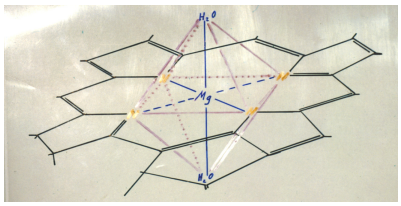
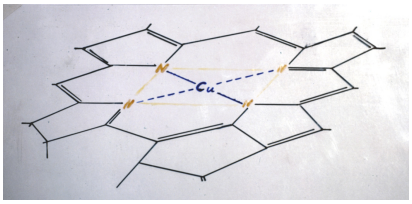
# Metals, Plants and People

## (Kovy, Rostliny a Lidé - KOROLID)



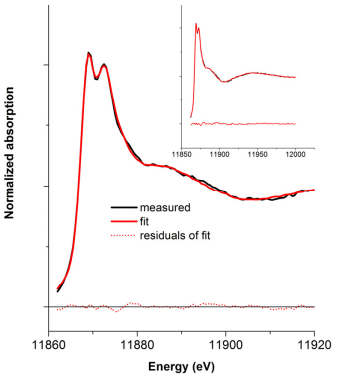
# Mini-Symposium



**on metals in photosynthetic organisms**

**- aimed at bringing together existing and (potential) future members and cooperation partners on the KOROLID grant**

**17 - 19 August 2017**
**Location: Biology centre of the Czech Academy of Science, Institute of Plant Molecular Biology (main lecture hall)**

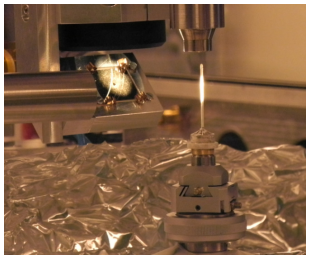


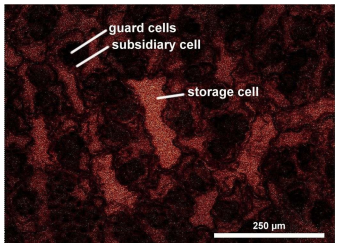

Programme:

**17 August**

15:00 to 17:00: Registration

18:00: "Get together"- party (free for all registered participants)






**18 August**

8:30-10:00 Lectures

10:00-10:30: Coffee break, poster viewing

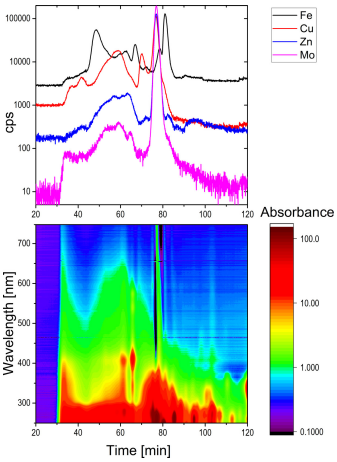
10:30-12:30 Lectures

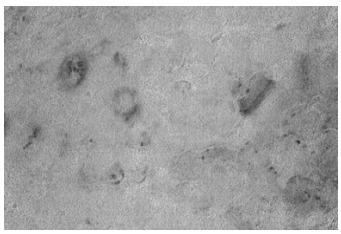
12:30-13:30 Lunch (free for all registered participants)

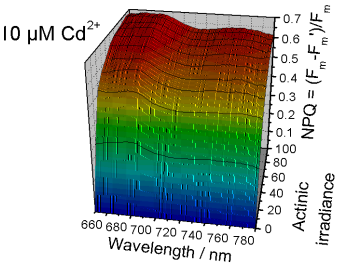
13:30-15:30 Lectures

15:30-16:00 Coffee break, poster viewing

16:00-18:00 Lectures



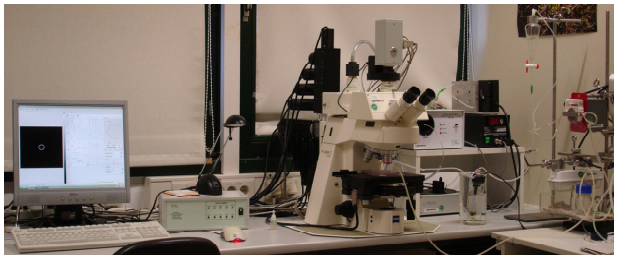


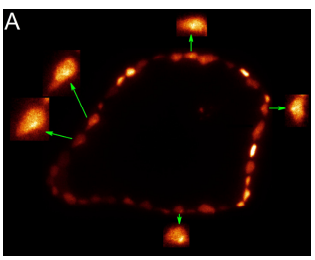

**19 August**

9:00-11:00: Planning of collaborative projects, in particular COST "Metal Metabolism in Plants"

11:00-11:30: Coffee break

11:30-13:00 (if necessary continued after lunch): Planning of collaborative work






# List of talks and posters

**Talks**

| Time | Speaker, affiliation | Title |
|---|---|---|
| 8:30-8:40 | Prof. Libor Grubhoffer<br>(director of the Bioloby Centre of the Czech Academy of Sciences = BCAS, České Budějovice, Czech Republic) | Welcome |
| 8:40-9:20 | Prof. Hendrik Küpper<br>(BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Introduction to the KOROLID project |
| 9:20-9:40 | Dr. Lyudmila Lyubenova<br>(BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Pollutants and health supporters. Both sides of the coin regarding the investigation of metal(loids) in plants |
| 9:40-10:00 | Dr. Filis Morina,<br>(BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Understanding the mechanisms of metal-induced oxidative stress through comparative analysis of populations from contaminated and un-contaminated areas |
| 10:00-10:30 | ***coffee break, posters*** | |
| 10:30-11:00 | Prof. Nathalie Verbruggen<br>(Université Libre de Bruxelles, Faculté des Sciences, Laboratoire de Physiologie et de Génétique moléculaire des Plantes, Belgium) | Plant tolerance to cadmium: study of *Arabidopsis halleri* |
| 11:00-11:20 | Dr. Aimone Porri<br>(Ruhr-Universität Bochum, Lehrstuhl für Molekulargenetik und Physiologie der Pflanzen, Germany) | *Arabidopsis halleri* as a model to uncover the molecular basis of metal hyperaccumulation |
| 11:20-11:40 | Dr. Stefanie Hoeller<br>(Martin Luther University Halle-Wittenberg, Inst. of Agricultural and Nutritional Sciences, Plant Nutrition Laboratory, Germany) | Impact of mtp8 on metal accumulation and localization in seeds of *Arabidopsis thaliana* |
| 11:40-12:00 | Dr. Ana Mijovilovich,<br>(BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Combination of X-ray spectroscopies (XRF, XANES, EXAFS, HERD XES) with UV/VIS/NIR absorption and emission spectroscopies to study metal metabolism in plants |
| 12:00-12:30 | Dr. Radek Litvin<br>(BCAS, IPMB, Dept. of Photosynthesis, Czech Republic) | Light harvesting architecture of eukaryotic algae with plastids of secondary endosymbiotic origin |
| 12:30-13:30 | ***lunch break*** | |

