COST Action 859

Uptake, sequestration and detoxification – An integrated approach

ABSTRACT BOOK

WG1 & WG2 WORKSHOP AND MC MEETING

April 16-17, 2009
Szeged, Hungary

Local organizer and venue of the MC meeting and Workshop

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Dr. Ferenc Horváth, Lecturer
Ernő Homolya, System Administrator
Ms. Erika Pál, Secretary

SCIENTIFIC PROGRAM

Thursday 16th April 2009 morning

8.30 – 9.00	**Registration**
9.00 – 9.20	**Opening, welcome and practical information** Local organizers and Action chair

9.20 - 12.30
SESSION 1: UPTAKE, TRANSFER AND SEQUESTRATION OF METALS
Chairs: Charlotte Poschenrieder (ES), Stefan Shilev (BG)
Rapporteur: Flavia Navari-Izzo (IT)

9.20 – 9.40	**Borbala Biro (HU)** Soil-characteristics as key factors in metal translocation and rhizosphere properties of sewage-sludge treated green-pea
09.40 – 10.00	**Paula Pongrac (SI)** Quantitative analyses of trace elements in environmental samples: options and (im)possibilities
10.00 – 10.20	**Stanislaw Gawronski (PL)** Some insights into physiological and molecular basis of Arabidopsis thaliana L. plants response to platinum
10.20 – 10.30	Discussion
10.30 – 11.00	**Coffee Break and Poster Session**
11.00 – 11.20	**Erika Nehnevajova (DE)** Increased tolerance of sunflower mutants to cadmium and excess zinc on hydroponics and metal-contaminated soil
11.20 – 11.40	**Pierre Saumitou-Laprade (FR)** Genetic and microfluorescence analysis of Zn and Cd hyperaccumulation in Arabidopsis halleri
11.40 – 12.00	**Hélène Molins (FR)** Analysis of the mechanisms of Cadmium tolerance in plants using Cadmium sensitive Arabidopsis thaliana mutants
12.00 – 12.20	**Xiaoe Yang (CN)** Stem and leaf sequestration of zinc at the cellular level in the hyperaccumulator Sedum alfredii
12.20 – 12.30	Discussion
12.30 – 14.10	**Lunch**

Thursday 16th April 2009 afternoon

14.10 – 18.20
SESSION 2: MOLECULAR ASPECTS OF DETOXIFICATION OF POLLUTANTS
Chairs: Antonella Furini (IT), Stanislav Smrcek (CZ)
Rapporteur: Jean-Paul Schwitzguébel (CH)

14.10 – 14.30	**Tomas Macek (CZ)** Accumulation and transformation of chlorinated xenobiotics in plants
14.30 – 14.50	**Valérie Page (CH)** The role of cytochromes P450 and peroxidases in the detoxification of sulphonated anthraquinones in plants
14.50 – 15.10	**Christian Huber (DE)** Influence of diclofenac on plant anti oxidative stress defense systems
15.10 – 15.30	**Melissa Brazier-Hicks (UK)** Control of plant detoxification systems by herbicide safeners: the role of metabolic activation
15.30 – 15.50	**Ann Cuypers (BE)** Gene expression profiles of antioxidative enzymes and related miRNAs as a tool to identify metal-specific effects in Arabidopsis thaliana
15.50 – 16.00	Discussion
16.00 – 16.30	**Coffee Break and Poster Session**
16.30 – 16.50	**Maura Cardarelli (IT)** Comparative analysis of cadmium tolerance between tobacco and Arabidopsis plants in relation to the levels of phytochelatins
16.50 – 17.10	**Sylwia Wojas (PL)** Detoxification of cadmium and arsenic in tobacco plants overexpressing phytochelatin synthase and AtMRP7
17.10 – 17.30	**Juan Pedro Navarro-Avino (ES)** Overexpression of TaPCS1 in Populus tremula x tremuloides enhances tolerance and accumulation for Pb
17.30 – 17.50	**Zaigham Shahzad (FR)** Genetic characterisation of the MTP1 zinc transporter family in Arabidopsis halleri reveals a large functional and genetic diversity
17.50 – 18.10	**Damien Sudre (CH)** Characterisation of AtABCB29 (ATH12), a new member of ABC transporter proteins family involved in metals transport across chloroplast membrane
18.10 – 18.20	Discussion

Friday 17th April 2009 morning

9.00 – 12.30
SESSION 3: INTEGRATED APPROACH TO PHYTOTECHNOLOGIES
Chairs: Agnieszka Sirko (PL), Jean-Paul Schwitzguébel (CH)
Rapporteur: Hassan Azaizeh (IL)

9.00 – 9.40	**Keynote Lecture** **Laszlo Erdei (HU)** From the laboratory till practice: heavy metals *en route*
9.40 – 10.00	**Giovanna Visioli (IT)** Significance of Ni supply for growth in the Ni-hyperaccumulator *Thlaspi caerulescens*
10.00 – 10.20	**Avi Golan (IL)** Halophilic interplay between *Pistacia* spp. and *Salsola inermis*
10.20 – 10.30	Discussion
10.30 – 11.00	**Coffee break and Poster Session**
11.00 – 11.20	**Rachel Olette (FR)** Enzymatic basis for the removal of the fungicide, dimethomorph, from water bodies by aquatic macrophytes (STSM-859-03760).
11.20 – 11.40	**Bernadett Bartha (DE)** Comparison of detoxification responses of acetaminophen in Indian mustard (Brassica juncea L. Czern.) and Broadleaf cattail (Typha latifolia L.)
11.40 – 12.00	**Martina Novakova (CZ)** Introduction of bacterial genes for dioxygenases into plant genome to improve phytoremediation of aromatic xenobiotics
12.00 – 12.20	**Martina Mackova (CZ)** Potential of plants and plant compounds to stimulate PCB degradation activities of rhizosphere bacteria
12.20 – 12.30	Discussion
12.30 – 14.10	**Lunch**

Friday 17th April 2009 afternoon

Field trip

Field trip - Visit to the Bugac National Park on Friday afternoon

Details

14.00 Departure (after lunch). Travel by bus (about 80 km).
15.00 Arrival to Bugac Guest House
15.15 Visit Bugac ecosystems
17.30 Introduction of the original sustainable agricultural system
18.15 Dinner
22.00 Return to Szeged

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LECTURES

WG1 & WG2 WORKSHOP AND MC MEETING

April 16-17, 2009
Szeged, Hungary

Soil-characteristics as key factors in metal translocation and rhizosphere properties of sewage-sludge treated green-pea

Borbála Biró[1], Anna Füzy[1], Marianna Makádi[2], Balázs Morvai[3], Imre Kádár[1]

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Sewage sludge depositions or injections in the arable fields are increasing agricultural practices. After the long-term use adverse effects are known, however due to the accumulating heavy metals. Soils with their versatility might have a great impact on the element allocation in the food chain. The most representative four Hungarian soil-types was studied for the sludge application and the element accumulation potential in a pot experiment with green pea (*Pisum sativum* L), by involving the assessment of micro(rhizo)biological colonizations on a long-term basis.

The effect of various sewage sludge doses (0, 2.5, 5.0, 10.0 and 20.0 g/dry soil, i.e. the 7.5, 15, 30 and 60 $t.ha^{-1}$ rates) of municipal and industrial origin (enhanced Zn-and Cr-content, 28450- and 5225 $mg.kg^{-1}$ dry sludge, respectively) was investigated on some Hungarian representative soil-types in a four-years periods. Soils were selected according to their texture and pH-levels, so both sandy or loamy and acidic or neutral types were involved, such as the calcareous sand- and chernozem (CS and CC) or the acidic sand- and forest soils (AS and AF) in Hungary. Parameters, such as the dry-matter production of green-pea (var. "Rajnai törpe") and the element accumulations and some of the microbial (rhizosphere) colonizations (*Rhizobium* nitrogen-fixers and arbuscular mycorrhizal fungi and some of the food-safety important microbes were assessed annually.

Increasing doses of sewage sludge could produce a fertilizer effect more particularly on the sandy types of soils (CS, AS) and at the lowest doses when it was studied on a long-term level. Acidic soils responded on the most sensitive way for the sludge doses and for the heavy metal translocation towards the shoot biomass of the pea. Translocation was enhanced more particularly from the less compacted soils, but it was also realized, that the seed-yields of the pea was particularly protected from the heavy metal accumulations. The high Cr-content and the greatest applied rates of sludge have resulted adverse effects on the green-yield of pea after the 2nd years of applications. Such adverse effect of the accumulating Zn-content could be developed in the 4th years, only. Colonization of microsymbionts proved to be highly retarded both by the enhanced nutrient-availability and also by the increasing heavy metal accumulation in the later periods of the project.

The permanent soil-physical, -chemical and biological monitoring is highly suggested from the study in case of the permanent sewage sludge application. The acidic-type sandy soils with low OM content needs a particular attention.

Supports of the Hungarian research funds (OTKA T046610, EN 064310), the EU-FP7 Soil-CAM project, the bilateral (CSIC-HAS, RUS-HAS) agreements and the COST Action 8.59 are highly acknowledged.

Keywords: soil-types, heavy metals, sewage sludge, element uptake, rhizosphere

Quantitative analyses of trace elements in environmental samples: options and (im)possibilities

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The main aim of contemporary ecotoxicological studies is to determine the bioavailability, toxicity and risk relationships of trace-element contaminants in ecosystems. Discussions thus focus primarily on the element concentrations in soils, their dynamics, and the impact they have on microorganisms, plants, animals and the ecosystem as a whole. However, before the basic ecotoxicological principles can be discussed, the appropriate analytical methods for particular question(s) need to be chosen among the broad array of physico-chemical analytical methods that are available. Together with the sampling strategy and preparation, these are key issues that affect the final outcome. Here we present micro-proton-induced X-ray emission (micro-PIXE) that is able to provide simultaneously information on spatial distribution and concentration of all elements encompassing the list of macro- and micro-nutrients, as well as elements considered as toxic pollutants. Maps of element distribution can provide valuable information that it is not possible to obtain using point analyses or linear scans. The localization and quantification data on toxic elements acquired with this technique can indicate possible pathways of detoxification mechanisms, and can therefore improve our understanding of plant adaptations to various environmental conditions. However, tracking element distribution can only be achieved through the appropriate preparation of specimens, making this the most important step in the analysis (Mesjasz-Przybyłowicz and Przybyłowicz 2002; Schneider et al. 2002; Vogel-Mikuš et al. 2008a, b). In the talk preparation of samples along with several case studies will be presented.

