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ORTA DOĞU TEKNİK ÜNİVERSİTESİ
MIDDLE EAST TECHNICAL UNIVERSITY

Plant Metals Conference and MC Meeting

Proceedings Book

Trace metal metabolism in plants

COST Action 19116

ANKARA , TURKEY

BIOLOGY
CENTRE
CAS

CA19116
Plant Metals

ISBN: 978-625-00-9037-4

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30 AUGUST - 2 SEPTEMBER 2022

Location: Biological Sciences Building, METU

PROCEEDINGS BOOK

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Plant Metals Conference and MC Meeting

Trace metal metabolism in plants

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30 AUGUST - 2 SEPTEMBER 2022

Location: Biological Sciences Building, METU

Location: Culture & Convention Center, METU

30th August

15.00-17.30: Registration
18.30-23.00: Welcome dinner

31st August

09.00-18.45: Lectures with coffee breaks and one lunch break
free evening

1st September

09.00-12.30 Poster session and talks
12.30-14.30 Lunch break
14.30-18.00 Lecture and MC meetings
19.00-22.00 Gala dinner

2nd September

09.00-12.30 Work group planning
12.30-13.00 Concluding remarks

BIOLOGY CENTRE CAS

DETAILED PROGRAMME

30th August, 2022 (Tuesday)	
15:00 – 17:30	Registration (@Biology Department*)
18:30 – 23:00	Welcome Dinner (@Zeytin Restaurant**)

31st August, 2022 (Wednesday)	
09:00 – 09:40	Opening ceremony (Speakers: Local organizer (Seckin Eroglu), COST action chair Hendrik Kuepper)
09:40 – 10:10	**WG1 Lecture:** Nathalie Verbruggen, Contrasting metal accumulation in *Arabidopsis halleri* populations
10:10 – 10:40	Break (Mounting posters @ Atrium) (Coffee+cookies)
10:40 – 12:25	**WG1 selected talks**
10:40 – 10:55	Célestine Belloeil, Root transport mechanisms involved in metal hyperaccumulation in *Noccaea caerulescens*
10:55 – 11:10	Deon Mandebere, Iron determines seed germination speed by weakening endosperm through ROS
11:10 – 11:25	Feixue Liao, Regulation of the zinc deficiency response in the legume model *Medicago truncatula*
11:25 – 11:40	Short break
11:40 – 11:55	Amir Maqbool, Transcriptomics insights of IDC-tolerant soybean varieties under high pH indicates a sub-family of amino acid transporters are linked to iron homeostasis in plants
11:55 – 12:10	Matthias Wiggenhauser, Stable isotope fractionation: a tool to identify major processes that separate cadmium from zinc in plants?
12:10 – 12:25	Santiago Alejandro Martinez, Characterization of metal tolerance proteins in *Beta vulgaris* reveals diversity of micronutrient homeostasis mechanisms in dicotyledons
12:25 – 12:35	Group photo
12:35 – 14:15	**Lunch break**
14:15 – 14:45	**WG2 Lecture:** Marie Theres Hauser, Does peptide mediated cell wall signaling detect metal ions?
14:45 – 15:00	Short break
15:00 – 16:45	**WG3 selected talks**
15:00 – 15:15	Irena Macek, Plants play a crucial role in the development of soil fungal communities in remediated substrate after EDTA washing of metal contaminated soils
15:15 – 15:30	Hendrik Küpper, Trace metals at the frontline of pathogen defence responses in plants
15:30 – 15:45	Giovanni dal Corso, DNA methylation in the hyperaccumulator *Noccaea caerulescens* "Ganges" prevents ROS damages thus increasing Cd hypertolerance
15:45 – 16:00	Short break (Coffee+cookies)
16:00 – 16:15	Florian Delerue, Leaf metal exclusion or accumulation is related to soil resource exploitation strategy in European Calaminarian species: Evidence for a leaf elemental and economic spectrum?
16:15 – 16:30	Michel Mench, Advancing in the application of innovative phytomanagement strategies in contaminated areas of the SUDOE space (the Phy2SUDOE Project)
16:30 – 16:45	Speaker absent/Free slot
16:45 – 17:00	Short break

17:00 – 18:45	**WG4 selected talks**
17:00 – 17:15	Ephrem Habyarimana, Identification of genetic factors governing grain Fe and Zn contents in sorghum
17:15 – 17:30	Faheem Shehzad Baloch, Genetic and genomic resources for biofortifying the crops grains with few examples
17:30 – 17:45	Hagai Yasuor, Biopolymeric Nano-vehicles for zinc application in plants
17:45 – 18:00	Short break
18:00 – 18:15	Valerie Bert, Characterization of *Arabidopsis halleri* and *Urtica dioica* responses to Zn and Cd: Soil management practices to help biofortification?
18:15 – 18:30	Levent Öztürk, Interactive effects of climate change, nitrogen and zinc nutrition on growth and yield performance in wheat
Free evening	

1st September, 2022 (Thursday)	
09:00 – 09:15	**WG5 selected talk**
09:00 – 09:15	Seçkin Eroğlu, Iron Localization in Everyday Fruits
09:15 – 09:45	**WG5 Lecture:** Robert Dulfer, EU Projects - Dissemination, Communication, and Exploitation of Achievements
09:45 – 12:30	**Poster session (@ Atrium)** (Coffee+cookies)
12:30 – 14:00	**Lunch**
14:00 – 14:30	**WG6 Lecture**: Muhammed Imran, Intellectual property rights (IPR): importance for young scientists to learn
14:30 – 15:45	MC meeting Report of activities by the leaders of the Action: chair / STSM coordinator, vice chair / ITC conference grant coordinator, science communications officer, WG leaders incl.
15:45 – 16:00	Short break (Coffee+cookies)
16:00 – 17:30	MC meeting Discussion and decision of goals and budget of the third grant period
17:30 – 19:00	Free time/Unmounting posters
19:00 – 22:00	Gala dinner (@Zeytin Restaurant**)

2nd September, 2022 (Friday)	
09:00 – 09:30	WG1 – Metal transport: Activity planning
09:30 – 10:00	WG2 – Metalloproteins: Activity planning
10:00 – 10:30	WG3 – Environment: Activity planning
10:30 – 11:00	Break (Coffee+cookies)
11:00 – 11:30	WG4 – Agronomy: Activity planning
11:30 – 12:00	WG5 – Dissemination: Activity planning
12:00 – 12:30	WG6 – IPP: Activity planning
12:30 – 13:00	Concluding remarks, farewell

*Biology department: https://goo.gl/maps/pR4jCSxpF6NXbeCKA
** Zeytin Restaurant: https://goo.gl/maps/wqRkT8Kqp2otmWjU8

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List of Proceedings

Effect of Biochar, Farmyard Manure (FYM) on Fenugreek Growth and Yield

Abdel Rahman M. Al Tawah

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Fenugreek (*Trigonella foenum-graecum*) is an important Fabaceae family legume herb. The objective of this study was to assess the capacity of biochar and farm yard manure to improve fenugreek growth, yield, yield components, and mineral content. Therefore, fenugreek plants were exposed to varying concentrations of biochar and farmyard manure in diverse soil and ecological environments. The experimental design was factorial, with three biochar concentrations (0, 0.5, and 1 percent w/w) and manure treatments (with and without farm yard manure). The variables that were measured were seed yield plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, plant height (g), pod length, number of branches per plant, number of leaves per plant, seed oil percentage, seed oil yield/plant, seed nitrogen, seed total carbohydrates, Potassium (K) percent, Phosphorus (P) percent and Iron (Fe) percent. According to the findings, fenugreek responded significantly better in the red Mediterranean silt loam soil in terms of yield and related traits, as well as mineral accumulation, in comparison to plants grown in the yellow Mediterranean sandy loam soil. The highest rate of farmyard manure and biochar application in the red Mediterranean silt loam soil had a positive effect on the phenology of the fenugreek plant as well as the yield it produced. However, there was no discernible difference in the concentration of seed carbohydrates across any of the application rates of farmyard manure or biochar, regardless of the two locations. It was determined that the soil diversity and ecological condition of both localities prompted fenugreek phenology, morphology, oil concentration, oil yield, N digestion, Potassium (K) percent, Phosphorus (P) percent, and Iron (Fe) percent but did not provoke carbohydrates concentration in the plant. This was the conclusion reached.

Keywords: Biochar, Farmyard Manure, FYM, Fenugreek

Does Silicon Affect Antioxidant Performance, Enzyme Phenylalanine Ammonia-Lyase and Concentration of Anthocyanins in Maize Exposed to Arsenic Toxicity?

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Both arsenic (As) and silicon (Si) are metalloids - while the first one is toxic in general, the latter one is considered as beneficial for plants suffering from various kinds of stresses. The aim of our work was to monitor the response of young maize plants (*Zea mays* L.) under stress induced by arsenic and find whether silicon helps the plant in defence at the molecular and biochemical level. Experiments were focused on the visualisation of free radicals along with monitoring of some antioxidant enzymes activity in roots and also monitoring activity and expression of PAL (phenylalanine ammonia-lyase) enzyme in leaves. The results showed that Si significantly decreased the presence of both superoxide and hydrogen peroxide in roots that suffered from As toxicity, which was also confirmed by detailed monitoring of the activity of APX, G-POX and SOD enzymes. Also, the PAL enzyme showed increased activity with increasing concentration of As, and expression of genes encoding this enzyme was significantly upregulated almost in all treatments containing Si. Arsenic negatively affected the concentration of anthocyanins, which are very important non-enzymatic antioxidant molecules and play an important role in plant protection against reactive oxygen species, but addition of Si significantly improved it. We assume that Si might affect the functionality of key antioxidant enzymes and enzymes which probably increases the production of phenolic substances, and in this way at least partially help to overcome negative effects of As on maize.

Keywords: Anthocyanins, Antioxidant enzymes, Arsenic, PAL, Silicon

Acknowledgements: This work was supported by the Grant Slovak Research and Development Agency under the contract No. APVV-17-0164, APVV SK-CN-21-0034 and Grant Agency VEGA, No. VEGA 1/0472/22.

Transcriptomics Insights of IDC-Tolerant Soybean Varieties Under High pH Indicates a Sub-family of Amino Acid Transporters are Linked to Iron Homeostasis in Plants

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Iron (Fe) is an essential transient metal required for proper plant growth and development. Due to its non-bioavailable form in alkaline soils, iron deficiency in plants is a widespread global constraint affecting many crops. For instance, It results in poor nutritional quality and millions of tons of yield losses every year in soybean (*Glycine max* L.). In an initial screening of 20 soybean varieties under high soil pH, two tolerant ones showed distinctive biochemical responses under Fe deficiency, suggesting the involvement of different tolerance mechanisms in these two varieties. Although several transcriptomic studies have been done to compare the sensitive and tolerant soybean varieties, no transcriptomic studies have been done before to compare two tolerant cultivars with different biochemical responses under Fe deficiency. Therefore, RNA sequencing (RNA-seq) of roots and leaves was done under Fe deficiency to determine the genetic mechanisms and players behind the distinctive tolerance mechanisms among these two varieties. As a result, a major difference was found in the number of differentially expressed genes (DEGs) and their families between the two varieties. The gene ontology (GO) enrichment results combined with hierarchical clustering showed the activation of different biochemical pathways to explain the differences between the varieties. Following further bioinformatic analyses of RNA-seq data, several families of novel transporters and transcription factors were identified that can be involved in Fe homeostasis in plants. Among them, especially a sub-group of amino acid transporters were highly enriched in IDC-tolerant soybean roots and a totally of 21 transporters from the family were differentially expressed with high fold change. In silico analyses of their Arabidopsis 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 these transporters in *Arabidopsis thaliana*, which may enable us to develop IDC-tolerant crop species in the future.

Keywords: Arabidopsis, amino acid, iron deficiency, RNA sequencing, soybean, transporters

Differences in Ionome of *Pistia Stratiotes* (L.) as a Response to Environmentally Relevant Concentrations of Copper and Zinc

Anna Kokavcová[1], Filis Morina[2], Ana Mijovilovich[2], Syed Nadeem Hussain Bokhari[2], Zuzana Lukačová[1], Jana Kohanová[1], Alexander Lux[1] and Hendrik Küpper[2,3]

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Pollution caused by increased concentrations of toxic elements, such as Sb, as well as microelements that are essential in lower concentrations, like Cu or Zn, is nowadays widespread across the world mainly due to agricultural activities. We investigated the potential of aquatic macrophyte *Pistia stratiotes* (L.) for phytoremediation of a metal(loid) polluted environment. *P. stratiotes* (L.) is an invasive species common in standing waters of Central Europe that can effectively accumulate elements like Cd or Cr into its tissues. Here, we treated the plants with environmentally relevant concentrations of Cu (redox active) and Zn (redox inert) with the aim to study their interaction and distribution at the root tissue level, as well as reveal the tolerance mechanisms against excess metals. The concentrations used were 0.1, 0.3, or 1 μM of Cu or 0.3, 1, or 3 μM of Zn. We determined the ionome of the roots and leaves of the plants and the chlorophyl fluorescence kinetics (OJIP). The general translocation of Cu and Zn to plant leaves decreased with increasing concentrations of these elements in the nutrient solution, thus the translocation factor was low mainly in the Cu 1 μM and Zn 3 μM treatments. The overall accumulation of other mineral elements was affected. Significant differences were found in the accumulation of Cu, Ni, P, S and Zn. Interestingly, the concentration of Cu increased with increased Zn supply. Concentrations of P and S decreased in roots of treated plants and the concentration of Ni increased in both roots and leaves with increaed Cu supply. In addition, direct imaging measurements of fast chlorophyll fluorescence induction (OJIP) showed photosynthesis inhibition only in the Cu 1 and Zn 3 treatments.

Keywords: aquatic plants, elemental analysis, ionome, photosynthesis, *Pistia stratiotes*

Acknowledgements: The project was supported by CZ.02.1.01/0.0/0.0/15_003/0000336, RVO: 60077344, CA 19116, VEGA 1/0472/22 and APVV-17-0164 grants.

Trace Element Uptake of Oriental Tobacco Varieties Fertilized with Different Nitrogen Rates

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Oriental tobacco (*Nicotiana tabacum* L.) is characterized as an effective accumulator of trace elements that are most concentrated in its aboveground organs i.e., leaves. This study presents field experiments that are carried to study the influence of increasing nitrogen fertilization on the trace elements content on four varieties of oriental tobacco. Fertilization was done using inorganic mineral fertilizer NPK and 27% KAN with different nitrogen rates (0, 20, and 30 kg/ha), with constant amount of phosphorus (60 kg/ha) and potassium (40 kg/ha). Two domestic (P-23 and P-79) and two introduced (Basma and Elenski) tobacco cultivars were collected from two different soil types on two different locations with typical non contaminated agricultural soil. Following elements were determined in soil and plant samples: Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, Sr, V and Zn analyzed by atomic emission spectrometry with inductively coupled plasma (ICP-AES). The plant available fractions were analyzed to assess the element bioaccumulation. Higher doses of nitrogen fertilizers lead to linear increase on the content of Ni, Fe and Co in all tobacco varieties. Zn and Cu content of the oriental tobacco leaves of all four variants decreased upon the lower nitrogen rate and decreased upon the higher dosage.

Keywords: trace elements, nitrogen fertilizer, soil, oriental tobacco

Root Transport Mechanisms Involved in Metal Hyperaccumulation in *Noccaea caerulescens*

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Transition metals such as iron, zinc or nickel are essential for living organisms but become toxic at high concentration. Remarkably, about 500 plant species are able to accumulate tremendous amounts of metals in their leaves. The large majority of these plants hyperaccumulate nickel. The Brassicaceae is one of the family containing the largest number of nickel hyperaccumulators, including the species *Noccaea caerulescens*. To identify the mechanisms involved in nickel hyperaccumulation, we compared several accessions of *Noccaea caerulescens* with contrasted capacity to accumulate nickel and closely related non-accumulator species. Reciprocal grafting experiments between *Noccaea caerulescens* accessions indicated that nickel hyperaccumulation mainly depends on mechanisms taking place in roots. To identify these mechanisms, we looked for differentially expressed genes in intra- and inter-specific comparative transcriptomic analyses using our newly generated *Noccaea caerulescens* genome as reference. We also searched for polymorphisms in coding sequences to identify genetic variations that might affect genes involved in metal homeostasis. Among differentially expressed genes, Heavy Metal ATPases *HMA4* and *HMA5* and Nicotianamine Synthase *NAS3* are more expressed in the roots of nickel hyperaccumulator accessions. We also identified a deletion in the broad-spectrum metal transporter *NcIRT1* in the La Calamine accession, that is not able to hyperaccumulate nickel. Further analysis in yeast indicated that NcIRT1 can transport nickel and that the deletion identified in La Calamine leads to a non-functional transporter. Finally, the expression of a functional NcIRT1 in the roots of La Calamine accession partially restored the capacity to accumulate nickel, showing that NcIRT1 is involved in nickel transport in *Noccaea caerulescens*. Taken together, our results suggest that NcIRT1 could be at the origin of nickel hyperaccumulation in *Noccaea caerulescens* and potentially in other species.

Keywords: Hyperaccumulator, IRT1, Metal, Nickel, RNA-seq

From Soil to Bean: Tracing Cd Pathways in Cacao Trees

Hester Blommaert[1], Anne-Marie Aucour[2], Matthias Wiggenhauser[3], Claudia Moens[4], Philippe Telouk[2], Sylvain Campillo[1], Caleb Lewis[5], Umaharan Pathmanathan[5], Erik Smolders[4], Géraldine Sarret[1]

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The accumulation of the toxic metal Cd in cacao beans has recently become a subject of intense research after the European Union lowered its limits in chocolate. Large differences (factor 13) in cacao bean Cd concentrations were found between cultivars grown in the field in conditions with comparable soil Cd. Genetics-based techniques may thus offer sustainable strategies for Cd mitigation in cacao. Nonetheless, this reduction potential is yet to be exploited since there is a lack of knowledge on how Cd is transported from soil to the cacao bean. Here, we aimed to compare the mechanisms that control Cd transfer in two genotypes 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. Three replicate trees of a low (L) and a high Cd accumulating (H) cultivar were selected from a conservatory of cacao cultivars in Trinidad (soil Cd: 0.3 mg Cd kg-1). Cadmium concentrations in leaves and beans of H were factors 3.5-4 higher than in L. Both cultivars showed a similar partitioning strategy, i.e. Cd concentrations increased in the order: placenta < nib < testa < pod husk < root < young leaf - old leaf < branch. In the branches, Cd was mostly bound to oxygen ligands, and more specifically to carboxyl ligands (70-100%). In the nib Cd was mostly associated to oxygen-ligands (60-100%), with phosphate as most plausible ligand. Roots, branches and fruits were enriched in light isotopes compared to the leaves, indicating more control points between branches and leaves than branches and fruits. This enrichment was more pronounced in L, suggesting different Cd loading from branches to nibs/leaves between genotypes, and confirming a genetic difference in the accumulation of Cd between the cultivars.

