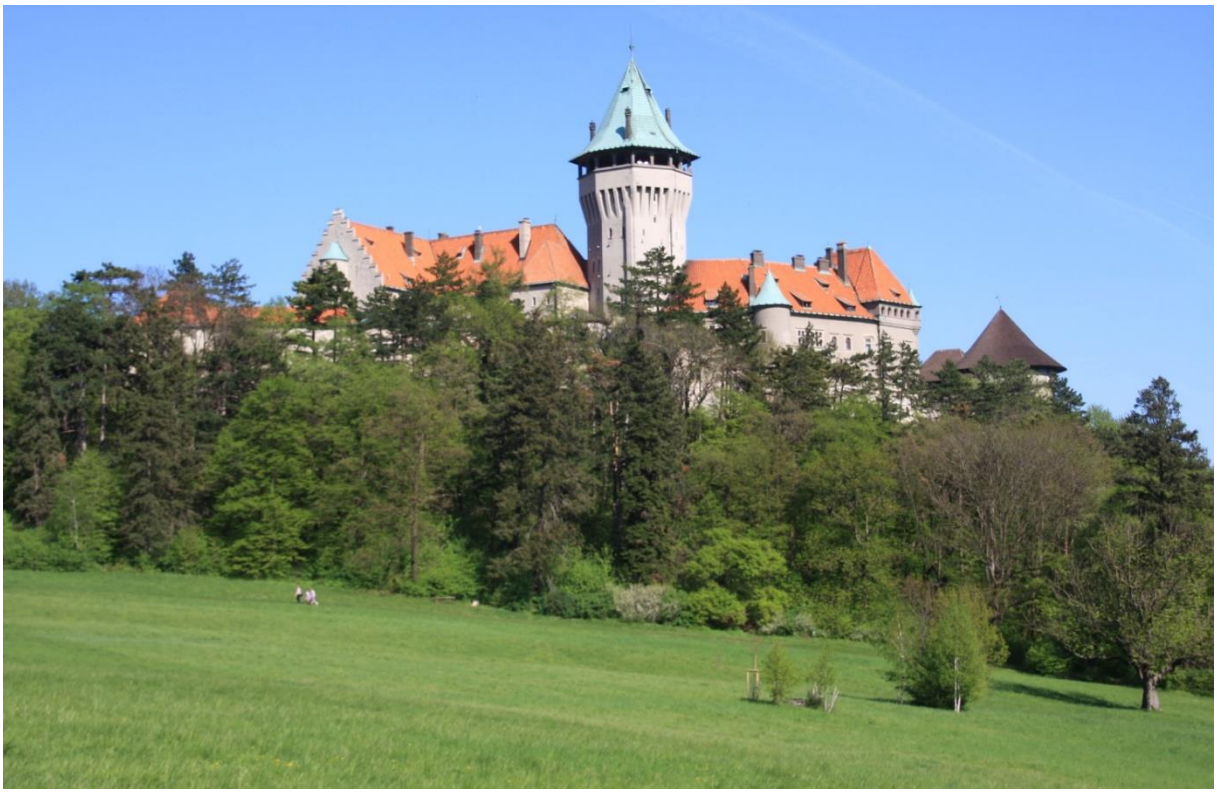

PLANTS, METALS & ENVIRONMENT

Smolenice Castle, Slovakia; April 13-14, 2023

Book of abstracts

COST Action 19116 meeting open for members of all workgroups organized with special focus to Action´s PhD students as organisers and participants

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COST Action CA19116

Plants, Metals & Environment

Spring meeting open for members of all workgroups organized with special focus to Action's PhD students.

Date: April 13-14, 2023

Location: Smolenice Castle near Bratislava, Slovakia

Deadline for registration: March 12, 2023
Abstract submission: March 12, 2023
Selection of talks: until March 20, 2023

- Key note speakers: Marie-Theres Hauser (AT) & Mark Aarts (NL)
- Lectures
- Poster session
- Workshop about manuscript and thesis writing
- Workshop about STSMs
- Conference field excursion to old contaminated mining site

More details on: https://plantmetals.eu

Organizing Institutions:

Plant Science and Biodiversity Centre of Slovak Academy of Sciences, Bratislava, Slovakia

&

Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia

&

Slovak Society of Botany, Slovakia

CA Action 19116 Grant holder Institution:

Biology Centre of the Czech Academy of Sciences,

Institute of Plant Molecular Biology, České Budějovice, Czech Republic

Organizing team:

Anna Kokavcová, Adriana Mišúthová, Jana Kohanová, Alexander Lux, Hendrik Küpper, Robert Dulfer & Marek Vaculík

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Conference program

Thursday, April 13, 2023

8:30 – 9:30 Registration

9:30 - 9:50 Opening

9:50 – 10:50 Keynote lecture 1 – Marie-Theres Hauser

10:50– 11:10 Anna Kokavcová

11:10 – 11:30 Hester Blommaert

11:30 - 14:00 Lunch break (including visit of Castle tower and Castle park)

14:00 - 14:20 Mohammad Imran

14:20 – 14:40 Mohammad Mukarram

14:40 – 15:00 Adriana Mišúthová

15.00 – 16:00 Poster flash talks

16:00 – 16:30 Coffee break

16:30 – 17:30 Poster session

17:30 – 18:30 Workshop – How to prepare a good scientific paper and PhD thesis (lead by Hendrik Küpper)

Friday, April 14, 2023

9:00 – 10:00 Keynote lecture 2 – Mark Aarts

10:00 – 10:20 Amir Maqbool

10:20 – 10:40 Marek Vaculík

10:40 – 11:30 – Workshop – Short Term Scientific Mission (STSM) – a good tool for young scientist to find a host lab (experiences from previous STSM grant holders and hosts and suggestion for future candidates – lead by Filis Morina)