References:
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Schneider et al. (2002) A method for cryosectioning of plant roots for proton microprobe analysis. Int J PIXE 12, 101-107.
Vogel-Mikuš et al. (2008a) Spatial distribution of Cd in leaves of metal hyperaccumulating *Thlaspi praecox* using micro-PIXE. New Phytol 179, 712–721
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Some insights into physiological and molecular basis of *Arabidopsis thaliana* L. plants response to platinum

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Modern cars equipped with catalyzer emit platinum into environment. Helmers (1997) estimated that between 0.5-0.8 μg of Pt can be emitted by one car per 1km of the road, most of which is found in a distance of 1 to 3m from the road edge. Although platinum is a noble metal it can be a source of pollution if in high concentration and its oxides are recognized as allergenic or carcinogenic. The goal of this work was to: (i) evaluate of platinum uptake by *A. thaliana* plants and its distribution between roots and rosette (ii) check whether platinum affects selected physiological processes, and (iii) monitor profile gene expression in plants as influenced by Pt ions in growing medium.

Plants were grown in continuously aerated hydroponic culture with Hoagland's nutrients solution weekly changed. After six weeks of plant growing in Pt free medium they were - exposed for14 days to $Pt(NH_3)_4(NO_3)_2$ in concentrations: 5, 50, 500, 1000, 5000, 10 000 and 20 000 μg dm^{-3}, added during nutrients change. Data on plant gas exchange, chlorophyll content, chlorophyll *a* fluorescence, membrane integrity were collected weekly. At harvest sub-samples for relative water content, platinum accumulation and distribution were collected and fresh and dry weights were recorded. For the profile gene expression samples from plants exposed to 500 and 10000 μg dm^3 exposed for 24 hr were collected and micro-array technology was applied using cDNA microarray (*Arabidpopsis thaliana* Genome Oligo Set, Version 3.0) according to manufacture.

Amount of Pt in plants increased along with its concentration in medium and up to 14 % of total Pt taken up by plants was translocated to rosette. At lower concentrations of Pt, either no effects or even slight stimulation of some processes was recorded while at the higher concentrations negative impact such as lowered biomass accumulation, chlorophyll content, photosynthesis, transpiration, relative water content or membrane integrity was noted. Presence of Pt ions changed profile gene expression and both induction and suppression as well as up- and down regulation of the level of expression were recorded. Higher number of genes differentially expressed was noted at the higher Pt concentration.

References: Helmers E. (1997): Pt emission rate of automobiles with catalytic converters. ESPR- Environ. Sci. Poll. Res. 4(2), 100-103

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Increased tolerance of sunflower mutants to cadmium and excess zinc on hydroponics and metal contaminated soils

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Key words: sunflower mutants, metal tolerance, metal accumulation, antioxidative enzymes

Deficit or excess of plant nutrients and exposure to pollutants inhibit the growth and development of plants. Different methodologies have been proposed to improve plant yields and crop quality under stressful conditions. Plants with enhanced tolerance to trace elements, such as cadmium and zinc, can be obtained by chemical mutagenesis, *in vitro* breeding or genetic engineering (Nehnevajova et al. 2007a, 2007b). The effects of Cd and Zn on plant growth, chlorophyll content, production of carotenoids, activities of antioxidative enzymes and metal accumulation in sunflower seedlings were investigated in the 5th generation of sunflower mutant lines with improved metal extraction traits.

Eight sunflower mutant lines and control inbred line were grown on hydroponics with or without Cd and Zn (0.6 mg/l Cd and 3.3 mg/l Zn; pH 6.0) for 2 weeks. In addition, metal tolerance and accumulation capacity in young and adult sunflowers were investigated in two mutant lines on soil contaminated mainly with Cd and Zn (10 mg/kg Cd and 1110 mg/kg Zn, pH 6.3) in the greenhouse. Sewage sludge contaminated soils were obtained from Biotop Schladitz (DE), kindly provided by Dr A. Gerth (Bioplanta GmbH, Leipzig, DE). Sunflower seedlings tested on hydroponics and in pots in greenhouse were separatelly harvested into roots and shoots. Pigment concentrations and activity of antioxidative enzymes were measured by spectrophotometry. Cd and Zn concentration in plant tissues were determined by flame-AAS.

Root elongation test indicated an increased tolerance of some mutants. Sunflower mutant lines with improved growth on the control medium and on the medium with Cd and Zn also showed a higher tolerance index than sunflower inbred line. In four sunflower mutant lines a minor effect of cadmium on chlorophyll content was observed. Sunflower mutant lines grown on medium with Cd and Zn showed a similar or an increased carotenoid concentration than on control medium. An enhanced carotenoid content after metal exposure revealed a possible protective mechanism of this mutant line against oxidative stress caused by Cd and excess Zn concentration.

M_5 sunflower mutants showed improved growth on a metal contaminated soil, as compared to control inbred lines. They showed 2.5 times higher Cd and Zn extraction by leaves and 1.8 times higher Cd and 2 times higher Zn extraction by roots, as compared to inbred line. The root enhancement can lead to improved access to water, minerals as well as toxic metals therefore plants with well developed roots and high shoot biomass can be useful for phytoremediation.

References:

Nehnevajova E, Herzig R, Federer G, Erismann KH, Schwitzguébel JP (2007a) Int J Phytorem 9: 149-165.
Nehnevajova E, Herzig R, Erismann, K.H., and Schwitzguébel, J.P. (2007b) Plant Cell Rep 26: 429-437.

Genetic and microfluorescence analysis of Zn and Cd hyperaccumulation in *Arabidopsis halleri*

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A. halleri is a pseudo-metallophyte species in which zinc (Zn) and cadmium (Cd) tolerance seem to be constitutive traits. Moreover, *A. halleri* shows the ability to concentrate these Zn at very high levels in the aerial parts and also Cd in some populations. The close phylogenetic relationship of *A. halleri* with the biological model system *A. thaliana* contributed to the emergence of *A. halleri* as one of the model systems for the study of heavy metal tolerance and hyperaccumulation.

The genetic analysis of Zn tolerance and accumulation in *A. halleri* was initiated by the development of interspecific crosses between *A. halleri* and *A. lyrata* (*1*) through which the constitutive nature of Zn tolerance in *A. halleri* could be bypassed. Based on the segregation of Zn tolerance and accumulation in F2 progenies, it was concluded that both traits were dominant and genetically independent. Recently, an *A. halleri* × *A. lyrata* first-generation backcross progeny was used to elucidate the genetic architecture of Zn and Cd tolerance in *A. halleri*. Three Quantitative Trait Loci (QTL) of comparable additive effect were identified to govern Zn tolerance in *A. halleri* (*2*) and three QTL with a major one to govern Cd tolerance (*3*). Interestingly an independent Zn accumulation analysis performed on the same BC1 (in which all individuals exhibited very low accumulation capacities), showed a partially recessive component of the trait and detected a QTL that co-localizes with Zn and Cd tolerance (*4*). Recently, a comparative genomic analysis with *A thaliana* demonstrated that HMA_4 was the single candidate gene included in the *A. halleri* QTL region co-localizing with Zn and Cd tolerance, and Zn accumulation (*5*). A functional analysis performed on this gene (*6*) showed that it contributed significantly to Zn tolerance and hyperaccumulation and Cd tolerance in *A. halleri* but it is not sufficient to perform to completion Zn hyperaccumulation.

Based on an F2 generation produced by interspecific crosses we showed a wide transgressive segregation for Zn accumulation. Four independent phenotyping have been performed to detect QTL: two Zn and two Cd concentrations have been applied to the F2 progenies. Three QTLs could be identified in the analysis of Zn accumulation while only one was detected for Cd accumulation. The QTL for Cd accumulation co-localizes with one Zn accumulation and HMA_4 gene confirming the role of the protein in Zn and Cd accumulation. Interestingly, the additional QTLs detected for Zn accumulation explain the major part of genetic variance of the trait when plants are cultivated in weakly contaminated growth substrate. In addition to this genetic approach, a microfluorescence localization of Zn

was performed on a subsample of the F2. The vein/tissue fluorescence ratio was negatively correlated with Zn accumulation supporting the importance of Zn unloading in the shoot.

Literature cited

1. M. R. Macnair, *et al*. *Proceedings of the Royal Society, Biological Series* **266**, 2175 (1999).
2. G. Willems *et al.*, *Genetics* **176**, 659 (2007).
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4. N. Roosens, G. Willems, C. Godé, A. Courseaux, P. Saumitou-Laprade, *Plant and Soil* **306**, 105 (2008).
5. N. Roosens, G. Willems, P. Saumitou-Laprade, *Trends in Plant Science* **13**, 208 (2008).
6. M. Hanikenne *et al.*, *Nature* **453**, 391 (2008/05/15/print, 2008).

Analysis of the mechanisms of Cadmium tolerance in plants using Cadmium sensitive *Arabidopsis thaliana* mutants

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The mechanisms of cadmium toxicity in plants are not yet fully understood but it is well established that a tight control of metal homeostasis at the cellular level is required to limit the stress induced by cadmium (Cd). Metal homeostasis is maintained by different ways including sequestration of metals into the vacuole and their efflux out of the cell. These processes involve metal transporters.

The NRAMP gene family encodes hydrophobic membrane proteins that act as metal transporters. *Arabidopsis thaliana* has six *NRAMP* genes and among them *AtNRAMP3* and *AtNRAMP4* genes encode transporters that can export iron, manganese or zinc from the vacuole to the cytosol. These proteins can also transport cadmium (Oomen et al. New Phytol. 181: 637-50). AtNRAMP3 and AtNRAMP4 protein levels are increased after Cd stress. Whereas *nramp3* and *nramp4* single knockout display a moderate increase in Cd tolerance, the double mutant *nramp3nramp4* is strongly hypersensitive to Cd (Oomen et al. New Phytol. 181: 637-50). We established that *nramp3nramp4* double mutants display a level of Cd hypersensitivity comparable to that of the mutant *cad1-3* which is known in the literature as the most cadmium sensitive mutant (Cobbett et al. Plant Cell 11: 1153-64).

We have investigated the basis for *nramp3nramp4* Cd hypersensitivity and compared it to *cad1-3*. We hypothesized that *nramp3nramp4* cadmium hypersensitivity is due to decreased tolerance to oxidative stress generated by this heavy metal. In agreement, *nramp3nramp4* displays a growth defect on H_2O_2 and shows elevated levels of H_2O_2 production compared to the wild type, even in the absence of Cd stress. In contrast, *cad1-3* mutant accumulates lower levels of H_2O_2 and is not hypersensitive to H_2O_2. We will further analyze the redox status of these two Cd sensitive mutants by measuring the production of $O_2^{\cdot-}$ and the balance between oxidized and reduced metabolites such as glutathione, ascorbate, NAD and NADP. Because *nramp3nramp4* mutant plants display increased sensitivity to H_2O_2 and elevated levels of H_2O_2, we study the activities of reactive oxygen species (ROS) detoxifing enzymes such as superoxide dismutase, ascorbate peroxidase, catalases, and peroxidases in this mutant. We will compare the ROS detoxifying enzyme activities in the *nramp3nramp4* mutant with the wild type and the *cad1-3* mutant.