Keywords: Cacao, Cadmium, Metal Stable Isotopes, XAS, genotype

Acknowledgements: This study is supported by French National Research Agency program ‘Investissements d'avenir’ (ANR-15-IDEX- 02), and the CNRS through the INSU/EC2CO program. We thank the staff of the Geochemistry Mineralogy platform at ISTerre, and ICP MS platform at ENS Lyon.

Mercury Resistance Strategies in a Rhizosphere Associated Fungal Isolate

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Mercury is a highly toxic element posing sever risks to human and wildlife health as it readily bioaccumulates through the food chains. As physical and chemical remediation strategies for the Hg polluted environments are invasive and extremely costly, bioremediation approaches are explored. We have isolated a filamentous fungus, identified as *Fusarium oxysporum*, from the rhizosphere soil of a plant species growing on the premises of a severely Hg-contaminated decommissioned facility. The fungal isolate exhibited remarkable Hg, Cu, Pb, or Zn resistance, according to the minimum inhibitory concentrations assayed in solid medium. The remediation potential of fungal live biomass was established in aqueous solution supplemented with 100 mg kg^{-1} Hg^{2+}, under shaking conditions: 62% removal efficiency and biosorption capacity of 37 mg Hg g^{-1} of dried mycelium in 48h. To identify the processes (i.e., adsorption, uptake) responsible for the high resistance to Hg^{2+}, the F. oxysporum isolate was grown in nutrient medium supplemented with Hg^{2+}. The concentrations of Hg^{2+} in mycelium and culture filtrate were quantified by CV-AAS. The fungus showed 81.53% total Hg^{2+} removal capacity. The processes involved in the removal of Hg^{2+} were adsorption (47.58%), uptake (30.4%) and interestingly, 22.02% biovolatilization. Reduction of Hg^{2+} to Hg^{0} is known in bacteria, being due to the presence of a mercuric reductase encoded by the merA gene. Bioinformatic analysis of publicly available *F. oxysporum* genome revealed that the fungus might possess a similar mercuric reductase. A fragment of 1437 bp, encoding for the putative merA gene, was amplified from the *F. oxysporum* cDNA and confirmed by sequencing. It was found to be constitutively expressed, suggesting distinct regulation from the bacterial mer operon induction mechanism. Underpinning the strategies and eventually the mechanisms underlying the mercury resistance in filamentous fungi could be a promising pathway to develop eco-friendly environmental decontamination strategies.

Keywords: bioremediation, fungi, *Fusarium oxysporum*, mercury

Acknowledgements: The research is funded by the Romanian National Authority for Scientific Research and Innovation, CNCS—UEFISCDI, project number PN-III-P2-2.1-PED-2019-5254, contract no. 390PED/2020.

Influence of Iron Fertilization on Yielding and Alkaloids Concentration in California Poppy (*Eschscholzia californica*)

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California poppy is a plant with great healing potential due to the high content of alkaloids. Alkaloids are substances used to treat a very wide range of diseases (e.g., cardiovascular or digestive system diseases). Those detected in the california poppy prove the legitimacy of its use by the indigenous peoples of North America to treat insomnia and mild anxiety states. In the region of Europe and other continents, this plant is almost exclusively decorative, although it is sometimes used in sleep aids. Isoquinoline alkaloids were detected as synthesized in the california poppy. One of them is californidine - an alkaloid whose content in this plant is one of the greatest and this is the cause of this alkaloid name. The design of the pot experiment was based on the literature data on the reaction of opium poppy to fertilization with iron(II) sulfate. The aim of the research was to investigate the impact of iron fertilization on the plant biomass of california poppy and to check how fertilization would affect the alkaloid content in this plant. The following treatments were created in the experiment: control without iron application and five combinations fertilized into the soil with iron(II) sulfate in the form of a solution in the following concentrations: 0.02, 0.10, 0.50, 1.00 and 2.00 mmol Fe/L. Satisfactory results were obtained, proving a positive response of plants to the applied iron fertilization with dose from 0,02 to 0,10 mmol/L cause the highest total alkaloid amount, but there are differences between individual alkaloids i.e. allocryptopine, californidine, chelerythrine, codeine, corydine, noscapine, papaverine, protopine, (S)-reticuline, sanguinarine, scoulerine, thebaine. The highest plant biomass was obtained for iron fertilization 0.50-1.00 mmol Fe/L. The total iron concentration in plants was increased from 78.7 mg/kg d. w., to more than 210 mg/kg by a raising iron(II) sulfate dose.

Keywords: alkaloids, *Eschscholzia californica*, fertilization, iron(II) sulfate, yielding

Phenolic Secretion of Plants under Fe Deficiency and High pH

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Iron (Fe) is an essential trace metal for the normal growth of plants. Fe is severely limited in calcareous soils which may have an impact on natural distribution of plant species. Plants use different Fe acquisition strategies for Fe solubilization and uptake to combat Fe deficiency, strategy I and II; namely. Recently, in Strategy I plants, an additional component for Fe acquisition that relies on secretion of phenolics from the plant roots, has been identified as a response to Fe deficiency in alkaline soil. Phenolics are secondary plant metabolites produced via the phenylpropanoid pathway and possess a benzene ring moiety that makes them fluorescent under UV light. Their secretion increases with Fe deficiency. This study aimed to screen various plants under Fe sufficient and limiting conditions in agar medium to discover novel phenolic secretion patterns. To achieve this, we collected plant seeds from common crops, seed banks and Arabidopsis T-DNA insertion mutants. The results showed most plants secrete phenolics to a higher extent upon Fe deficiency; but exceptions exist. Two Arabidopsis mutants showed differential phenolic secretion patterns. Plants that show differential fluorescence emission profiles will be further characterized for their Fe deficiency-related pathways. This study may help us to understand the extend and essentiality of the phenolic secretion strategy among plants in natural settings.

Keywords: Fe deficiency, Phenolic secretion, Fluorescence, coumarins

Selenium Biofortification and Cadmium Accumulation in Field-Grown Spinach as Affected by Soil pH

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Spinach belongs to the cadmium-accumulating vegetables. By applying selenium, the uptake and translocation in rice plants could be counteracted. Therefore, the aim of the present study was to investigate whether this approach could also be considered for reducing the cadmium content in spinach and, at the same time, whether selenium could be biofortified in this way. For this purpose, a field trial was conducted on a loamy sandy soil that had been adjusted for several decades to five different pH values between 4.5 and 6.5. Sodium selenite and sodium selenate were applied as soil drench (50 g Se/ha three days after sowing) and as combined soil and foliar application (the latter with additional 25 g Se/ha three weeks before harvest). The selenium treatments (n=4) did not affect fresh matter shoot yield and external quality of spinach regardless of application technique and selenium form. The selenium content of spinach leaves was below 0.4 µg/100 g FM in the control plots. While soil fertilization with selenite did not affect the selenium content of spinach, application with selenate resulted in an increase to 3.9 - 7.4 µg/100 g FM depending on soil pH. A combined foliar and soil fertilization increased the selenium content even further, up to 11.9 µg/100 g FM with selenite and 28.7 µg/100 g FM with selenate. The cadmium content in the spinach leaves ranged between 0.06 and 0.15 mg/kg FM. As expected, the highest cadmium accumulation was observed in the plots with soil pH ≤ 5.0. However, none of the selenium treatments tested had any effect on the cadmium content in the spinach. This could be due to the fact that both the cadmium content of the soil and the selenium fertilization rate were much lower than in previous studies on rice. Further investigations are required in this regard.

Keywords: Cadmium, Selenite, Selenate, Soil Drench, Foliar Spray, Soil pH

Acknowledgements: This work was supported by the COST action CA19116 ´Trace Metal Metabolism in Plants - PLANTMETALS.

Changes in Heavy Metals Fractions Distribution after Organic Amendments Incorporation into the Acidic Soil

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Biochar can be produced from a wide range of organic wastes via pyrolysis, where due to its versatility it has been widely applied in many different research areas, i.e., for carbon sequestration in soils as physicochemical soil amendments and to improve soil productivity. Biochar like lime and fly ash increases soil pH and hence can act as an immobilizing agent for heavy metals. This study aimed to investigate biochar produced from different organic by-products effects on heavy metals immobilization into the soil. Three types of biowaste, such as corn stalks, degistates from sewage sludge biogas, and rapeseed residues of biodiesel production, were used for biochar preparation via pyrolysis and used as soil amendments. Moreover, conventional organic amendment sewage sludge was tested as well. Vegetation experiment with buckwheat was instaled to determine biochar and sewage sludge effect as an immobilization agent. Obtained results demonstrate that biochar is a prospective agent for heavy metals immobilization and could decrease its availability to plants. Organic amendments incorporation increased soil pH and reduced heavy metals availability to plants. Biochar additive increased bond to organic matter nickel and copper content. Biochar application on soil decreased bond to carbonates and bond to manganese and iron oxides content of copper. The hugest changes were observed with zinc. In the soil without, organic amendments around 62 percent of zinc were found in bond to Fe and Mn oxides. While after organic amendments application residuals fraction increased and only around 20 percent of the total determined amount of zinc was found as other fractions which possess higher bioavailability.

Keywords: Heavy metals fractions, sequential leaching, biochar, sewage sludge

Acknowledgements: This study is supported by the South Baltic Interreg program Baltic Phytoremediation (BAPR) No. STHB.02.02.00-SE-0155/18.

Searching for Potential Candidates for Phytoremediation and Microorganism Assissted Phytoremediation of Heavy Metals

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All organisms require metals for their physiological processes. Metal homeostasis needs to be strictly maintained for both deficiencies and excess of essential metals or presence of non-essential ones can have detrimental effects. The current research focuses on identifying i) the mechanisms that plant species and their associated microorganisms employ to inhabit heavy metal contaminated soils and ii) suitable microorganisms (bacteria and fungi) that can assist plant growth in heavy metal contaminated substrates for phytoremediation purposes. We are interested in identifying plant species within the spontaneous flora growing on high concentration of metals, especially Hg. and understanding their molecular mechanisms of resistance/tolerance. Different plant species, inhabiting highly Hg contaminated sites, are screened for their Hg and other metals content in the root and aboveground organs. Rhizosphere microorganisms like bacteria and fungi may facilitate plant survival under heavy metal contamination of substrate either through their direct action on the metal bioavailability or by synthesis of compounds that promote plant growth. Culturable rhizosphere microorganisms, bacteria and fungi, are selected based on their phenotypic resistance to mercury, cadmium, copper, lead and zinc. Bacterial isolates are further investigated for their abilities to produce auxin, siderophores and ammonia. Selected bacteria and fungi are screened for their capacity to assist plant growth on Hg contaminated soil being added either independently or as partners. One fungal isolate of *Fusarium oxysporum* is currently investigated for its Hg resistance strategies (see Cristina L. Văcar poster).

Keywords: mercury plant growth promoting compounds, heavy metals, bacteria, fungi

Acknowledgments: The research is funded by the Romanian National Authority for Scientific Research and Innovation, CNCS—UEFISCDI, project number PN-III-P2-2.1-PED-2019-5254, contract no. 390PED/2020. Travel costs are supported by COST Action 19116.

GATA12-DELLA Shunt Regulates Iron Deficiency Signalling in the Roots

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Iron (Fe) is one of the essential micronutrients for plants, and Fe deficiency is among the most widespread nutritional deficiencies. Although mechanisms involved in Fe uptake are well known, transcription factors (TFs) controlling these mechanisms are limited to some families. bHLH is the predominant family of TFs regulating the Fe deficiency signalling in the roots. GATA-type zinc finger TF family is known to function in various developmental processes. However, its roles in Fe deficiency signalling was not studied before. Our RNA-sequencing analysis showed that GATA12 is associated with hormonal control, DELLA proteins, Fe response network and root development. GATA12 regulate the expression of important Fe deficiency signalling genes by binding to their promoters. Detailed bioinformatics analyses indicated that GATA12 and its close paralogs may function in Fe deficiency signalling by interconnecting the hormonal development of roots via regulation of DELLA proteins. These data suggest essential roles of GATA12 in Fe uptake in plant roots.

Keywords: Arabidopsis, iron deficiency, signalling, transcription factor, root development

Acknowledgements: This research is supported by The Scientific and Technological Research Council of Turkey (project number 119Z149) and the European Union (COST project number 19116).

Identification of Genetic Factors Governing Grain Fe and Zn Contents in Sorghum

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Malnutrition due to intake of poor nutritional quality food i.e., lacking crucial micronutrients like iron and zinc (Fe/Zn), was recognized as a global health threat, more so among children, women of reproductive age, and pregnant and lactating women. Hunger and malnutrition affect one-sixth of the world's population, while a substantial part of the world's population eats rice and wheat as a major staple, which is unacceptably unhealthy. Wheat and rice are deficient in mineral micronutrients which are required for fundamental metabolism in the human body. Biofortification is a sustainable and cost-effective approach to combat micronutrient malnutrition and complements dietary diversification, food fortification and supplementation that are currently used to address micronutrient deficiency in human diets. Genomics-driven biofortification of sorghum to enhance Fe/Zn concentrations in the grain is being implemented at ICRISAT. Biofortifying sorghum would represent a game changer as this crop is climate-resilient and global. Previous studies identified minor-effects Fe/Zn QTLs with little breeding applications. We are set to discover QTLs/genes with meaningful breeding applications as our research design included whole-genome resequencing information (WGRS) and a high-diversity population for the traits of interest (19-73 ppm Fe; 14-54ppm Zn). The following objectives of our research works are discussed: (1) to evaluate a large sorghum diversity panel with several temporal replications in several target environments, (2) carry out Genome-Wide Association Analysis for the grain Fe/Zn contents using WGRS, (3) conduct expression analysis to identify the putative candidate genes and their functions (4) develop single nucleotide polymorphism (SNP) assay for the identified loci and validate the SNPs for their downstream routine use to expedite sorghum breeding for Fe/Zn biofortification, (5) genomics-driven development of superior Fe/Zn biofortified sorghum cultivars in a cost-effective way.

Keywords: Biofortification, Iron, SNP markers, Sorghum, Zinc

Genetic and Genomic Resources for Biofortifying the Crops Grains with Few Examples

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Turkey, part of fertile crescent, is most important center of domestication and diversity of the most of many crops particularly cereals and legumes. Genetic resources from its of diversity, i.e. Turkey, represent the novel source of genetic variation for traits of interest. Micronutrient deficiencies globally affect more than two billion people particularly in developing countries where population is characterized by consumption of staple crops with low mineral concentration/bioavailability, which can result in the impairment of many metabolic processes. Biofortification represents an important strategy to reduce mineral deficiencies, especially in areas of the world where this crop has natural diversity. Thus, identification of the natural germplasm with required mineral concentration is vital for effective utilization in breeding programs to improve the quality of crops that will ultimately contribute to mitigate the world malnutrition problem. We had huge wild and cultivated germplasm of many crops such as wheat, bean, lentil, sorghum, sesame, peanut, soybean etc. We characterize these germplasms for increased mineral elements and many nutritional aspects as well as decreased heavy metal concentrations. Recent advances in genomics, the ability to generate SNP datasets and the whole genome sequencing of the crops have enabled genome-wide association study to be an attractive approach for examining the genetic architecture of polygenic traits. The approach is based on the phenotyping and genotyping of diverse germplasm and relies on historical recombination events that have accumulated during crop evolution, domestication, and crop improvement. We performed whole-genome resequencing of 172 sorghum populations under ERA-NET project and we are identifying the genomic regions for micro-macronutrients under limiting condition of H_2O and N to develop climate resilient elite biofortified sorghum lines. We will also provide here few examples of DNA marker use for identification of linked markers in crops and marker assisted selection for desirable micronutrients contents in some crops.

Keywords: Cadmium, GWAS, Genetic Resources, Se, Micronutrients, Biofortified Crops, Genetic Variations

Acknowledgements: This study is supported by TÜBİTAK (TOVAG:2150630) and ERANET (220N276).

Regulation of the Zinc Deficiency Response in the Legume Model *Medicago truncatula*

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Zinc-deficient soils are widespread globally and human zinc (Zn) malnutrition affects about one-third of the world's population, being more prevalent in populations that rely on cereal grains as staple food. Nevertheless, micronutrient deficiencies are also becoming an issue in developed countries. Fundamental knowledge on the Zn deficiency response and Zn homeostasis regulation in crop species will contribute to developing crops with improved Zn nutritional content (biofortified) and improved resilience to Zn deficient soils. Plant F-bZIP transcription factors play a central role in the transcriptional regulation of the Zn deficiency response, with evidence of evolutionary conservation of this regulatory network in land plants. Our recent work revealed that the Arabidopsis F-bZIPs, bZIP19 and bZIP23, the central regulators of the Zn deficiency response, also function as Zn sensors, through a direct binding of Zn^{2+} ions to their Zn-sensor motif (ZSM). These results indicated that it is plausible that the activity of F-bZIP homologues from crop species can be modulated, through modifications in the ZSM, to improve Zn content in plants. Legumes are generally protein-rich crops, resilient in nutrient-poor soils, used worldwide as part of traditional diets and as animal forage, being therefore an attractive target for micronutrient biofortification. Here, we present the identification of the F-bZIP homologs in the model legume *Medicago truncatula* and their gene expression analysis, performed within a short-term-scientific-mission (STSM). We identified two *M. truncatula* F-bZIP proteins and investigated their function in the Zn deficiency response. Our results suggest conservation of this regulatory network in *M. truncatula*. In addition, we performed a phylogenetic analysis with F-bZIP homologs from representative legume species. Fundamental knowledge on the Zn deficiency response in *M. truncatula* and legume crops will contribute to develop plant-based strategies to address the problems of Zn deficiency in soils, crops and human diets.

Keywords: F-bZIP transcription factors, legume, *Medicago truncatula*, ZIP transporters, Zn deficiency response

Acknowledgements: This study was supported by the Independent Research Fund Denmark (DFF-FTP, grant no. 9041-00182B), by the Chinese Scholarship Council (Grant No. 201907940009), and by the EU-COST CA19116 "PLANTMETALS" through a STSM grant.

DNA Methylation in the Hyperaccumulator *Noccaea caerulescens* "Ganges" Prevents ROS Damages Thus Increasing Cd Hypertolerance

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In recent years, epigenetic studies have garnered attention and hold great potential in both improving the understanding of metal tolerance and hyperaccumulation in plants as well as revealing candidate mechanisms for possible applications. The cruciferous plant *Noccaea caerulescens* is considered a model to study the mechanisms of metal hyperaccumulation and hypertolerance. Among *N. caerulescens* populations, the Ganges ecotype has shown superior Cd accumulation and hyper-tolerance when compared to other populations studied so far. In the presented study, we report the application of the comet assay to investigate the induction of DNA damage occurring in response to Cd treatments in *N. caerulescens* Ganges vs the non-accumulator and close-relative species *Arabidopsis thaliana*. The methy-sens comet assay and semiquantitative Real Time RT-PCR were also performed to associate the metal induced variations in nucleoids to possible epigenetic modifications. Cadmium treatment induced an increase in DNA damage in nuclei of *A. thaliana* and a smaller increase in DNA migration in the Ganges ecotype. Moreover, *N. caerulescens* Ganges showed higher CpG DNA methylation upon Cd treatment when compared to control conditions, and an up-regulation of *MET1* gene, involved in symmetric DNA methylation. In addition, upon Cd treatment, *N. caerulescens* Ganges does not show any oxidative stress while *A. thaliana* Cd treated plants showed an up regulation of transcripts of the respiratory burst oxidase, accumulation of ROS and enhanced SOD activity. Our data suggest a possible role of epigenetic modifications in the *N. caerulescens* to face high Cd shoot concentrations while preserving genome integrity, limiting ROS production and contributing to Cd tolerance. The differences between *N. caerulescens* Ganges and *A. thaliana* regarding the DNA damage and the expression of genes coding for enzymes involved in DNA methylation support the hypothesis of different mechanisms to prevent the Cd-induced DNA damage which evolved in the hypertolerant and hyperaccumulator species.