**...talks continued...**

| Time | Speaker, affiliation | Title |
|---|---|---|
| 13:30-14:00 | Prof. Fangjie Zhao<br>(Nanjing Agricultural University, Nanjing College of Resources and Environmental Sciences, PR China) | Arsenic in rice: mechanisms of arsenic uptake and detoxification |
| 14:00-14:30 | Dr. Manuel Gonzalez-Guerrero<br>(Universidad Politécnica de Madrid, Centro de Biotecnología y Genómica de Plantas, Spain) | Molybdate transport in *Medicago truncatula* root nodules |
| 14:30-15:00 | Dr. Vojtech Lanta / Dr. Milada Vitova<br>(Institute of Microbiology CAS, Algatech Centre, Laboratory of Cell Cycles of Algae, Czech Republic) | Growth responses of the alga *Desmodesmus quadricauda* to lanthanides – New insights for bioremediation projects |
| 15:00-15:30 | Dr. Stephan Wawra<br>(Universität zu Köln Institut für Genetik, CEPLAS, Germany) | Signaling in plant-fungus interactions |
| 15:30-16:00 | ***coffee break, posters*** | |
| 16:00-16:20 | Dr. Ricardo Giehl<br>(Leibniz Institute of Plant Genetics & Crop Plant Research, Germany) | Heavy metals induce iron-deficiency responses at different hierarchic and regulatory levels |
| 16:20-16:40 | Dr. Sowmya Shreedhar<br>(BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Microbes – expanding the horizon |
| 16:40-17:10 | Dr. Petr Šimek<br>(BCAS, Analytical Biochemistry & Metabolomics, Czech Republic) | Mass spectrometry based metabolomics approaches, metabolomic analytical platforms and metabolite coverage in biological research |
| 17:10-17:40 | Prof. Ursula Fittschen<br>(Institut für Anorganische und Analytische Chemie, TU-Clausthal, Germany) | *In vivo* elemental imaging of plant samples using XRF |
| 17:40-17:50 | Prof. Josef Špak<br>(BCAS, director of the Institute of Plant Molecular Biology = IPMB, České Budějovice, Czech Republic) | Closing remarks to the talks |
| 17:50-18:00 | Prof. Hendrik Küpper<br>(BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Update on details concerning the session for collaboration planning on Saturday |

**Posters**

| Number | Presenter, affiliation | Title |
|---|---|---|
| 1 | Dr. Elisa Andresen (BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Mechanisms of cadmium toxicity investigated on physiological and biophysical level under environmentally relevant conditions |
| 2 | Dr. David Bina (BCAS, IPMB, Dept. of Photosynthesis, Czech Republic) | Light harvesting using red-shifted forms of chlorophyll a |
| 3 | Dr. Nadeem Bokhari (BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Inductively coupled Plasma Mass Spectrometry |
| 4 | Dr. Gerald Falkenberg (Deutsches Elektronen-Synchrotron, Photon Science, Germany) | Cryogenic X-ray fluorescence tomography using the Maia detector and a cryo-stream at beamline P06 |
| 5 | Dr. Archana Mishra (BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Zn distribution, homeostasis and regulation of Cadmium/Zinc pumping ATPases during Turnip yellow mosaic virus (Tymv) infection in the hyperaccumulator *Noccaea caerulescens* |
| 6 | Prof. Ursula Fittschen (Institut für Anorganische und Analytische Chemie, TU-Clausthal, Germany) | X-ray fluorescence elemental imaging, and micro analysis of plant tissue samples |
| 7 | Nermeen Ashraf (BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic) | Genetic transformation of *Lycopersicon esculentum* using an abiotic stress tolerance gene. |
| 8 | Tomáš Hubáček; Iva Tomková; Prof. Jakub Borovec (BCAS, SoWa Research Infrastructure, Czech Republic) | The IC - ICP-QQQ - a useful tool for the description of biogeochemical processes |
| 9 | Dr. Ansgar Gruber (University of Konstanz, Department of Biology, Germany) | Intracellular reallocation of metabolic pathways in cells with complex plastids |
| 10 | Prof. Tomas Macek (University of Chemistry and Technology (Prague), Faculty of Food and Biochemical Technology, Czech Republic) | Plants for bioremediation need increased resistance to stress and expression of specific transgenes |

# Abstracts

## Talks

Hendrik Küpper

BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic

### Introduction to the KOROLID project

The project will enhance research of the Biology Centre CAS concerning environment, agriculture and health safety. The primary aim of this project is basic research aimed at better understanding of the response of photosynthetic organisms to metal deficiency as well as metal(loid) toxicity & detoxification. This is required for the following challenges.
(a) For environmental risk assessment. We will evaluate the relevance of different proposed mechanisms of metal-induced inhibition under environmentally relevant conditions and compare the results with those obtained under conditions that were used in identifying the putative toxicity mechanisms
(b) For improved agriculture through more targeted fertilization, plus breeding crops that grow in adverse conditions where current crops either suffer from metal deficiency or toxicity and/or contain an elemental composition that is suboptimal for human health. Further, in a collaborative project with partners in various EU countries we will investigate the role of metals in plant immunity against pathogens, which is an exciting emerging field because many proteins involved in plant immunity need metal binding for their function.
(c) For phytoremediation of polluted soils and aquifers. Our project will contribute to the understanding of physiological and biochemical mechanisms of hyperaccumulation by revealing mechanisms of metal uptake, transport and detoxification.
(d) For understanding evolutionary processes. The analysis of differences in photosynthesis biophysics and metal/acid resistance of Zn-BChl containing bacteria compared to their Mg-BChl containing relatives will help in understanding why almost all organisms utilize the Mg-complexes, and under which exact conditions it becomes advantageous to use alternatives.
(e) For more realistic estimation of the productivity of ecosystems, especially the oceans. This will be tackled by a project on regulation of photosynthesis and nitrogen fixation under Fe limitation stress. This project will furthermore study how the oxygen-sensitive Fe centers in the nitrogenase are protected from damage.

These aspects will be studied by the new Plant Biophysics and Biochemistry Department, founded by Hendrik Küpper and Elisa Andresen in 2014. Cooperating with current teams working on photosynthesis and remediation of polluted soil and water it will be exploiting a unique sector field ICP-MS machine and additional instruments that will allow ultratrace analyses of metals and other elements. The ICP-MS facility will be open to collaboration with research and government institutions as well as industries.

Lyudmila Lyubenova

BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic

**Pollutants and health supporters. Both sides of the coin regarding the investigation of metal(loids) in plants**

Institute of Plant Molecular Biology, Department of Plant Biophisics and Biochemistry, Biology Centre CAS, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic

As a consequence of the negative input of human activities on terrestrial and aquatic ecosystems metal(loid)s are mobilized from their natural reservoirs to the atmosphere, soil and water, but often the underlining biological mechanisms remain unknown.

When metal(loid)s accumulate in plants they often cause toxicity - oxidative stress by cell damaging or replacement of essential nutrients. Metal(loid)s uptake leads to reduction of the biomass production, enzyme inhibition, changes in hormonal and water status. Changes in enzyme activities are plants response to environmental stress. Following the patterns of these changes may shed light on the mechanisms of plant tolerance respectively of plants sensitivity to pollutants. As an example, the uptake capacity of *Typha latifolia* for cadmium (Cd) will be presented as well as its detoxification enzymes response to this toxic metal.

A number of metal(loid)s are known to have health supportive functions for humans. Some research of the trace elements contribution for human well-being and health will be presented. These types of studies are notably important for the population stratum suffering from multielemental malnutrition.

An additional part of the metal(loid)s study, as a part of the KOROLID project, is focused on toxicity mechanisms at environmentally relevant, nano molar concentrations of cadmium. Preliminary results will be presented regarding the identification of target proteins of Cd-binding at nano molar Cd concentrations, based on Cd-peaks chosen after analytical metalloproteomics.