Finally, we will analyze the Cd sensitivity of the *nramp3nramp4cad1-3* triple mutant. As *cad1-3* and *nramp3nramp4* are impaired in distinct mechanisms of Cd tolerance, this should clarify the interaction between different Cd tolerance mechanisms.

Stem and leaf sequestration of zinc at the cellular level in the hyperaccumulator *Sedum alfredii*

X.E. Yang[1]* S. K. Tian[1], L.L. Lu[1], J. M. Labavitch[2], Y.Y. Huang[3], P. Brown[2], and Z.L. He[1]

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The hyperaccumulating ecotype (HE) of *Sedum alfredii* Hance, reported as a new Zn/Cd co-hyperaccumulating plant native to China, can accumulate 2.9% Zn in shoots without toxicity symptoms, with the highest concentration of the Zn in stems. Research on *S. alfredii* root compartmentation revealed that approx 2.7-fold less Zn was stored in the vacuoles of root cells of the HE than in the nonhyperaccumulating ecotype (NHE). Thus, the Zn accumulated in roots of the HE was more available for xylem loading and subsequent translocation to shoots. By using leaf tissue fractionation, preferential distribution of Zn in the apoplast (cell wall space) of leaves and stems of the *S. alfredii* HE was also reported. However, the detailed cellular and subcellular characteristics of stem and leaf sequestration of Zn in *S. alfredii* are unclear. Thus, the objectives of this study were to investigate the characteristics of Zn distribution in stems and leaves of both HE and NHE *S. alfredii* by using SRXRF microprobe analysis and the Zn-fluorophore, Zinpyr-1 and to use SRXRF microprobe analysis to examine the relationship of Zn cellular distribution patterns in stems and leaves with distribution patterns of other elements in these organs.*Sedum alfredii* is a fast-growing, high-biomass zinc (Zn) hyperaccumulator native to China. Here, the characteristics of *in vivo* Zn distribution in stems and leaves of the hyperaccumulating (HE) and nonhyperaccumulating ecotypes (NHE) of *S. alfredii* were investigated by synchrotron radiation X-ray fluorescence (SRXRF) analysis, together with a Zn probe. • Preferential Zn accumulation in leaf and stem epidermis was observed in both ecotypes, but to a much greater extent for HE. Epidermal Zn increased largely in leaves and stems of HE as exposure time was prolonged, while Zn saturation occurred relatively early in HE leaf mesophyll cells and stem vascular bundles. A second peak of Zn enrichment in stem and leaf vascular systems was shown in both ecotypes. However, the proportion of Zn accumulated in stem vascular bundles relative to other tissues was much greater for HE than for NHE. Leaf and stem distribution patterns of phosphorus (P) and sulphur (S) in the HE were very like that for Zn, while the calcium (Ca) distribution pattern was the reverse of that for Zn. No such relationship was observed in NHE. Our study mainly suggested that epidermal layers serve as important storage sites for accumulated Zn in the *S. alfredii* HE.

Accumulation and transformation of chlorinated xenobiotics in plants

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Some microorganisms and plant species are able to grow on areas with high level of different types of contaminants, some being able to metabolize xenobiotics to less phytotoxic ones or accumulate pollutants. To study transformation of PCBs black nightshade SNC-9O culture was cultivated individually with 12 dichlorinated, 7 trichlorinated, 5 tetra- and one pentachlorinated PCB congeners. Free hydroxylated PCB metabolites were identified based on the mass spectra characteristics after GC separation. Dichlorinated PCBs lead always to at least two metabolites (isomers). Trichlorinated PCBs mostly lead to lower number of metabolites, tetrachlorinated and pentachlorinated PCBs mostly didn't give any metabolites. 3 of 5 detected metabolites of PCB 9 were identified by comparison with available standards. The 2',5'-dichloro-2-biphenylol, 2',5'-dichloro-3-biphenylol and 2',5'-dichloro-4-biphenylol, present as the main metabolite, were found in biomass of SNC-9O culture. Tobacco WSC-38 callus culture was individually cultivated with 6 dichlorinated PCB congeners. Lower number of hydroxy metabolites was found in this culture comparing to the number of metabolites of appropriate PCBs in SNC-9O culture. PCB 9 lead to only 2 metabolites. These were identified as 2',5'-dichloro-3-biphenylol and 2',5'-dichloro-4-biphenylol (main metabolite). Methoxy-PCBs and hydroxy-methoxy-PCBs (1 or 2 isomers per PCB congener) were detected as additional metabolites. Exudation of unbound biphenylols from the cells into cultivation media is not expected neither in SNC-9O culture nor in WSC-38 culture as only traces of metabolites were found in the media. In the experiments performed in real long-term contaminated soil tobacco and black nightshade plants were cultivated for several months. In plants different previously identified products were detected together with original PCBs which accumulated in roots, stalks, leaves and even in berries. Other compounds which can occur together with PCBs are chlorobenzoic acids, products of bacterial PCB degradation. First the transformation of the CBA was tested using plant cells of three species cultivated *in vitro*, cell culture of *Armoracia rusticana* K54 (horseradish), *Nicotiana tabacum* WSC 38 (tobacco) and *Solanum nigrum* SNC 9O (black nightshade). In bacteria degradation efficiency depends on microbial species, number of chlorine atoms and their position. In plants we found the similar phenomenon. The efficiency of CBA transformation differed by tested plant species. Cells of *S.nigrum* showed high degree of 2-, 3-, 4-, 2,3-di, 2,4-di, 2,5-di and 2,3,5-triCBA conversion (almost 100%) in 14 days. Methylester of 2,3-diCBA and methylester of 2,4-diCBA were identified by GC-MS analysis in medium after cultivation of *S. nigrum* with 2,3-diCBA and 2,4-diCBA. In biomass after cultivation of *S. nigrum* with 2,5-diCBA hydroxyderivates of 2,5-diCBA and 2,5-diCBA together with 3-hydroxy-4-methoxy-benzaldehyde and 4-hydroxy-3-methoxybenzylalcohol were identified. 2,3,5-trichloro-benzoate was transformed to 3-hydroxy-4-methoxybenzaldehyde and two dichlorobenzoates.

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The role of cytochromes P450 and peroxidases in the detoxification of sulphonated anthraquinones in plants

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In the development of a phytotreatment for effluents from dye and textile industry contaminated with sulphonated aromatic compounds, some plants producing (rhubarb and red sorrel) or not (maize and celery) natural anthraquinones, were tested for their ability to metabolise sulphonated anthraquinones. The aim of this work was to elucidate the detoxification mechanisms of sulphonated aromatic compounds in plants, to investigate the possible roles of cytochrome P450 monooxygenases and peroxidases in the detoxification of these pollutants and to characterise the response of different plant parts to penetrated sulphonated anthraquinones.

Plants were cultivated in a greenhouse or in growth chamber under hydroponic conditions in the presence or absence of a mixture of five sulphonated anthraquinones (0.2 mM each). Plants were harvested at different times and microsomal and cytosolic fractions were prepared. The activity of cytochrome P450 mono-oxygenases, key detoxification enzymes also involved in other biochemical processes, was measured in different parts of plants exposed or not to sulphonated anthraquinones. For the measurement of cytochromes P450 activity, a new method based on the fluorimetric detection of oxygen consumed during P450-catalysed reaction with NADPH and sulphonated anthraquinones as substrates was used. The peroxidases activity was assayed spectrophotometrically at 470 nm by the guaiacol oxidation rate in the same plant parts exposed or not to the pollutants.

A significant activity of cytochromes P450 was detected in rhubarb leaves, while no (rhizome) or low (petioles and roots) activity was found in other parts of the plants. An induction of this enzyme was observed at the beginning of the exposition to sulphonated anthraquinones. The results also indicated that cytochromes P450 were able to accept as substrate the five sulphonated anthraquinones, with a higher activity toward AQ-2,6-SS and AQ-2-S. An activity of the cytochromes P450 was also found in the leaves of maize, red sorrel and celery, but no induction of the activity occurred after the exposition to the pollutants for red sorrel and celery. The activity of peroxidases increased when rhubarb was cultivated in the presence of the five sulphonated anthraquinones. Peroxidases activity was also detected in the leaves of red sorrel, maize and celery but in these plants no significant difference was found between plants cultivated with and without sulphonated anthraquinones.

Results indicated that the activity of cytochromes P450 and peroxidases increased in rhubarb in the presence of sulphonated anthraquinones and were involved in their detoxification mechanisms. Further investigation should be performed to find the next steps of this detoxification pathway. Besides these promising results for the phytotreatment of sulphonated anthraquinones, it will be of high interest to develop and test at small scale an experimental wastewater treatment system to determine the efficiency of such a treatment.

Influence of diclofenac on plant anti oxidative stress defense systems

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Over the last decade numerous reports on residues of pharmaceuticals in the environment have been published but only recently scientists begun to study them in more detail. Although the reported concentrations of these compounds are in ng-μgL^{-1} range, pharmaceuticals are believed to pose a significant burden on the environment and a potential threat to human health. Till now very limited knowledge exists on long term effects under chronical exposure to these low concentrations, especially on non target organisms. Diclofenac is one of the compounds that can be regularly found in the European water cycle and is detected in low μgL^{-1} levels in influents and effluents of waste water treatment plants and surface waters. It's a non steroidal anti-inflammatory drug that is extensively used in human medication and tends to be relatively persistent in the environment.

Biological wastewater treatment in constructed wetlands is an effective and low cost alternative to conventional wastewater treatment plants. In this situation plants get in contact with pharmaceutical compounds and might contribute to their removal and degradation. However, till now there is literally no information around how plants react, cope with and metabolize the most commonly used pharmaceuticals.

Our study aims at evaluating plant stress responses towards diclofenac as well as the formation of diclofenac related metabolites or degradation products.

First experiments were carried out using a cell culture of *Armoracia rusticana* and *Hordeum vulgare* plants as two model systems. The cell culture was cultivated in MS-Medium under defined growth conditions in the lab whereas barley was grown in a hydroponic system in full strength Hoagland medium under greenhouse conditions. Diclofenac treatments were carried out using concentrations from 10 to 1000µM over a period of up to one week. Enzyme extractions of leaf and root samples were performed to determine specific enzyme activities of Glutathione S-Transferases (GST), Glutathione Reductase (GR) and Peroxidases (POX) as plant stress markers. To answer the question if dilcofenac serves as a potential substrate for plant P450 monoxygenases, a fluorometric oxygen sensor system was used to spectrophotometrically follow the oxygen consumption of P450 catalyzed reactions in a fluorescent photometer at an excitation of 485nm and an emission of 590nm.The content of the tripeptide glutathione that plays an essential role in anti oxidative stress defense reactions was measured in its reduced (GSH) and oxidized form (GSSG) via HPLC using the fluorescent dye monobrombimane for derivatisation. Qualification and quantification of diclofenac and its metabolites was carried out on an HPLC or LC-MS system, respectively.

