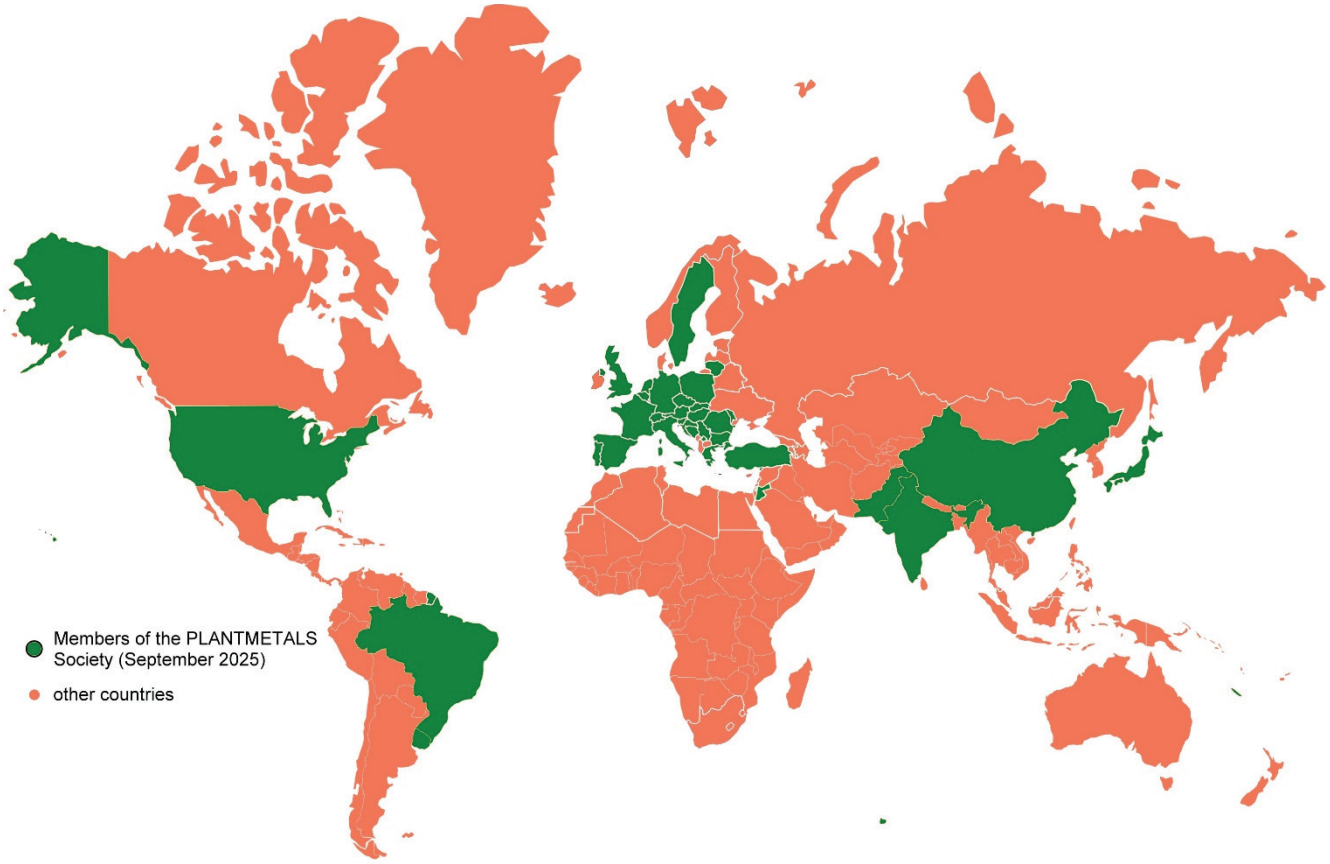
The BC is about 2 km from the historical centre of the town, to the main square it is 2.3 km on foot (36 min according to mapy.cz) via the shortest route or 2.8km (43 min) via a more scenic route along the "Stromovka" park.

Alternatively, you can use the local public transport; several lines regularly pass the BC (stopping point University):