Filis Morina

BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic

**Understanding the mechanisms of metal-induced oxidative stress through comparative analysis of populations from contaminated and un-contaminated areas**

Although redox non-active, $Zn^{2+}$ can induce oxidative stress, and damage cellular components. In this study, Verbascum thapsus L. was used for revealing the mechanism of Zn-induced oxidative damage in the cell wall. Zinc preferentially accumulated in the root and leaf cell wall, accompanied by $H_2O_2$ production and increased EPR signals of •OH and endogenous charge transfer complex, quinhydrone (•CH3). Quinhydrone exhibits both antioxidant and prooxidant activities, and its accumulation is a result of Zn2+ stabilization of phenoxyl radicals. Analysis of antioxidative system in response to Zn2+ indicated a crucial role of cell-wall bound MnSOD and POD/Phenolics/Ascorbate cycle in the apoplast, and APX and MDAR (ascorbate recycling) in the symplast. Moreover, Zn stimulated ascorbate accumulation in the leaves which was also evident by increased activity of L-GalLDH, enzyme involved in the last step of ascorbate biosynthesis, while total and reduced glutathione content declined without changes in redox state.

*Verbascum* species are early colonizers of degraded soils, and several reports point to their metal tolerance traits in the field. In order to investigate adaptive capacity of *Verbascum* to excess $Zn^{2+}$ and $Cu^{2+}$ under controlled conditions four populations were chosen: MET1-*V. thapsus*, from Zn- and MET2-*V. lychnitis* from Cu-contaminated soils, and NMET1 and NMET2 –two *V. thapsus* populations from un-contaminated areas. MET populations showed higher tolerance to $Zn^{2+}$ and $Cu^{2+}$ than NMETs observed by higher growth and net photosynthesis rate, and lower electrolyte leakage and $H_2O_2$ accumulation, yet they had higher $Zn^{2+}$ concentrations in the shoot. Constitutively higher POD activity and ABA content was observed in the roots of the most Cu and Zn tolerant population (MET2). The results indicated that *Verbascum* populations can become tolerant to excess metal in soil after relatively short time of exposure (10-20 years), and have potential for use in phytostabilization due to fast growth rate and well developed rooting system.

Within the KOROLID project, my research is focused on revealing the beneficial effects of low, environmentally relevant Cu concentrations on soybean in relation to pathogen resistance (*Phomopsis longicolla*). In addition, the mechanisms of Cu tolerance in Cu-hyperaccumulating algae *Prasiola crispa* will be investigated. The research will cover subcellular response to stress in relation to antioxidative metabolism, ROS accumulation and metal sequestration, and changes in photosynthetic apparatus determined by chlorophyll fluorescence kinetics and gas exchange measurements, as well as identification and analysis of metal binding to proteins by HPLC coupled with ICP-MS.

Nathalie Verbruggen

Université Libre de Bruxelles, Faculté des Sciences, Laboratoire de Physiologie et de Génétique moléculaire des Plantes, Belgium

## Plant tolerance to cadmium: study of *Arabidopsis halleri*

Arabidopsis halleri is a pseudometallophyte and close relative of *Arabidopsis thaliana*. This species is a model to study metal homeostasis and evolutionary ecology. Although Zn hyperaccumulation & Zn and Cd hypertolerance are constitutive in *Arabidopsis halleri*, substantial intraspecific variation in these traits and in Cd accumulation has been observed between metallicolous (M) and non-metallicolous (NM) *A. halleri* populations. We have previously published that M populations were more tolerant to Cd than NM populations and that Cd hyperaccumulation was not constitutive in the species. We will show how populations respond differently to Cd and will highlight mechanisms that are likely associated with local adaptation.

Aimone Porri, Justin E. Anderson, Veronica Preite, Lara Syllwasschy, Gwonjin Lee, Ute Krämer

Department of Molecular Genetics and Physiology of Plants, Ruhr University Bochum, Germany

## *Arabidopsis halleri* as a model to uncover the molecular basis of metal hyperaccumulation

The genetic and molecular alterations underlying extreme phenotypic traits is still poorly understood. Metal hyperaccumulation and associated hypertolerance constitute extreme traits requiring vast physiological alterations in the metal homeostasis network that is comparably highly conserved across a broad range of organisms. Among the established metal hyperaccumulator model plants, we study the Zn/Cd hyperaccumulator species *Arabidopsis halleri* in the sister clade of *A. thaliana*. Genome-wide cross-species comparative approaches including closely related non-hyperaccumulator species have led to hypotheses on the molecular mechanisms and the evolution of metal hyperaccumulation. The functions of a few key metal hyperaccumulation genes were demonstrated through the stable transformation of *A. halleri* with RNA interference constructs. Next we aim to address the genetic basis of the vast within-species phenotypic diversity in *A. halleri* (Stein et al., 2017). This work is based on a large field survey of *A. halleri*, with analysis of leaf samples and rhizosphere soil samples of about 2,000 individuals in their natural habitats. Genotyping-by-sequencing data for a subset of 850 individuals in our *A. halleri* biodiversity resource has allowed initial genome-wide association studies (GWAS). To expand this approach, phenotyping of field-collected individuals under controlled standardized growth chamber conditions is in progress. We will present results from ongoing experimental approaches to validate the loci and polymorphisms identified so far. Our results are relevant for the development of phytomining, phytoremediation and crop bio-fortification technologies.

Stein RJ, Höreth S, de Melo JR, Syllwasschy L, Lee G, Garbin ML, Clemens S, Krämer U (2016) Relationships between soil and leaf mineral composition are element-specific, environment-dependent and geographically structured in the emerging model Arabidopsis halleri. New Phytol. 213(3): 1274-86.

Stefanie Höller[1], Seckin Eroglu[2,3], Ricardo F. H. Giehl[3], Bastian Meier[1], Elisa Andresen[4], Konstantin Ignatiev[5], Jan Garrevoet[6], Gerald Falkenberg[6], Hendrik Küpper[4], Edgar Peiter[1], Nicolaus von Wirén[3]

1 Plant Nutrition Laboratory, Institute of Agricultural and Nutritional Sciences, Faculty of Natural Sciences III, Martin Luther University Halle-Wittenberg, Halle (Saale), GERMANY (stefanie.hoeller@landw.uni-halle.de)
2 Department of Genetics and Bioengineering, Izmir University of Economics, Izmir, TURKEY
3 Molecular Plant Nutrition, Leibniz-Institute for Plant Genetics and Crop Plant Research, Gatersleben, GERMANY
4 Biology Centre of the Czech Academy of Sciences, Institute of Plant Molecular Biology, Department Plant Biophysics and Biochemistry, České Budějovice, CZECH REPUBLIC
5 Diamond Light Source Synchrotron, Didcot, UK
6 Deutsches Elektronen-Synchrotron (DESY), Photon Science, Hamburg, GERMANY

**Impact of mtp8 on metal accumulation and localization in seeds of *Arabidopsis thaliana***

In dry seeds of *Arabidopsis thaliana*, metals are not evenly distributed but accumulate in specific tissues. Manganese (Mn) localization is defined to subepidermal cells at the abaxial side of the cotyledons, while iron (Fe) accumulates mainly around the vasculature conferred by the vacuolar Fe transporter VIT1. In this study, we used synchrotron X-ray fluorescence (µ-XRF) to investigate the impact of the vacuolar Mn transporter METAL TOLERANCE PROTEIN 8 (MTP8) on Mn and Fe localization in mature seeds. A knockout of *MTP8* disrupted this Mn distribution pattern completely, thus we concluded that Mn localization is conferred by MTP8 in seeds. Besides Mn, MTP8 is able to transport Fe, which was indicated by yeast complementation assays and tomograms of *vit1* seeds, in which Fe was localized at the same sites as Mn. A dispersed distribution of Fe throughout the embryo in a *mtp8vit1* double knockout mutant confirmed the impact of MTP8 on Fe localization. Unexpectedly, in seeds overexpressing *MTP8* (35S:MTP8) Mn and Fe accumulated at the same sites as in wild type seeds whereas overall Mn concentrations were decreased. This is in contrast to the expected homogenous distribution, since the cauliflower mosaic virus *35S* promoter is not tissue-specific. 35S:MTP8 plants accumulated more Mn in roots, as compared to wildtype plants, with a tendency to lower Mn levels in shoots. Thus, Mn loading into seeds might be deteriorated in these lines, explaining the absent effect of *MTP8* overexpression in seeds. However, overexpression of MTP8 enhanced Mn tolerance of germinating seeds, pointing to an additional role of this transporter in Mn detoxification at this early developmental stage.