Diclofenac caused stress reactions in the plants. Thiol contents and homeostasis was significantly altered and GR and POX activities were induced. Diclofenac exerted also strong effects on detoxification enzymes. GST and P450 enzyme activities were modulated under diclofenac exposure, which may influence the detoxification of the compound itself or other xenobiotics. Plant survival and remediation success are critically discussed.

Control of plant detoxification systems by herbicide safeners: the role of metabolic activation

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Herbicide safeners are used commercially to increase crop tolerance to herbicides in cereals by enhancing their ability to detoxify xenobiotics, but their mode of action at the molecular level is not understood. Fenclorim, which 'safens' chloroacetanilide herbicides, induces detoxifying glutathione transferases (GSTs) in rice and shows similar activity in the model plant *Arabidopsis thaliana*. As one experimental strategy to identify how Arabidopsis recognizes safeners, we are studying how metabolism regulates the activity of fenclorim. When fenclorim was fed to Arabidopsis cell suspension cultures it was rapidly metabolized by GST-mediated glutathionylation. The GSTs responsible were identified and shown to be induced by fenclorim exposure. The glutathione conjugates underwent rapid catabolism to the respective cysteine derivative, which could be processed by *N*-malonylation, *N*-acetylation followed by a second round of *S*-glutathionylation or, C-S lyase and *S*-methyltransferase mediated conversion to *S*-methyl-fenclorim. Whereas the glutathione and cysteine derivatives of fenclorim were inactive as safeners, the *S*-methyl derivative induced GSTs in Arabidopsis and safened against herbicide injury in rice. Our results demonstrate a) that fenclorim induces its own detoxification through the selective enhancement of detoxifying GSTs; b) that fenclorim can undergo two rounds of glutathionylation and c) that catabolism of the primary conjugates can result in the reactivation of the safener. In addition to providing novel insight into how plants process xenobiotics, these results also show the potential of how metabolism can play an active role in regulating the biological activity of widely used safener chemistries.

Gene expression profiles of antioxidative enzymes and related miRNAs as a tool to identify metal-specific effects in *A. thaliana*

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Both Cd and Cu are known to induce oxidative stress in organisms exposed to either metal. Obviously, the interplay between production and scavenging of ROS (Reactive Oxygen Species) is balanced, and the role of the cellular redox state in intracellular signalling is considered of major importance. MiRNAs function in post-transcriptional gene regulation by guiding mRNA degradation or translational repression and their expression can be influenced by the redox state of the cell.

Regarding the antioxidative defence system, metal-specific patterns were observed in the gene expression of superoxide-quenching enzymes. In addition, a role of miRNA398 in the metal-induced regulation of copper zinc superoxide dismutases was identified. Underlying regulation mechanisms of the oxidative stress related miRNA398 expression were explored by measuring their altered expression levels in MAPK-related mutants during Cd and Cu stress. Transcription rates were estimated by real-time RT PCR using intron-specific primers that amplify the primary transcript.

Because organisms are almost always exposed in a multipollution context, metal-specific responses for single element exposures were compared with these occurring after exposure to the combination of both metals. This way, interesting metal-specific markers were detected in the gene expression profile of superoxide quenching enzymes, and their related miRNA398 profiles.

Comparative analysis of cadmium tolerance between tobacco and Arabidopsis plants in relation to the levels of phytochelatins

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Phytochelatins (PCs) are metal binding peptides enzymatically synthesized from reduced glutathione (GSH) and the reaction is catalyzed by phytochelatin synthase (PCS). We have previously shown that in tobacco *AtPCS1* overexpression causes an increase in Cd tolerance and accumulation directly related to the availability of GSH (Pomponi et al. 2006). It has also been reported that *AtPCS1* overexpression enhances metal tolerance in *Nicotiana glauca* and *Brassica juncea*. In contrast, PCS1 overexpression seems to confer Cd hypersensitivity to *Arabidopsis thaliana* plants, maybe due to the toxicity of PC supraoptimal levels. PCs form stable complexes with metals that are subsequently sequestered into the vacuoles. In *Saccharomyces cerevisiae,* the ABC protein YCF1 transports Cd-GSH complexes into the vacuoles. MRP3, an arabidopsis ABC transporter highly homologous toYCF1 protein could have a role in mediating the transport of PC-Cd complexes.

Aim of this study was to verify cadmium tolerance and accumulation of *AtPCS1* overexpressing Arabidopsis plants (pcs plants) in relation to their levels of PCs and to the availability of GSH. To this end, we analysed fresh weight of seedlings, primary root length, number of lateral roots and alterations in root anatomy of pcs seddlings grown at different concentrations of cadmium in the presence and in the absence of GSH. Cd accumulation was measured by ICP analysis and by using BTCN-5 AM fluorochrome. We also compared Cd tolerance of Arabidopsis and tobacco pcs plants in relation to their PC levels and to the availability of GSH. In addition, we analysed Cd tolerance of *mrp3* Arabidopsis mutants, defective in the MRP3 transporter. In our hands, Arabidopsis pcs seedlings were not hypersensitive to Cd and exogenous GSH had a limited effect on Cd tolerance of these lines, whereas *mrp3* mutant lines showed cadmium hypersensitivity. In addition Arabidopsis and tobacco overexpressors showed differences in PC content. Taken together these results indicate that Arabidopsis pcs lines are much less tolerant to cadmium toxicity than tobacco *AtPCS1* overexpressing seedlings.

Pomponi M., Censi V., Di Girolamo V., De Paolis A., di Toppi L.S., Aromolo R., Costantino P. and Cardarelli M. PLANTA 223(2):180-90 (2006)

Detoxification of cadmium and arsenic in tobacco plants overexpressing phytochelatin synthase and *AtMRP7*

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Phytochelatins are small heavy metal and metalloid complexing thiol peptides, synthesized by phytochelatin synthase (PCS) from glutathione. In order to engineer plants for phytoremediation, different *PCS* genes were overexpressed in various plant species; however, obtained results were contradictory. Although PCS transformants were more tolerant to arsenic, they exhibited a range of responses to cadmium, from higher sensitivity to higher tolerance.

In present work, we demonstrated that the *PCS* gene used for plant transformation strongly influences the cadmium tolerance of transgenic plants. We used a novel approach- two different *PCS* genes were introduced into one model species – tobacco (*Nicotiana tabacum* var. Xanthi). We found that plants overexpressing the *AtPCS1* gene from *Arabidopsis* were hypersensitive to Cd^{2+}, whereas the tobacco expressing *CePCS* from *C.elegans* was more Cd-tolerant. The differences in Cd-tolerance were accompanied by substantial changes in thiol metabolism. There was only a moderate and temporary increase in phytochelatin levels due to *AtPCS1* and *CePCS* expression. Moreover, we detected in AtPCS1 plants: (i) increased oxidative stress level in the presence of cadmium and (ii) decreased Cd detoxification capacity; which probably partially explains the increase in Cd-sensitivity. The substantially increased PCS activity in AtPCS1 plants probably leads to the imbalance between the formation of PC–Cd and their transport to the vacuole, and may lead to degradation of PC-Cd in the cytosol and to the release of "free" cadmium ions, leading to Cd-hypersensitivity. To test this hypothesis, *AtPCS1* plants were crossed with tobacco expressing AtMRP7 - a member of MRP family of ABC transporters, possibly involved in the transport of heavy-metal complexes, localized in tobacco both the plasma membrane and tonoplast. We demonstrated the increase in Cd-tolerance of AtPCS1/MRP7 plants relative to AtPCS1 transformants.

A previous study by Li *et al.* (2004) on *Arabidopsis* demonstrated contrasting responses to Cd and As due to *AtPCS1* overexpression: Cd-hypersensitivity along with an increased As-tolerance. Therefore, to address the mechanisms underlying this phenomenon, we analyzed the As response of AtPCS1 and CePCS transformants. We showed that it strongly depends on the external As(V) concentration. At the less toxic 50 μM As(V), AtPCS1 and CePCS transformants accumulated more arsenic in roots and leaves than WT and an increase in PC production was detected. In contrast, at 200 μM As(V), we found significant changes in thiol metabolism and the decrease in PC levels of transgenic plants relative to WT. We demonstrated that expression of both genes resulted in an increase of As-tolerance, with CePCS plants most tolerant. These results strongly suggest the existence of metabolic pathways/ regulation mechanisms specifically associated with phytochelatin function in As-tolerance, different from those involved in cadmium detoxification. However, further research is needed to identify the mechanisms underlying the observed differences in response to As and Cd and the role of phytochelatins in this phenomenon.

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Overexpression of *TaPCS1* in *Populus tremula x tremuloides* enhances tolerance and accumulation for Pb.

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The response of *Populus tremula x tremuloides* cv. Etrepole transgenic lines overexpressing the phytochelatin synthase *TaPCS1* for Pb tolerance and accumulation under both hydroponic and Pb/Zn-contaminated soil conditions has been studied. In the hydroponic experiment, no significant differences in Pb concentrations were found between the 10 transgenic lines assayed and the wild type (WT) plants at any of the Pb solutions tested. However, total biomass, tolerance indexes and Pb accumulation were significantly greater in 3 (PTa3, PTa5, PTa10) transgenic lines with respect to the control (WT) line when the plants were grown on 0,75 and 1,5 mM Pb solutions. The PTa3 and PTa5 lines have accumulated around 1400 µg of Pb against 813 µg of the WT plants at the end of the experiment (18 weeks). No differences in any of the parameters recorded were found when the aspen plants were subjected to 0,38 mM Pb, and the concentration of 3,0 mM Pb is toxic for both transgenic and WT plants.

Biomass production was significantly greater in the transgenic lines PTa3 and PTa5 than in the WT plants growing in M4 mining soil and the Pb and Zn accumulated in these two lines is 2-fold and 1,75-fold, respectively, than the WT plants. However, due to the low values of concentration factors recorded for Pb, aspen plants tested should be considered more interesting for phytostabilisation of this metal than for phytoextraction. When the plant material was growing in soil M15, no significant differences in any of the parameters recorded were found between the transgenic and WT plants. The differential response of the two transgenic lines in soils M4 and M15 indicated that the physic-chemical properties of the soil play a determinant role in their phytoremediation pontential.

Genetic characterisation of the MTP1 zinc transporter family in *Arabidopsis halleri* reveals a large functional and genetic diversity

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MTP1s, the members of the Cation Diffusion Facilitator (CDF) family, encode proton antiporters effluxing zinc out of the cytoplasm and thus mediating zinc detoxification. In *A. halleri*, which is a wild metallophyte relative of *A. thaliana* showing both zinc and cadmium hypertolerance and hyperaccumulation, *MTP1* is the only zinc CDF transporter that is highly expressed in comparison to its *A. thaliana* orthologue. It was proposed that *A. halleri* harbours three independently and differentially regulated *MTP1* genes (there is only one *MTP1* gene in *A. thaliana*), two of which co-segregating with zinc tolerance in a backcross between *A. halleri* and its non-accumulator non-tolerant relative *A. lyrata*.