11:30 Coffee break

12:00 Closing ceremony

13:00 – 18:00 Conference excursion to contaminated old mining site

(Locality with previous mining and processing of As-Sb-Au ores, bus will depart from the Castle – lead by Marek Vaculík)

WELCOME ADDRESS

Dear friends and colleagues,

It is our pleasure to welcome you at COST CA 19116 Spring meeting held at marvellous place of Smolenice Castle, Congress centre of Slovak Academy of Sciences, located 50 km from Bratislava at slopes of Malé Karpaty Mts. This meeting is open for members of all workgroups within our COST Action and is being organized with special focus to Action´s PhD students - both as organisers and participants. I am pretty sure that this meeting will be a perfect place for all attendees to present their results, discuss with other Action members and it will be a good place to get together people fascinated by world of metals and metalloids in plants and environment. Together with other members of local organizing team I wish you a pleasant stay in Smolenice and enjoy our early spring 2023 meeting.

Marek Vaculík

SMOLENICE, APRIL 12, 2023

THE ODYSSEY OF CADMIUM IN CACAO TREES

Blommaert Hester [1], Aucour Anne-Marie [2], Wiggenhauser Matthias [3], Moens Claudia [4], Telouk Philippe [2], Campillo Sylvain [1], Beauchêne Jacques [5], Landrot Gautier [6], Testemale Denis [7], Pin Serge [8], Lewis Caleb [9], Umaharan Pathamanathan [9], Smolders Erik [4] and Sarret Géraldine [1]

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Key words: cacao, cadmium, stable isotopes, XANES, imaging

Cadmium (Cd) concentrations in cacao often exceed food limits. The research on strategies to reduce Cd accumulation in cacao beans is currently limited by a lack of understanding of the Cd transfer pathways within the cacao tree. We elucidated the mechanisms that control Cd transfer in a high Cd accumulating cultivar by a combination of Cd stable isotope analyses and X-ray absorption spectroscopy. The latter is providing insights on Cd speciation whilst isotopic fractionation is increasingly used to infer Cd pathways in plants. Vegetative (roots, branches, leaves) and reproductive (placenta, nib (seed), testa (seed envelope), pod husk) tissues were collected from three genetically identical trees in a conservatory of cacao cultivars in Trinidad (soil Cd: 0.3 mg Cd kg-1). The plant Cd concentrations were 10-28 higher than the topsoil Cd concentrations and increased as placenta < nib < testa < pod husk < root < leaf < branch. Mass balance estimations indicated that the largest fraction (57%) of total plant Cd was present in the branches where it was primarily bound to carboxyl-ligands (60-100%) and mainly localized in the phloem rays and phelloderm of the bark. The retention of Cd in the roots was low, and light Cd isotopes were retained in the roots while heavier Cd isotopes were transported to the shoot, as observed in annual crops. The isotope fractionation data further revealed that the main contribution of nib Cd was from the phloem tissues of the branch rather than from leaf remobilization, contrastingly to annual crops. In the nibs, Cd was mainly bound to oxygen ligands (60-90%), with phytate as the most plausible ligand. This study extended the limited knowledge on Cd accumulation in perennial, woody crops and revealed that the Cd pathways are markedly different than in annual crops, which has implications for mitigation strategies.

Acknowledgements: This work was financially supported by the French National Research Agency program ‘Investissements d’avenir’ (ANR-15- IDEX- 02), and in the CNRS/INSU/EC2CO project CACAO. GS, HB and ES are working in the framework of the Program Hubert Curien “TOURNESOL” 2020–2021 (project n° 44274TC). HB, MW, ES, and MW are members of the COST Action CA19116 PLANTMETALS (COST, European Cooperation in Science and Technology, www.cost.eu). We acknowledge the review committees for the provision of beamtime at SAMBA (Soleil) and BM30 (ESRF).

ACCLIMATION TO MEDIUM-LEVEL NON-LETHAL IRON LIMITATION: ADJUSTMENT OF ELECTRON FLOW AROUND THE PSII AND METALLOPROTEIN EXPRESSION IN *TRICHODESMIUM ERYTHRAEUM* IMS101

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Key words: *Trichodesmium*; photosynthesis; HPLC-ICP-MS; PSII; iron; metalloproteins; µXRF

The aim of this study was to investigate how acclimation to medium-level, long-term, non-lethal iron limitation changes the electron flux around the Photosystem II of the oceanic diazotroph *Trichodesmium erythraeum* IMS101. Fe availability of about 5x and 100x lower than a replete level, i.e. conditions common in the natural environment of this cyanobacterium, were applied in chemostats. The response of the cells was studied not only in terms of growth but mechanistically via imaging fluorescence kinetic microscopy (FKM) with 0.3 ms time resolution and measurement of metal binding to proteins via online coupling of metal-free HPLC (size exclusion chromatography SEC) to sector-field ICP-MS. General increase of fluorescence has been observed, with the consequent decrease in the quantum yields of the PSII, while the efficiency of the electron flux between PSII and the PSI remained surprisingly unchanged. This indicates the ability of *Trichodesmium* to cope with a situation that makes assembling the many iron clusters in Photosystem I a particular challenge, as shown by decreasing ratios of Fe to Mg in these proteins. That Fe limitation had a negative effect on PSII at the same time may be because of its fast turnover. A broader view was obtained from metalloproteomics via HPLC-ICP-MS, revealing a differential protein expression pattern under iron limitation with a drastic down-regulation especially of iron-containing proteins and some increase in low MW metal-binding complexes.

Acknowledgements: The authors are grateful for being funded by the Ministry of Education, Youth and Sports of the Czech Republic with the co-financing from the European Union (grant "KOROLID", CZ.02.1.01/0.0/0.0/15_003/0000336), the COST Association (grant “PLANTMETALS”, CA19116) and the Czech Academy of Sciences (RVO: 60077344). Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA CZ LM2018140) supported by the Ministry of Education, Youth and Sports of the Czech Republic. We thank Bojan Vujic for 3D printing of sample holders and sample preparation tools for benchtop µXRF.