Keywords: Hyperaccumulators; Cadmium toxicity; Methy-sens Comet assay; epigenetic regulation

Biopolymeric Nano-vehicles for Zinc Application in Plants

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Delivery of highly hydrophilic nutritive microelements via hydrophobic cuticle of plant foliage is one of the major challenges in modern agriculture. In traditional nutrition via roots, up to 80% of microelements permeate to soil and get wasted, therefore foliar treatment is an environmentally and economically preferable alternative. Carboxymethyl cellulose (CMC) was modified to amphiphilic N-octylamide-derivative (CMC-8), which spontaneously self-assemble to nanovehicles. It was found that hydrophobic substituents endow biopolymer with unexpected affinity toward hydrophilic payload. CMC-8 nanovehicles effectively encapsulated ionic zinc ($ZnSO_4$) and delivered it upon foliar application to pepper (Capsicum annuum) and tomato (Solanum lycopersicum) plants. Zinc uptake and translocation in plants were monitored by SEM-EDS and fluorescence microscopic methods. In planta monitoring of carrier was done by labelling nanovehicles with fluorescent carbon dots. Three-dimensional (3-D) structural modeling and conformational dynamics shed light on CMC-8 self-assembly mechanism and explained the zinc coordination phenomenon upon introduction of hydrophobic substituents. The reported experimental and theoretical findings may become a platform for the much-awaited breakthrough in foliar nutrition and could lead to advancements in other biotechnological fields.

Keywords: carboxymethyl cellulose, foliar nutrition, polysaccharide nanovehicles, microelements, zinc, delivery, 3-D structural modeling

Acknowledgements: This study is supported by Chief Scientist of the Israeli Ministry of Agriculture (Grant Number 20-01-0161.).

Organic Acids and Phenolic Compounds of the Fe-Resistant and Susceptible Tomato Genotypes

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Organic acids and phenolic compounds of the root play an important role in Fe efficiency of plants. Organic acids and their concentrations in root tissues of Fe-resistant and Fe-susceptible tomato parents and their F1 hybrid plants were determined. The major organic acids; citric acid, malic acid, fumaric acid and trans aconitic acid were determined in the root extracts. It is seen that especially the amounts of citric and malic acid were higher in resistant parent Roza (P2) than susceptible parent Line No. 227/1 (P1) in conditions of Fe deficiency. Also, for these two organic acids, there are notable differences between reciprocal hybrid plants. It can be said that hybrid combinations are directly influenced by the female parent (maternal effect) in terms of citric and malic acid concentrations. It was noted that the hybrid combinations were low in P1 x P2 and high in P2 x P1, and the difference between them was significant. Under iron deficiency conditions, the root of the resistant parent Roza (P2) accumulated more phenolic compounds than that of the susceptible parent Line No. 227/1 (P1). The phenolic compound concentrations of the hybrid combinations in root tissues were similar as in the case of the organic acids.

Keywords: Iron (Fe) efficiency, *Solanum lycopersicum*, citric acid, malic acid, phenolic compound

Plants Play a Crucial Role in the Development of Soil Fungal Communities in Remediated Substrate after EDTA Washing of Metal Contaminated Soils

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We investigated the importance of plant cover for secondary succession and soil fungal community development in remediated substrate after EDTA washing of metal contaminated soils. Molecular methods were used to quantify the abundance of fungal marker genes (internal transcribed spacer - *ITS* region) in in two soil types (calcareous and acidic) with seeded perennial ryegrass (*Lolium perenne* L.) and without plant cover (bulk soil). Our results show that 120 days after the start of the experiment, *ITS* fungal markers in the soil clearly showed that the presence of plants is the main driver of *ITS* gene copy abundance, which increased in rhizosphere soil in most treatments, while remaining at a low level in bulk soil (no plants present). Interestingly, the addition of environmental inoculum (rhizosphere soil from a semi-natural meadow) had no positive effect on fungal *ITS* copy number. While soil fungal *ITS* copies could be detected at the end of the first vegetational season, arbuscular mycorrhizal (AM) structures were not found in Lolium roots in any of the treatments throughout the first season. However, in the second season, one year after establishing the experiment AM fungal colonisation could be detected in Lolium roots in virtually all treatments, with the frequency of colonised root length ranging from 30% to >80% in some treatments, the latter also in the remediated soil. This study demonstrates the importance of plant and rhizosphere in the development and secondary succession of fungal communities in soil with some important implications for revitalisation of remediated soil and regenerative agriculture.

Keywords: Biodiversity, Fungi, Remediation, Revitalisation, Soil

Rumex acetosella Growing on Different Metal-Contaminated Soils: Uptake of Zinc, Lead and Copper and Response of the Leaves

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Rumex acetosella L. is a pseudometallophyte that grows in areas with normal and high soil concentrations of zinc (Zn), lead (Pb), and copper (Cu). Questions remained if and where metals are absorbed and if the plants' morphology and the cells' resilience respond to the toxic metals in their environment. We therefore compared plants from different areas in Slovakia where soils are contaminated with Zn and Pb (populations Terézia, Lintich) or Cu (populations Špania Dolina, Staré Hory) with those from non-contaminated soil (Dúbravka). Leaf structure and physiology were analysed by light microscopy (LM) and transmission electron microscopy (TEM), and metals were located within leaves and their cells by energy-dispersive X-ray analysis (EDX) in the scanning electron microscope (SEM) and by specific fluorescence dyes. Leaves of R. acetosella are amphistomatic and contain capitate glandular trichomes. Compared to the control, the metallicolous populations had a higher density of both stomata and trichomes. In addition, the normally four-celled glandular heads of trichomes varied and contained fewer cells (down to two) or more cells (five or more, up to ten). These deviations were more frequent in leaves from the metalliferous sites. The ultrastructure of secretory cells revealed fine wall ingrowths bordered by plasma membrane protruding into the cytoplasm. The metallicolous populations had higher contents of Zn and Cu in the epidermal and glandular cells, suggesting secretion of toxic metals. Leaf cells of metallicolous populations tolerated significantly higher concentrations of Zn and Cu compared to plants from the control site Dúbravka. Our findings locate specific accumulation of metals in glandular cells, and they suggest physiological and structural reactions of plant leaves to heavy metals; in particular, the abnormal number of head cells in glandular trichomes and elevated metal content propose effects of heavy metals, especially of Cu, on mitosis and cell plate formation.

Keywords: Metallicolous populations, Leaf epidermis, Glandular trichomes, Cytoplasmic tolerance, Copper (Cu), Zinc (Zn)

Acknowledgements: This work was supported by the Grant Agency VEGA (grant no. 25/5086/05), Grant Agency APVV (grant no. APVV-0432-06), Aktion Österreich–Slowakei (grant no. 46s5], and the Austrian OEAD (grant PL 07_2018). CIUS is a member of EuroBioImaging and of VLSI.

Accumulation of Heavy Metals in the Grains of Differents *Triticum* Species

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Although they are considered heavy metals, Fe and Zn are essential elements of all organisms. As plants are a very important source of these elements for human and animal diet, accumulation of these elements in edible parts of plants is very important. The ability of plants to accumulate mineral elements is very different and depends on many factors, and it also differs between varieties within the species. This study aimed to assess differences in the accumulation of different metals (Fe, Zn, and Al) in the whole grain of 17 *Triticum* species that have different genomic structures and levels of ploidy. The diploid (AA; 3 different genotypes), tetraploid (BBAA; 5), and hexaploid (BBAADD; 9) genotypes of wheat were included in the experiment. The experiments were set up in the Institute of Field and Vegetable Crops in Novi Sad, in calcareous gleyic chernozem. The concentration of Fe, Zn and Al in the grain of tested genotypes was analyzed at crop maturity. The differences are obvious both between different species and between different genomes. The highest accumulation of Fe, Zn, and Al was found in the wild primitive diploid genotypes, where the concentration of Fe and Zn was up to 2.3 times higher than in some tetra and hexaploid genotypes. For this reason, primitive genotypes can be used as an excellent breeding material to create genotypes that could enrich the human diet with these elements. On the other hand, as these genotypes may accumulate to a higher extent undesirable elements as well (i.e. Al), it is difficult to perceive the extent to which a larger amount of accumulated elements in these grains would be health-safe.

KEYWORDS: wheat, di- tetra- and hexaploid, Cd, Zn, Al

Acknowledgements: We thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support (451-03-68/2022-14/200117).

Phenolic Compounds and Antioxidative Capacity in Obligate Serpentinophyte *Halacsya sendtneri* (Boiss.) Dörfl. (*Boraginaceae*)

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Halacsya sendtneri (Boiss.) Dörfl. (Boraginaceae) is an obligate serpentinophyte of the Balkan Peninsula. Ultramafic habitats are specific for unfavourable conditions for a plant development due to high concentrations of heavy metals and deficiency of N, P, K, Ca. Plant material was collected in May 2022 on two ultramafic localities, Ljubić and Pribinić (Republic of Srpska, B&H). Phenolic compounds play an important role in the adaptation of plants to habitat challenges and increased metal concentrations as important components of antioxidative metabolism. The content of phenolic compounds in ethanol extracts of various tissues (rhizome, stem and leaves) of H. sendtneri from both localities was measured. Antioxidative capacity was determined by the following methods: ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), Fe chelation ability and ability for Cu reduction. The results showed that the contents of phenolic compounds were in the next order: rhizome stem leaves stem rosette leaves from Pribinić and stem leaves rhizome rosette leaves stem from locality Ljubić. The ABTS radical scavenging capacity follows the order of the phenolics concentrations from both localities, except from stem and rosette leaves from Pribinić. The greatest ability to reduce Cu was shown for stem leaves at both localities. Samples from Pribinić locality had a higher ability to chelate Fe for all organs, with the most for rhizomes. The results were discussed in terms of the possible important role of phenolic compounds in adaptation of H. sendtneri to the challenges of life on metal-rich ultramafics.

Keywords: ABTS method, Balkans, Cu reduction, Fe chelation, ultramafics.

Acknowledgments: This work was funded by the Ministry of Scientific and Technological Development, Higher Education and Information Society of the Republic of Srpska (Project No. 19.032/961-95/19).

Bacterial-inoculum-assisted Phytoremediation of Cr(VI)-polluted Leachates by Aquatic Hyperaccumulator *Callitriche cophocarpa*

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Callitriche cophocarpa is a macrophyte widely distributed in aquatic systems of temperate zone, proved to be a model-hyperaccumulator toward chromium under laboratory conditions. Ten morphologically-different bacterial isolates were obtained from *C. cophocarpa* and identified. Three bacterial isolates: *Ct1/Microbacterium* sp., *Ct4/Aeromonas* sp. and *Ct 7/Acinetobacter* sp. showed the highest resistance to Cr(VI), i.e. up to 2 mM (104 mg/L). Moreover, these isolates were also able to effectively detoxify Cr(VI) by its reducing to Cr(III) what we detected applying a spectrophotometric assay with 1,5-diphenylcarbazide and electron paramagnetic resonance spectrometry (L-band EPR). We assumed that the inoculation of plants with a consortium consisted of Ct1, Ct4 and Ct7 may improve the phytoremediation of Cr(VI)-loaded wastes if the plants are implemented into real-world conditions. Therefore, the aim of that work was to find out if the inoculation affects: (1) phytoextraction of Cr(VI), (2) plans' physiological status. We treated the plants for 10 days with leachates of a Cr landfill, containing 10.7 mg/L of Cr(VI) - the amount exceeded the standards more than 530 fold. We examined the accumulation of Cr by plants (ICP-OES). Plants' physiological status was evaluated based on analysis of chlorophyll fluorescence and many parameters were considered, e.g.: ABS/RC, TR0/RC, ET0/RC, DI0/RC and others. The average content of Cr in inoculated and non-inoculated plants was at the statistically similar level: 1040.4 and 1135.7 mg/kg d.w., respectively. We found that Cr(VI) influenced significantly the photosynthesis, but the presence of bacterial consortium removed the negative effects of Cr(VI) on plants. Moreover, the inoculated plants incubated without Cr exhibited sometimes even better status than control. We can conclude that *Callitriche* should be inoculated by the above bacterial consortium before introducing into constructed wetland; though inoculation does not lead to an increase in Cr accumulation, it shows very positive effect on efficiency of photosynthesis.

Keywords: *Callitriche*, Cr(VI), macrophytes, photosynthesis, phytobial remediation

Acknowledgements: This study was supported by "Incubator of Innovation 2.0" under the Intelligent Developmental Operational Program 2014-2020 (Measure 4.4) and subsidies of the Ministry of Science and Higher Education of the Republic of Poland (in years 2020 and 2022).

Iron Determines Seed Germination Speed by Weakening Endosperm through ROS

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Iron (Fe) is an essential micronutrient for all living organisms. In plants, it functions as a cofactor in a range of metabolic processes, towards growth and development. Although many processes in which Fe is involved are well-known, whether germination is one of them is yet to be explored. Endosperm weakening is a developmental process controlled by interactions between the endosperm cap and the radicle. It is a crucial step in germination that allows quiescent tissues surrounding the embryo to become active allowing radicle protrusion. Previous studies showed that it is regulated through GA and ethylene-ABA interactions. In our lab, we observed a late germination phenotype in a mutant tomato, the infamous chloronerva, that cannot synthesize nicotianamine (NA), an essential chelator for Fe acquisition in plants which could be partially rescued by supplementation of NA. The present study investigated the possible mechanisms that involved NA in determining the germination speed of tomato. Our results surprisingly showed NA's stimulatory effect on germination speed is not restricted to the chloronerva mutant but extends also to wild-type tomatoes. In tomato, germination speed is tightly dependent on the weakening of the endosperm, a process previously shown to be mediated by enzymatically produced ROS. Perl's staining showed that Fe is preferentially accumulated in the endosperm around where root protrudes in tomato. Deferoxamine, a chemical that chelates Fe to repress Fenton-related ROS production was sufficient to postpone germination. Effects of NA and deferoxamine on seed germination were conserved in Arabidopsis but not its endosperm-missing close relative Brassica napus. Taken together, the results linked Fe for the first time to germination by providing a mechanistic explanation.

Keywords: Deferoxamine, endosperm weakening, iron, nicotianamine, ROS

Interactive Effects of Climate Change, Nitrogen and Zinc Nutrition on Growth and Yield Performance in Wheat

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Agricultural sustainability requires addition of macro (e.g., nitrogen: N) and micro (e.g., zinc: Zn) nutrients exported from soils by harvested products. World's changing climate is hindering the nutrition and hence the yield of staple food crops. Here we investigated the interactive effects of predicted climate change, N and Zn nutrition on the performance of bread wheat (*T. aestivum* cv. Ceyhan-99) cultivated with adequate or low N and Zn under ambient (ambient CO_2 and temperature) or predicted climate (700 µmol CO_2 mol^{-1} and 3°C temperature rise) conditions. The predicted climate scenario (PC) caused early onset of successive growth stages and reduced the maturation time. Under both PC and ambient climate (AC), adequate N and Zn supply significantly improved straw and grain yield through increased number of spikes $plant^{-1}$ and grains $spike^{-1}$. However, PC treatment significantly reduced straw and grain yield due to reduced spikes $plant^{-1}$, particularly in plants supplied with adequate N. Adequate Zn and PC treatments were significant only under adequate N supply. Adequate N increased grain protein and Zn, particularly under adequate Zn treatment. Although the grain protein concentration did not vary significantly, grain protein yield (i.e., total mass of protein in whole grains of a single plant) was severely reduced by the PC treatment. Our results demonstrated a dramatic reduction in maturation period of wheat plants cultured under the PC scenario. Although supplied with ample fertilization (with N, Zn and other mineral nutrients), plants cultured under the PC scenario produced lower straw and grain yield. While the PC and adequate Zn treatments enhanced main spike gain yield and grains $spike^{-1}$, PC declined overall grain yield, particularly due to severe reduction in spikes $plant^{-1}$. We conclude that sustaining a higher number of spikes per plant and ensuring an adequate N and Zn nutrition are essential to minimize the adverse effects of rising temperatures on yield and quality of wheat, and to exploit the elevating CO_2 in the atmosphere.

Keywords: Climate Change, Nitrogen, Zinc, Nutrition, Wheat

Acknowledgements: This study was fully supported by Sabanci University's personal research funding (PRF) system.

Genes Involved in mRNA Surveillance are Induced in *Brachypodium distachyon* under Cadmium Toxicity

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Cd accumulation in plant cells results in dramatic problems including oxidative stress and inhibition of vital enzymes. It also affects mineral uptakes by disrupting membrane permeability. Interaction among Cd and other plant nutrient elements changes the nutritional contents of crops and reduces their yield. In the present study, Cd stress in *Brachypodium distachyon* led to the upregulation of some heavy metal transport genes (influx or efflux) encoding cation-efflux proteins, heavy metal-associated proteins and NRAMP proteins. The Arabidopsis orthologs of the differentially expressed *B. distachyon* genes (DEGs) under Cd toxicity were identified, which exhibited Bradi4g26905 was an ortholog of *AtALY1*-2. Detailed co-expression network and gene ontology analyses found the potential involvement of the mRNA surveillance pathway in Cd tolerance in *B. distachyon*. These genes were shown to be downregulated by sulfur (S) deficiency. This is the first transcriptomic study investigating the effect of Cd toxicity in *B. distachyon*, a model plant for genomic studies in Poaceae (Gramineae) species. The results are expected to provide valuable information for more comprehensive research related to heavy metal toxicity in plants.

Keywords: *Brachypodium distachyon*, cadmium toxicity, mRNA surveillance, heavy metal stress, RNAseq

Acknowledgements: The study was funded by Akdeniz University Scientific Research Projects Coordination Unit. Grant number: FBA-2018-3532.