Ana Mijovilovich

BCAS, IPMB, Dept. of Plant Biophysics and Biochemistry, Czech Republic

**Combination of X-ray spectroscopies (XRF, XANES, EXAFS, HERD XES) with UV/VIS/NIR absorption and emission spectroscopies to study metal metabolism in plants**

With a combination of spectroscopies it is possible to determine the location, binding, oxidation state, and ligands of metals in biological intact tissue as well as extracted proteins. UV-VIS and NIR are used during protein extraction and characterization. FKM (Fluorescence Kinetic Microscopy) gives insight on the photosynthetic mechanism. XANES together with DFT calculations gives information about electronic structure at the metal site and oxidation state, while EXAFS gives structural information about the ligands. HRFD XANES unveils the transition lines at the pre-edge with sub-lifetime resolution, revealing the electronic structure in full detail. The valence-to-core transition in HRFD XES allows to determine one proton bound to a metal. Nano-fluorescence tomography down to a few hundred nm makes possible a 3D view at sub-cellular level. All these combined with proteomics to isolate the proteins involved in the metal trafficking, enables to study the metabolic paths of metals in the plants.

Nano-tomography allows to leave the "inference" to get the "foot print" of the phenomena. In Mishra et al (2016) it was concluded that As was mainly localized in the nucleus because only one brilliant dot was measured in each cell, and the nucleus happens to be the only singular organelle. of the observed size and location. This is an example of how imaging at sub-cellular resolution enables to think the physiology at the level of "one cell".

A new possibility to make time resolved studies is opening with the development of X-ray Free Electron Lasers (XFELs) as user facilities. It is possible to get data with femtosecond time resolution as well as structural data from a single molecule. HRFD XES is specially suited for XFELs because of the use of analyzer crystals for detection that allow collecting spectroscopy on one shot.

The ability to cover a wide energy range with "in-house" spectroscopy (UV-VIS-NIR and hard x-rays) with the acquisition of the μXRF BRUKER M4 Tornado in a biology-optimized configuration brings an important improvement in the group work flow. Plants and plant extracts can be monitored at all stages of development and *in vivo*, bringing a plethora of information about the physiology changes. The possibility to have in-house both a very accurate mass spectroscopy as well as space resolution down to 20 μm with the μXRF enables to determine the metal distribution at the tissue level. It also provides the best screen for suitable samples for the synchrotron for the sub-cellular metal distribution and spectroscopy for metal ligation.

Seema Mishra, Matthias Alfeld, Roman Sobotka, Elisa Andresen, Gerald Falkenberg and Hendrik Küpper. Journal of Experimental Botany, Vol. 67, No. 15 pp. 4639–4646, 2016

Radek Litvin

BCAS, IPMB, Dept. of Photosynthesis, Czech Republic

**Light harvesting architecture of eukaryotic algae with plastids of secondary endosymbiotic origin**

Thylakoid membrane architecture of the eukaryotic algae from the group *Stramenopila* differs from that of higher plants and represents a very interesting case of independent evolution. The result is a diversity of pigments and supramolecular assemblies used in photosynthetic light harvesting processes. In terms of structure, stramenopile algae lack analogs of monomeric antenna components of PSII - CP24, CP26 and CP29, resulting in altered supramolecular structure of the PSII supercomplex. Likewise, the light harvesting antenna of PSI is weakly bound in stramenopiles in contrast to the situation in plants. Results of a structural study of PSI in stramenopile algae by means of electron microscopy will be presented.

In terms of pigment composition, stramenopile algae utilize a range of carotenoid and chlorophyll types that differ from those in plants yet provide efficient means of light harvesting and photoprotective abilities. First, a light harvesting system using chl-a and violaxanthin will be presented as an example of a light harvesting antenna which uses the same pigment (violaxanthin) for light harvesting and also for photoprotection. Second, light harvesting systems with absorption extended to the far-red region will be shown. These red-shifted light harvesting systems demonstrate the flexibility of the basic LHC protein structure and also the influence of protein structure on cofactor (chl-a) properties.

Fang-Jie Zhao

Nanjing Agricultural University, Nanjing College of Resources and Environmental Sciences, PR China

**Arsenic in rice: mechanisms of arsenic uptake and detoxification**

Rice is the staple food for about half of the world population and is also a major dietary source of inorganic arsenic (As), a class-one carcinogen for humans. Rice accumulates As much more efficiently than other cereal crops. This results from a combination of an elevated As bioavailability in anaerobic paddy soil and efficient uptake of As by rice roots. Paddy soils in many areas in Asia are contaminated with As due to mining activities and irrigation of As-laden groundwater, leading to phytotoxicity in rice crop and substantial yield losses. Understanding the As biogeochemistry in paddy environments and the mechanisms of As uptake and transport in rice plants is important for both food security and safety. In flooded paddy soils, arsenite [As(III)] is the predominant species of As, with arsenate [As(V)] and methylated As species also being present. Arsenite is taken up mainly by the silicon transporters in rice, whilst As(V) is taken up by phosphate transporters (e.g. OsPT8). As(V) reduction followed by As(III) efflux is a major mechanism of As detoxification in microorganisms. This has been found to be true in plants as well. Recent studies have identified a new class of As(V) reductases, named HAC or ATQ, in Arabidopsis thaliana. Our recent studies have identified OsHAC1;1, OsHAC1;2 and OsHAC4 as new As(V) reductases in rice that play a key role in As(V) tolerance and As accumulation in rice plants. These enzymes reduce As(V) to As(III); the latter is then effluxed to the external medium to avoid excessive build-up of As in the cells. Mutation in the OsHAC genes resulted in greatly increased accumulation of As in the above-ground tissues of rice, whereas overexpression of OsHAC genes decreased As accumulation. Methylated As species DMA is taken up by rice roots partly via the silicon transporter Lsi1 and transported to rice grain via the peptide transporter OsPTR7. DMA is more toxic to plants than inorganic As. This is because DMA cannot be detoxified by complexation with phytochelatins and, as a result, is highly mobile during the long-distance translocation in rice plants. The high mobility and high toxicity of DMA explains its high potency in causing spikelet infertility in rice panicle, the key symptom of the straight-head disease.

Manuel Tejada-Jiménez[1], Patricia Gil-Diez[1], Javier León Mediavilla[1], Juan Imperial[1,2], Manuel González-Guerrero[1]

1 Centro de Biotecnología y Genómica de Plantas (UPM-INIA). Madrid, Spain.
2 Consejo Superior de Investigaciones Científicas. Madrid. Spain.

## Molybdate transport in *Medicago truncatula* root nodules

Symbiotic nitrogen fixation (SNF) requires molybdenum for nitrogenase to work, as an integral element of the FeMo cofactor. This metal has to be delivered from the host plant to the bacteroids in order to synthesize the cofactor. However very little is known about the transporters mediating molybdenum supply to nodules of legumes in connection to SNF. MOT1 family has been found to transport molybdate in plants, what maked them good candidates to be involved in molybdenum delivery to nitrogen-fixing nodule cells.