CHARACTERIZATION OF THE ACTIVITY OF THE IRT1 TRANSPORTER INVOLVED IN METAL UPTAKE IN THE HYPERACCUMULATOR SPECIES *NOCCAEA CAERULESCENS*

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Key words: heavy metals, hyperaccumulator plant, IRT1, transporter, ZIP

Transition metals like Fe, Ni and Mg are essential micronutrients for cell development because they are cofactors for many important proteins. However, they become highly toxic at high concentrations, leading to the increment of oxidative stress and the competition with other nutrients. That is why organisms need to tightly regulate metal uptake and accumulation. Recently, the discovery of IRT1 (Iron Regulated Transporter 1) in *Arabidopsis thaliana* led to the definition of a new metal transport family, ZIP, whose members have been also identified in mammals and bacteria. Some members of this family have been characterized, like ZIP4 from mammals, which couples divalent metals transport with protons. Nevertheless, little is known about IRT1. This transporter is localized at the plasma membrane of the epidermal cells of the root, and it is known to transport different divalent metals, but the transport mechanism is still unidentified. Based on transcriptomic data from the hyperaccumulator species *Noccaea caerulescens*, the putative orthologue from *A. thaliana* IRT1 has been proposed to be linked with the metal hyperaccumulation trait. The advantage of using *N. caerulescens* instead of *A. thaliana* as a model is that IRT1 activity is expected to be higher. Also, IRT1 is expressed constitutively, while in *A. thaliana* it is only expressed under Fe deficiency. Therefore, it is an interesting tool to decipher whether IRT1 transports divalent metals using the proton gradient force generated between the apoplast and the cytosol by the plasma membrane proton ATPases. The goal of this PhD project is to analyse the biophysical characteristics of IRT1 using hyperaccumulator plants as a model. This will set up the bases to elucidate the transport mechanism of the ZIP family.

EFFECT OF SILICON ON THE ANTIOXIDANT DEFENSE IN YOUNG MAIZE PLANTS UNDER ANTIMONY STRESS

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Key words: antimony, ascorbate peroxidase, catalase, glutathione reductase, silicon, Zea mays *L*.

Silicon (Si) is not considered as an essential element for plants, but it has a beneficial effect on plants under different types of stresses such as drought, high temperature, salinity, or heavy metals like cadmium, copper, or antimony (Sb). Sb can cause growth retardation, inhibition of photosynthesis, decrease in the uptake of essential elements and synthesis of certain metabolites. Uptake of toxic elements is usually connected with overproduction of reactive oxygen species. For their scavenging plant cells employ antioxidant defense systems comprising non-enzymatic and enzymatic antioxidants, like ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6), or glutathione reductase (GR; EC 1.8.1.7). Here we compared two maize (Zea mays L.) cultivars Luciana and Tweetor, sensitive and resistant to cold and drought. The plants grew hydroponically in the Hoagland solution (control) supplemented by 25 or 50 mg.L^{-1} Sb ($K_2(SbO)_2C_8H_4O_{10}$), with or without the combination with 1.25 mM Si (27% SiO_2 dissolved in 14 % NaOH). Enzymatic activities were determined spectrophotometrically in the apical segments of roots (R) of 12-day-old plants. Sb alone increased the GR activity in roots of both cultivars and addition of Si to Sb-treated plants reversed this trend. The presence of Sb decreased APX activity in Luciana, while in Tweetor we observed the opposite effect. Addition of Si tended to lower this activity in both hybrids. The influence of Sb on CAT activity compared to the control was manifested by a decrease in CAT activity. Addition of Si caused lowering CAT activity in both hybrids. The presence of Sb decreased POX activity in Luciana and Tweetor compared to the control, but addition of silicon had different consequences for activity in Luciana, where increase was detected and for Tweetor, where we observed the opposite effect These results indicate that Si might have a potential to alleviate Sb-induced stress in maize influencing enzymatic defense systems.

Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-17-0164 and project VEGA 2/0103/21.

CONTRIBUTION OF THE CATION DIFFUSION FACILITATOR MTP10 TO FE HOMEOSTASIS IN *ARABIDOPSIS THALIANA*

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Key words*:* deficiency, iron, iron translocation, metal tolerance protein

Members of the Cation Diffusion Facilitator (CDF) family are involved in manganese (Mn) and iron (Fe) translocation in Arabidopsis, but the role of MTP10 in Fe homeostasis has not been elucidated yet. Promotor-GUS studies showed expression of *MTP10* in the pericycle of roots and vasculature tissue of shoots, and Yeast complementation assays showed that MTP10 was able to rescue Fe- and Mn- hypersensitive strains of *S. cerevisiae*. Expression of IRT1 and FRO2 is decreased in *mtp10* plants, and Fe accumulates in older leaves to a higher extent in the mutant compared to the wild type. These results indicate a role of MTP10 in Fe homeostasis and a missregulation of the Fe deficiency response in *mtp10* mutants. To investigate Fe translocation by MTP10, histochemical Perls/DAB Fe staining was applied to wild type and *mtp10* plants under different Fe regimes. Perls/DAB staining showed a higher accumulation of Fe in the vasculature of *mtp10* leaves compared to the wild type under sufficient Fe supply, while seedlings grown under Fe deficiency did not show an altered Fe pattern. To investigate whether MTP10 is responsible for root-to-shoot translocation of Fe, four-days-old seedlings grown on Fe-deficient media were treated for 5 h with an excess of Fe. Perls/DAB staining showed a high Fe accumulation in the wild type while no Fe staining was present in the *mtp10* mutant. Taken together, these results indicate a role for MTP10 in Fe translocation from root to shoot as well as in unloading Fe from the xylem into cells of the mesophyll. Since the Fe deficiency response is disrupted by knockout of *MTP10*, we will measure expression levels of genes associated with Fe deficiency and long-distance Fe deficiency signalling.