Zinc Priming to Enhance Waterlogging Stress Tolerance in Cucumber

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The research was focused on the analysis of the physiological, molecular and metabolic responses of Zn primed cucumber under waterlogging stress. It is known that some natural products or synthetic chemicals can protect crop plants against abiotic stresses through induction of molecular and physiological defense mechanisms and moreover some of these compounds can also stimulate plant growth. It has been proved that Zn may improve abiotic stress tolerance mainly by boosting physiological and enzymatic antioxidant defence mechanisms. However, our knowledge of the effects of internal Zn concentration on the performance of plants under waterlogging stress is limited. This study hypothesized that plants with higher Zn concentration could improve cucumber performance under waterlogging stress. The major objective of this study was to investigate the effect of foliar-applied Zn as ADOB® 2.0 Zn IDHA and $ZnSO_4 \cdot 7H_2O$ on waterlogging tolerance in cucumber. Chlorophyll fluorescence measurements, followed by OJIP analysis, were used for studies of plant reactions under stress. The H_2O_2 content and the level of antioxidants such as CAT, APX, POX, and ascorbate (ASC) have also been studied. Using “omics” approaches, including RNA-seq analysis, HPLC-ICP-MS QQQ and LC-MS/MS QUTRAP metabolomics we identified genes, metabolites, and biochemical pathways with a possible role in the molecular priming induced by Zn as a biostimulant of the waterlogging stress tolerance.

Keywords: cucumber, priming, waterlogging, zinc, Zn

Acknowledgments: This study was subvented by the Polish Ministry of Science and Higher Education in 2021.

Zinc and Selenium Biofortification of Broccolini: Effects on Plant Growth and Mineral Accumulation

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Millions of people have Zn and Se-deficient diets, and agronomic biofortification is one effective way to alleviate these inadequate intakes. The aim of this study was to evaluate the use of agronomic Zn and Se biofortification of broccolini-a new hybrid crop variety derived from a cross between kalian cabbage and broccoli. Plants were grown in pots using a Zn and Se deficient soil. Four fertiliser treatments were tested for Zn: (1) control (2) soil application of 5 mg $ZnSO_4 \bullet 7H_2O$ kg^{-1} soil (3) foliar application at the early flowering stage of 0.5% (w/v) $ZnSO_4 \bullet 7H_2O$ (4) combined soil and foliar treatments; and three foliar applications in the Se study: 0, 0.05% or 0.10% (w/v) sodium selenate, applied at the early flowering stage. Florets were harvested in four sequential harvests, showing that both yield and nutritional composition were better in the early harvests, producing fewer but heavier and nutrient-richer florets. Soil Zn application increased floret production. There were increases in the Zn concentration stem+leaves and florets of 12- and 2.5-fold in foliar and soil+foliar treatments respectively. The increase in total Se concentrations was more than 80- and 170-folds in both root and shoot of 0.05% and 0.10% Se, respectively, and by 270- and 600-fold in the florets. Although, boiling reduced concentration by 40% and >50% in Zn and Se, respectively, an intake of 100 g of fresh weight boiled broccolini florets biofortified with soil+foliar Zn treatment and with 0.05% Se, would deliver ~49 mg Zn, a 46% increase than in the non-biofortified broccolini, and about 590 µg Se day^{-1} (upper of the tolerable upper limit for Se intake, and lower application rates would need to be determined to be used for human food purposes).

Keywords: Selenium, sodium selenate, zinc, zinc sulphate, agronomic biofortification

Comparison of the effects of cadmium on white and black mustard

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Higher concentrations of heavy metals in the soil may harm plant metabolism, quality, and yield. Cadmium is very toxic for humans and animals and it can often reach the food chain through plant-based foods. In addition, it is important to find genotypes that will be adequate for phytoremediation, which is the best way to reduce Cd in slightly and moderately contaminated soils. The ability to synthesize sulfur-containing compounds, which is a feature of plants of the family Brassicaceae, is important for plant tolerance to the presence of heavy metals. Before sowing, mustard seeds (*Sinapis alba* (L.) and *Brassica nigra* (L.) Koch) were for 24 h submerged in deionized water (control) or $CdCl_2$ dissolved in water to final concentrations of 10 and 50 µmol $CdCl_2$ dm-3. Subsequently, seeds were germinated in the quartz sand, in an incubator, at 26ºC. By the end of the experiment, the plants were grown in pots containing ½ strength Hoagland nutrient solution (control) or the same solution containing 10 or 50 µmol $CdCl_2$ dm^{-3}, in a greenhouse. The concentration of 50 µmol dm-3 Cd affected growth, nitrate metabolism, the concentration of free proline, and the content of photosynthetic pigments in the leaves of both black and white mustard. The highest difference between the two species was in the content of free proline in the presence of 50 µmol Cd dm^{-3}, where white mustard had 2.7 and black 6.1 times higher content of free proline in the leaves than the control. The total leaf area in white mustard was only 6.7% and of black mustard 13% relative to control and activity of nitrate reductase was 30% and 67% of the control, respectively. Based on these results, black mustard has a greater potential to be used in phytoremediation than white mustard.

Keywords: cadmium (Cd), *Sinapis alba* L. and *Brassica nigra* L. chloroplast pigments, transpiration, leaf area

Acknowledgements: We thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support (451-03-68/2022-14/200117).

Stable Isotope Fractionation: A Tool to Identify Major Processes that Separate Cadmium from Zinc in Plants?

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Metal isotopes fractionate in plants, which results in systematic differences of isotope ratios in distinct plant tissues. The drivers of isotope fractionation in plants are biological and physicochemical processes such as sorption, complexation, membrane transport, diffusion and redox processes. For the non-essential trace element Cd, grains of cereals contain isotopically heavy Cd while light Cd isotopes are retained in roots and leaves. The Cd isotope fractionation patterns correspond with a strong retention of Cd in roots and leaves, which causes that only a minor fraction of Cd is transported into grains. In contrast to Cd, a large fraction of the essential trace element Zn is transported to grains, which are enriched in light Zn isotopes compared to all other plant tissues. The opposing isotope fractionation of these chemically similar elements may be driven by thiol (R-SH) containing organic ligands such as glutathione, phytochelatins or metallothioneins. These ligands tend to favorably complex light Cd and Zn isotopes at equilibrium while Cd has a higher affinity to thiols than Zn. Hence, strong complexation of Cd to thiol ligands and their subsequent sequestration to e.g., vacuoles may control the separation of Cd from Zn and drive the distinct isotope fractionation of these elements in cereals. To further test this hypothesis, a consortium of PLANTMETALS members will conduct Cd and Zn binding experiments in vitro with a small thiol containing model protein (metallothionein), conduct uptake experiments with recombinantly metallothionein producing *Escherichia coli* cells, and ultimately link the speciation of Cd and Zn with their pathways in plants by measuring species-specific Cd and Zn isotope ratios in plants.

Keywords: Zinc, Cadmium, stable isotopes, thiols, crops

Advancing in the Application of Innovative Phytomanagement Strategies in Contaminated Areas of the SUDOE space (the Phy2SUDOE Project)

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Phytomanagement is a set of phytotechnologies combining (1) phytoremediation options based on the use of plants, associated microorganisms, biostimulants and amendments to control the pollutant linkages due to contaminant excess in soils, (2) the production of valuable biomass that can be locally processed to supply e.g., wood, resin, essential oils, bioenergy, ecomaterials, biosourced chemicals, ecocatalysts, etc., and (3) the remediation / supply of ecological functions to enhance ecosystem services (C sequestration, erosion control, habitats, biodiversity conservation, etc.). The Phy2SUDOE project aims to value sites contaminated by metal(loid)s and organic compounds in the South-west European region (SUDOE) through the use of phytomanagement. It also aims to conserve endemic biodiversity typical of some contaminated sites (e.g., metallophytes, plant growth-promoting bacteria, mesofauna, etc.) due to their intrinsic and utilitarian value (e.g., biotechnological applications) and role in the ecosystem life cycle. The PhytoSUDOE network has been extended to 15 contaminated sites distributed over southwest France, Portugal and Spain. Its human capital has been expanded with various partners (site managers, universities, R&D centers, companies, and administrations) to stimulate the creation of phytomanagement strategies and the transfer of results. Each site has its own action plan and management: i.e. conceptual model, option appraisal, remediation strategies, protocols, monitoring, etc., while following harmonized practice guidelines. All sites total an area of 350,000 m² where the various phytomanagement strategies are applied. The diversity of the site properties, as well as the surface area treated, makes it possible to improve the ecological quality of these SUDOE areas, but also to develop protocols, tools and management models that can be implemented in many contaminated sites of the SUDOE zone and in the world. This presentation will highlight examples of phytomanaged sites in Nouvelle-Aquitaine, Occitanie, Basque Country, Galicia, and Northern Portugal.

Keywords: Metal(loid), Phytomanagement, Excluder, Hyperaccumulator, Ecosystem services

Acknowledgements: The Phy2SUDOE project (SOE4/P5/E1021) is funded by the Interreg Sudoe Programme.

Status and Distribution of Heavy Metals in Soils of Bosnia and Herzegovina

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Soil pollution is defined as presence of toxic chemicals in high enough concentrations to pose a risk to the ecosystems and human health. A literature analysis was performed according to the available papers listed in the Web of Science concluding with 17.02.2022. Keywords used for the search were "heavy metal soil Bosnia and Herzegovina" and „trace elements soil Bosnia and Herzegovina". Additionally published articles in regional journals including papers published in Bosnia and Herzegovina and data obtained from relevant institutions (Federal institute for Agropedology in Sarajevo, Agronomy Institute Sarajevo, Agronomy Institute of Republic of Srpska Banja Luka, Faculty of Agriculture and Food Science Sarajevo, Faculty of Forestry Sarajevo) have been included in this review. According to available data detected pollution with heavy metals was usually around urban areas and was a result of anthropogenic activities industry, agriculture and mining. Depending on the type of heavy metal pollution spikes in heavy metal, concentrations are detected in the soil of industrial cities such as Tuzla, Kakanj, and Zenica. Some pollution was found also in agricultural soils far away from industrial centers. In some cases, detected elevated concentrations of Ni, Cr and Mn are inherent to the substrate especially in areas where littoral substrate is serpentine origin. High soil concentrations of heavy metals because of human activities, such as As, Cd, Hg and Pb are identified in cities Zenica, Mostar, Tuzla, Vareš. The shown contamination is according to New Dutch list (limitations according to B&H legislations). For large portion of B&H data are still missing and future research should be oriented toward completing the map of soil pollution in B&H.

Keywords: Cd, Heavy metals, Industrial region, Pb, Soil pollutions

Cadmium Content on Corn and Transfer Factor in Polluted and Unpolluted Sides of Kosovo

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Cadmium as a trace metal is considered as one of the soil and plant contaminants, due to its toxicity and persistence in the soil. Its transfer from soil to plants is a public concern as it enters the food chain and can be a risk to human health. The main purpose of this research is to look at the transfer factor to corn plants in contaminated and non-contaminated soils. According to standard procedures, 20 average samples of soil and corn plants were taken (10 soil and plant samples for each side), and the cadmium level was measured in the analyzed samples. The Cd content on the contaminated side showed values up to 10.27 in soil (mean 4.27), respectively 4.43 mg kg^{-1} in plants (mean 1.06), while on the non-contaminated side these values showed up to 0.12 in soil (mean 0.1), respectively up to 0.33 mg kg^{-1} in plants (mean 0.14). The Cd transfer factor showed opposite values, respectively on the contaminated side showed an average of 0.25 (up to 0.49), while in non-contaminated side the average was 1.33 (up to 2.84). Based on the results we can conclude that on the contaminated side the amount of Cd both in soil and in plants was high, while the transfer factor was low and vice versa on the non-contaminated side, low amount in soil and plants but the transfer factor was high. Therefore, we suggest to be careful when using the transfer factor because it does not show the real state of contamination.

Keywords: Trace metals, polluted, risk, food chain, transfer factor

Interactive Effect of Chelated and Non-chelated Micronutrients with Fe-EDTA: Micronutrients Uptake and Growth of *Vicia faba* under Acidic and Alkaline Conditions

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Chelated iron (e.g. Fe-EDTA) is widely used in hydroponics / greenhouse farming system, as it is more efficient Fe source than the non-chelated Fe. Due to high affinity of chelating agents, free Cu, Zn and Mn ions can replace Fe from Fe-EDTA in a standard nutrient solution. Under high pH and alkaline conditions, competition between metal ions (Fe, Cu, Zn and Mn) for chelating agents could be even stronger. Therefore, the interaction of chelated and non-chelated forms of Zn, Mn and Cu with Fe-EDTA was studied in *Vicia faba* for micronutrients uptake under different levels of alkalinity (0, 5 and 15 mM $NaHCO_3$). Na-free chelated micronutrients were used in treatments to avoid any additional Na in nutrient solution. Plants were harvested after four weeks of growth in nutrient solution. The shoot dry weight remain unchanged at 0 and 5 mM $NaHCO_3$, but reduced by half at 15 mM $NaHCO_3$, whereas, the shoot dry weight was uninfluenced by chelated and non-chelated micronutrients. The results revealed that the concentrations of Fe, P and Zn in shoots supplemented with chelated micronutrients increased at-least by 21, 16 and 14% respectively, as compared to non-chelated micronutrients at all alkalinity levels. Moreover, the application of chelated micronutrients increased the Mn concentration in shoots by 20 and 26% at 0 and 5 mM $NaHCO_3$ respectively, but reduced Mn concentration by 13% at 15 mM $NaHCO_3$ as compared to non-chelated micronutrients. Based on available results, it is concluded that Fe availability from Fe-EDTA to plants can be enhanced by using chelated forms of other micronutrients as well. In addition, chelated micronutrients also facilitates the uptake of P and Zn by plants, which could be attributed to reduction in their precipitation.

Keywords: Alkalinity, Chelated micronutrients, Fe-EDTA, Non-chelated micronutrients, Precipitation

The Potential of *Brassica napus* for Phytoremediation of Multielement Contaminated Soils – A Field Test

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Heavy metals are known as contaminants present in the environment as a consequence of industrialization and other human activities. When present in an aquatic and terrestrial environment they could cause harmful effects on plants, animals, and humans. Polluted soils are often contaminated with more than one heavy metal due to the origin of the contamination (industry, mining, etc.). Phytoremediation of multielement contaminated soils is challenging since plants have different potential for uptake of different metals. In this study, the ability of energy plant rapeseed (Brassica napus) for phytoremediation of soil contaminated with Cd, Ni, Cu, Cr, and Pb was assessed through a field test. Polluted soil was located at Itebej, Vojvodina, Serbia. The concentrations of heavy metals present in the soil were 194.4 mg/kg, 45,3 mg/kg, 129,8 mg/kg, 4,1 mg/kg, and 105 mg/kg for Cr, Ni, Cu, Cd, Pb, respectively. The winter rapeseed variety Zlatna was grown to full maturity from September 2021 to June 2022. The plant samples were taken five times during growth (after 10, 26, 29, 32, and 38 weeks), and the concentration of heavy metals in the below- and above-ground parts was monitored. The phytoextraction efficacy was assessed with parameters, such as bioconcentration and translocation factor. Among all heavy metals investigated, the highest bioconcentration and translocation factor were observed for Cd. Bioconcentration factor is the measure of the ability of a plant to take up heavy metals from soil and it was higher than 0.7 for Cd, whereas translocation factor indicates the ability of a plant to translocate heavy metals from the roots to the aboveground part, and at some point, it was higher than 1. All the above-mentioned suggest that the rapeseed has the potential for phytoextraction of Cd in contaminated soils and translocating it to above-ground parts.

Keywords: Cadmium, Heavy metals, Rapeseed, Soil, Translocation

Acknowledgements: This study has funded by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 101006912-Phy2Climate.

Insights on Zn deficiency and Zn excess effects on the Root Apical Meristem in Arabidopsis

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Zinc, an essential cofactor to many enzymes, is an important micronutrient in plants. Crop culture on Zn deficient soils, which are widespread worldwide, is limiting productivity and quality and is considered as a concern for human nutrition. In contrast, with industrialisation, soils were contaminated in large areas with toxic metal concentrations including Zn. To address both issues of Zn deficiency and Zn excess, it is required to better understand the responses to variation in Zn supply in plants, which will enable the design of efficient biofortification or phytoremediation strategies, respectively. Root growth is regulated at the root apex, where mitosis, as well as cell elongation and differentiation occur. Zn deficiency and excess are known to have an effect on root growth, for instance by affecting auxin production and Reactive Oxygen species (ROS) levels in the Root Apical Meristem (RAM). However, a detailed investigation on the delicately balanced regulation of the root meristematic activity by Zn is still missing. In this study, root responses to Zn deficiency and Zn excess were examined in Arabidopsis. We re-assessed RAM activity upon both Zn deficiency and Zn excess and observed distinct effects of both treatments on cell elongation and differentiation, as well as on the cell cycle itself. To better pinpoint which specific processes are affected by altered Zn nutrition, RNA-Seq datasets were generated for root tips versus differentiated roots comparing normal Zn supply to Zn excess or Zn deficiency. We observed root tip specific responses and identified potential candidate genes (e.g. transporters, hormonal response, cell-wall) for involved in these responses Zn supply imbalance. Furthermore, linking the RNA-Seq datasets with Zn localization in root tissues by Laser Ablation-ICP-MS experiments and publicly available single-cell transcriptional maps provided a detailed assessment of the RAM response to Zn supply.

Keywords: zinc, root apical meristem, zinc imaging, development

Measuring the Concentration of Heavy Metals in Leaves of Oriental Plane (*Platanus orientalis* L.) in urban area of the capital of Albania

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Air pollution is an important concern in urban environments. Plants are capable of reducing air pollution through absorbing heavy metals in their tissues. The aim of this study is to measure the concentration of heavy metals in the leaves of oriental plane (*Platanus orientalis* L.) trees situated in heavy urban traffic sites. The oriental plane (*Platanus orientalis* L.) is a large, deciduous tree of the Platanaceae family that lives long, grows up to more than 30 m and widely spreads the crown. The study area was selected in the urban city of Tirana, capital of Albania, where around 200,000 vehicles circulate in a 24 hours interval. The urban area of Tirana has been divided in three different strata: (i) heavy vehicle traffic, (ii) heaviest vehicle traffic and (iii) no vehicle traffic within a radius of around 1 km. Sample of plane tree leaves have been collected in all the three strata in three seasons: spring, summer and autumn. Concentrations of cadmium (Cd) and chromium (Cr) on the leave tissues have been measured through atomic absorption spectrometry with electrothermal atomizer with graphite furnace. Obtained results revealed that cadmium and chromium content varied between 0.10 mg/kg to 0.14 mg/kg and 1.63 to 3.50 mg/kg, respectively. Both elements, Cd and Cr had the highest concentration in station 2 (heaviest vehicle traffic area), during summer and the lowest during autumn.

Keywords: Albania, Heavy metals, Leaves, Plane tree, Urban area

Introduction

Tirana, the capital of Albania, has the largest number of inhabitants (more than 906'166 or 31.8 % of the population of Albania). Around 200,000 vehicles circulate in a 24 hours interval in Tirana and this number is increasing day by day (INSTAT, 2020). Important as they are, motor vehicles pollute the environment.

The pollutants released from the motor vehicles are carbon monoxide, carbon dioxide, hydrocarbons, nitrogen oxides, sulfur oxides, soot in particle form, and some heavy metals. The main heavy metal pollutants caused by traffic are lead (Pb), nickel (Ni), mercury (Hg), cadmium (Cd), zinc (Zn), chromium (Cr), and copper (Cu) (Bilge & Çimrin, 2013). Heavy metal, which can be released from various anthropogenic sources (e.g., combustion of fossil fuels and industrial activities), can have adverse effects on living things (Sawidis et al., 2011). Plants can be used as bio-monitors of environmental pollution (Sawidis et al., 2011).