We have identified two *Medicago truncatula* MOT1 family members (MtMOT1.2, and MtMOT1.3) as critical elements in molybdenum transport ito rhizobia-infected cells. Heterologous expression in *Saccharomyces cerevisiae* shows that both proteins are able to transport molybdate toward the cytosol. *MtMOT1.2* is expressed in roots and nodules, and *MtMOT1.3* is nodule-specific. Immunolocalization studies show that MtMOT1.2 is located in the plasmam membrane in the endodermis in roots and nodules, while MtMOT1.3 is located in the plasma membrane of infected and non-infected nodule cells. A loss of function mutant of either gene exhibited reduced growth under symbiotic conditions, associated with a decreased nitrogenase activity compared to wild-type plants. This phenotype was rescued by the addition of molybdate to the nutritive solution or by genetic complementation with a wild-type copy. These results point to a role of MtMOT1.2 and MtMOT1.3 in molybdenum supply nodule cells, being a first step to understand Mo homeostasis in the legume-rhizobium symbiosis.

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**Growth responses of the alga Desmodesmus quadricauda to lanthanides – New insights for bioremediation projects**

The presented study deals with utilization of the micro-alga *Desmodesmus quadricauda* to recycle valuable lanthanides used in industrial applications and agriculture. In our pilot project, we focused on ability of the alga to recycle lanthanides from red mud, a highly alkaline waste by-product, produced in large quantities during the extraction of alumina from bauxite. The results indicated that the alga performs well on red mud-amended medium and that its biomass indeed contains lanthanides, as analyzed by ICP-MS. This points out the potential of the model alga to be utilized as active biological agent.

Further, we used a stratified batch culture approach to test growth responses of the model alga to two concentrations of four lanthanide elements (Ce, La, Nd, Gd). The procedure included chemical mutagenesis allowing to produce mutant strains which would be resistant to presence of lanthanide elements at high doses. The results showed that the mutants greatly varied in responses to lanthanides added to the growth medium. However, several strains (7 out of 42) clearly showed enhanced growth rate at low concentration of lanthanides (10 μM), indicating a beneficial effect, and were insensitive to the adverse effect of a high dose of lanthanides (100 μM; toxic in wt). This procedure allowed to identify the candidates most suitable for bioremediation projects concerning, for example, cleaning up wastewaters containing lanthanides at high concentrations.

In the ongoing project, beneficial and toxic effects of the lanthanides for algal growth in the experiments optimized to simulate natural conditions will be studied.

Stephan Wawra

Universität zu Köln Institut für Genetik, CEPLAS, Germany

**Signaling in plant-fungus interactions**

In natural ecosystems, plants often benefit from the presence of root symbiotic fungi. However, in order to colonize the plant root system the symbionts need to overcome the host immunity and have to be able to manipulate the metabolic processes. For this purpose fungi have evolved an array of effector molecules which often are small secreted proteins. In order to identify and characterize these compatibility factors, we use in our studies the root endophyte Serendipida indica. S. indica is a generalist able to colonize the roots of monocots and dicots including the experimental host Arabidopsis thaliana and the crop barley. Our studies revealed that S. indica implemented in its genome a bacterial-derived gene encoding for a histidine rich protein we named SiDLD1 that likely originally evolved to tolerate cell stress mediated by transition metal ions. DLD1 expression is induced during colonization of barley at early time points and the protein can directly interact with a variety of transition metal ions. Several lines of evidence suggest that DLD1 is used by the fungus to counter iron mediated cereal immune defense responses at the side of root cell penetration. Furthermore, we identified additional mechanisms utilized by S. indica to overcome host defenses including manipulations of extracellular ATP levels and dampening of β-glucan induced MAMP recognition by SiFGB1. Here we will present our latest data.

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**Heavy metals induce iron-deficiency responses at different hierarchic and regulatory levels**

In plants, the excess of several heavy metals mimics Fe deficiency-induced chlorosis indicating a disturbance in Fe homeostasis. To examine at which level heavy metals interfere with Fe-deficiency responses, we carried out an in-depth characterization of Fe-related physiological, regulatory and morphological responses in *Arabidopsis thaliana* exposed to heavy metals. Enhanced Zn uptake closely mimicked Fe deficiency by leading to low chlorophyll but high ferric-chelate reductase activity and coumarin release. These responses were not caused by Zn-inhibited Fe uptake via IRT1. Instead, Zn stimulated the transcriptional response of typical Fe-regulated genes, indicating that Zn affects Fe homeostasis at the level of Fe sensing. Excess supplies of Co and Ni altered root traits in a different way as Fe deficiency, induced only transient Fe-deficiency responses, which were characterized by a lacking induction of the ethylene pathway. Cadmium showed a rather inconsistent influence on Fe deficiency responses at multiple levels. By contrast, Mn evoked weak Fe deficiency responses in wild-type plants but strongly exacerbated chlorosis in *irt1* plants, indicating that Mn antagonized Fe mainly at the level of transport. These results show that the investigated heavy metals modulate Fe-deficiency responses at different hierarchic and regulatory levels and that the interaction of metals with physiological and morphological Fe deficiency responses is uncoupled. Thus, our results emphasize not only the importance of assessing heavy metal toxicities at multiple levels but provides also a new perspective on how Fe deficiency contributes to the toxic action of individual heavy metals.

Sowmya Shreedhar

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**Microbes – expanding the horizon**

Microbes inhabit every niche on the planet including hot springs, glaciers, deserts and many other extreme environments. These tiny machineries of the invisible world are known to play significant roles in the evolution of earth and its life forms. Furthermore, microorganisms have versatile applications in industrial, medical, agricultural and environmental technologies.

Phosphate solubilizing bacteria belong to one such class of organisms that has found immense application as biofertilizers in the agricultural world. In addition, research in the past decade has focused on the phosphate solubilizers from uranium mining sites that contain additional characteristic of uranium tolerance. These entities find prospective applications in non-reductive mineralization of uranium into uranyl phosphate mineral that can aid in long-term management of uranium wastes.

In recent years, the microbial world has expanded its horizon into previously under-explored areas such as civil and geotechnical engineering. Present day geotechnical problems favour biological interventions to develop eco-friendly and sustainable technology. In this aspect, microorganisms are studied for prospective applications such as self-healing cement, slope stabilization, ground development, foundation engineering and municipal solid waste treatments.

As mentioned above, microbes are cosmopolitan in distribution owing to their adaptability to any environmental conditions. One such interesting adaptation is witnessed in the Zn2+-containing bacteriochlorophyll (BChl) of the genus *Acidiphilium*. Magnesium ($Mg^{2+}$) is the ubiquitous metal ion involved in energy harvesting reaction centers in entire photosynthetic organisms. Nevertheless, $Zn^{2+}$ as the metal ion in the BChl of *Acidiphilium*, which thrives in acidic metal-rich environments, makes an exception. Understanding this unique presence of an alternative metal ion will lead to major insights into the evolution of the photosynthetic machinery and in understanding the survival mechanism of bacteria under adverse stress conditions in their environment.

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**Mass spectrometry based metabolomics approaches, metabolomic analytical platforms and metabolite coverage in biological research**

Metals and metalloid species inevitably play a distinct role in the life cycle of each cell and tissue by effecting on multiple endogenous molecular processes, including the metabolome homeostasis. The principal metabolomics research is based on comparative analysis of natural and challenged state of the studied organism. Here this omics approach is discussed in the field of biological research with particular attention to current mass spectrometric based analytical platforms for the comprehensive metabolite coverage. Some illustrative applications are further presented with the aim of a critical evaluation of the approach which promises to shed more light into the metal – metabolite interactions and thus into their action on the phenotype and plasticity of the examined organism.