PERCEPTION OF TRACE ELEMENTS BY CELL WALL MONITORING RECEPTORS AND THEIR LIGANDS

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Key words: Arabidopsis, *Cr*RLK1L, homogalacturonans, pectin methylesterases, RALFs

The regulation of growth and modifications of the plant cell wall is intimately linked with responses to biotic and abiotic stresses. Hence, the cell wall acts as a link between the external environment and the internal cell functions and can be seen as signaling platform. Members of the cell wall integrity sensors of the *Catharanthus roseus* RECEPTOR LIKE KINASES 1 LIKE (*Cr*RLK1L) family have been shown to be involved in many stress signaling responses including growth reactions to excess of trace metal ions. The seminar will present the current knowledge and experimental approaches to determine the specificity of recently identified upstream factors of the *C*rRLK1L cell wall sensors.

Acknowledgement: This work would not been possible without the help of J. Richter, M. Ploderer, S. Strobl, P Stasnik, A. Ünal, S. Afşar, P. Stasnik, S. Haghani, J. Kelner, D. Merz, M Berger, D. Keck and F.N. Apon. The project was supported by grants of the Austrian Science Fund and the French National Research Agency project ANR-FWF I 1725-B16 to MTH and the COST Actions 859 and 19116.

EFFICIENCY OF DIFFERENT FOLIAR-APPLIED ZN SOURCES IN COMBINATION WITH P IN MAIZE GROWN UNDER ZN DEFICIENCY

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Keywords: chelates; foliar fertilization; phosphorus; zinc

Foliar application of zinc and phosphorus is a common practice in maize to ensure sufficient supply of these nutrients. However, mixing $ZnSO_4$, a common water soluble foliar Zn fertilizer. with P in the foliar spray tank results in precipitation of zinc phosphate which may compromise the application and efficiency of both the nutrients. This problem could be addressed by using chelated Zn sources, owing to their unique chemical properties. To test this possibility, three weeks old maize plants grown in Zn-deficient nutrient solution were foliar fertilized with three Zn sources ($ZnSO_4$, Zn-EDTA and biodegradable Zn-chelate @ 0.03% Zn) alone and in combination with P (200 mM KH_2PO_4, pH 4.4). Foliar spay solution containing KH_2PO_4 and $ZnSO_4$ resulted in white precipitates, which were not observed for other Zn sources. A simulation model study showed that more than 20% of the total Zn got precipitated at pH below 4 when mixed solution of $ZnSO_4$ and KH_2PO_4 was made. Foliar applied chelated Zn sources visibly retrieved Zn deficiency symptoms in maize plants, whereas, the effect was not prominent with $ZnSO_4$ foliar fertilization. Compared to untreated Zn deficient plants, foliar Zn application improved SPAD values in all treatments. Application of Zn-EDTA+P to Zn-deficient maize plants increased shoot dry matter by 36%. Moreover, Zn concentration was significantly increased only by foliar fertilization of Zn-EDTA and biodegradable-Zn-chelate in combination with P. Hence, it is concluded that although foliar application of all Zn sources retrieved growth of Zn-deficient maize plants but Zn-EDTA was the most appropriate Zn source for foliar application to maize in combination with P fertilizer.

Acknowledgement: Thanks to the DAAD & HEC for PhD fundings.

IS THE RECIPE OF NUTRIENT SOLUTION FOR ALKALINITY EXPERIMENTS IN HYDROPONICS NEED MODIFICATIONS?

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Micronutrients such as iron (Fe) and zinc (Zn) are crucial to the health of plants and crops in any cropping system. In salinity or alkalinity experiments conducted in hydroponics or nutrient solution, Fe-EDTA is the most commonly used Fe source to ensure it supply. But Fe-EDTA is not a stable Fe source at high pH (>6.5) therefore its ability to supply Fe for plant growth under high pH nutrient solution conditions is limited. In standard nutrient solution, presence of phosphate may cause Fe precipitation as Fe-phosphate which may leads to Fe deficiency in plants. Furthermore, at high pH levels in the nutrient solution e.g., salinity or alkalinity experiments, presence of carbonates can cause severe Fe deficiency. Therefore, experiments were planned to study the ability of different Fe chelates to keep it in soluble form in the nutrient solution at different pH level due to $NaHCO_3$. Furthermore, (i) time dependent Fe precipitation in high and optimal pH nutrient solution was studied and (ii) Fe-EDTA was compared to Fe-DTPA and Fe-EDDHA as a suitable source of Fe in alkalinity experiments.

For this purpose, nutrient solutions prepared by using different Fe-chelates with variable levels of $NaHCO_3$ were incubated for 24 hours, 3 and 6 days at room temperature in 3 replicates. Nutrient solutions were aerated during the whole incubation period.

At the end of incubation period, nutrient solutions were filtered by using MN 619 G ¼, Macherey-Nagel filter papers. For this purpose, filter papers were fitted in the funnels and well shaken nutrient solutions were filtered through it. Clear filtrates from all the treatments were analyzed for soluble Fe contents by using ICP-OES.