WHO (1996) permissible limits for some heavy metals in plant are as follows: Cd (0.02 mg/kg), Zn (0.60 mg/kg), Cu (10 mg/kg), Cr (1.30 mg/kg), Pb (2 mg/kg), Ni (10 mg/kg).

Heavy metal pollution leads to stress in the plants. It can directly or indirectly affect the physiological functions and biochemical events in plants, especially after certain doses. High levels of heavy metal accumulation in plant tissues negatively affects many things such as mineral nutrition intake, photosynthesis, transpiration, enzyme activity, nucleic acid structure, chlorophyll biosynthesis, and germination (Duru et al., 2011).

Air pollution is the most important element of the environmental pollution. It has been shown that in many urban areas of the world a major source of air pollution is motor vehicles traffic which contributing 57–75% of total emissions (WHO 2006).

Most respiratory tract diseases leading to death are caused by air pollution. The effects of air pollution on human health arise due to the high levels of harmful materials in the atmosphere. In order for humans to live healthy and comfortable lives, the air must be absolutely clean.

It was revealed that 70-90% of carbon monoxide, 40-70% of nitrogen oxide, 50% of hydrocarbons, and 100% of lead emissions originate from motor vehicles (Çelik & Çelik, 2013). In Albania, 68 of 100'000 persons die every year because of air pollution (WHO, 2019).

In urban cities, trees that grow alongside the main roads and highways pay an important role in regulating services, such as the air quality, through filtering and taking up gases and particles (Beckett et al., 2000). Plane tree (*Platanus orientalis* L.) is originally native to South Western Asia. It can be planted from different elevations up to nearly 2500 meters above sea level and the species is growing up to 30 m tall. Plane trees are widely planted in Tirana city, in parks and along streets. The aim of this study was to measure the concentration of heavy metals (Cr-chromium and Cd - cadmium) in the leaves of oriental plane (*Platanus orientalis* L.) trees situated in different urban traffic sites.

Materials and Methods

The fieldwork for the plane leaves collection was carried out in three seasons, spring (second week of May), summer (second week of July) and autumn (second week of October). The locations chosen for taking samples were in three stations of the city of Tirana; according to different levels of road traffic (sampling stations are shown in the map of figure 1). The first station represents a crossroads with a high concentration of road traffic. The second station represents the largest intersection at the western entrance to the city of Tirana. This is an area with heavy traffic throughout the day. The third station is located in the biggest park of Tirana, a park only for the movement of pedestrians and not cars, but very close to some of the roads with heavy traffic. The number of plane leaves (*Platanus orientalis* L.), for each station, in each season, was about 10 leaves, so a total of 90 plane leaves were used for this study.

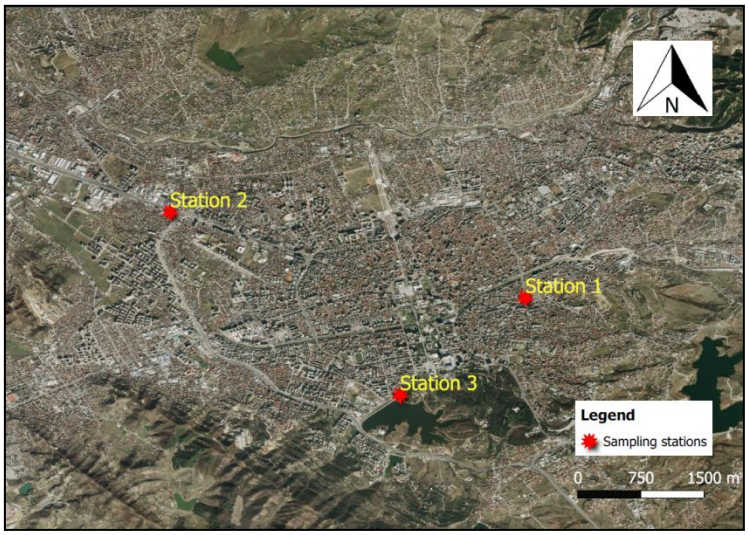

Figure 1: Sampling locations of *Platanus orientalis* L. tree leaves in Tirana city

After cutting leaves from trees, immediately they were placed in plastic bags and were imparted on ice to the laboratory. After being washed with distilled water, the leaves were completely dried, then homogenized and placed in polypropylene bags with closure and accompanied with the corresponding labels. The next step was weighing through the analytical scale, about 0.5 gram for each sample. We made 2 replicates weighing for each sample.

Before starting with the digestion process, in a graduated cylinder, we added 60 ml of hydrochloric acid and 20 ml of nitric acid, in a ratio of 3:1. In each of the glassware with the pre-weighed samples, was poured 15 ml of this preparation, and they were left to act overnight with watch glasses placed on top. The digestion process: All the samples were placed in the oven (starting at 90˚C and ending the last hour at 200˚C for finishing the whole digestion). This process continued for 3 hours and at the end, when the digestion was completed, the material from each glassware was passed into 50 ml flasks, being filled with distilled water up to the mark. The work continued with the vacuum filtration process through 0.45 micrometer glass filter of 47 mm in diameter.

The determination of heavy metals, Cd and Cr was done using an atomic absorption spectrometer with an electrothermal atomizer with a graphite furnace. In atomic emission spectrometry, the source of radiation is absent and the sample is excited by temperature.

Sample analysis is done in 5 steps:

• First drying, or dehydration of the sample, which is done at 90 °C temperature.
• Second drying, takes place at a temperature of 100-120 °C, where the solvent is removed from the sample solution injected into the oven.
• Calcinations take place at a temperature of up to 600 °C, where all possible components are removed from the element that we will analyze.
• Atomization takes place at temperatures up to 2000 °C; the element passes into a gaseous state.
• Cleaning, where the temperature goes to 2400 °C and the evaporation of all sample residues from the graphite tube takes place (Trikshiqi, 2016).

Before starting to analyze the samples, a calibration procedure is required, which consists in finding the relationship between the measured analytical signal and the concentration of the analyte. For this purpose, a series of standard analyte solutions of known concentrations was prepared accuracy covering the entire work area. Measurements of the standard solutions signals are made and the dependence curve of the signal values is built. After calibrating the method, heavy metal concentrations were measured in sample solutions. To find the concentrations of heavy metals in the samples, we used the calculation method based on the corresponding equations of the calibration curves.

Results and discussion

The present investigation showed the relationship between levels of metal pollution and seasons, also the impact coming from the traffic road. Plane tree (*Platanus orinetalis* L.) is a deciduous tree, leaves reach their maximum size during summer, and thus the heavy metal accumulation is higher during summer, for both metals (Cd and Cr).

Cadmium- Cd

Cadmium (Cd) is one of the most toxic metals in the environment, and has noxious effects on plant growth and production, such as; leaf chlorosis, a delay in the growth rate, and inhibition of respiration and photosynthesis (Navarro et al., 2019), increased oxidative damage, and decreased nutrient uptake ability (Mohamed et al., 2012). Cadmium is usually known as a very labile metal, which can be easily transported by plants roots to the leaves. On the other hand, the uptake of metals, by leaves increases with increasing metal concentration in the external medium, such as dust and gases in air.

In the table below are presented the results obtained from the measurements. Results are expressed as the average value of two replicate samples.

Table 1. Cadmium (Cd) concentration in mg/kg in leaves of *Platanus orientalis* L. tree

Season/ Location	Spring (Sp)	Summer (Su)	Autumn (Au)
Station 1 (S1)	0.10	0.14	0.11
Station 2 (S2)	0.10	0.13	0.11
Station 3 (S3)	0.11	0.11	0.12

Station 1(S1) represents a heavy vehicle traffic site, station 2 (S2) represents the heaviest vehicle traffic site of this study and station 3 (S3) represents a no vehicle traffic site within a radius of around 1 km.

In the graphic below (Figure 2) is shown the interval plot of Cd concentration in selected plant species in three seasons and in three selected stations. Evaluation of the experimental data was carried out by using the MINITAB 17 program as well as by Excel.

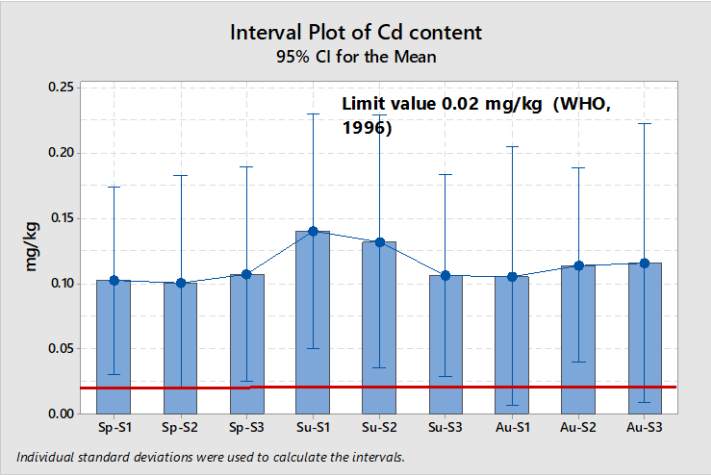

Figure 2: Interval plot of Cd content

The obtained results show that concentration of Cd varied from 0.10 mg/kg in stations Station 1 (heavy vehicle traffic area) and Station 2 (heaviest vehicle traffic area) during spring to the highest values 0.13 and 0.14 mg/kg found in Station 3 (no vehicle traffic area) and Station 1 (heavy vehicle traffic area), respectively during summer.

Based on the recommended value of Cd concentration in plant species, according to WHO, 1996 "Permissible limits of heavy metals in plant and soil" it was concluded that the content of Cd in plant species *Platanus orientalis* L., in the urban area of the capital of Albania exceeds the recommended limit by more than 5 times.

Probability plot of Cd concentration in all samples was used to evaluate the distribution of Cd in plant species (Figure 3). According to the obtained data, it was concluded that Cd concentration in selected

plant species (in three different seasons and three different stations) can be considered to be "normally distributed". This is confirmed by the P-Value which has resulted P-Value>0.05 (which suggests a 95% of confidentiality) as well as the low value of Anderson-Darling test, (AD). Normally distributed data suggest that concentration of Cd in plant species is mainly of natural origin, depending on the content of Cd in soil and not from any anthropogenic input. Higher concentrations of Cd registered during summer can be related mainly to the higher temperatures (lack of rainfall) and dust content in the air, which can contribute to higher accumulation of the element.

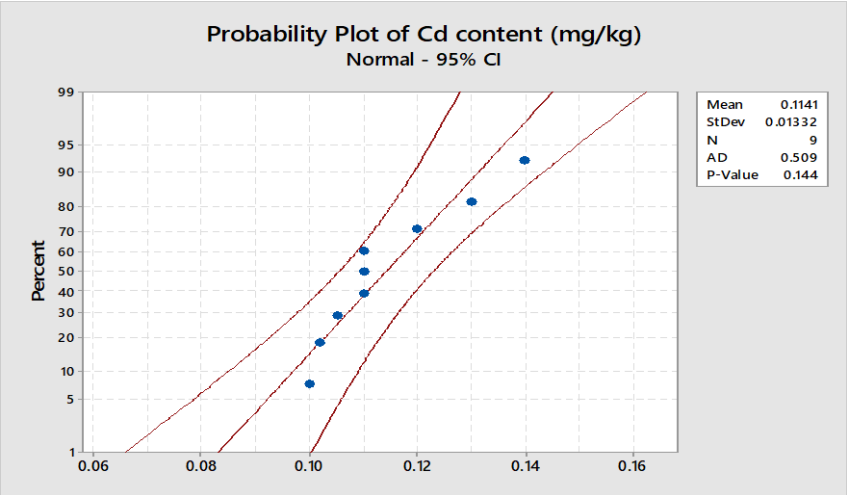

Figure 3: Probability plot of Cd

Chromium - Cr

Chromium (Cr) is usually known to have a low liability from soil to plants, but its content in plants is closely related to the concentration in soils. Based on the data published by Gjoka et al., 2011, the content of Cr in soils of Tirana city is 174.2 mg/kg, which is about twice of the value recommended by the WHO, 1996.

In the table 2 are presented the results obtained from the measurements. Results are expressed as the average value of two replicate samples.

Table 2. Chromium (Cr) concentration in mg/kg in leaves of *Platanus orientalis* L. tree

Season/ Location	**Spring (Sp)**	**Summer (Su)**	**Autumn (Au)**
Station 1 (S1)	2.36	3.04	1.63
Station 2 (S2)	2.15	3.50	1.90
Station 3 (S3)	3.07	2.82	1.85

Station 1 (S1) represents a heavy vehicle traffic site, station 2 (S2) represents the heaviest vehicle traffic site of this study and station 3 (S3) represents a no vehicle traffic site within a radius of around 1 km.

In Figure 4 is shown the interval plot of Cr concentration in selected plant species in three different seasons and in three selected stations. Evaluation of the experimental data was carried out by using the MINITAB 17 program as well as by Excel.

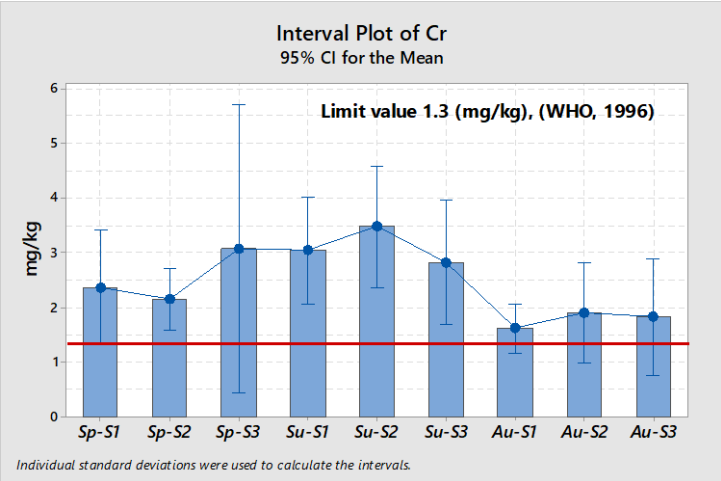

Figure 4: Interval plot of Cr

The obtained results show that concentration of Cr varied from 1.63 mg/kg in Station 1 (heavy vehicle traffic) during autumn to the highest values 3.50 and 3.07 mg/kg found in Station 2 (heaviest vehicle traffic) and Station 3 (no vehicle traffic), respectively during summer and spring.

Based on the recommended value of Cr concentration in plant species, according to WHO, 1996 "Permissible limits of heavy metals in plant and soil" it was concluded that the content of Cr in plant species *Platanus orientalis* L., in the urban area of the capital of Albania exceeds the recommended limit (1.3 mg/kg), about 3 times.

Probability plot of Cr concentration in all samples was used to evaluate the distribution of Cr in plant species (Figure 5). According to the obtained data, it was concluded that Cr concentration in selected plant species (in three different seasons and three different stations) can be considered to be "normally distributed". This is confirmed by the P-Value which has resulted P-Value>0.05 (which suggests a 95% of confidentiality) as well as the low value of Anderson-Darling test, (AD). Normally distributed data suggest that concentration of Cr in plant species is mainly of natural origin, depending on the content of Cr in soil and not from any anthropogenic input. Higher concentrations of Cr registered during summer can be related mainly to the higher temperatures (lack of rainfall) and dust content in the air, which can contribute to higher accumulation of the element.

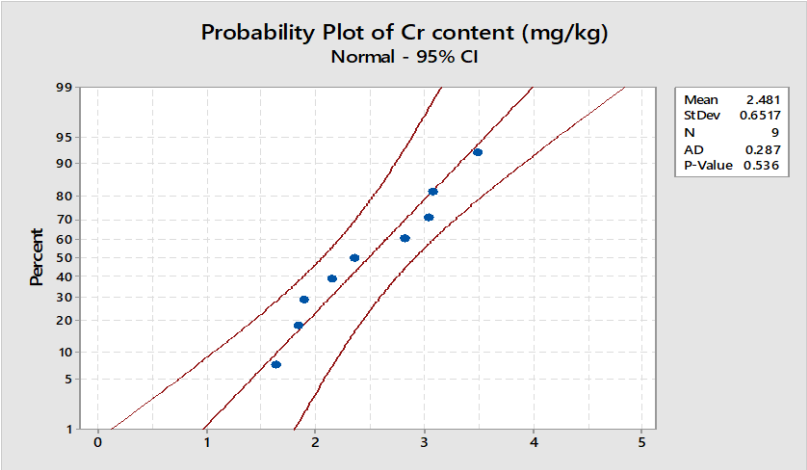

Figure 5: Probability plot of Cr

Environmental contamination of Cr has recently become a serious concern because global emissions of Cr exceed that of Pb and Cd (Dixit et al., 2002). High concentrations of Cr can decrease chlorophyll pigments and increase activities of antioxidant enzymes (Qin et al., 2014). Since the soil and air of the urban area contained considerably greater amounts of Cd and Cr, the higher concentrations of these two heavy metals in urban leaves likely originates from atmospheric sources.

Analysis of Variance, ANOVA was used to evaluate if there exist any significant difference of Cd and Cr concentration in different stations as well as in different seasons (Table 3).

The obtained results show that no significant difference was found for Cd concentration between selected stations as well as in different seasons.

While, regarding to Cr metal, it was concluded that significant differences in Cr concentration were found between different seasons which suggests that weather conditions can contribute to Cr accumulation by plant species *Platanus orientalis* L.

Table 3. Analysis of Variance (ANOVA) of Cd and Cr

ANOVA						
Source of Variation	*df*	*F (Cd)*	*P-value (Cd)*	*F (Cr)*	*P-value (Cr)*	*F crit*
Between seasons	2	2.41	0.21	**8.07**	0.039	6.94
Between stations	2	0.21	0.82	0.27	0.78	6.94

Acknowledgement

This study has been carried out under the PhD work: "Using the Floristic Diversity of the City of Tirana for Urban Pollution Control". We would like to thank the Department of Analytic Chemistry in the Faculty of Natural Sciences of the University of Tirana, in which we carried out the measurement of heavy metals. We also would like to thank The COST Action 19116 (Trace metal metabolism in plants – PLANTMETALS) and Middle East Technical University (METU), Ankara, Turkey, for organizing and supporting the "*Plant Metals Conference and MC Meeting*", where this study was presented.