In this work, the *MTP1* family was characterised in greater details in the *A. halleri* Auby accession, a metallicolous population from the North of France. Two new *MTP1* paralogues were identified from the screening of a BAC clone library. The 5 *AhMTP1* had more similarities to each other than to their unique *A. thaliana* orthologue. Two of them, which were in direct tandem, were found to be 99.9% identical. A new *MTP1* locus was identified. Amazingly, this new *MTP1* was present in 14 *A. halleri* accessions chosen to represent metallicolous and non-metallicolous sites from the whole geographic area of this species, but it was not present in ~25% of 188 plants from the Auby accession, suggesting that this paralogue might be currently being lost. Gene expression studies indicated that the *MTP1* genes that were co-segregating with zinc tolerance in the *A. halleri* X *A. lyrata* BC1 progeny showed a much stronger transcript accumulation than their *MTP1* paralogues that were not co-segregating with zinc tolerance. Functional analyses in the *S. cerevisiæ zrc1 cot1* mutant were more surprising. The 5 *AhMTP1s* displayed a significant variation in their ability to complement the zinc hypersensitivity of the mutant yeast. In particular, one of the genes that co-segregated with zinc tolerance in the *A. halleri* X *A. lyrata* progeny displayed the least ability to complement the mutant yeast.

In conclusion, we showed that the zinc tolerant and hyperaccumulating species *A. halleri* harbours 5 paralogues (and not 3 as previously thought) of the zinc tonoplastic transporter *MTP1*, whereas *A. thaliana* displays only one *MTP1*. Interestingly, this gene pentaplication, which enabled an increase in functional as well as gene expression diversity, is not completely stabilised. Our analyses may reveal different evolutionary fates taking place for this MTP1 family in *A. halleri*. We bring important tools to study this process.

Characterisation of AtABCB29 (ATH12), a new member of ABC transporter proteins family involved in metals transport across chloroplast membrane

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ABC (ATP Binding Cassette) transporters constitute one of the largest families of proteins in living organisms ranging from bacteria to humans. ABC proteins are involved in the transport of a wide variety of substances, including ions, carbohydrates, lipids, xenobiotics, antibiotics, drugs and heavy metals.

Among plant ABC transporters, AtATM3 is a mitochondrial ABC protein involved in the biogenesis of Fe/S clusters, and iron homeostasis in plants. Moreover, it has been suggested to transport Cd^{2+}. AtMRP3 partially restores cadmium resistance when expressed in the ycf1 mutant, a mitochondrial ABC transporter and it confers cadmium and lead resistance in *Arabidopsis thaliana*.

The analysis of gene expression in *Arabidopsis thaliana* using cDNA-microarrays and RT-PCR showed that ATH12 transcript levels are induced by Cd^{2+} treatment.

In yeast, we performed drop test analysis after complementation of a cadmium sensitive yeast strain by ATH12 with and without the chloroplastic transit peptide. These results showed a partial complementation of Cd tolerance using ATH12 without transit peptide, suggesting that ATH12 may act as a Cd exporter.

In plants, to verify its sub-cellular localization, AtATH12 was fused (C-terminal) with EYFP and expressed under the control of the CaMV 35S promoter in Arabidopsis. The observed localization was in the chloroplast with is congruent with in silico and previous proteomic data .

The *atath12* T-DNA insertion mutants were more affected than wild type plants by Cd^{2+}. Under hydroponic culture conditions, leaves from wild-type Arabidopsis plants took up more cadmium than *atath12* and the mutant exhibited a marked chlorotic phenotype when exposed to 0.5 µM $CdCl_2$ for 7 days. A similar phenotype was observed in Fe-deficiency condition.

Ferritin content was monitored in WT and *atath12* seedlings by Western blot analysis: A higher signal was detected in *atath12*, supposing that ATH12 is involved in iron homeostasis.

The role and the function of this ABC protein in Cd tolerance and detoxification and iron homeostasis are under investigation and will be presented.

From the laboratory to practice: Heavy metals *en route*

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The aim of our 3-year-long project was to establish a complex phyto(bio)remediation technology for the removal of heavy metals from the contaminated environment and for the safe handling of the harvested plant material. To fulfill the project goals, both basic and applied research were carried out.

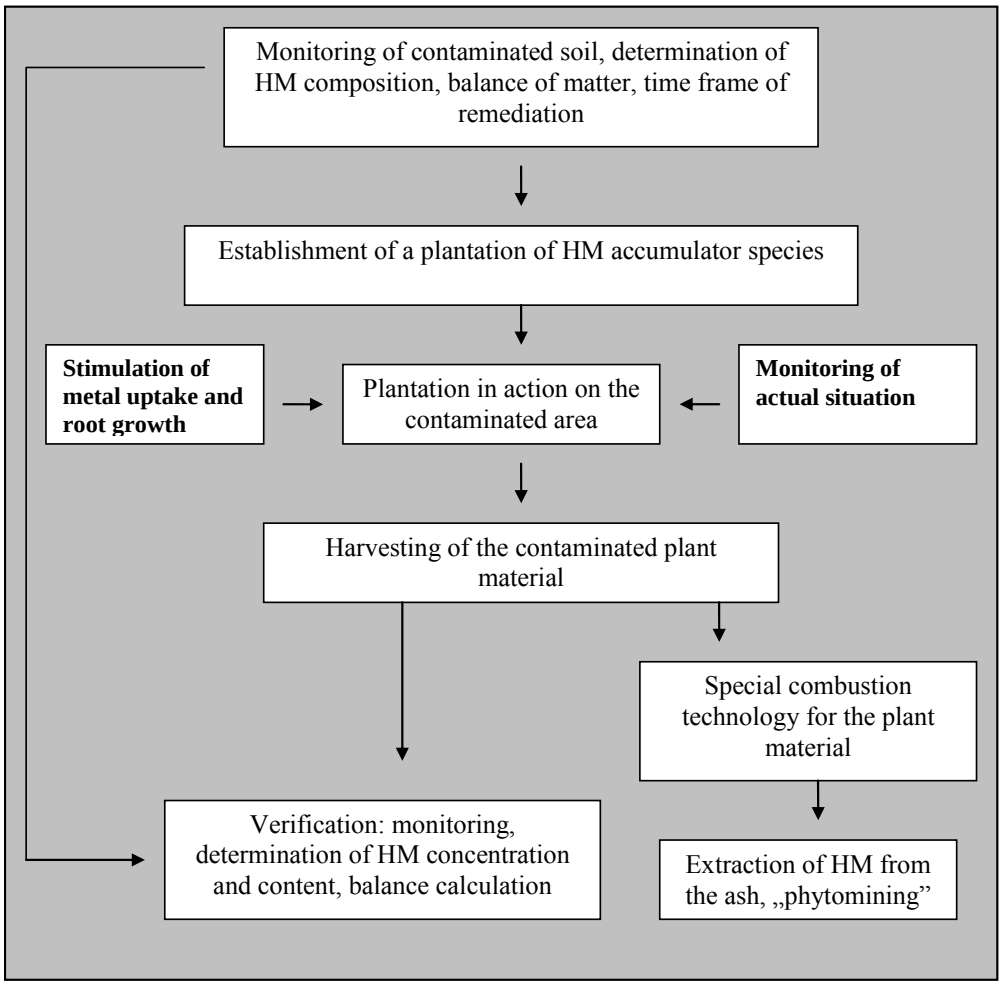
We have studied those molecular, biochemical and physiological processes which are the bases of the stress tolerance of the plant species applied. First of all the effects of heavy metals (Cu^{2+} and Cd^{2+}) on the elements of the antioxidant defence mechanism and on the gene expression of glutathione S-transferase (GST) and ABC transporter were determined in *Brassica juncea* (Gallé et al., in this issue). Concerning the signalization, we have found that a fast nitric oxide (NO) transient is involved in de Cu^{2+}-induced pathway while Cd^{2+} does not evoke NO-response (Bartha et al. 2005). It seems that the presence of a transient metal ion is necessary for the formation of NO, since its chelated form is ineffective (Kolbert et al., in this issue).

Both for basic and applied point of view it was important to work out a biosensor construction using the transgenic cyanobacterium Synechocystis which well responded to the presence of Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Cr^{6+}, As^{3+} ions (Kós et al., in this issue).

In the contaminated soil, indigenous bacterium species contribute to mobilization/chelation of metal ions and often they have a positive effect on root development. We have isolated bacteria consortium from the rhizosphere of willow grown on the contaminated soil, and found that they significantly promoted rooting of willow cuttings from nil to intensive. The effects of different phytoextraction treatments to the HM content of moderated contaminated soils were also examined in fields experiments on plots formed near the Tisza river (Farsang et al., in this issue).

The contaminated biomass was incinerated in a special, technically improved incinerator and from the ash heavy metal concentrations were determined and recovery was calculated.

As a summary, the following technology was worked out in details and applied:



The flow diagram of the phyto(bio)remediation technology

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Significance of Ni supply for growth in the Ni-hyperaccumulator *Thlaspi caerulescens*

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Nichel (Ni) is one of the nine transition metal elements required for nutrition in higher plants. Plants take up the essential metals from soil, transport them to various organs and use them for many enzymatic activities. Among them, Ni is used as co-factor for many enzymes such as hydrogenase, urease, Ni-superoxide dismutase, and so acts to maintain cell activity. However, at excess concentrations, this metal become toxic for a majority of plant species, causing inhibition of growth, chlorosis, necrosis and wilthing. Interestingly, some species named hyperaccumulators have the ability to accumulate large amount of metals without suffering symptoms of toxicicy. Among them some *Thlaspi spp.* are known to be Ni hyperaccumulators (Baker and Brooks, 1989). In recent years several studies on *Thlaspi* species have investigated the molecular and physiological mechanisms involved in heavy metal hyperaccumulation. In particular profiling technique have extensively applied at trascriptome level (Weber et al., 2006; van de Mortel et al., 2008). On the contrary, little attention was paid to explore the potential of proteomics to investigate possible genetic functions involved in determine the hyperaccumulator phenotype.