Results: The soluble Fe fraction was significantly variable in $NaHCO_3$ containing alkaline nutrient solutions after adding different Fe chelates. At, 5 mM $NaHCO_3$ concentrations, after 24 hours, FeEDTA and FeDTPA containing nutrient solution had soluble Fe fraction of 22.7 % and 44.6 % respectively, of the total applied Fe. And at 15mM $NaHCO_3$ concentrations, only 15.2 % and 42 % soluble Fe fraction was calculated. After 3 days of incubation at 5 mM $NaHCO_3$ concentrations, in FeEDTA and FeDTPA containing nutrient solutions, soluble Fe fraction was increased to 51.4 % and 52.8 %, respectively. Similar trend was found for soluble Fe fraction at 15 mM $NaHCO_3$ concentrations after 3 days. After 6 days of incubation, in FeEDTA containing nutrient solution at 5 mM and 15 mM $NaHCO_3$ concentrations, a significant increase (80.8 % and 52.2 %, respectively) in soluble Fe fraction was recorded. For FeDTPA containing nutrient solutions, at 5 mM $NaHCO_3$ concentration, soluble Fe fraction was increased to 62.9 %, while it was slightly decreased to 42.6 %

in 15 mM $NaHCO_3$ containing nutrient solution as compared to 3 days of incubation. Iron-HBED resulted in the maximum soluble Fe fraction > 98 % at all $NaHCO_3$ concentration levels during the whole incubation duration.

Based on the results it is concluded that FeHBED is the most stable Fe source in the nutrient solution at high pH due to $NaHCO_3$. Increased soluble Fe fraction in FeEDTA (after 3 and 6 days) and FeDTPA (after 3 days) containing solutions at high pH could be due the breaking Fe bonding with chelate in the beginning but at later times Ca in the solution may have replaced Fe from Fe-phosphates. This free Fe from phosphates could be chelated again with EDTA or DTPA to become solubilized but needs further experiments for better understanding.

EFFECTS OF ROOT CAP REMOVAL ON ELEMENT UPTAKE AND PLANT RESPONSES TO ENVIRONMENTAL STRESSES IN *PISTIA STRATIOTES* (L.)

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Key words: abiotic stress, antimony, antioxidant enzymes, *Pistia stratiotes*, root cap

The role of the root cap of terrestrial plants is well known. In some aquatic plant species, including the floating aquatic macrophyte *Pistia stratiotes* (L.), a specific type of root cap known as a root pocket is present. It is slightly different in comparison to the root caps of terrestrial plants due to its adaptation to the aquatic environment. The root cap of *P. stratiotes* is relatively large and easy to remove, making it an optimal model structure for studies regarding the potential of the root cap. The purpose of this study was to determine whether the effect of the root cap removal on *P. stratiotes* roots influences the uptake of selected elements and to determine the anatomical, morphological, and physiological changes of the plant as a result of root cap removal and the presence of an increased concentration of selected elements. Partial results from the experiments with antimony (Sb), copper (Cu), and zinc (Zn) at various concentrations are presented. The specific role of the root cap was determined by the μXRF when the differences in the distribution of iron (Fe) and manganese (Mn) in the roots of Cu and Zn treated plants were present. One of the results of different environmental stresses, including the abundance of toxic elements in the substrate, is oxidative stress. Therefore, in the case of the Sb experiment, we determined the activity of antioxidant enzymes since these are the compounds responsible for the scavenging of reactive oxygen species. Visualisation of the lignin deposition was performed in the root tips, since lignin is a phenolic compound responsible for the formation of Casparian bands. The experimental period was set to seven days, but some of the defence mechanisms could already be observed after three days of the treatment by Sb.

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DETERMINATION OF MINERAL ELEMENT CONCENTRATIONS OF DIFFERENT FLAXSEED CULTIVARS (*LINUM USITATISSIMUM* L.)

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Key words: diversity, flax seed, field, nutrient concentration, biofortification

Flaxseed is cultivated in many parts of world for fiber, oil as well as for medicinal purposes and also as nutritional product. Flaxseed contains relatively high contents of ash (4 %), fat (30–40 %), protein (20–30 %), and dietary fiber (20 %). However, despite its high oil content, due to the potential of flax seeds to reduce the risk of certain diseases, interest in this plant has increased in recent years. Because flaxseed has an excellent nutritional profile, it has become an attractive ingredient in diets formulated for certain health benefits. In this study, it was aimed to determine the nutrient concentrations of different flaxseed cultivars. This study was conducted to assess for 9 mineral element content of 16 different flaxseed cultivars. The N, P, K, Ca, Mg, Fe, Zn, Mn and Cu concentrations of flaxseed cultivars were varied between 1.25-2.98%, 0.129-0.358%, 1.41%-1.73%, 0.32% -1.33%, 0.25%-0.51%, 7.24-65.63 mg kg-1, 45.4-76.0 mg kg-1, 32.7-81.7 mg kg-1 and 14.6-22.5 mg kg-1 respectively. When the research findings were evaluated in general, it was determined that flaxseed Kaolin cultivar had the highest and flaxseed LS Koral cultivar had the lowest concentrations of nutrients.

MECHANISMS OF MANGANESE AND ZINC PRIMING EFFECTS IN *CAPSICUM ANNUUM* CHALLENGED WITH *BOTRYTIS CINEREA*

Kuvelja Anđela[1,3], Morina Filis[1], Mijovilovich Ana[1], Bokhari Syed Nadeem Hussain[1], Konik Peter[3], Koloniuk Igor[2], Küpper Hendrik[1,3]

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Key words: gas-exchange parameters, metabolites, metal distribution, OJIP, plant defence responses

The importance of micronutrients in pathogen defence responses in crops has been recently emphasized. However, the mechanisms are not fully known. To reveal the priming effects of Zn and Mn, *Capsicum annuum* plants grown at deficient (0.1 µM =Zn0, 0.02 µM =Mn0), control (1 µM Zn, 0.6 µM Mn) and surplus metal levels (5 µM Zn, 3 µM Mn) were challenged with *Botrytis cinerea*. Zn surplus reduced the lesion size and preserved net CO_2 assimilation rate and stomatal conductance on a higher level than other treatments. Direct imaging of chlorophyll fluorescence kinetics (OJIP, quenching analysis) showed undisturbed efficiency of PSII only in Zn5 and Mn3 infected plants. MicroXRF imaging of infected Zn5 leaves showed increased Zn accumulation in mesophyll, and Zn and Fe accumulation in the veins. Increased Zn binding to membrane proteins in infected Zn5 was confirmed by size-exclusion HPLC/ICP-MS and a number of Zn-binding candidate proteins were identified. Common metabolic response to infection in Zn5 and Mn3 treatments was accumulation of acetophenone, a strong antifungal agent in particular when binding Zn, and corchorifatty acid F, a signalling compound, as well as increased activity of class III peroxidases. Metal-specific response to infection included accumulation of phenolics and amino acids (Mn3) and Zn-ligand isocitrate (Zn5). An *in vitro* toxicity assay showed higher sensitivity of *B. cinerea* to Zn than Mn. The results indicate that pathogen inhibition may be achieved by localized Zn mobilization, accumulation of Zn-chelating compounds and Zn toxicity. Under our experimental conditions Mn priming was not as efficient.