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The Effect of Various Phosphate Forms on Thorium Accumulation by Plants

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Thorium, which may be a new potential source of nuclear energy, is both a toxic and radioactive element. Leakage of this metal into the environment would mean environmental and economic damage. A cheap solution to such a situation could be phytoremediation, which, however, with current knowledge lacks a plant with sufficient ability to accumulate thorium. This work therefore focuses on the potential of various forms of phosphates to increase the accumulation of thorium by plants. Thale cress (*Arabidopsis thaliana*) including selected mutant lines were chose as model plants. The plants were hydroponically cultivated in media containing thorium and various forms of phosphates (ammonium dihydrogen phosphate, hydroxyapatite nanoparticles). Subsequent analyzes showed that plants had an inhibitory effect of thorium on the uptake of biogenic elements and reduced biomass production. Hydroxyapatite nanoparticles reduced the toxic effect of thorium on the plant, but especially in *A. thaliana* plants reduced the intake of this metal into the aboveground parts. The highest measured thorium accumulation was recorded in *A. thaliana* growing in Hoagland's solution and reached a concentration of 26 μg/g dry matter. These experiments ruled out the suitability of nano-hydroxyapatite as a stimulant of thorium accumulation, but pointed to the possibility of its possible use for chemical stabilization of this radioactive metal.

Keywords: Thorium, Heavy metal, Phosphates, Nanoparticles, Accumulation

Acknowledgements: The work was supported by the Ministry of Education, Youth and Sports of CR from European Regional Development Fund-Project ""Centre for Experimental Plant Biology"": No. CZ.02.1.01/0.0/0.0/16_019/0000738 and by COST Action CA19116.

Trace Metals at the Frontline of Pathogen Defence Responses in Plants

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Trace metals (TMs) are essential for organisms as active centres of enzymes and in proteins involved in signal transduction and gene regulation - about one third of all proteins are metalloproteins. In this way, but also as low MW complexes, TMs are involved in plant immunity to pathogens. We are investigating this on fungal (*Botrytis cinerea, Phomopsis longicolla*) and viral pathogens (Turnip Yellow Mosaic Virus, TYMV), both in metallophytes (*Noccaea caerulescens, Noccaea ochroleucum*) and agriculturally relevant species (*Capsicum annuum, Glycine max*). Our work on this topic is fundamental research aiming to improve the mechanistic knowledge about the role of trace elements in plant defence response to pathogens and its relation to metal metabolism in general. In metal hyperaccumulators, high leaf metal contents are an exclusive evolutionary trait contributing to efficient defence against biotic stress. The defence can be based on direct metal toxicity or joint effects of metal and organic compounds. Research of ourselves and others pointed at direct toxicity of metals to pathogens in hyperaccumulators, and this still could apply to N. ochroleucum as a metal tolerant but not really hyperaccumulating species and its defence against TYMV. The role of TMs in the pathogen immunity of non-accumulator plants is far less understood. New findings showed that in metal non-hyperaccumulating plants, localized hot spots of Zn, Mn and Fe increase plant immunity, but likely not by direct toxicity to the pathogen. Manipulation of nutrient availability may be used for priming against subsequent pathogen attack, but recent findings also showed a narrow line between deficiency and toxicity. Our results indicated host-pathogen nutrient competition and synergistic effects of simultaneous metal-biotic stress. The suitability of direct defence and joint effect hypotheses in the case of non-hyperaccumulating plants, and involvement of metals as active centres of immunity-related enzymes is discussed.

Keywords: manganese, metabolites, metal distribution, metalloproteomics, µXRF, OJIP, plant defence responses, priming, TYMV, zinc

Acknowledgements: This study is 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), and the Czech Academy of Sciences (RVO:60077344) and COST Action (CA 19116 “Trace metal metabolism in plants-PLANTMETALS).

Characterization of the Activity of the IRT1 Transporter Involved in Metal Uptake in the Hyperaccumulator Species *Noccaea caerulescens*

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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.

Keywords: heavy metals, hyperaccumulator plant, IRT1, transporter, ZIP

Characterization of Metal Tolerance Proteins in *Beta vulgaris* Reveals Diversity of Micronutrient Homeostasis Mechanisms in Dicotyledons

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Plant micronutrients are important in diverse physiological processes inside different plant cell compartments. Therefore, their transport and cellular homeostasis have to be tightly controlled by diverse transport proteins. Among them, plant metal tolerance protein (MTP) transporters have been shown to mediate Mn, Fe, and Zn transport to several compartments in Arabidopsis and rice. To get further insights into this gene family in a dicot plant phylogenetically distant to Arabidopsis, we identified and functionally characterized the MTP transporter family in sugar beet (*Beta vulgaris*). BvMTPs were localized in endomembrane compartments, vacuoles, as well as in chloroplasts and mitochondria, whereby the localization did not always coincide with their Arabidopsis orthologs. Expression profiling of *BvMTP* genes in root and shoot showed a specific expression of splice variants depending on the plant's developmental stage. Upon expression in yeast, BvMTPs complemented Mn, Fe, and Zn transport-deficient mutants. Intriguingly, besides the subcellular localization, regulation of gene expression under micronutrient starvation of some BvMTPs differed greatly to their closest homologs in Arabidopsis. Taken together, the results suggest that the localization, regulation, and hence role of homologous MTP proteins is highly diverse amongst dicots, which has implications on the general applicability of metal homeostasis models derived from Arabidopsis.

Keywords: Manganese, MTP, Sugar beet, Transporter

Acknowledgements: This work was supported by the European Regional Development Fund (ERDF) within the State Research Focus Program "Molecular Biosciences as a Motor for a Knowledge-Based Economy" (ZS/2016/06/79740 to E.P.) and by the German Research Foundation (DFG, grant INST271/371-1 FUGG to Ingo Heilmann).

Iron Localization in Everyday Fruits

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Iron (Fe) is an essential element for human nutrition. Human nutrition depends mainly on edible plant organs for Fe intake which includes seeds, stems, roots, leaves and fruits. Among those, only the fruits "aim" to be eaten and digested by animals as a part of plants reproductive strategy. Thus, only fruits come in many different colours, textures, flavours and scents that are attractive to us but difficult to explore with a unified method. Seeds offer humans a lot of Fe but as in the example of rice, the majority of Fe is lost because it almost solely localizes to the very outer part of the grains that are lost by the milling process indicating not only the quantity but also the localization of Fe in the organs is of utmost importance for human consumption. We know Fe patterns in seeds well but not in fruits due to the technical limitations. Previously, Fe localization studies in plant organs extensively used a staining method called Perls which has been difficult to apply for the soft and colorful tissues of fruits. To be able to apply Perls staining in fruits, we employed a vacuum dry method that preserves the structure and a decolorization protocol to remove the existing colors. This allowed us to apply Perls staining to most of the fruits we obtained from the market. Results showed preferential Fe localization patterns in the fruits including regions around specific veins, endosperm of seeds fand funiculus. These results may impact the preference of humans on eating fruit parts. From the scientific view, preferential Fe localizations point out new physiological roles yet to be determined.

Keywords: Iron, fruit, localization

Acknowledgements: This study is supported by TUBITAK (grant number: 118Z788).

Content of Trace Metals and Antioxidative Capacity in the Leaves of Selected Medicinal Plants

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Medicinal plants have been traditionally used in our region for prevention and treatment of various types of diseases. However, the mechanisms of their action and active components have not been sufficiently explored. There is also a lack of knowledge about the content of trace metals in plants and their contribution to medicinal properties. In this paper, we examined the content of Cu and Fe in the leaves of eight flowering plants and one fern from territory of Republika Srpska (B&H). In addition, antioxidative capacity was determining as ability of plant to chelate Fe and reduce Fe and Cu. Quantification of metal contents was done by atomic absorption spectrophotometry. The highest Fe content was measured in the Asplenium ceterach (164.57 mg/kg) while the highest Cu concentration was measured in Menyanthes trifoliata (11.61 mg/kg). The highest content of phenolic compounds as well as the highest ability to reduce Fe and Cu were measured in Punica granatum leaves. Statistically significant positive correlations between the content of phenolic compounds and the ability of the extract to reduce Fe and Cu indicate an important role of phenolic compounds in maintaining redox homeostasis in the cell. On the other hand, the obtained results indicate that the content of phenolic compounds does not correlate with the ability of extract to chelate Fe for the investigated plant species.

Keywords: Chelate, Copper, Ethnobotany, Iron, Phenolic compounds

Acknowledgment: This work was funded by the Ministry of Scientific and Technological Development, Higher Education and Information Society of the Republic of Srpska (Project No. 19.032/961-104/19 and 19.032/961-95/19).

The Role of Manganese in Light Stress Induced Tipburn of Lettuce

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Tipburn is a physiological disorder visible as necrotic spots or lesions on the edge of outer older leaves or young inner leaves. It is a common injury of lettuce and leafy vegetables cultivated in protected environment and reduces the marketable yield and causes severe food loss. Light stress is known to increase the severity of tipburn. We found that increased irradiance triggered accumulation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) in leaf tips of lettuce (*Lactuca sativa* L 'Frillice'). To mitigate the effect of ROS, plants have antioxidant defense mechanisms which have the function of removing and transforming ROS into non-toxic substances. Manganese (Mn) is involved in redox reactions as a cofactor for enzymes like superoxide dismutase (SOD) known to mitigate ROS. Hence, we investigated the role of Mn in the development of tipburn and hypothesized that increased manganese content alleviates tipburn incidence in plant exposed to high irradiance. The results showed that increased Mn concentrations in the nutrient solution increased Mn content, SOD activity and reduced the incidence of tipburn in young inner leaves. However, old outer leaves showed Mn toxicity symptoms. The results suggest that accumulation of Mn in lettuce is dependent on leaf age and light level, and deficiency and toxicity can occur in the same plant.

Keywords: Light stress, Lettuce, Manganese, ROS, SOD, Tipburn

Acknowledgements: This study is supported by Grofondet and Norwegian Research Council (grant number 282290)

A vascular CDF Transporter Participates in Iron and Manganese Distribution in *Arabidopsis thaliana*

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Since Mn and Fe both play critical roles in photosynthesis and as cofactors of many enzymes, maintaining metal homeostasis especially under conditions of metal deficiency is essential. Mn catalyses the water-splitting reaction during photosynthesis, resulting in the evolution of oxygen, while Fe cofactors are an important part of the photosynthetic electron transport chain. In *Arabidopsis thaliana*, members of the Cation Diffusion Facilitator (CDF) family are involved in manganese (Mn) transport, like Metal Tolerance Protein (MTP) 11, a Golgi-localized transporter that mediates the detoxification of excessive Mn, or MTP8, a Mn transporter that is involved in securing iron (Fe) acquisition by sequestering Mn into the vacuole. Although these transporters are classified as Mn-CDFs, MTP8 has been shown to transport Fe as well. We found that besides MTP8 another member of the CDF family is involved in the homeostasis of both elements. Yeast drop assays confirmed that this transporter can complement the Mn-sensitive yeast strain *pmr1Δ* and the Fe-sensitive strain *ccc1Δ*. Plant histochemical GUS studies showed a promoter activity in the vascular system of roots and shoots. In roots, expression was specific to the pericycle. Based on this expression pattern, the transporter was presumed to have a function in the elemental translocation from roots to shoots. Intriguingly, these mutants showed tolerance to Mn toxicity and sensitivity to Mn^{2+} deficiency. Additionally, knockout mutants were also impaired in Fe translocation in leaves. The altered metal homeostasis led to an impaired photosynthetic performance under Fe-deficient conditions, supporting the importance of Fe localization within the plant. Our results strongly indicate that altered distribution of both metals impacts plant tolerance to changing nutrient supply, and that a CDF transporter is involved in these processes.

Keywords: CDF transporter, manganese, iron, metal translocation

Transcriptomic and Metabolic Aspects of Interaction Iodine and Iodosalicylates in Lettuce Plants

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For biofortification studies, there is necessity to know the molecular, biochemical or physiological basis underlying iodine response in plants. The aim of this study was to determine the process of uptake, transport, and metabolism of I applied to lettuce plants 'Melodion' cv. by fertigation in NFT hydroponic system. KIO_3, KIO_3+salicylic acid (KIO_3+SA), 5-iodosalicylic acid (5-ISA) and 3,5-diiodosalicylic acid (3,5-diISA) were applied. Each I compound was applied at a dose of 10 µM and SA at a dose of 10 µM. To identify differentially expressed genes (DEGs) in roots and leaves under SA and KIO_3, RNA-Seq assay was employed. Enrichment analysis of DEGs related to I metabolism was performed with the Gene Ontology and KEGG which indicated pathways specific only for supplementation with KIO_3 such as ubiquinone and other terpenoid-quinone biosynthesis, protein processing in endoplasmic reticulum, mRNA surveillance pathway, plant-pathogen interaction, starch and sucrose metabolism, phenylpropanoid biosynthesis, galactose metabolism, cyanoamino acid, arginine and proline metabolism, vitamin B6 metabolism, endocytosis and spliceosome, flowering induction as well as plant-derived thyroid hormone analogs (PDTHA) potential metabolic pathway. qRT-PCR of *per64-like, samdmt, msams5*, and *cipk6* genes revealed their participation in regulation of physiological processes. The performed analyzes (including ICP-MS/MS, ICP-OES, LC-MS/MS, capillary electrophoresis techniques) showed different chemical composition of plants. The plants treated with 5-ISA accumulated the most iodine. Instead 3,5-diISA fertilization caused the highest accumulation of: dry matter, vitamin C and B3, sucrose, glucose, fructose, phenolic compounds, SA; iodobenzoic, chlorogenic, sinapic, p-coumaric, ferulic, 3-hydroxybenzoic and hypuric acids; triiodothyronine, thyroxine, Fe, Mo, Al as well as the higher antioxidant activity of DPPH, ABTS, FRAP in lettuce plants. In these plants, however, a decrease in the accumulation of Cu, Zn was found.

Keywords: iodine, salicylic acid, iodosalicylic acids, biofortification, micronutrients

Acknowledgements: This study is supported by the National Science Centre, Poland (grant number: UMO-2017/25/B/NZ9/00312).

Characterization of *Arabidopsis halleri* and *Urtica dioica* Responses to Zn and Cd: Soil management Practices to Help Biofortification?

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

Keywords: Cadmium, Foliar accumulation, Phytoextraction, Phytostabilization, Zinc

Acknowledgements: This study is supported by national funds through EXTRA-Zn and EVALPHYTO projects (ADEME - Agence de la transition écologique, conventions n° 2019-2022 and 1672C0005). A. Grignet received grants from ADEME (n° TEZ17-21) and the Hauts-de-France Region.

The Biostimulant Super Fifty Prime Enhances the Yield and Influences the Nutrient Distribution in Eggplant and Pepper

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Super Fifty Prime® is a commercially available biostimulant that contains highly concentrated *Ascophyllum nodosum* (brown seaweed) extract. Previously, it has been demonstrated that in the field, it successfully reduces abiotic stress and increases plant performance in some crops like tomato, rice and wheat. However, its effect on other economically important species, such as eggplant and pepper, is still unknown. Furthermore, the improvement of nutrient assimilation and distribution in crops by Super Fifty Prime® is not well studied. To address these issues, we have investigated the influence of Super Fifty Prime® on eggplant and pepper plants grown in open field conditions. In the flowering stage, they were sprayed two times with an optimal concentration of the biostimulant. To monitor the progression of fruit development in primed and unprimed plants, fruit diameter was measured at the cell division, cell expansion, and maturation stages. Our results demonstrate that Super Fifty Prime® improves the overall plant growth, increases the fruit number, and enhances the fruit size in both analysed crops. In addition, various macro- and microelements, such as Mg, K, Ca, Fe, Mn, Zn and Cu, were quantified by ICP-MS with the aim to reveal differences in their distribution patterns in Super Fifty Prime® treated and untreated leaves and fruits of eggplant and pepper.

Keywords: Biostimulant, Eggplant, Microelements, Pepper, Yield

Acknowledgements: This study was supported by the European Union's Horizon 2020 research and innovation programme, projects PlantaSYST (SGA-CSA No. 739582 under FPA No. 664620). V.P. and T.G. acknowledge the COST Action 19116 PLANTMETALS. N.S. acknowledges the Horizon 2020 project RESIST (Grant No. 823746).

RAPD-PCR Based Evaluation of Genotoxic Influence of Metal Stressors in Plant Model Systems

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The presented study was designed to get the possibility to measure the outcome of long term exposition of high concentrations of different metal stressors on DNA damage in plant model systems. We used different plant species as models, like *Taraxacum officinale* L. (Asteraceae), *Matricaria recutita* L. (Asteraceae), *Robinia pseudoacacia* L. (Fabaceae), and *Urtica dioica* L. (Urticaceae). Metal contents (cadmium, lead, copper, nickel, and zinc) in the samples was determined by using ICP-AES technique. DNA damage was investigated by a Random Amplified Polymorphic DNA (RAPD) technique, and RAPD profiles of plants exposed to metal stress and control plants (non-exposed) were compared. Agarose-gel electrophoresis reveal total of 37 bands with molecular weights ranging from 1250 to 5000 bp. Distinctive polymorphism of 72.97% (27 bands) total in all plant species investigated was estimated. The dendrogram constructed using NTSYSpc programme, showed that there is grouping in separate clusters of the same plant model collected from two different areas (metal-exposed and control samples). The number of polymorphic bands observed in samples exposed to metals suggests that long term metal-exposition in high doses can cause mutations on genomic level in investigated model plants. These bands are unique and distinctly differentiated the samples, and can act as markers for evaluation of the environmental metals exposition. Encounter the fact that plants are used as food or in medical purposes, the issue of possible genotoxicity initiated by metal contamination must be concerned.

Keywords: DNA damage, Heavy metals, Plant model, Genotoxicity

Common Bean a Potential Food Crop: Germplasm Characterization and Identification of Genomic Regions Associated with Seed Minerals and Vitamins Contents

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The problem of deficiencies in essential vitamins and minerals called 'hidden hunger' continues to pose serious threats to populations and economies around the world. Therefore, biofortification of staple crops with target micronutrients is essential. However less efforts has been in the biofortification of legumes compared to other crops. Common bean (*Phaseolus vulgaris* L.) is a nutrient-dense legume crop serving as a source of food for millions of people. Characterization of unexplored common bean germplasm to unlock the phenotypic and genetic variations is still needed to explore the breeding potential of this crop. Current study aimed to dissect the genetic variations and genomic regions having association with seed macro, microelements, vitamin A, B1, B2 and folic acid. A total of 188 common bean accessions collected from 19 provinces of Tükiye were used as plant material under four environments and two locations (Sivas and Bolu). Genotype by environment interaction was studied and most superior and stable common bean accessions were evaluated. Genotyping be sequencing resulted DArTseq markers (7900 markers) were used for association analysis. We successfully investigated marker-trait association for seed magnesium, iron, zinc, vitamin A, B1, B2 and folic acid contents. Genetic and genomic resources identified though this study will serve as a starting point for the breeding community to develop nutritional enriched common bean cultivars to overcome hidden hunger problems not only for Turkey but for all over the world.