In this work we analysed the proteomic variations which accompanied the functional modifications occurring in the hyperaccumulator plant species *Thlaspi caerulescens* originating from a Ni-enriched serpentine soil (Monte Prinzera). In particular *Thlaspi caerulescens* 3 months plants grown in hydroponic were exposed or not for 21 days to 0.01 mM $NiSO_4$ This Ni concentration was sufficient to lead hyperaccumulation of Ni in the shoot and in addition plants treated with this moderately high concentration of Ni clearly evidenced a better physiological performances than in the control condition. Leaf crude protein extracts were analysed by a 2D liquid chromatography technique. Qualitative and quantitative differences between protein profiles of treated and untreated samples were evidenced by using DeltaVue software. Among the 500 polypeptides detected in shoot tissues, very few were found to change in abundance. In particular only some proteins involved in transport and metal chelation resulted over-expressed in the treated samples. Interestingly in the condition of absence of Ni many proteins involved in sulphur metabolism, protection against reactive oxygen species or stress response were evidenced. Considering these data, we can hypotheses that even if the concentration of Ni for plant nutrition is very low for normal plants, for the Ni hyperaccumulator *Thlaspi caerulescens* (MP), naturally adapted to grow on a metalliferous soil, a certain concentration of Ni in the medium could be necessary for a better growth performance for this population.

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Halophilic interplay between *Pistacia* spp. and *Salsola inermis*

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This report is based on an observation at the *Pistacia spp*. germplasm collection (http://www.bgu.ac.il/pistacia), that the growth of *Salsola inermis* is inhibited in the vicinity of the evergreen *Pistacia lentiscus*, but not around deciduous *P. atlantica* and *P. chinensis*. Irrigation of trees during the summer months increases soil salinity around the trees. It was therefore hypothesized that inhibition of *S. inermis* around *P. lentiscus* is related to depletion of salt in the vicinity of the latter species.

A multi-approach experimental scheme was carried out which included, soil edaphic characterization, and germination tests. To test salt tolerance of *P. lentiscus*, plants were grown in a hydroponic system for a month in a medium containing NaCl, and physiological and growth parameters were measured.

Conductivity measurements in summer, during the growth season of *S. inermis*, indicated that soil salinity beneath deciduous *Pistacia* trees was significantly higher than that below *P. lentiscus*. Germination of *S. inermis* seeds on filter paper moistened with *P. lentiscus*-low conductivity soil filtrate was twice as high as that of the deciduous trees-high conductivity soil filtrates. Nevertheless, fresh and dry weights of mature *S. inermis* growing next to *P. atlantica* and *P. chinenis* was 2.9 to 4.8 times higher than that of plants growing in the vicinity of *P. lentiscus*. In hydroponic system, no significant differences were found in growth parameters and stomatal conductance between *P. lentiscus* growing in control and salt treatments. It is therefore proposed that salt depletion in the vicinity of *P. lentiscus* inhibits its growth, but not germination, confirming the halophylic characteristics of *S. inermis*.

The nature of *Salsola-Pistacia* interactions cannot be explained by alleloptahic effects, hence plausible salt-driven interactions were considered. Our data showed that *Salsola inermis* accumulated salt and has halophytic characteristics. Interestingly, germination of *S. inermis* was inhibited in medium containing salt, but the salt was obligatory for further growth, development and fast biomass production. These results explained the observation of large biomass accumulation in the more saline soil around the deciduous *P. atlantica* and *P. chinensis* and the lack of development in the salt depleted soil around the salt-tolerant-accumulator *P. lentiscus*.

Soil salinity around *Pistacia* trees affects critically the growth of *S. inermis*. Inhibition of Salsola plants growth, but not germination, around the evergreen *P. lentiscus*, stems from its higher salt tolerance compared to the deciduous *P. atlantica* and *P. chinensis*. The results indicated that *P. lentiscus* is able to tolerate and accumulate salt, which we assume contributes to *P. lentiscus* wide distribution along the Mediterranean coast in Israel.

The phytoremediation potential of *Salsola inermis* has not been fully explored yet. We contend that germination of seeds in tap water and transfer to saline soil could hasten salt removal and biomass production for energy production. The evergreen salt-tolerant *Pistacia lentiscus* is recommended for forestation and soil stabilisation in relatively saline conditions.

Enzymatic basis for the removal of the fungicide, dimethomorph, from water bodies by aquatic macrophytes (STSM-859-03760).

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Aquatic plants can absorb a diversity of natural and man-made toxic compounds for which they have developed diverse detoxification mechanisms. However, little is known about the detoxification of fungicides in plants. It is known that plants are able to metabolize and detoxify a wide array of xenobiotics by oxidation, sugar conjugation, glutathione conjugation and more complex reactions. In this study, uptake and detoxification mechanisms of dimethomorph (DMM) as a fungicide currently found in aquatic media, were studied in *Lemna* and *Elodea*. The results showed that cytochrome P450 (CYP - enzyme catalyzing phase I reactions of the detoxification process) were induced in *Elodea* exposed to DMM suggesting a role in the metabolisation of this fungicide. Our finding of CYP activation following exposure to DMM is the first evidence in aquatic plants (*Lemna* and *Elodea*).

The activity of Guaiacol Peroxidase (GPOX), an enzyme involved in plant defense and catalyzing phase I reactions of detoxification processes, was also analysed in both plants. In *Elodea* GPOX activity was found to increase after 96 h in the presence of DMM 600 $\mu g.L^{-1}$, whereas in *Lemna* an induction was earlier and transient. These results indicate that these peroxidases may play a role as detoxification enzymes of DMM in aquatic plants.

Glutathione-S-transferase (GST), known as an enzyme involved in phase II reactions of detoxification and an anti-oxidative enzyme, was also assayed in our system. In *Elodea*, 600 $\mu g.L^{-1}$ DMM inhibited the GST activity, while in *Lemna* the same concentration of DMM increased its activity. Similar trends were observed with ascorbate peroxidase (APOX) activities in plants exposed to DMM, suggesting that the GST activation is a likely response to an oxidative stress caused by DMM.

Moreover, results assessing the degree of O- and N-glucosyltransferase activities (enzyme catalyzing phase II reactions) in the metabolisation process of DMM will be presented.

Comparison of detoxification responses of acetaminophen in Indian mustard (*Brassica juncea* L. Czern.) and Broadleaf cattail (*Typha latifolia* L.)

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The appearance and increasing concentrations of pharmaceuticals in domestic wastewater were detected in the last decade. Because their elimination during the water treatment process is not complete and from the treated plants they enter to our aquatic environment or via agricultural use of sludge to soils, they received major attention. The risk of these chemicals for the environment, mainly to aquatic ecosystems is well known, but information about the pharmaca-plant interactions: uptake, transport and metabolic pathways is scarce. However, this knowledge could help elucidate the detoxification capacity of the plants in wastewater treatment processes. Eliminating pharmaceutical pollution would improve water quality and avoid pollution of the food chain.

In our study the uptake and effects of acetaminophen were investigated (one of the mainly used painkiller in human medication) in Indian mustard (*Brassica juncea* L. Czern.) and Broadleaf cattail (*Typha latifolia* L.) plants. These species are well investigated in phytoremediaton processes and well known as heavy metal tolerant species with substantial accumulation ability. However, little is known about the uptake metabolism of micro pollutants like pharmaceuticals.

Plants grown in hydroponic system were treated with 1 mM concentration of acetaminophen. After 24, 72 and 168 hours treatment root/leaf samples from *Brassica* and rhizome/leaf samples from *Typha* were collected to study the effects of acetaminophen. Activities of enzymes involved in antioxidative stress responses like Guiacol peroxidase (POX), Ascorbate peroxidase (APX), Catalase (CAT) and Glutathione reductase (GR) were measured. Furthermore to complete the picture, Glutathione S-transferase and cytochrom P450 enzymes were assayed which are involved in Phase I and Phase II reaction against organic xenobiotixs.

Oxidative stress responses were detected in case of both plants, but the activity changes of the antioxidative defence enzymes (POX, APX and GR) showed differences in the two species. The rhizomatous perennial cattail plant seems to regulate its defence in different way than the annual *Brassica*. The results underline also the important role of Glutathione S-transferases in the detoxification process, as like as cytochrom P450 enzymes.

Introduction of bacterial genes for dioxygenases into plant genome to improve phytoremediation of aromatic xenobiotics

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The aim of this work is to construct genetically modified plants with increased capabilities to degrade organic pollutants such as polychlorinated biphenyls, toluene, TCE and other organic pollutants. Phytoremediation using transgenic plants can thus provide a useful, cheap and effective method for decontamination of the environment.

Therefore bacterial genes were chosen to clone into plant of *Nicotiana tabacum* and *Linum ussitatissimum*. Chosen genes of environmental importance are genes of bacterial dioxygenases – *bphC* and *todC1C2* genes. *BphC* gene encodes 2,3-dihydroxybiphenyl-1,2-dioxygenase which cleaves the aromatic ring of dihydroxybiphenyl and was cloned in fusion with gene for beta-glucuronidase (GUS), luciferase (LUC) and with histidine tail, under the CaMV 35S promoter. The *todC1C2* genes were chosen to clone into plants to produce oxygenase ISP_{TOL} (with histidine tail), a component of bacterial toluene dioxygenase that can oxidize toluene and other organic pollutants, both genes either under the constitutive CaMV 35S promoter or inducible RbcS promoter. Several genetic constructs were designed and prepared and the possible expression of desired proteins in tobacco plants was studied by transient expression via agrobacterial infiltration. Expressed oxygenases His/BphC, BphC/GUS, BphC/LUC and His/ISP_{TOL} were then detected by Western blot or histochemically. The next step involved preparation of transgenic plants. *BphC* gene was transferred into plant genome of *Nicotiana tabacum* by agrobacterial infection. The presence of transgenic DNA and expressed proteins was studied using several techniques. The growth experiments on increased concentrations of PCB metabolites showed higher resistance of transgenic plants to 2,3-dihydroxybiphenyl. *TodC1C2* genes are transferred nowadays into plant genome of *Nicotiana tabacum* and *Linum ussitatissimum*.

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Keywords: phytoremediation, tobacco, toluene dioxygenase, biphenyldioxygenase, transgenic plant

Potential of plants and plant compounds to stimulate PCB degradation activities of rhizosphere bacteria

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One of the most effective and most ecologically-sound ways of removing PCBs from contaminated sites is rhizoremediation. This method exploits the interactions between plants and the indigenous bacteria in the rhizosphere. So far, many aerobic bacterial strains that are able to degrade PCBs have been isolated and characterized. These strains are members of the genera *Pseudomonas*, *Alcaligenes*, *Achromobacter*, *Burkholderia*, *Comamonas*, *Sphingomonas*, *Ralstonia* or *Acinetobacter* (Gram-negative strains) and *Rhodococcus*, *Corynebacterium*, *Arthrobacter* and *Bacillus* (Gram-positive strains) [3]. Plants contribute to remediation by aerating soil or releasing compounds that selectively foster indigenous PCB-degrading bacteria. Until now, several compounds have been identified that stimulate bacteria degrading PCBs, such as flavonoids. We studied the effect of different phenolic compounds of plant origin to serve as growth and degradation stimulators, first in laboratory conditions in liquid culture with single degraders, then compounds were added to contaminated soil to stimulate indigenous microflora. Chosen compounds were generally better inducers of bacterial PCB degradation than biphenyl. The most effective inducers were naringin, 4-hydroxycoumarin, caffeic and ferulic acids. *R*-(+)-limonen, coumarin and morin proved very poor induction activity. Further experiments followed the growth of plants in vegetated contaminated soil and the effect of individual plants (tobacco, black nightshade and horseradish) on degradation activity and diversity of rhizosphere bacteria. Microbial diversity and composition of microbial populations in rhizosphere and bulk soil was evaluated using metagenomic approach and stable isotope probing method which identifies functionally active species using ^{13}C labelled substrates. 16S rRNA gene clone libraries constructed from ^{13}C-DNA following 1, 3, 7, and 14-day incubation of the matrices with ^{13}C-biphenyl suggest that biphenyl is catabolized mainly by *Hydrogenophaga* spp. in the horseradish rhizosphere and by *Paenibacillus* spp. in the non-vegetated soil.