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## *In vivo* elemental imaging of plant samples using XRF

Research in plant physiology focuses on understanding plant function on a molecular level to improve plant health and productivity in the long term. This include the goal to design or bread more stress tolerant plants, which for instance can cope better with the increasing threat of drought and salt stress. Metal ion gradients across biomembranes play a fundamental role in cellular function, such as energy storage, nutrient distribution, signaling pathways and enzymatic reactions. However, ion gradients are not static and thus can change depending on leaf age, nutrient supply, light availability or external abiotic stress factors, e.g. soil salinity. Studying these spatial ion fluxes in correlation to factors like leaf age and genotype provides insights into ion transport and its significance for plant function Accordingly, research efforts to analyze the elemental composition of plants designated as ionomics has been intensified in a similar fashion like proteomics and genomics [1]. However, generally these ion studies focus on the macro-scale content e.g. pooled shoot ionome. We have started investigating the plant ionome on a more microscopic level by studying ion gradients corresponding to leaf age *Arabidopsis thaliana* in wild-type and mutant plant leaf tissue using total reflection X-ray fluorescence (TXRF) [2]. TXRF analysis enabled sampling of minute amounts of plant leaf tissue which was sufficient to unveil ion gradients between individual leaves. However, even within one leaf ions are not homogeneously distributed but accumulate in certain structures like plant hairs (trichome) or veins. This phenomenon has been studied by Synchrotron micro-XRF by several groups on chemically fixed clipped tissue samples numerous times [3]. However, some ions like K are known to be highly mobile in the plant tissue. Accordingly, we followed a new approach to study the micro ionome in plants using laboratory based micro-XRF *in vivo*. Using chlorophyll fluorescence to determine plant vitality throughout the measurements, we could show that plants were not damaged by X-ray exposure [4]. Here, we will present the developed set up and its evaluation. Additionally, we will report about first evaluations of quantification procedures that will eventually give accurate result on ion tissue concentrations.

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## Posters

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### Mechanisms of cadmium toxicity investigated on physiological and biophysical level under environmentally relevant conditions

The heavy metal cadmium (Cd) is highly toxic to most organisms. We used the aquatic shoot model plant *Ceratophyllum demersum* and the agriculturally important crop *Glycine max* (soybean) to study the mechanisms of chronic Cd-toxicity under environmentally relevant conditions.

In both plants, toxicity symptoms appeared quickly when treated with lethal concentrations (up to 200 nM for the macrophyte, 1-10µM for the crop) while exposure to sublethal Cd (about 20-50 nM for both species) had negative effects only towards the end of the treatment.

In both species, thresholds of incorporation of Cd into proteins could be analysed by size exclusion chromatography coupled to a photodiode array detector and an inductively coupled plasma mass spectrometer (ICPMS). Replacement of $Mg^{2+}$ by $Cd^{2+}$ in the major antennae of photosystem II (LHCII) starting already below 5nM Cd2+ (apparent KD for LHCII monomers: 10.5nM) and leading to dissociation of LHCII trimers was most likely the reason for the decreased photosynthetic activity in low light, as determined oxygen exchange. Fluorescence kinetics revealed Cd-induced inhibition of the photosystem II reaction centre in high light.

Micro X-ray fluorescence of *C. demersum* leaves revealed a changing distribution pattern of Cd and Zn with increasing Cd concentrations: Applied in low concentrations (2nM), Cd was homogeneously distributed in the whole section of shoot, indicating to be either useful or not harmful. Sublethally (20nM) and lethally toxic (200nM) Cd concentrations led to sequestration of Cd in the vascular bundle and the epidermis cells, where Cd does not affect photosynthetic molecules.

Consistently, phytochelatins (PCs) showed different responses to the different concentrations of Cd treatment, with some of the being induced already by low nanomolar concentrations. Altogether, the results presented here indicate a different time and dose dependent manner of Cd toxicity in plants than known from various studies with unnaturally high Cd concentrations.

David Bina

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**Light harvesting using red-shifted forms of chlorophyll a**

Organisms inhabiting bottom layers of a stratified phototrophic community face the effect of shading: incident radiation depleted by wavelengths absorbed by chlorophyll-a and carotenoids, leading to relative enhancement of the far-red component of the spectrum. Only recently it has become apparent that this spectral window is utilized by a diverse group of eukaryotic algae using specialized chlorophyll-a based light-harvesting complexes (LHC). The main light-harvesting antenna of the freshwater eustigmatophyte alga *Trachydiscus minutus* is a prime example of this unique class of LHC complexes. A wide range of steady-state and time-resolved spectroscopic approaches was deployed to investigate this pigment-protein complex, providing insight into structural and functional aspects of the light harvesting strategy based on red-shifted spectral forms of chlorophyll a.

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## Inductively coupled Plasma Mass Spectrometry

The science of metrology is important for different disciplines e.g. environmental, bio-geochemical, pharmaceutical, industrial, nutritional, clinical and nuclear for precise and accurate elemental investigation of trace metals. Reliable data is required for understanding the mechanism of the metal uptake, its fate, pathways through the different environment components, the toxicity or advantages of certain metals i.e. As in food samples. Several measurement procedures have been suggested over the years but the quantitative analysis requires techniques that are even more sophisticated when concentrations are at ppq-ppt level.

Inductively coupled plasma mass spectrometry (ICP–MS) is the current state of the art technique for the trace elemental analysis but the separation of analyte ions from spectral interferences are its main limitations. The matrix elements, argon gas, acids and water can introduce new polyatomic interferences in spectra presenting background concentrations of the analyte of interest. Mathematical corrections, special sample introduction systems such as collision/dynamic reaction cells are recently used to neutralize the interferences in quadrupole mass spectrometers. High mass resolution with a sector-field mass spectrometer, however, is more reliable for this task as it simply distinguishes the analyte from the interferences by difference in mass.

The Department of Plant Biophysics and Biochemistry at IPMB, BCAS has recently purchased, to be installed end of August 2017, the sector-field ICP-MS "Element XR" from Thermo Fisher Scientific Germany. With a new detection system, which is a combination of a single Faraday collector with the SEM, the linear dynamic range of the Element2-XR is increased to 12 orders of magnitude ($10^{12}$). The maximum measurable concentration achievable with the Element2-XR is over 1000 μg/g (ppm) with Counting, Analog and Faraday detector modes. Thus, simultaneous analysis in mg/l to sub pg/l concentration range and ppq range is possible with desolvating injection, allowing for analysis of ultra-trace and matrix elements. This instrument is essential for the several tasks in the department, a focus of which is analysis of metal binding to biological ligands, in particular proteins ("metalloproteomics"). For this purpose, the ICP-MS will be coupled online with a metal-free HPLC system. It will be capable of providing interference free measurement for the range of analytes across the periodic table for ultra-trace, with high mass resolution and high precision isotope ratios analysis.

The instrument will support many running projects at the institute, but it will furthermore be available to support the scientific community, environmental agencies, NGO's and industry for provision of trace elemental analysis.

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## Cryogenic X-ray fluorescence tomography using the Maia detector and a cryo-stream at beamline P06

The Microprobe at the PETRA III beamline P06 is a versatile experiment for scanning X-ray microscopy with X-ray fluorescence, X-ray absorption spectroscopy and X-ray diffraction contrasts. A KB system focusses a beam of $10^{11}$ photons/s down to 300 nm focus size in the energy range 5 - 21 keV. Advanced detector technology, namely the Maia X-ray fluorescence detector 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 biological applications including frozen hydrated tissue samples measured in a cryo-stream. The capability of the P06 Microprobe for cryogenic X-ray fluorescence tomography of tissue samples with sub-cellular resolution are demonstrated in a study on the Arsenic toxicity in the aquatic plant Ceratophyllum demersum.