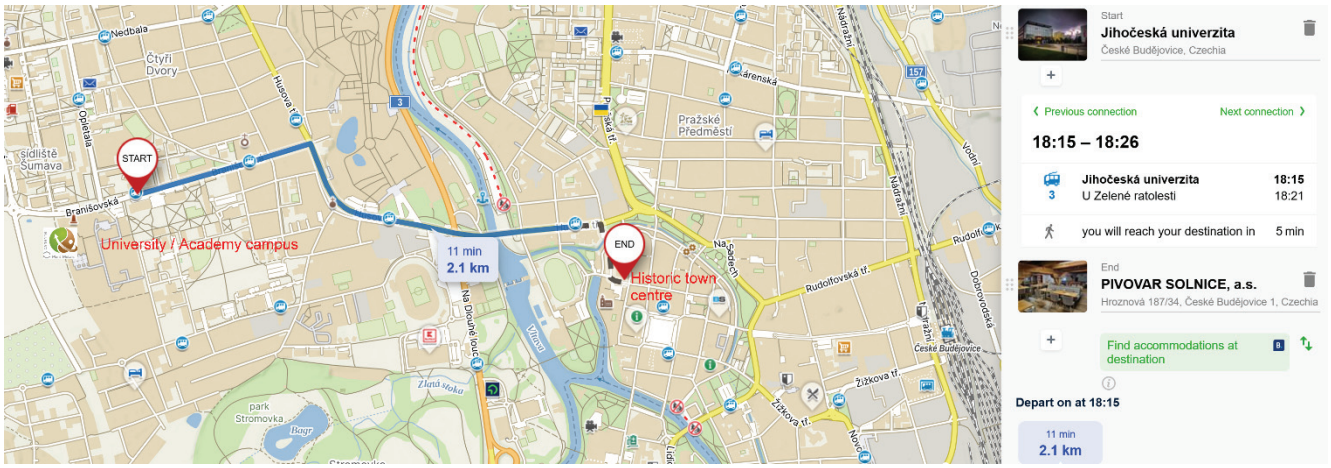
Autobus and Trolleybus

These two lines are going from the railway station to the western outskirts, passing the university stop. You can join this bus just a short walk to outside the historic center ring. The main connection is Line 3 to Máj, Antonína Barcala (to Nadrazi if going to town). This goes very frequently from early morning until late evening. Alternatively, or additionally depending on the location of your accommodation, you can also take lines 7, 15, or 45 and some long-distance buses to/from BC. Your accommodation provider can give you more information on this, or you can search for connections on https://www.seznam.cz/jizdnirady/.

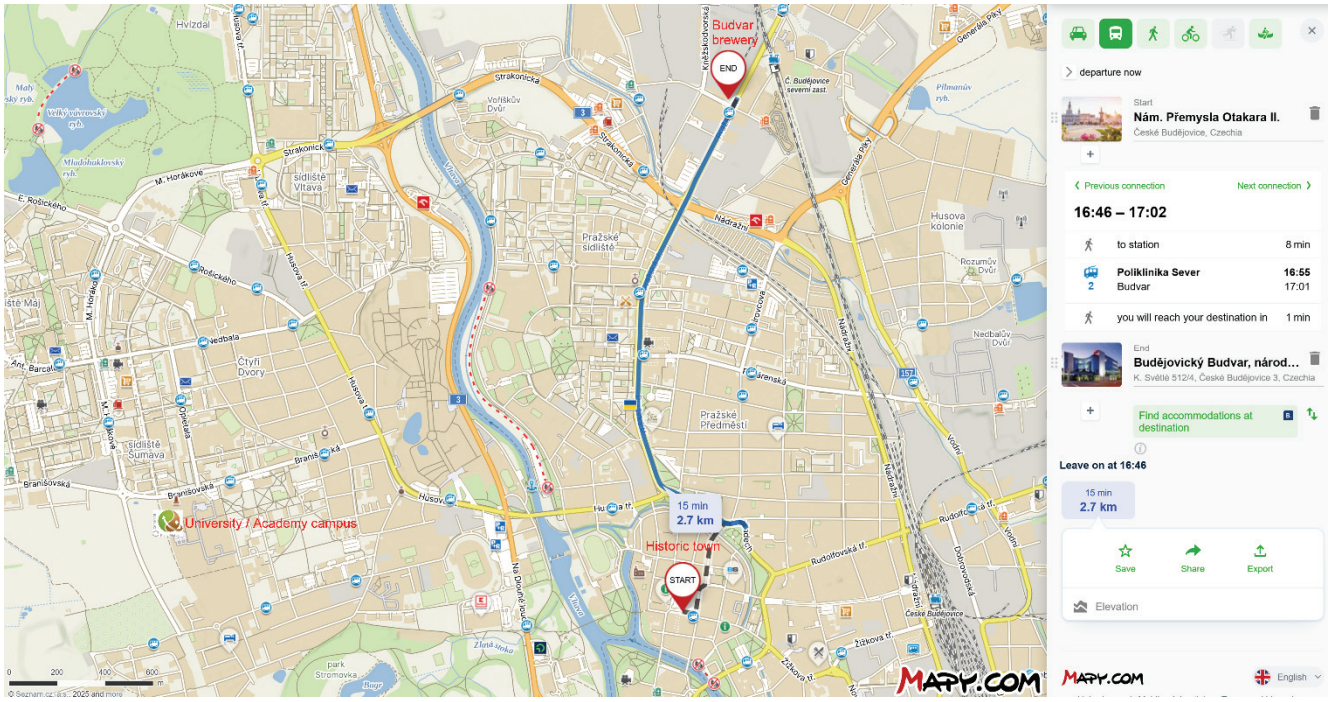
For coming to BC from town, private cars are NOT recommended due to limited parking space.


Members of the PLANTMETALS Society (September 2025)
other countries

Dinner on 10th of September: Transfer to Restaurant / Brewery “Solnice” from campus by bus #3



Excursion 12th of September: Start at the Tourist information, after tour of the historic town (2h) transfer by public bus #2 to Budvar Brewery




BIOLOGY CENTRE ASCR
Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice
Bus stop
Lectures & posters
Welcome party
Plant Metals
CA19116
Emy Destinové
J. Opletala
Větrná
Branišovská
Studentská
K. Fleischmanna
Buzulucká
Na Zlaté stoce
Lipová
Na Sádkách
Šípková
V Březinách
knihovna
menza
vysokoškolské koleje
baseball
Jihočeský vědeckotechnický park
Biologické centrum AV ČR
Zkušební pozemky Fakulty zemědělské a technologické Jihočeské univerzity
JČU Botanická vila
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