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COST Action 859

Uptake, sequestration and detoxification – An integrated approach

POSTERS

WG1 & WG2 WORKSHOP AND MC MEETING

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P1 - Assimilation of Arsenic and impact on plant physiology Study of three species: *Agrostis tenuis, Holcus lanatus* and *Solanum nigrum*

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Arsenic (As) is a metalloid element naturally present in soil with an average concentration evaluated at 2 $mg.kg^{-1}$ and can reach 200 $mg.kg^{-1}$ in the calcareous and phosphate deposits. However, human activities (industrial, mining, pesticide, etc...) are responsible for a significant accumulation of this element in the environment, mainly as As_2O_3.

This study is one part of a phytoremediation program at an industrial wasteland. This site, located in Auzon (Haute-Loire, France), presents an As pollution caused by manufacturing and storage of fertilizers and herbicides (an average of 4000 mg $As.kg^{-1}$ in the soil).

This work focuses on the assimilation and toxicity of As on three plants, *Agrostis tenuis, Solanum nigrum* and *Holcus lanatus,* selected following a botanical survey of the site. The plants are cultivated in control conditions, on two soils which present an increasing of As concentrations (respectively, 249 and 3782 mg $As.kg^{-1}$ in the soil) and a compost. The physiological and biochemical parameters are measured, notably, the gas exchange, photosynthetic rate and lipid peroxidation.

These three species are able to maintain themselves and to accumulate the As present in the soil, in particular, *A. tenuis* and *S. nigrum*. The major part of the As uptake is stored in the roots. The As transfer towards the leaves increases with the As concentration in the soil, particularly for *S. nigrum,* which possesses a significant aerial biomass and so a better capacity of storage. *H. lanatus* shows a smaller uptake's ability.

The As accumulation causes some physiological disorders in the plant, such as a depression of photosynthetic activity, less significant in *A. tenuis*. The stomatal conductance and the transpiration rate are also affected by the metalloid. The limited plant growth is also a sign of As toxicity, possibly associated with senescence. In fact, As interferes with the processes of phosphorus absorption and transport which are essential for the growth and the development of plants. Furthermore, the composition of membranous lipids in leaves plays an important role in photosynthetic activities as well as in the structure and function of cellular membranes. The As would be responsible for lipid peroxidation through an oxidative stress which affects the composition in membranous lipid in plants, changing their integrity, their fluidity and, consequently, their permeability.

This study will be useful to select tolerant plants for the phytoremediation of polluted sites. The physiological and biochemical parameters can be considered as biomarkers of As pollution in the soil.

P2 - Removal capacity of constructed wetland for enteric pathogens and protozoan parasites from treated wastewater used in irrigation

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The use of treated domestic and municipal effluents in agriculture is an efficient way to conserve water resources and recycle nutrients (N & P) while minimizing pollution of receiving water bodies. There is crucial health and environmental risks associated with using partially or improperly treated wastewater and the official policy has therefore been to provide adequate treatment of sewage before its reuse. The utilization of treated wastewater for agricultural restricted and/or unrestricted irrigation in semi-arid/arid/desert regions constitutes both a blessing and a major concern. On the one hand, the advantages of such water reuse in view of the shortage of water and scarcity of natural potable water resources in such regions are obvious. On the other, the possible irreversible contamination of the irrigated soils and vicinal "hydrological sinks" (wells, river basins, and lower soil formations), surface-water and aquifers should not be underestimated. Health risks rise sharply with the ingestion of unsafe water. Diseases related to water sanitation are estimated to account for 4.0% of all deaths and 5.7% of the total disease burden worldwide. Wastewater treatment is needed in order to reduce the risks associated with improper sanitation, particularly in terms of effluent reuse for crop irrigation. Wetlands are used to reduce the organic load and heavy metals, as well as detoxification organic pollutants and other contaminants in wastewater. Only limited attention, however, has been paid to examining their efficacy for the removal of enteric pathogens from domestic sewage. Moreover, information regarding removals and correlations between indicators is still lacking.

In general, increased levels of fecal coliforms (FC) provide a warning of the failure in water treatment, a break in the integrity of the distribution system, or the possible contamination of pathogens. High levels indicate an elevated potential risk of waterborne gastroenteritis. Constructed wetlands (CWs) receiving primary municipal wastewater were found to reduce the total numbers of coliforms in the effluent from a level of 6.7×10^5 ml^{-1} by 99%. Other studies demonstrated 31–91% removal of *E. coli* in the rhizosphere, but only 0–35% in the un-vegetated controls. Removals of 2.4–5.3 orders of magnitude for *Salmonella* and FC were not enough to meet current regulations for unlimited irrigation.

Whilst CW systems facilitate efficient removal of chemical and bacterial contaminants, they may not provide substantial protozoan pathogen remediation, despite reported high removal rates. This occurs because CWs also have the potential to act as reservoirs supporting both the propagation and transmission of zoonotic protozoan enteropathogens entering these wetlands. CWs implemented into municipal wastewater treatment may not provide substantial remediation for human protozoan enteropathogens such as *Cryptosporidium*, *Giardia*, and human-virulent microsporidia.

In previous and ongoing studies, we found that the removal of pathogens and FC in a CW system used for treatment of municipal wastewater from sedimentation ponds was partial and affected by seasonal sampling and flow rate through the CW. Despite high removal efficiency rate (>96%) of FC, treated wastewater still contained high counts of FC (ranging from 3.33×10^2 to 6.17×10^4) and *Salmonella* (9×10^2-1.1×10^4). These findings demonstrate that wastewater polishing using CW provides no guarantee for the wastewater microbial safety despite high removal rate of microbial indictors. Additional treatments, such as disinfection, are apparently required for using CW effluents in crop irrigation.

P3 - The influence of road maintenance substances on graminaceous vegetation

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Technical granular sodium chloride treated with potassium hexacyanoferrate, a road maintenance material most-widely applied in wintertime in Lithuania, was used for the experiment. As an alternative to it, an organic material obtained on the basis of molasses, patented under the name of *Safecote* in Europe, was selected for the experiment. In Lithuania, the first trials to use this material for street slipperiness reduction were made during the winter season of 2005/2006 in Vilnius. 18 tons of *Safecote* were used in Vilnius during the winter season of 2006/2007.

Safecote is an organic material, which is a derivative from sugar making process, also known as molasses. This organic material contains minerals, including calcium, phosphorus, sulphur, chloride, sulphate, potash, soda. This material is of dark brown colour, liquid and can be mixed with all technical road maintenance salts and their solutions and less frequently is used purely alone.

The aim of research: to analyse how road maintenance materials affect the growth process and main parameters (the height of an above-ground part and a phytomass) of the graminaceous plants in question.

Three species of graminaceous plants, most frequently used for roadside planting in Lithuania when building new and reconstructing or renovating the exiting motor roads, were analysed: perennial ryegrass (*Lolium perenne L.*), fescue-grass (*Festuca pratensis Huds.*), meadow-grass (*Poa pratensis L.*).

The experiment's findings show that the perennial ryegrass had the biggest and the meadow grass – the smallest phytomass of the analysed graminaceous plants in contaminated soil. During the experiment it was again the perennial ryegrass that reached the biggest, while the meadow grass – the smallest height of the above-ground part. The experimental findings also show that the perennial ryegrass has the highest resistance to a toxic effect of salts while the meadow-grass – the lowest as it does not germinate in soils with big concentrations (5–8 g/kg) of NaCl. It has been proved experimentally that NaCl content in soil has a negative effect on the growth process of graminaceous vegetation, i.e. it retards the increment of above-ground part's height and reduces a phytomass. When inserted into soil, *Safecote* has a positive effect on the growth process of graminaceous vegetation, i.e. accelerates the increment of the above-ground part's height and increases a phytomass. The higher is *Safecote* concentration in soil the bigger are above-ground part's height and phytomass of graminaceous vegetation. In some cases graminaceous plants in soil with *Safecote* content showed better parameters than those in control soil. When NaCl application is reduced by 10 % replacing it with the same rate of *Safecote*, the soil condition is improved with mineral substances and favourable conditions for plant development are created.

Keywords: graminaceous vegetation species, molasses, road maintenance salt.

P4 - Molecular breeding of poplar for phytoremediation purposes

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Plants are exposed to several forms of biotic and abiotic environmental stresses, such as drought, cold temperatures, air pollution, heavy metals, herbicides, and pathogen infections. Most of them are coupled with oxidative damage due to the formation of reactive oxygen species (ROS), such as superoxide, hydroxyl radical, and hydrogen peroxide. The development of new plant varieties that are resistant to oxidative stresses might result in an improved phytoremediation capacity. Therefore, many breeding programs are focusing on the improvement of lines resistant to oxidative and general abiotic stress by means of either genetic transformation using genes responsible for oxidative stress tolerance, or by the in vitro selection of somatic cells and calli.

Nowadays, the most widely used *in vitro* breeding technologies for phytoremediation includes genetic engineering providing GM (genetically modified) plants with a very high capacity for cleaning up contaminated soils. However, most environmentalists reject the use of GM plants and urge the use of alternative technologies for the production of non-GM plants with elevated phytoremediation capacity for soil remediation.

In this work the *in vitro* selection and analyses of stable paraquat-tolerant poplar (*Populus* × *canescens*) clones is reported. Paraquat is a non-selective contact herbicide that targets the plant chloroplasts and induces the formation of ROS. Paraquat tolerance has developed spontaneously in fields exposed to long term herbicide treatment as reported for horseweed, wild barleys and brome fescue, but not in woody plants.