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## Zn distribution and regulation of Cadmium/Zinc pumping ATPases during Turnip yellow mosaic virus (TYMV) infection in the hyperaccumulator *Noccaea caerulescens*

We investigated the regulation of Zn transport protein expression and related physiology during Turnip yellow mosaic (TYMV) infection in the hyperaccumulator model plant *Noccaea caerulescens* under Zn deplete ("0" $Zn^{2+}$) and Zn replete i.e. control (10 µM $Zn^{2+}$) condition. The mRNA levels of key Cd/Zn transporting proteins (ATPases HMA3, HMA4, ZIP transporter ZNT5) at cellular (QISH) and tissue level (QPCR) in response to Tymv infection were analyzed in shoots.

Our results using QPCR indicate that under Zn-deficient ("0" $Zn^{2+}$) and Zn replete (10 µM $Zn^{2+}$) conditions the expression of the Cd/Zn transporting ATPase NcHMA3 and NcHMA4 was up-regulated in response to virus attack. Virus infection furthermore caused higher Zn accumulation in the shoots of *N. caerulescens*. Our QISH analyses with Zn-deficient ("0" $Zn^{2+}$) condition revealed the virus-induced cellular expression (mRNA accumulation) pattern of ZNT5 in epidermal metal storage cells suggesting an active defense mechanism in shoots. The kinetic representation (mean value) of the OJIP stages showed regular pattern with the control (-virus) condition while significantly changed fluorescence curve with the infected (+virus) leaves, indicating heterogeneity in relative chlorophyll fluorescence as a result of impaired function of virus infected plant photosystem. These alterations were different at deficient vs. replete zinc nutrition. We expect that the results of this project will provide fundamentally new insights into host-pathogen- metal interactions and the evolution of the hyperaccumulator phenotype.

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**X-ray fluorescence elemental imaging, and micro analysis of plant tissue samples**

The resolution of our new laboratory based Micro-X-ray fluorescence (um@KeV) is sufficient to make it a powerful tool in quantitatively visualizing changes in elemental distribution on the microscale. Changes caused by plant development effects of by loss or gain-of-function mutations in the plant genome. Imaging elements that are highly mobile like K and Rb redistribution, due to preparation and environmental changes need always be considered. We have shown previously using TXRF that micro-analysis (7.1 mm2, 300 µg) presents a powerful tool to understand complex physiological processes in context such as the link between the plant ion homeostasis and photosynthetic efficiency. With the micro-XRF instrument we will be able to probe even smaller areas. Recently, we finished the design and assembly of our first laboratory-based X-ray microscope at WSU. Here, we will present our first results obtained on several plant samples from the model plant *Arabidopsis thaliana*. Our preliminary data provide insights regarding the spatial ion distribution in plants and provide high data resolution which represents a fundamental requirement for all the fine-tuned biochemical processes occurring in different plant tissues simultaneously.

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## Genetic transformation of Lycopersicon esculentum using an abiotic stress tolerance gene

In my Master's thesis, abiotic stress tolerant gene *JUB1* isolated from *Arabidopsis thaliana* was used. *Lycopersicon esculentum* Mill. (Tomato) was used as an agricultural crop to express the gene for revealing its response among dicots through genetic engineering. Plants of tomato were regenerated on shoot regeneration medium containing different concentrations of plant growth hormones to check the organogenesis which was further subjected to transformation by *Agrobacterium tumefaciens*. Moreover, optimization of plant selectable marker gene i.e. hygromycin B was done for selection of explants. It was observed that SRM3 (Zeatin 1mg/l + IAA 0.5mg/l) medium is best for direct organogenesis from nodal explants. Lethal dose of hygromycin B for the control explants were 3mg/L that was proved to be optimum for selection of transgenic plants. The six transgenic lines generated were confirmed using PCR and a fragment of 93bp was amplified from transgenic resistant varieties.

*E.coli* and *Agrobacterium tumefaciens* transformation was conducted by heat shock method involving $Ca^{2+}$ competent cells and transmission was confirmed by PCR. Morphological characteristics of both transgenic lines and non-transformed control were also observed, which showed true leaves in control while transgenic lines showed expanded leaves that are intense green in colour. *JUB1* gene is involved in cold stress so plants were subjected to it. Transgenics shows more tolerance than wild type. This study indicates that abiotic stress resistant varieties of tomato can be produced through genetic transformations that are more resistant to abiotic stresses as compared to the un-transformed plants.

Within the KOROLID project, my research as a PhD student will be based on providing solid evidence for or against the use of Rare Earth Elements mainly (La and Nd) as fertilizers in plants (*Glycine max* etc) and green algae (*Desmodesmus quadricauda*). This research will be done by biochemical and biophysical analysis along with the subcellular localization, metal-binding proteins and as well as verification of physiological roles of REEs complexes.

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## The IC - ICP-QQQ - a useful tool for the description of biogeochemical processes

The aim of the newly developed IC - ICP-QQQ analytical pipelines is to provide a sufficiently accurate tool for the monitoring of small changes in organic and inorganic composition of studied systems and, consequently, in the different biogeochemical processes taking place. We utilise standard Ion Chromatography (IC) techniques together with the Inductively Coupled Plasma Mass Spectrometry (ICP-QQQ). The resulting chromatographic pipeline allows wide range of possibilities in analyte separation and the ICP-QQQ system serves as a universal, but highly selective detector with a high dynamic range and with the ability to determine very low concentrations of metals and hard-to-analyze biotic elements, such as sulphur and phosphorus.
3 examples IC-ICP-QQQ biogeochemical applications will be presented:
1) determination of Mg, Co, Cu, Zn, As, and Cd in plant tissue extracts using size exclusion chromatrography, with NH4CO3 as the mobile phase.
2) determination of P, Fe, and Al in sediment pore-water and detritus leachates using size exclusion chromatography, with water as mobile phase.
3) determination of ferric and ferrous iron in acid-preserved pore water using separation on the ion-exchange column.

Ansgar Gruber

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**Intracellular reallocation of metabolic pathways in cells with complex plastids**

Plastids of diatoms and related algae evolved through the uptake of a eukaryotic alga into a eukaryotic host cell. This process involved a number of gains and losses of intracellular compartments and metabolic capacities, for instance the gain of photosynthesis to the metabolism of the host cell, or the loss of most cytosolic processes of the endosymbiont cell. While for these metabolic pathways gains and losses are closely connected to the structure and function of the organelles, genomic and experimental data provides evidence that also other reallocations of metabolic pathways took place during the evolution of complex plastids. Examples are the presence of glycolytic enzymes in mitochondria or the absence of the oxidative pentose phosphate pathway from the plastids in diatoms. To complete the picture of the intracellular distribution of metabolic pathways in cells with complex plastids, combinations of existing protein targeting prediction tools, which have been evaluated for the use with the respective organisms, as well as newly developed prediction tools were applied to whole genomes and transcriptomes of diatoms and related organisms with complex plastids of the red lineage, with these customized approaches highly accurate predictions are possible.