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NEWS INSIGHTS ON THE NICKEL HYPERACCUMULATORS *BORNMUELLERA EMARGINATA*

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Key words: Bornmuellera emarginata, phenotyping, physiology, nickel, agromining

Bornmuellera emarginata (Brassicaceae) is a wild species highly adapted to grow on nickel-rich ultramafic soils in the Balkans and a promising candidate for nickel agromining. Six populations of *B. emarginata* were used for phenotypic characterization to provide more information on phenotypic variability and thus to analyze the accessions for plant breeding. Some of these accessions weresubjected to an ecophysiological investigation to determine the responses to exposure to different concentrations of nickel (control (no Ni added), 1, 10 and 100 µM Ni) in hydroponic solution. Biomass components were recorded and nickel accumulation was analyzed by ICP-AES. Nickel hyperaccumulation (>1000 ppm dry weight) was confirmed in most genotypes but differed significantly among them. Foliar nickel accumulation was strongly correlated (r = 0.99) with solution nickel concentration. This confirms the enhanced ability of *B. emarginata* to accumulate nickel over a wide concentration range. The results show that nickel exposure up to 100 µM Ni induces only mild physiological stress symptoms. *Bornmuellera emarginata* reduces the relative water content of the leaves by 6.88 % in response to nickel, as this parameter was significantly different when compared to control plants. This mechanism allows this species to maintain the same root and shoot biomass in all nickel treatments, which did not differ significantly for this parameter. The highest Ni concentration was found in the Metsovo group (Metsovo-1 , 6250 ppm) and the lowest in the Mantoudi population (Mantoudi-4, 290 ppm). Nickel yield, biomass time Ni shoot concentration, showed wide variation within the Mantoudi and Pefki genotypes, but only very little within the Asios Stefanos and Codris genotypes. The development of breeding program for efficient nickel agromining could be focused on the genotypes Pefki 3, Mantoudi 7, Mantoudi 12, Melia 1 and 4.

RNA-SEQ ANALYSES OF IRON DEFICIENCY CHLOROSIS TOLERANT SOYBEAN VARIETIES INDICATES A SUB-FAMILY OF AMINO ACID TRANSPORTERS ARE LINKED TO IRON HOMEOSTASIS IN PLANTS

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Key words: Arabidopsis, amino acid, iron deficiency, RNA sequencing, soybean, transporters

Iron is an essential trace metal involved in fundamental metabolic activities in almost all life forms. Due to low its low phytoavailable form in alkaline growth environments, iron deficiency leads to severe yield losses and poor nutritional crops. Soybean is an important legume severely affected by low iron availability and yield losses are recorded up to 30% in different soybean varieties. Keeping in view this scenario, RNA-seq analyses of two iron deficiency tolerant soybean cultivars had revealed an amino acid transporter family linked to iron homeostasis. In silico analyses of their *Arabidopsis thaliana* orthologs predicted them to be affected by Fe deficiency and interact with proteins involved in Fe uptake, translocation, and signaling networks. Currently we are characterizing members of the identified transporter family in *A. thaliana* and *S. cerevisiae*. Mutants and overexpression lines of the family members had showed distinctive growth under low iron availability. On the other hand, *S. cerevisiae* strains defective in Mn, Fe and Zn uptake had shown interesting results when transformed with selected transporter from the family. Details of the study will be discussed briefly in the presentation.

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TRACE METAL RESEARCH IN STRAWBERRY: A BIBLIOMETRIC ANALYSIS

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Key words: bibliometrix, performance analysis, science mapping

We performed bibliometric research on scientific documents regarding trace metal research in strawberry to provide a general overlook of the extant literature and research progress over time. We have extracted the topic literature published until 2023 from Web of Science database. Bibliometric analysis was conducted using Bibliometrix package and Biblioshiny interface in R statistical language. A total of 437 documents on the area were identified, published in 268 sources. The first document was released in 1987 based our data collection, and there was around 16% annual growth of publications with fluctuations over time. The core group consisted of 31 out of 268 journals with Journal of *Plant Nutrition* being the most productive in the field (19 documents), followed by *Food Chemistry* (9 documents). The USA published most in the area (126 documents), followed by Türkiye (112 documents) and China (99 documents). These were also the most cited three countries. The most frequent keywords projected after strawberry were *Fragaria x ananasa*, antioxidant activity, growth, yield, heavy metals, minerals, nutrients and fruit quality which imply the current research path of the field. Other important keywords were oxidative stress, polyphenols, chlorophyll, compost, bioactive compounds, hydroponics, iron deficiency and arsenic. Collaboration network analysis was run to display collaboration groups on the topic between countries, institutions and authors which resulted in five countries and institutions, as well as ten author collaboration networks with at least two collaborative papers on the area.

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DOES SILICON AFFECT THE METABOLIC PROCESSES INVOLVED IN THE PHENYLPROPANOID PATHWAY IN MAIZE (*ZEA MAYS* L.) UNDER ARSENIC STRESS?