Keywords: Food legumes, Germplasm characterization, GWAS, micro-nutrients, Vitamins

Biological Imaging with Synchrotron Radiation at Beamline P06

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The Microprobe experiment at the PETRA III beamline P06 is a versatile setup for scanning X-ray microscopy with X-ray fluorescence, X-ray absorption spectroscopy and X-ray diffraction/scattering contrasts. A KB system focusses a beam of 10^{10} photons/s down to 300 nm focus size in the energy range 5 - 21 keV. Compound refractive lenses allow to extend the energy range towards to K-edge energies up to REE (44 keV). Advanced detector technology, namely multi-element SDD X-ray fluorescence detectors (Ardesia and Vortex ME4) and the EIGER X 4M hybrid photon counting detector, enable on-the-fly scanning schemes with millisecond dwell times per scan pixel. The ability to collect megapixel images in less than an hour facilitates series of 2D images for full 3D fluo-tomography, spectro-microscopy, time-resolved in-situ microscopy or other multi-dimensional microscopic experiments. The microprobe setup is frequently applied for various bio-imaging applications such as element mapping of metals in soft tissues (plants, animals or human), bone, teeth or single cells.

Samples can be chemically fixed, freeze dried or even fresh (unfixed). The golden standard, however, is shock-freezing and measurement in the frozen-hydrated state in order to avoid beam damage and artefacts of element re-distribution. A cryogenic sample transfer protocol is available both for measurements under a nitrogen cryo-stream or in a cryogenically cooled UHV chamber.

Examples are presented for various sample types (*tissues, bone, teeth, cells*), scanning modes (*fast 2D, tomographic 3D*), and sample preparation techniques (frozen-hydrated, unfixed, chemically fixed).

Keywords: Bio-imaging, X-ray fluorescence**,** scanning X-ray microscopy**,** heavy metals, tomography

Does Peptide Mediated Cell Wall Signaling Detect Metal Ions?

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Cell walls do not only protect the protoplasts but serve as signaling platform between the extracellular environment and the intracellular physiology. Ions of heavy metals and trace elements bind to cell wall components, trigger their modification, and provoke growth responses. The goal of our study was to determine whether metal ions trigger cell wall sensing receptor like kinases (RLKs) of the Catharanthus roseus RLK1-like (CrRLK1L) family and their ligands, the RAPID ALKALINIZATION FACTOR (RALF) peptide family. Growth inhibition assays of roots and etiolated hypocotyls of CrRLK1L mutants and overexpressors on cadmium (Cd^{2+}), copper (Cu^{2+}), nickel (Ni^{2+}), lead (Pb^{2+}), and zinc (Zn^{2+}) revealed a complex pattern of CrRLK1L specific, overlapping, and antagonistic responses. For example, THESEUS1 (THE1) seem to inhibit hypocotyl on Ni^{2+}, since the loss-of-function mutants were more tolerant while the gain of function mutants were hypersensitive. In contrast, hypocotyl elongation of the *FERONIA* mutant, *fer-4*, and the quadruple mutant of *MEDOS1-4* (*mds1-4*) were hypersensitive to Ni^{2+}. These data indicate an antagonistic action between THE1 and FER in relation to hypocotyl elongation upon excess of Ni^{2+}. Several CrRLK1L function as receptor for the secreted, cysteine-rich RALFs. Most of RALFs alkalinize the medium, inhibit growth, many induce reactive oxygen species (ROS) and the majority modulate bacterial Pathogen-associated molecular patterns (PAMPs) triggered ROS. Here we show that RALFs are also important for regulating hypocotyl growth inhibition upon Ni^{2+}. Overall, our findings establish a molecular link between metal ion stress, growth, and the cell wall integrity sensors of the CrRLK1L family with their peptide ligands.

Keywords: *Arabidopsis thaliana*, CrRLK1L, hypocotyl elongation, RALF, root growth

Acknowledgements: This study was supported by grants of the Austrian Science Fund I 1725-B16 and the DOC 111 grant.

Potential of Maize Breeding Germplasm for Biofortification to Fight with Hidden Hunger Challenges

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Due to the increase in the world population, "Food Safety" emerges as the most important problem in the near future. Corn is the second most grown cereal for animal feed, grain for human consumption and industrial purposes. Due to its economic importance, Maize has become the focus of breeders' attention. In hybrid corn breeding, the first stage is to develop parent lines, and the second stage is to obtain hybrid varieties with good adaptation and high yield by using parent lines. The availability of genetic diversity is critical to the success of breeding programs. In fact, elite lines with good adaptation and hybrid performance are developed by combining materials from various gene sources in hybrid corn breeding studies conducted by the Eastern Mediterranean Agricultural Research Institute. These genetic resources exhibit wide variation for many desirable traits including plant mineral element concentration. Different classical and modern breeding tools are used effectively to enhanced the genetic variation for effective corn breeding at our institute. Previously, we studied the micro-macro element concentrations in maize landraces collected from Turkey. We witnessed the high variation for mineral element concentration in our germplasm. Currently, we perform genotyping by sequencing of 370 maize inbred lines and we have thousands of SNP and DArTseq markers through our TUBİTAK project (project no 121O313) and this germplasm is planned to phenotype for mineral element concentrations for identifying the candidate parents for crossing programs with desirable mineral concentrations, and using these genotypic and phenotypic data for micro-macro element concentration, genomic algorithms will be developed to identify linked markers for for increased Fe and Zn in maize grain to combat the mineral malnutrition problem in human population and to develop genomic selection database at our institute.

Keywords: corn, germplasm, breeding, inbredline, biofrotification

Four Selected Plant Species from Serpentine Substrates in B&H Revealed Heavy Metal Tolerance

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Heavy metal polluted soils present one of the major threats to ecosystem integrity. Mounting evidence suggests various plant species tolerate or accumulate heavy metals. However, for scientists, the selection of effective potential phytoremediators poses an old riddle (a great challenge). In this regard, the aim of our research was to investigate the potential of plant species present in Bosnia and Herzegovina (B&H) that grow naturally in soils rich in heavy metals. After the vegetation survey, we selected four species from serpentine substrates (*Plantago lanceolate* L., *Geranium robertianum* L., *Medicago lupulina* L. and *Sanguisorba minor* Scop.). The plant and soil samples were collected during the flowering phase at various serpentine localities throughout B&H, after which the concentration of heavy metals in the soil and the plant material was determined. Physiological and biochemical parameters of stress were also determined. Our result confirmed high level of nickel, chrome and cobalt in all soil samples as well as high level of Ni in examined plant samples. On the other hand, results of physiological and biochemical analyses revealed that all four species have a high tolerance to heavy metals present in the soil. Our results suggest that the four analyzed species have great potential to be further studied as candidates for phytoremediation.

Keywords: *Geranium robertianum* L., Heavy metal tolerance, *Medicago lupulina* L, *Plantago lanceolate* L, *Sanguisorba minor* Scop.

Phytosiderophore Analog Efficiently Alleviates Iron Deficiency Symptoms in Soybean

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Iron (Fe) bioavailability is dependent on soil pH. Its solubility, therefore bioavailability dramatically decrease in calcareous alkaline soils with high pH. Therefore, yields of soybean decrease when they are grown in alkaline soils, which cover 1/3th of the world's arable land. Inorganic micronutrient fertilizers with stabilizing chelates have been developed to rescue the micronutrient deficiency symptoms in crops grown in alkaline soils; yet, they bring a large economic burden to the farmers and accumulation of their by-products causes environmental contamination. Therefore, alternative biodegradable chelated fertilizers are required to be developed to increase crop yields in sustainable agriculture. Grasses secrete phytosiderophores into the rhizosphere which increases Fe uptake by the plants, and these chemicals can help dicots, such as soybean, to uptake Fe more efficiently. Although it is difficult and expensive to produce them in a stable form, phytosiderophore 2′-deoxymugineic acid (DMA) derivative PDMA was cost-effectively produced as a stable analog. Here, we show that PDMA application from the soil eliminates the Fe deficiency symptoms in sensitive soybean plants when they are grown in calcareous alkaline soil with a pH of 8.5. Several physiological and biochemical parameters related to Fe deficiency response in soybean were increased in a dose-dependent PDMA application when they reached at V3-4 stage. Taken together, PDMA application from the soil increases the Fe uptake and accumulation in Fe deficiency-sensitive soybean cultivar, leading it to become Fe-efficient and tolerant in calcareous alkaline soil. In future studies, PDMA supplemented with different Fe sources may be formulated in a new type of micronutrient fertilizer to alleviate the Fe deficiency symptoms in dicots.

Keywords: Alkaline soil, fertilizer, iron deficiency, phytosiderophore, soybean

Acknowledgments: This research is supported by Aichi Steel Corporation, Aichi, Japan.

Photosynthetic Activity and Element Distribution in *Capsicum annuum* Leaves Infected with *Botrytis cinerea* under Different Mn and Zn Supply

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Botrytis cinerea is a necrotrophic fungus causing grey mould disease and significant crop yield losses. To reveal how zinc and manganese priming contribute to plant pathogen defence responses, *Capsicum annuum* plants were grown hydroponically under Zn or Mn deficiency (Zn0, Mn0), control (replete, 1µM Zn and 0.6µM Mn) and surplus (5µM Zn or 3µM Mn) conditions. The leaves were subsequently inoculated with *B. cinerea* by agar disks and analysed after 48h. The percentage of infected area was the lowest in Zn5, 29% less than in infected controls. Direct imaging of fast chlorophyll fluorescence kinetics (OJIP) revealed diminished photosynthetic activity (Φ_{Po}, Φ_{Et2o}) in infected plants in deficiency and control treatments while normal functioning remained in Zn5 and Mn3 treatments. Both stomatal conductance and CO_2 assimilation rate were the least affected in Zn5 (40% and 15% decrease compared to non-infected plants, respectively). *In vivo* µXRF analyses revealed higher accumulation of Zn in the veins, bundle sheath cells and mesophyll at the infection site regardless of the metal treatment and Mn in mesophyll under Mn3 and control treatments. In addition, size exclusion HPLC-ICP-MS analyses showed increased Zn binding in the membrane protein fraction with infection in Zn5 leaves. *B. cinerea* infection enhanced the accumulation of many metabolites including acetophenone, amino acids, lipids and organic acids in Mn3 and Zn5 treatments. Overall, the results show that Zn priming significantly improves resistance of *C. annuum* to *B. cinerea*, while Mn surplus was less efficient. The differences between the two metals in relation to defence mechanisms and induction of specific metabolites are discussed.

Keywords: *Botrytis cinerea*, *Capsicum annuum*, manganese, metabolites, metal distribution, metalloproteomics, µXRF, OJIP, plant defence responses, priming, zinc

Acknowledgements: This study is 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 Academy of Sciences (RVO:60077344) and COST Action (CA 19116 “Trace metal metabolism in plants-PLANTMETALS).

Leaf Metal Exclusion or Accumulation is Related to Soil Resource Exploitation Strategy in European Calamine Species: Evidence for a Leaf Elemental and Economic Spectrum?

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Functional traits related to plant growth, survival and reproduction vary along two independent axes: the leaf economic spectrum opposing fast-growing nutrient-exploitative species and slow-growing nutrient-conservative species; and plant stature opposing short and tall plants. This work aimed at characterizing the unknown position of calamine species along these two functional axes in relation with leaf metal accumulation or exclusion. We assumed that species growing in highly polluted environments should be positioned similarly to species growing in other harsh ecosystems where both plant size and growth rate are limited. We studied height functional traits related to the leaf economic spectrum (Leaf Mass Area, Specific Stem Density, leaf Nitrogen concentration, Leaf Dry Matter Content, Twigg Dry Matter Content) and plant stature (Height, Leaf Area, Seed Mass) in 44 dominant species in nine calamine grasslands (Northern plain and Pyrenean range in France) and 42 dominant species in nine non-calamine grasslands as control. Leaves and roots Zn, Cd and Pb concentrations were characterized for 9 out of the 44 calamine species. Regarding plant stature, calamine species had small size like those observed for non-calamine species growing in harsh environments. However, calamine species showed an important variability for traits related to the leaf economic spectrum. This variability was strongly correlated with metal accumulation, fast growing species showing lower metal sequestration in roots and higher leaf metal concentrations. Fast-growing species exploiting soil resources are likely more exposed to soil contamination. In that case, metal exclusion from leaves and sequestration in roots may be less efficient than translocation to leaves procuring defense against herbivores, and leading to the observed Leaf Elemental and Economic Spectrum. Screening of plant traits including those related to metal accumulation in more diverse metalliferous plant communities is necessary to assess the generalization of this trade off coupling leaf metal accumulation/exclusion with soil resource exploitation/conservation.

Keywords: Functional Ecology; Leaf Economic Spectrum; Metallicolous grasslands; Metal accumulation; Metal translocation, Calamine species.

Acknowledgements: This work has been supported by the SIXP project and the French National Research Agency (ANR-19-CE02-0013-01).

Contrasting Cadmium Accumulation in *Arabidopsis halleri* populations

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Cd is one of the most toxic substances released into the environment. Even chronic low level of Cd exposure has been associated with severe health issues (Clemens et al. 2016). Cd is also toxic to plants. Cadmium can compete with essential nutriments (like Zn, Fe, Ca,..), displace Zn, Ca, Fe & other metals in proteins, react with thiol groups of proteins and glutathione, induce oxidative stress by depleting reduced glutathione, and by inhibiting photosynthesis and respiration. Cadmium can also inhibit DNA repair.

Plants growing on metalliferous soils, named metallophytes, mainly display two strategies, exclusion (which is the most common strategy) or hyperaccumulation (rare strategy). *Arabidopsis halleri* is a pseudo-metallophyte, i.e it grows on both metal-contaminated and non-contaminated soils. The close relationship with *Arabidopsis thaliana*, the broad distribution in Europe and Asia, the capacity to hyperaccumulate Zn in the whole species, and in some populations Cd too, the hypertolerance to Zn and Cd, make *A. halleri* a model species to study metal homeostasis and adaptation to an extreme environment.

In the last 20 years, our laboratory has studied populations of *Arabidopsis halleri*, with variable metal accumulation and tolerance. In this work, we have used two metallicolous populations from different genetic units, which have adapted to Zn and Cd contaminated soils by different mechanisms. I16 (North Italy) and PL22 (South Poland) have similar tolerance but contrasting Cd accumulation. In I16, there was a 2-times lower translocation of Cd from the root to the shoot than in PL22 (Corso et al. 2018). A transcriptomic study highlighted cell wall as a main category of differentially expressed genes between the two populations, especially in the root. Therefore, we investigated the role of cell wall in the contrasting Cd accumulation.

There is a diversity of polysaccharides that make up the cell wall, they are classified as cellulose, hemicellulose and pectin. Together pectin and hemicellulose form a matrix for cellulose fibrils. Pectins consist of four polysaccharide domains: homogalacturonan, xylogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II. The first domain, also being the most important in binding both divalent and trivalent metals.

Different analyses of cell walls were undertaken in I16 and PL22. First Cd concentrations were measured in pectins and hemicellulose extracted from root cell walls. Pectins of PL22 accumulated 3 times higher Cd than I16, without any significant difference in pectin content. No difference in Cd content was found in hemicellulose. However, changes in the content, and ratio between hemicellulose 1 and 2 were measured after Cd treatment in both populations, with higher contents of hemicellulose 1 and 2 in I16 after Cd treatment.

Cd treatment also induced a change in the structure of pectins in both populations, as measured by the homogalacturonan/rhamnogalacturonan (RG) ratio. In PL22 only, RG-I galactan sidechain and arabinogalactan sidechain were shortened by 14% and 19% respectively.

Epitopes of cell wall polysaccharides were analysed by CoMPP (comprehensive microarray polymer profiling), by using 20 antibodies raised against specific cell wall components.

Pectins and hemicellulose extracts were separately hybridized. Main differences between PL22 and I16 root cell walls were observed in pectins. In particular signals corresponding to galactan and arabinan,

which are generally found as side-chains of rhamnogalacturonans I (RGI), were higher in I16 than in PL22. RG-I sidechains are covalently and/or non-covalent linked to the xylan and xyloglucan (Broxterman & Schols, 2018), and can affect the viscosity and elasticity of the CW (Hwang *et al.*, 1993; Kaczmarska *et al.*, 2021)

In strong support with this observation, the expression of *GALS1* an, *ARAD2* which are involved in the synthesis of (1→4)-β-galactan, and (1→5)-α-arabinan, respectively, were both more expressed in I16 than PL22 upon Cd exposure. Arabidopsis *gals1* and *arad2* mutants also showed both altered Cd tolerance and accumulation.

In short, our results suggest new players in Cd accumulation, with a role for β-1,4-galactan and (1→5)-α-arabinan in cell wall architecture, which affects the capacity of the cell wall to retain metals.

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Effects of Foliar Urea, Potassium and Zinc Sulphate Treatments on Wheat (completed project) and Characterization of Wheat Cultivars Grown in Turkiye in terms of Microelements (will start soon)

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(Completed Project) Nitrogen and sulphur application has a significant effect on the quality and quantity of storage proteins of wheat which affect the bread making process. In this study, effect of foliar applications of urea, potassium sulphate (K_2SO_4) and zinc sulphate heptahydrate ($ZnSO_4$.7H2O), under field conditions at vegetative and generative periods of wheat, were investigated for their effects on protein quality (SDS sedimentation and STK-Lactic Acid values), Glutopic parameters [(PMT (s)), BM (BE), BEM (BE) and PM (BE)] and dough rheological properties [stability (min), degree of softening (BU)] of wheat. As a result, in the first trial, 0,5% Urea, 1% Urea+0,5% $ZnSO_4$ and 0,5% K_2SO_4; in the second trial, 1% Urea+0,5% K_2SO_4 were found the most prominent applications. According to the obtained results, the effect of urea, potassium sulphate and zinc sulphate applications on protein quality, gluten rheology and dough rheology was found to be significant.

Keywords: Dought rheology, Nitrogen, Sulphur, Wheat, Zinc

Acknowledgements: This study It was funded by the T.C. Ministry of Food, Agriculture and Forestry (Project Number: TAGEM/TSKAD/15/A13 /P04/05).

(New Project) In this project, will planned soon, commercial varieties, local varieties grown in Turkey from past to present, primitive wheats, spelt wheat, advanced lines and purple colored CIMMYT lines will be screened in terms of trace elements (especially Fe, Cu, Ca, Cd, Mo, Se, Zn, Pb, As etc.) molecularly and biochemically. For this purpose, the materials of our institute and the materials we have obtained from other institutes and universities will be used. The effects of grain hardness, grain color, different locations, growth habit and wheat species on the amount of microelements will be investigated. The trace metals detected biochemically and molecularly (using SSR and KASP markers) will be validated phenotypically.

Keywords: Wheat, trace metals, markers, phenotypic validation

EU Projects - Dissemination, Communication, and Exploitation of Achievements

Robert Dulfer

The practice of EU funded scientific (and other) projects was that at the end of the project, a document was produced that was registered in some obscure locations and maybe a few scientific articles were published in Journals most people would never find.

Often, high quality work was done, and significant results and innovations were achieved. However, the general public and even potential interested parties and other researchers rarely noticed these results. This was mainly due to project implementers not realizing the importance of dissemination and not knowing how to do this on a regular basis.