Jitka Viktorova, Katka Rehorova, Jachym Suman, Tomas Macek

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# Plants for bioremediation need increased resistance to stress and expression of specific transgenes

The presentation summarizes our efforts to the preparation of transgenic plants with improved properties for biological remediation. A range of tested transgenes included yeast metallothionein in fusion with additional metal binding domain, which led to increased metal accumulation and better translocation from root to shoot, bacterial genes for cleaving polychlorinated biphenyls (PCB), or fungal metal transporters. Large part of our group is studying the fate of organic xenobiotics in plants, and their toxicity changes, as well as performing metagenomic studies of microbial populations in rhizosphere at contaminated sites and changes of their composition following the effect of plant secondary metabolites.

Here we present preparation of plants resistant to osmotic stress or both organic and inorganic pollutants. Transgenic tobacco (*Nicotiana tabacum*) containing bacterial *bphC* gene (coding for dihydroxybiphenyl-1,2-dioxygenase, cleaving the biphenyl ring of PCB), and double transgenic tobacco containing bacterial the *bphC* gene and yeast gene *CUP* (coding for metallothionein). Transgenic tobacco containing plant *OSM* gene (coding for antimicrobial protein osmotin providing the resistance to abiotic stress as well) we prepared together with transgenic flax (*Linum usitatissimum* L.) and potato (*Solanum tuberosum* L.) plants transformed by the *OSM* transgene.

**Editorial**

Organiser of the minisymposium:
Prof. Hendrik Küpper,
principal investigator of the KOROLID project

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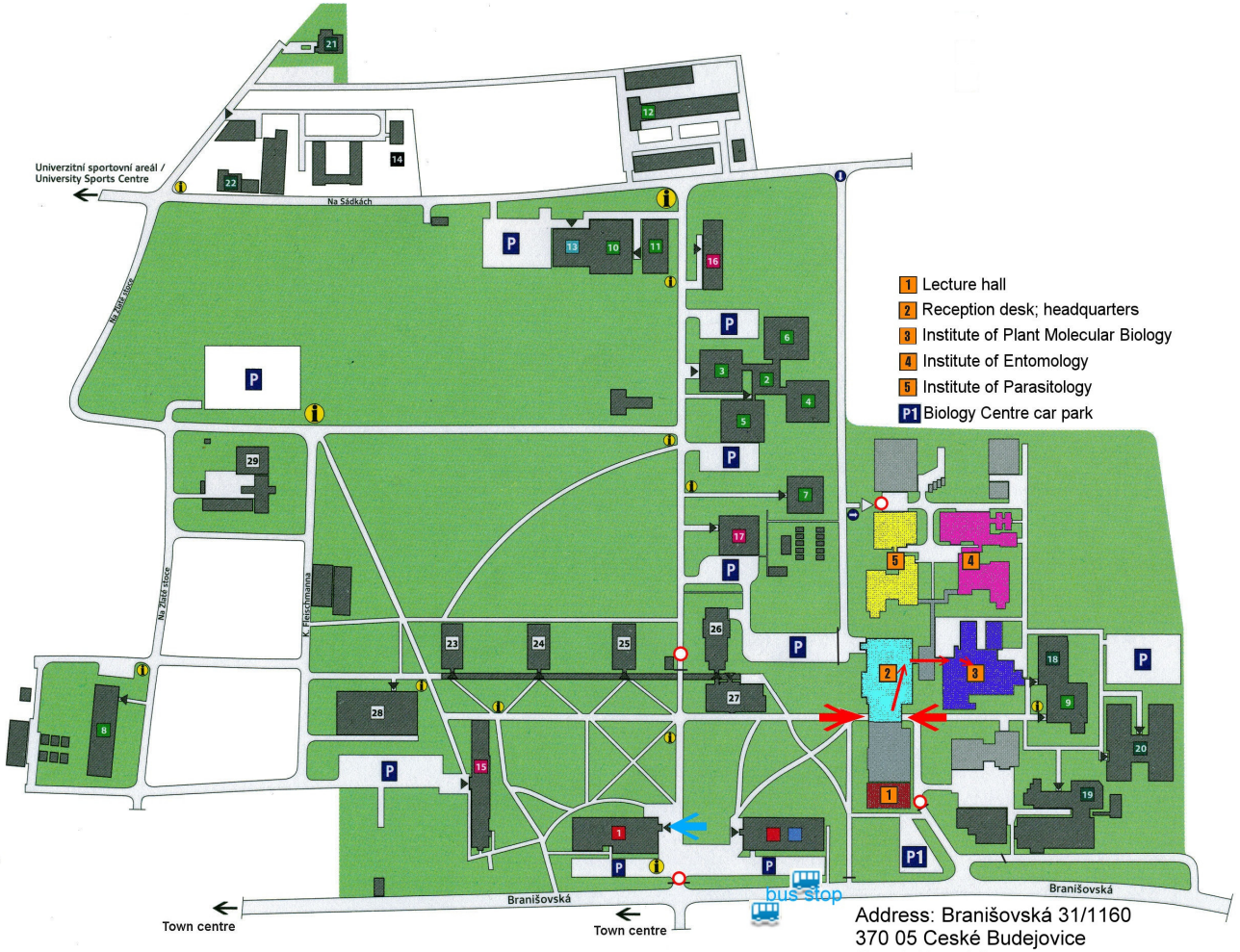
BIOLOGY CENTRE ASCR

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

Main entrance → way to KOROLID minisymposium lecture hall and poster area on 18+19 Aug

Registration and get together party on Thu 17 Aug




Address: Branišovská 31/1160
370 05 České Budejovice
Czech Republic

- **Rektorát / Rector's Office**
- 1 Akademická knihovna / Academic Library
- **Filozofická fakulta / Faculty of Philosophy**
- **Zemědělská fakulta / Faculty of Agriculture**
- 2 ZF – budova A, studijní oddělení / Building A, Student Affairs Office
- 3 ZF – budova B, katedry / Building B, Departments
- 4 ZF – budova C, centrum agroekologie / Building C, Centre for Agroecology
- 5 ZF – budova D, centrum zpracování produktů / Building D, Centre for Agricultural Product Processing
- 6 ZF – budova E, centrum hodnocení kvality / Building E, Quality Control Centre
- 7 ZF – budova M, batcentrum, učebny / Building M, BAT Centre, Classrooms
- 8 ZF – budova K 200, učebny / Building K 200, Classrooms
- 9 ZF – biologické centrum, učebny / Biology Centre, Classrooms
- 10 ZF – učebny, laboratoře / Classrooms, Laboratories
- 11 ZF – budova údržby / Service Building
- 12 ZF – sklady / Stock Rooms
- **Fakulta rybářství a ochrany vod / Faculty of Fisheries and Protection of Waters**
- 13 FROV – ústav akvakultury, ředitelství, učebny / Department of Aquaculture, Directorate, Classrooms
- 14 Školní zemědělský podnik / Agricultural School Farm
- Biologické centrum AV ČR / Biology Centre AS CR
- **Ekonomická fakulta / Faculty of Economics**
- 15 EF – budova A / Building A
- 16 EF – budova C / Building C
- 17 EF – budova F / Building F
- **Přírodovědecká fakulta / Faculty of Science**
- 18 PřF – budova A / Building A
- 19 PřF – budova B – Blažkův pavilon / Building B – Blazek Building
- 20 PřF – budova C / Building C
- 21 PřF – botanická vila / Department of Botany
- 22 PřF – centrum polární ekologie / Centre for Polar Ecology
- 23 Kolej K1 / Dormitory K1
- 24 Kolej K2 / Dormitory K2
- 25 Kolej K3 / Dormitory K3
- 26 Kolej K4 / Dormitory K4
- 27 Aula, hostel / Auditorium, Hostel
- 28 Menza / University Canteen
- 29 Vědeckotechnický park / Science and Technology Park

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