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Key words: arsenic, antioxidant enzymes, lignin, phenylpropanoid pathway, silicon

In recent decades, when investigating various biotic or abiotic stresses, it has been shown that the exogenous application of silicon (Si) can mitigate the impact of the given stress on globally important economic crops. One of the most important pathways that helps the plant with various types of stress is the phenylpropanoid pathway. It serves as a rich source of metabolites in plants, is required for the biosynthesis of lignin, and serves as a starting point for the production of many other important compounds.

The aim of our study was to find out how arsenic in combination with silicon affect the activity of phenylalanine ammonia-lyase (PAL), a key enzyme involved in the synthesis of flavonoids, such as anthocyanins and phenolic compounds, including lignin. At the same time, we aimed to quantify lignin and also to monitor the activity of other important enzymes such as peroxidase (G-POX) and polyphenol oxidase (PPO), which are associated with lignin polymerization. Our model organisms were 2 maize hybrids, Luciana and Tweetor, contrasting in their drought tolerance.

Results showed, that the activity of G-POX a PPO was significantly higher in the sensitive hybrid Luciana than in the more drought tolerant hybrid Tweetor. However, the phenotype and survival of the Tweetor in our experiment clearly indicated better growth of this tolerant hybrid. Tweetor contained higher content of lignin, as a product of phenylpropanoid pathway, so we can assume that it had an increase in enzyme activity earlier than on the 10th day of cultivation.

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IMPACT OF LONG-TERM COPPER EXPOSURE ON GROWTH, PHOTOSYNTHESIS, ANTIOXIDANT DEFENCE SYSTEM AND ARTEMISININ BIOSYNTHESIS IN SOIL-GROWN *ARTEMISIA ANNUA* GENOTYPES

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Keywords: *Artemisia annua*, Cim-Sanjeevani, Cim-Arogya, Copper, Heavy metal stress

The effects of copper (Cu) exposure on growth and physiological characteristics of three genotypes (CN-12, Cim-Sanjeevani and Cim-Arogya) of *Artemisia annua* L. were elucidated. The plants were grown under naturally illuminated greenhouse conditions and were harvested after physiological maturity (120 days after sowing). Results suggest that 10 mg kg^{-1} Cu significantly enhanced the growth and physiological parameters like enzyme activities, photosynthesis. At higher concentrations, Cu inhibited the growth, biomass, photosynthetic parameters; while increased lipid peroxidation in all the genotypes. The activities of antioxidant enzymes viz. catalase, peroxidase and superoxide dismutase were upregulated by the Cu stress. The highest applied concentration of Cu (60 mg kg^{-1}) proved most toxic for plants. Moreover, artemisinin content was increased upto 10 mg kg^{-1} of Cu treatment, compared with control, however, the artemisinin accumulation decreased at higher doses of Cu in all the genotypes. On the basis of studied parameters, Cim-Arogya was found to be most tolerant among all for Cu toxicity.

PHYTOMANAGEMENT STRATEGIES FOR A METAL-CONTAMINATED AGRICULTURAL SOIL TO PROVIDE BIOMASS FOR CLEAN BIOFUEL PRODUCTION – EXPERIENCE FROM A POT TRIAL

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Key words: Biofuel, biostimulants, lignocellulosic crops

Strategies to intensify biomass production using mycorrhizal fungi, biostimulants, and their combinations are poorly documented for lignocellulosic crops grown on metal-contaminated soils.

This study aimed to evaluate the yield of Miscanthus x giganteus and Cannabis sativa L. grown on a metal- contaminated agricultural soil amended with biostimulants and/or mycorrhizal fungi, and the plant Cd, Pb, Zn and Cu uptake.
A pot trial which lasted 90 days was conducted using soil collected from a field close to a former Pb/Zn smelter in France (Metaleurop Nord, Evin-Malmaison). Six treatments were applied: control (C), protein hydrolysate (B1), humic/fulvic acids (B2), arbuscular mycorrhizae (M), protein hydrolysate combined with mycorrhizae (B1xM), and humic/fulvic acids combined with mycorrhizae (B2xM). The arbuscular mycorrhizae used in the study was provided by Symbiom Company (Czech Republic). The humic/fulvic acids (Lonite 80 SP) was obtained from Alba Milagro International (Italy).

Metal concentrations in the soil pore water (SPW), soil pH, redox potential, and electrical conductivitywere measured over time. Plant parts of miscanthus and hemp were harvested at day 90 and analyzedfor metal concentrations.

The results showed that the use of protein hydrolysate (B1 and B1xM) led to high concentrations of Cd, Pb, and Zn in the soil pore water, 2.03, 1.97, 3.43 times higher for miscanthus and 7.8, 1.3, 23.4 times higher for hemp as compared to the control pots, respectively, causing phytotoxicity and decreasing biomass production for both hemp and miscanthus. Conversely, the use of humic/fulvic acids (B2 and B2xM) decreased concentrations of Cd, Pb, Zn, and Cu in the soil pore water, allowing for an increase in biomass production for both hemp and miscanthus.

Overall, this study suggested that humic/fulvic acids can be an effective biostimulant for increasing biomass production in a metal-contaminated soil. These results warrant further investigation in a fieldtrial setting.

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Different tissue-specific expression of *NtZIP4B* and *NtZIP5B* genes points to their distinct role in Zn homeostasis

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Key words: GUS, *Nicotiana tabacum*, transparent soil, zinc, *ZIP*

Zinc (Zn) is a key micronutrient for the proper growth and development of all living organisms. Among the whole group of microelements, it plays the most diverse role. The ZIP (ZRT- and IRT-like proteins) family transporters are mainly involved in Zn transport to the cell and when expressed in the epidermis its uptake from the soil. The ZIPs also control the Zn distribution within plants. As we know so far, ZIP expression occurs in various plant tissues at different developmental times and frequently it is increased during Zn deficiency.