The last years, EU programmes are trying to improve this situation by making this mandatory project achievements and a priority within the daily project management. The EU on-line manual on Funding and Tender Opportunities now mentions Dissemination and Exploitation and Communication as separate dedicated sections:

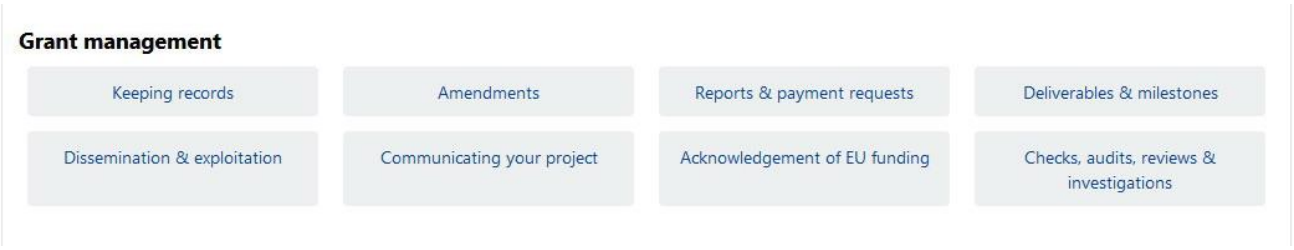

Image: https://webgate.ec.europa.eu/funding-tenders-opportunities/display/OM/Online+Manual

“Under Horizon 2020, beneficiaries should engage in dissemination and exploitation activities. As Horizon 2020 (now Horizon Europe) is financed by EU citizens, it should benefit to the largest number and the fruits of the research reach society as a whole.
Dissemination means sharing research results with potential users - peers in the research field, industry, other commercial players and policymakers. By sharing your research results with the rest of the scientific community, you are contributing to the progress of science in general.
Whereas exploitation is the use of results for commercial purposes or in public policymaking“.
Dissemination shouldn't be an after-thought. It should be an ongoing dialogue with potential users during your project. They can be your fellow researchers, but also companies, investors, regulatory bodies, sectoral organisations, NGOs, the education sector, the public sector, etc.

To help project implementers to fulfil these obligations, the EU has made guidelines and provides support tools and special services to assist project participants with dissemination and exploitation activities.
COST Actions are about networking and cooperation. As such, dissemination should be a very important and significant part of any COST Action as well. Dissemination, Communication, and Exploitation of the project and its achievements are legal obligations of the Grant Agreement.

Sources

Citations: https://ec.europa.eu/research/participants/docs/h2020-funding-guide/grants/grant-management/dissemination-of-results_en.htm
COST Video about dissemination: https://youtu.be/U4AMczwbySs

COST Action PLANTMETALS – Dissemination, Exploitation, and Communication during the COVID pandemic

Robert Dulfer

Dissemination, exploitation, and communication are considered integral and paramount activities within all COST Actions. The Scientific Communication Coordinator is now an obligatory leadership position.

The PLANTMETALS Memorandum of Understanding (**MoU**, p10) from 2020 specifically acknowledges this.

The Action's plan to maximise its impact includes: (i) good management, taking care of the interactions with the different stakeholders, (ii) broad communication through all media, specialised web sites, publication in open-site scientific journals, summer schools, (iii) invitation of different stakeholders at annual scientific meetings, (iv) active exchanges between participating laboratories (of researchers and of material), and (v) dynamic web-sites with open sections on analysis methods, collaborations, etc., and an active presence in social networks.

To further ensure the impact on the general public and farmers, targeted approaches will be used consisting of: (i) press releases after major discoveries will be directed to news agencies and to broadcasting programs interested in biotechnology/agriculture .. as well as distributed via social media such as twitter and Facebook (ii) technical notes in farmer bulletins, and (iii) participation in seminars, roundtables, meetings, fairs, exhibitions directed to the productive sector.

Unfortunately, we are behind schedule on this. The COVID pandemic stopped all planned live meetings, and virtual meetings were slow to develop. In 2021, COST did develop tools to increase virtual networking, but Actions took a waiting position and were slow to utilise this tool to its full potential due to the seemingly receding pandemic.

For PLANTMETALS, the lack of meetings, both live and virtual, seriously hampered the start of our Action. It slowed communications of tasks within workgroups, between workgroups, and with third parties. The available PLANTMETAL platforms (for chat and file-sharing) were not used either.

We are now almost at the end of the 2nd Grant Period. The dissemination, communication, and exploitation of PLANTMETALS and its achievements are far below the level it should be. These activities are legal obligations of the Grant Agreement and it is paramount that a renewed effort is needed to increase the level of communication significantly and fulfil our main objectives in that respect.

Determination of mineral element concentrations of different flaxseed cultivars (*Linum usitatissimum* L.)

Cemal KURT

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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.

Keywords: flax seed, field, nutrient concentration. biofortification

Venue

The congress will take place in the METU Culture and Convention Center, at the center of the METU campus.

Middle East Technical University (METU), Culture and Convention Center

Google maps link: https://goo.gl/maps/Jw7hMfxwUbktrtjy5

The meeting will take place in **Hall B.**

Arriving to the Venue

If you stay outside of the campus, you will probably first arrive at one of the gates of the university campus. From there, you can either take a taxi (use the yellow callbox in front of the ODTU metro station) or walk to the conference venue (20 min).

If you take a taxi, tell the driver that you want to go to KKM (which is the abreviavion for the Culture & Convention Center at METU).

Every time you enter the campus, security will check your names from the list provided to them.

Please see the METU campus map for the location of the venue (Culture & Convention denter – KKM), the Biology Building (for registration), guesthouses and the central restaurants on METU campus at the end of the book.

Accommodation

We offer 25 rooms for accommodation (20euro per room) at the campus, you can apply via our website before they are occupied.

Details for Aysel Sabuncu guesthouse:

Location https://goo.gl/maps/FLs35mmpnh2Eidrk6,

Pictures: http://stm.metu.edu.tr/tr/galeri/asym-resimleri

Details for Misafirhane guesthouse:

Location:https://goo.gl/maps/YXz2CahGGCNFZRdz7

Pictures: http://stm.metu.edu.tr/tr/galeri/misafirhane-resimleri

For campus accommodation, if you are staying in Aysel Sabuncu (https://goo.gl/maps/1nniPJo2f6EkoBUU8) taking taxi may be the best choice since it is 6 km away from the conference venue. If you are staying in Misafirhane, then it will be only 10 minutes walk.

Local Travel

Ankara is organized so that, wherever you wish to go, most of the time you must first go to the city center, “Kizilay”. You can then go almost anywhere from Kizilay by taking public buses, subway, dolmus (Small private buses) or taxis. Taxis and private buses may accept only cash, not credit card. Public buses and subways mostly accept special tickets. Single ride tickets can be bought in subway station. Alternative to single ride tickets is to buy Ankaracard and deposit

in some money. We highly recommend the latter, it provides benefits such as discounted transfers in an hour.

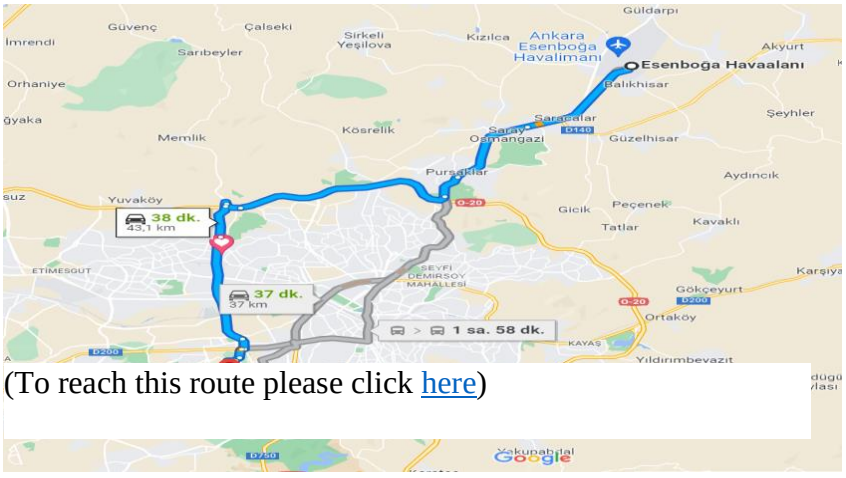
The meeting will take place in METU (Middle East Technical University). The Turkish name for the university is ODTÜ (Orta Doğu Teknik Üniversitesi). Note that you will mostly see the Turkish version ODTÜ on the signs instead of METU while you are in Ankara. METU has a big campus, from the various entrances to the conference hall, it approximately takes 20 minutes on foot.

For accommodation, there are various hotels around. We selected some of the hotels and provided transportation details in this guide. We recommend you use google maps to navigate anywhere, it works very well in Turkey. You can download the relevant maps before your arrival to use them without depleting your internet.

To arrive METU from the airport, you can directly take the taxi or to save some money you can take the bus to AŞTİ(last bus stop) first and then the taxi. Or you can go to the city centre, spend some time and then go to METU.

From Esenboğa Airport to METU (Middle East Technical University)

To travel from the airport to the METU campus, 3 ways can be followed.



(To reach this route please click here)

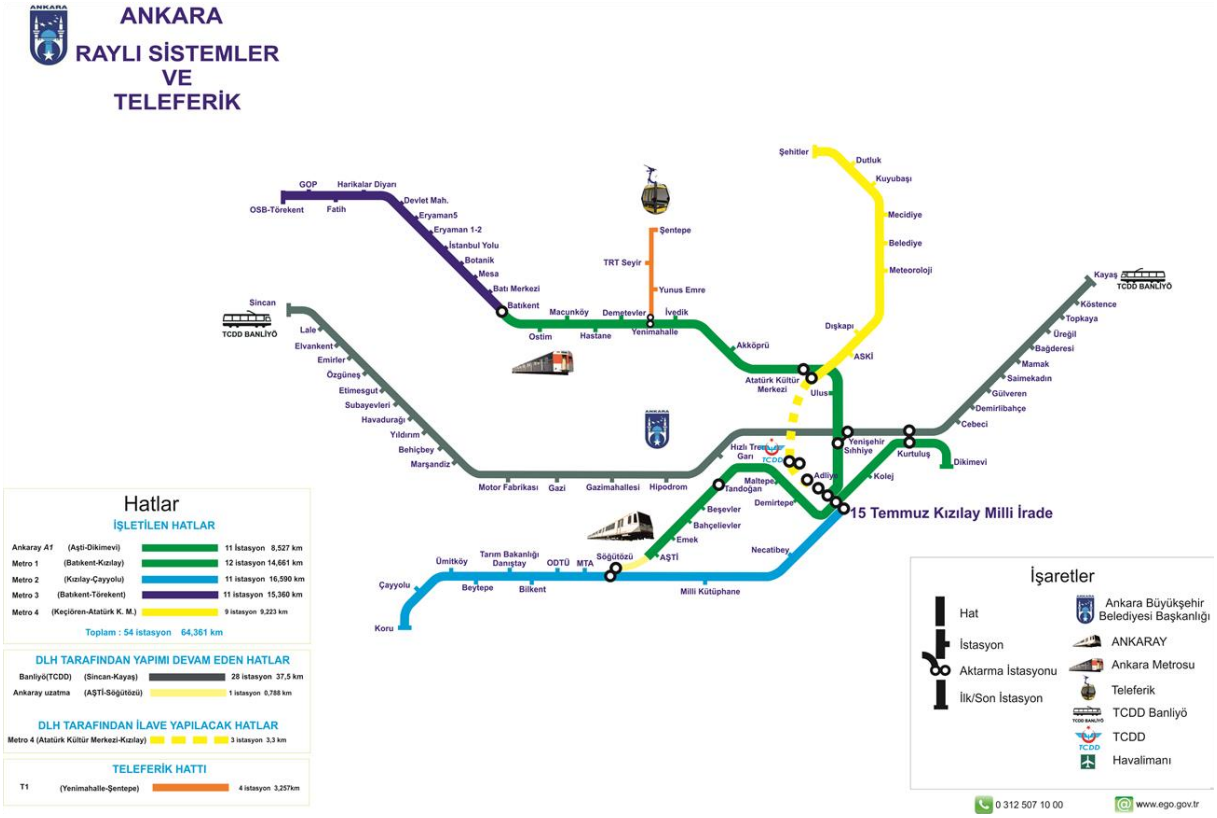
The first way of doing so is by taking a taxi directly to the campus. You can click here to open METU's location in google maps.

The second way of travelling from the airport to the campus is to take Havaş or BelkoAir to the last stop, AŞTİ, and then take the taxi. BelkoAir or HAVAŞ shuttles can be found in front of the domestic arrival and international arrival exit doors and depart every hour. They are also available at midnight but the schedule may vary (Belkoair: https://www.belkoair.com/en). BelkoAir and HAVAŞ shuttles that go from airport to AŞTİ cost 16 Turkish Liras, about 1€. Beware that they do not accept debit or credit cards as a form of payment. The commute takes about an hour, however it can fluctuate due to traffic jams and weather conditions.

Image 1.3 Ankaray Subway

The third way is to first go to the city centre by taking Havaş or BelkoAir and get off in Kızılay. From there, subway (red line) will go to ODTÜ station ("Koru" direction.).

Detailed travel information to and from the METU campus and the various hotels listed previously are available in the "METU Travel Guide" posted on our website. You can reach the guide here.



Ankara Rail Map

Calling a taxi at the campus: At the university campus, you can travel anywhere inside the campus for 20 tl. ODTU taxi number is 0090312 219 51 51. You need a local to help you to call the taxi, since the drivers don't speak English. Besides, every student at the university campus speaks English, as the official education language in METU is English. Unfortunately Uber is not available in Turkey.

VISA/Health registration

Please check the visa requirements for your country, Please trust to the official websites(with .gov).

For VISA requirements use https://www.mfa.gov.tr/visa-information-for-foreigners.en.mfa .

Please fill this form before entering Turkey: https://register.health.gov.tr/

Whatsapp group: This group's purpose is to help you about the conference related questions and to facilitate conversation among us.

https://chat.whatsapp.com/CdeLWkDkd991rdHq2qYpC7

Last minute poster printing: We talked to Dereagzi printing compant. This place will be open during the conference(opens daily at 9:30) and only 10 minutes walk from the conference venue. Choose non-glossy paper(otherwise waiting time will be longer), A0 size is 120tl, 50x70 size will be 50 TL and it will take around 20 min to print. You can send your file and communicate through whatsapp, they speak English.

Location: https://g.page/DEREAGZITEZCILTLEME?share

Whatsapp number: 0090545 212 24 15, **Name of the responsible person:** Zafer

Internet access: There will be free WI-FI in conference hall. You will get instructions about it when the conference starts.

Zoom link for the remote access

https://zoom.us/j/9601277185?pwd=bUMzWUZlZkg2QWtuN3V6bTZVVXpEZz09

Passcode: 668970

Conference will be hybrid, people in the conference hall will hear the remote participants and the remote participants will hear both the audience and the presenter.

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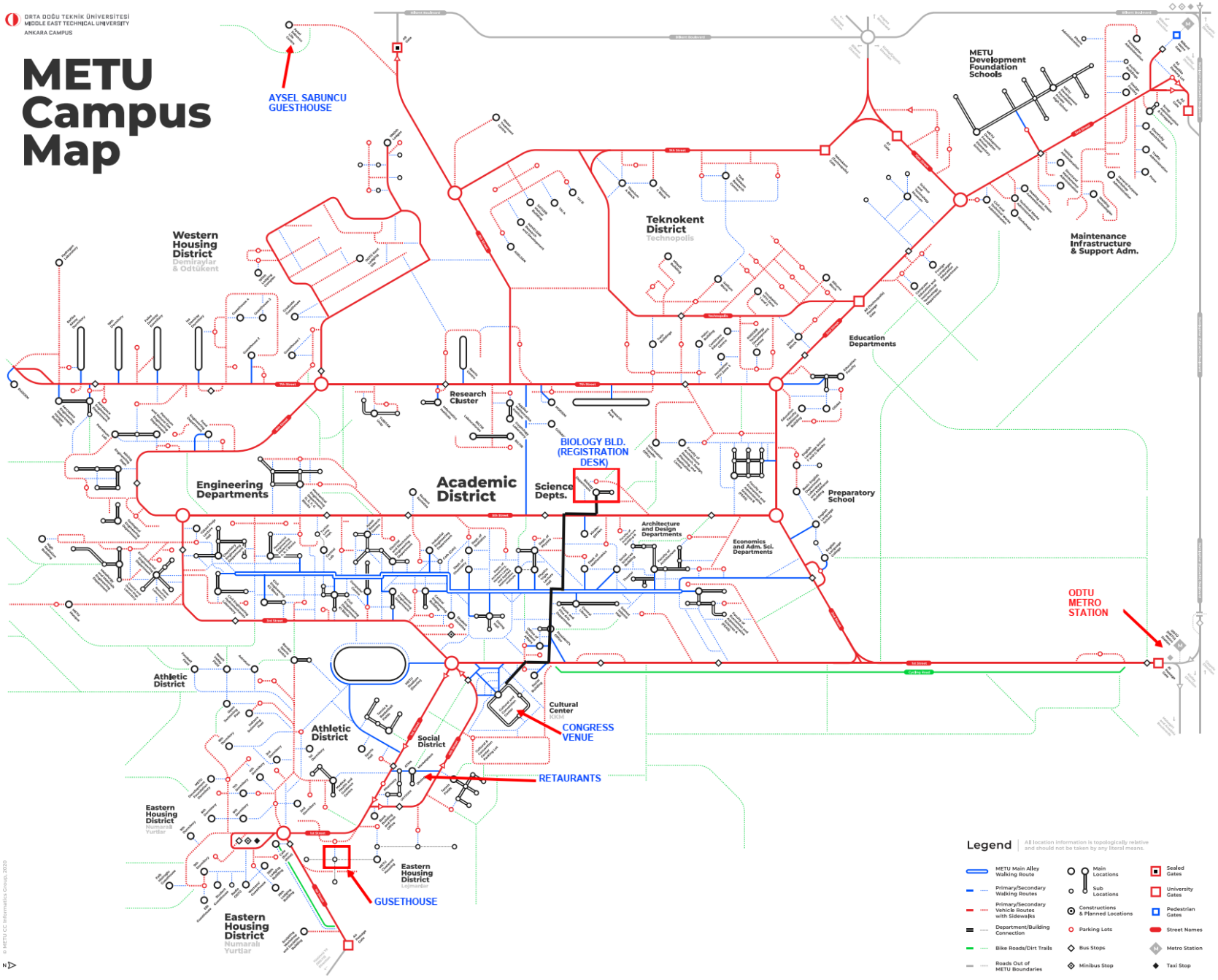
ORTA DOĞU TEKNİK ÜNİVERSİTESİ
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AYSEL SABUNCU GUESTHOUSE
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Demiraylar & Odtükent
Teknokent District
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RETAURANTS
Eastern Housing District
Numarali Yurtlar
GUSETHOUSE
Legend
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METU Main Alley Walking Route
Primary/Secondary Walking Routes
Primary/Secondary Vehicle Routes with Sidewalks
Department/Building Connection
Bike Roads/Dirt Trails
Roads Out of METU Boundaries
Main Locations
Sub Locations
Constructions & Planned Locations
Parking Lots
Bus Stops
Minibus Stop
Sealed Gates
University Gates
Pedestrian Gates
Street Names
Metro Station
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