I focused my research on the tissue-specific expression profile of the *NtZIP4B* and *NtZIP5B* genes in tobacco plants. To highlight the localization of the expression I used plants expressing the GUS reporter gene under the control of the respective gene promoter. Next, plants were grown in a Zn-deficient or Zn-sufficient hydroponic culture or transparent soil (TS). TS is hydrogel-based 3D medium that mimics soil. It has a modular grain size, access to the air pockets, holds water, and could be an adjustable nutrient source.

The initial results suggest that Zn deficiency led to increased expression of both *NtZIP5B* and *NtZIP4B* promoters. The difference was in the specific tissue localization of *NtZIP5B* promotor expression in the root apical region and *NtZIP4B* promotor in the basal parts of the roots. Differences in tissue-specific localization of these genes indicate a distinct role they play in the maintenance of Zn homeostasis in plants. It seems that NtZIP5B transporter is responsible for the upkeep of meristematic cells and that NtZIP4B plays a more general role in Zn uptake and possibly Zn relocation. These results complement the research on the mechanisms responsible for Zn uptake by plants and redistribution of this element between plant organs.

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PLANT COLONIZERS AT A FORMER CHLOR-ALKALI PLANT BROWNFIELD: TRACE METALS AND ASSOCIATED RHIZOSPHERE BACTERIA

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Keywords: *Diplotaxis muralis*, *Lotus tenuis*, mercuric reductase, phytoremediation, plant Hg tolerance

Mercury (Hg) is a highly toxic trace metal across all kingdoms of life, due to its global distribution, bioaccumulation and biomagnification properties. Here, we focused on the vegetation and associated rhizosphere bacteria colonizing a polymetallic contaminated brownfield with a median Hg topsoil concentration of 962 mg kg^{-1}. The aim of the study was to assess the suitability of an *in situ* phytomanagement approach by caring out a floristic survey of the native flora, and determining the metal concentrations retained in roots and shoots *via* PXRF. Trace metal substrate concentrations explained 28% of the total observed variance between plant species distribution and plant elemental content, with Hg as the main community driver, based on a redundancy analysis. Overall, the plant species were separated into two groups, driven by Mn or Hg-Pb-Cu. Out of the six plant species growing in more than one location at the site, *Diplotaxis muralis* was the dominant one. *Lotus tenuis* accumulated the highest concentrations of Hg, up to 10869 mg Hg kg^{-1} dry weight in roots and 1070 mg kg^{-1} in shoots, followed by *D. muralis* with up to 6049 and 1204 mg Hg kg^{-1} roots and shoots, respectively. High-throughput 16S rRNA amplicon sequencing served to estimate the relative abundance of bacteria from the rhizosphere soil of *D. muralis*. The dominant bacterial phylum for the rhizosphere communities was Proteobacteria. The high prokaryote diversity of the samples was likely dependent on the presence of merA gene encoding for mercuric reductase, a key enzyme in bacterial inorganic Hg detoxification. Collectively, this data suggests that *L. tenuis* is a suitable Hg indicator species, and together with *D. muralis*, may both be candidate species for *in situ* Hg phytomanagement approaches.

The associated rhizosphere bacteria may locally alleviate Hg toxicity in soil and thus facilitate plant colonization of the site.

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ZINC AMELIORATES COPPER TOXICITY IN LETTUCE GROWN IN SOIL IRRIGATED WITH CONTAMINATED SIMULATED WASTEWATER

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Key words: Copper, Lettuce, Simulated Wastewater, Zinc

Heavy metal (HM) pollution is increasing day by day due to different natural and anthropogenic factors. Wastewater contains various HM used for irrigation where freshwater availability is lacking. These HM negatively affect plant growth and quality. Copper (Cu) and zinc (Zn) are considered as vital micronutrients for plants. They play their role in plants growth and development at their lower concentration. But at higher levels Cu and Zn act as toxic elements for plant growth and development. A pot experiment was conducted to assess the effects of Cu and Zn on lettuce growth and mineral profile. Total four treatments were applied including control-uncontaminated simulated wastewater (SW), Cu 20 ppm (CuSW), Zn 100 ppm (ZnSW) and Cu and Zn together (CuZnSW) to the lettuce soil. The experiment was arranged following complete randomized design (CRD) with three replications of each treatment. Lettuce was harvested at maturity and growth and mineral parameters were recorded. Results showed that CuSW significantly decreased the growth and quality of lettuce. But irrigation with Cu and Zn contaminated SW (CuZnSW) retrieved Cu toxicity and increased root dry matter, shoot dry matters and root length by 13.5%, 46% and 19%, respectively. Moreover, CuZnSW improved lettuce quality with increased concentrations of Mg (30%), P (15%), Ca (41%), Mn (24%) and Fe (23%) as compared to control. Thereby, results suggest that presence of Zn in wastewater could mitigate the negative effects of higher levels Cu toxicity in crop plants.

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TOXICITY OF ANTIMONY AND ARSENIC FOR PLANTS AND ENVIRONMENT – LESSONS FROM THE LAB AND NATURE

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Key words: Arsenic, antimony, metalloid uptake, plant growth and development.

Arsenic (As) and antimony (Sb) are metalloids that enter the environment especially due to mining activities, ore processing and industry, traffic, shooting or intense agriculture. Despite non-essentiality of these metalloids for living organisms, plants take up and accumulate them in relatively high concentrations in their organs. They might show the phytotoxic effects already at lower levels in plant tissues, and increased accumulation of these elements in edible plant parts or medicinal herbs may cause health risks to humans and livestock. Although most of Sb and As is stored in root tissues, a relative small portion of these metalloids can be translocated to the shoot and cause several harmful effects on various levels. The present contribution aims to summarize some toxicity effects found on important agricultural plants, like maize or sorghum grown in laboratory conditions as well as present findings of As and Sb accumulation in plants grown on polluted sites after previous mining activity in Slovakia.

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