Paraquat tolerant poplar (*Populus* × *canescens*) clones (PQT) were selected in *in vitro* nodal segment cultures at the sublethal paraquat concentration (0.4 μM). PQT clones showed significantly higher glutathione (GSH) content and higher *gsh1* (γ-glutamylcysteine sinthase) gene expression activity than the wild type (WT) analyzed by qRT-PCR (quantitative reverse transcriptase real time PCR). Both the GSH content and the expression level of *gsh1* gene in the PQT clones showed 1.89-fold increments compared to WT. After testing on tissue culture media, regenerants were micropropagated, rooted and transplanted to a greenhouse followed by a field test at heavily contaminated fields of Chemical Company Fűzfőgyártelep (Hungary). Paraquat tolerant clones as powerful new stress tolerant and non-GMO plants can provide an effective tool for phytoremediation purposes where the pollutants cause oxidative stresses.

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P5 - Cadmium accumulation in tobacco cultivars

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Compared to other crop plants, tobacco naturally accumulates and concentrates relatively high levels of cadmium (Cd) in leaves. Cd bioavailability and environmental conditions are the main factors involved in Cd uptake and translocation in tobacco. The mechanism responsible for the transfer of Cd from root to shoot is largely unknown in plants, although some genes which are considered key players in the sequestration of Cd in leaves have been identified. This study is designed to determine whether genetic variation is also important in regulating Cd uptake in *Nicotiana* spp, and if so, to identify those genes responsible. Interestingly, not all *Nicotiana* species and *Nicotiana tabacum* varieties accumulate Cd in leaves at the same concentrations. For instance, *N. rustica* exhibits a higher Cd root/shoot ratio than *N. tabacum* under the same growth conditions, thereby suggesting that differential gene expression affects Cd transport and sequestration (Bovet et al., 2006, *Physiologia plantarum*, 128, 466-475). Furthermore, we recently found that *N. tabacum* cultivars also exhibit different Cd accumulation in leaves. Indeed, from independent field experiments in two different countries over two successive years, we observed that the same tobacco varieties accumulated similar relative amounts of Cd in leaves. These data were confirmed in a greenhouse environment with a selection of the same tobacco varieties. We can thus conclude from these growing tests that Cd accumulation in tobacco is dependent on the genetic variability of cultivars. We will also report on the specific genes in selected tobacco cultivars which are correlated with this trait from their expression patterns using Tobacco Exon Array chips.

P6 - Effect of EDTA on the Zn and Cd uptake and antioxidant enzyme activities of *Brassica juncea*

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Chelate-induced phytoextraction with high biomass plant species has been proposed for the clean-up of heavy metal polluted soils. Indian mustard *(Brassica juncea)* has been identified as a high biomass producing plant with the capacity to accumulate Zn and Cd at higher concentrations in the cells. Heavy metals enhance the generation of reactive oxygen species (ROS) and can cause oxidative stress. Incompletely reduced oxygen species can be extremely reactive and oxidize biological molecules, such as DNA, RNA, proteins and lipids, inactivate enzymes, decrease the rate of protein synthesis. EDTA (ethylene diamine tetraacetic acid) is a synthetic aminopolycarboxylic acid, a chelating agent commonly used for the assistance of phytoextraction processes. Although EDTA has been thought to be effective in enhancing phytoextraction, EDTA and EDTA-heavy metal complexes are toxic to soil microorganisms and to plants by severely decreasing shoot biomass.

In this work, the effects of the application of EDTA on the Zn and Cd uptake and on the antioxidant enzyme activities of Indian mustard *(Brassica juncea)* were studied. After imbibition in perlite, the plants were grown in Hoagland solution in pots for ten weeks. EDTA was applied at 50 and 100 μM concentrations alone or in combination with 50 and 100 μM zinc or cadmium, respectively. The heavy metal content, the levels of H_2O_2 and malonaldehyde (MDA), and the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), glutathione reductase (GR) and glutathione S-transferase (GST) were determined after one week treatments.

Applying EDTA to the Hoagland solutions decreased the accumulated zinc and cadmium content about half of the it both in roots and shoots. The treatments resulted in relatively low changes in the H_2O_2 and MDA levels in all cases which indicate the stress tolerance of *Brassica juncea*. Results of the H_2O_2 and the MDA content measurements revealed that EDTA also can be a stress agent for plants. The effect of EDTA on activity of some enzymes also suggests that EDTA affects the antioxidant defence mechanism. In those plants, where the zinc containing solutions were supplied with EDTA, the activities of CAT, POD and GR were elevated. In the case of experiments with cadmium, the GR and GST activities were enhanced in the presence of EDTA. Our results show that these enzymes can play important roles during EDTA application.

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P7 - Utilization of heavy metal ion polluted sites by biorefining of plants to utilizable base materials

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Biorefinery is a complex technology that can provide base materials for industry (cosmetic ingredients, pharmaceutical agents and precursors) without any by-products, ensuring the criteria of sustainability. Test systems were carried out to prove the applicability of biorefinery on heavy metal ion polluted fields originating from the previous mining activity. Due to the characteristics of test area, agricultural output is not feasible.

After the survey of the experimental area (contaminants – organic and inorganic; microbial florae and endemic vegetation) 88 plant species and variety were cultivated and tested for utilizable components.

The level of possible contaminants in the plants was monitored and the amount of different plant materials was also determined (mono-, bi- and oligosaccharides, proteins, organic acids, alcohols).

Crude plant extracts were tested as potential biologically effective components (antimicrobial and antioxidant) or as raw materials for fermentation processes (lactic acid fermentation or production of biomass containing carotenoids).

According to the results, numerous plant species were able to synthesize some biologically active compounds instead of high level of heavy metal contaminants. These results suggest that plants cultivated on industrial, heavy metal contaminated areas can serve as valuable sources of base materials for biorefinery.

As a next step, the development of a complex purification method of the active compounds is necessary, considering the presence of heavy metal contamination.

P8 - The Effects of Different Phytoextraction Treatments to the Heavy Metal Content of Moderated Contaminated Soils. Field Experiments in Hungary

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Phytoremediation is a relatively new method for decreasing soil and water pollution. This method includes numerous different technologies that can decrease the pollutants' concentration of the soil or can stabilize their transport in acceptable risk level with help of special plants and related microbes or chelatic agents (bacterial treatments and induced phytoextraction). These methods was tested and developed in field experiments in Mártély, SE Hungary.

The studied polluted material originates from one of the oxbow lakes of the Tisza river near Mártély. This slurry has moderated metal contamination (Zn, Cr, Cu) which can be derived from the natural sedimentation processes. Six experimental plots (10m x 30m) were formed and they were planted with *Salix alba*, *Brassica juncea* and *Brassica napus*. Passive phytoextraction experiments were processed in the study plots and these investigations were completed with induced phytoextraction in 1 m^2 large squares. The used chelatic agent was EDTA.

This presentation shows the changes of the critical metal contents in different soil horizons in special aspects to the root zones of the studied plants. The concentration differences give us evidence for the efficiency of the treatments and the average decrease of the element content gives us possibilities to calculate the duration which is necessary to press the concentration under the environmental limit values for each studied heavy metal.

Based on our experiments *Salix alba* proved the best accumulator plant from the studied species: even without any treatments 45-50 years are enough to clean up the upper 10 cm of the contaminated soil.

P9 - Proteomic analysis of *Arabidopsis halleri* shoot in response to the heavy metals cadmium and zinc and rhizosphere microorganisms

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Arabidopsis halleri is a zinc and cadmium hyperaccumulator species growing in heavy metal- contaminated as well as non-contaminated soils. The metal accumulation trait attracts researchers' attention for its potential use in phytoremediation of polluted sites. Within this context, *A. halleri* is a promising model species for molecular studies of metal tolerance and hyperaccumulation and the genetic closeness of this species with *A. thaliana* significantly facilitates the genetic analysis of metal hyperaccumulation. It is also known that plant metal uptake can be enhanced by rhizosphere microbial community. Therefore, shoot proteome analysis was carried out for the identification of differentially expressed proteins in plants i) grown in the solely nutritive solution, ii) with the addition of Cd and Zn or iii) maintained with these two metals plus soil rhizosphere microorganisms. Analysis of metal content suggested that populating the root zone with unselected microorganisms belonging to the autochthonous rhizosphere of a metal contaminated soil, is sufficient to greatly enhance plant metal uptake and transport to the shoot.

Conversely to what normally observed in non-accumulator plants, heavy metal treatment induced in *A. halleri* a consistent up-regulation of photosynthesis related proteins. Subunits of the complexes responsible for light harvesting (LHC, chlorophyll a/b binding protein) were overexpressed by metals plus microorganisms treatment: these might be required for an enhanced energy demand of the entire cellular metabolism. Similarly, an increment in the complexes responsible for electron transport was observed when plants were treated with Cd and Zn. Furthermore, the addition of these two metals caused a general down-regulation of proteins potentially involved in defence against herbivorous insects and pathogen attack. In particular, the expression of a myrosinase enzyme, two endochitinases and enzymes involved in the jasmonate biosynthetic pathway was strongly inhibited. This supports the view of a trade-off between metal hyperaccumulation and organic defences in in *A. halleri.*

In conclusion, metal uptake, transport and accumulation are energy-demanding processes, that can induce a general up-regulation of photosynthesis related proteins. This increased energy requirement is counteract by thrift defence system, therefore if high metal concentration in shoots provides a kind of protection system, other defence mechanisms are temporarily saved, highlighting a cross-talk between heavy metal signalling and defence signalling. Future proteome analysis will focus on cellular pre-fractionation of protein extract to obtain insights into the regulation of low expressed proteins such as transcriptional factors and kinases, and transmembrane proteins of the pathways that lead to heavy metal hyperaccumulation.

P10 - The effect of heavy metal stress on GST activities and transcript amounts of GST and ABC transporter genes in *Brassica juncea*

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An effective way of heavy metal detoxification is their conjugation with glutathione (GSH) or phytochelatins using the intracellular GSH pool. The GSH conjugation is catalysed by the glutathione transferase (GST) enzyme family, and the conjugate is transported to the vacuole by ABC transporters. After these processes the heavy metals are stored in a harmless way.Our aim was to detect the changes in the GST activity and gene expression of selected *GST* and *ABC transporter* genes after one week Cu^{2+} and Zn^{2+} treatments in *Brassica juncea*.Eight-week-old Brassica plants were exposed to 5, 10, 25 and 50 µM Cu^{2+} and Zn^{2+} treatments for one week. Whole leaf and root samples were harvested. Total GST activity was measured according to Habig et al (1974). Plant GSTs have seven soluble groups (Edwards and Dixon 2005), out of these phi and tau class are mostly responsible for the total GST activity measured with the substrate: 1-chloro-2,4-dinitrobenzene (Cummins et al 2003, Thom et al 2002). Besides the transferase activity, zeta group GSTs play a significant role in phenylalanine and tyrosine degradation. The maleylacetoacetate isomerase (MAAI) function suggests that GSTZ proteins are involved in the re-utilization and translocation of nitrogen metabolites. Brassica GST and ABC transporter sequences were found using an *in silico* approach from DDBJ/EMBL/GenBank sequence database. Two GST and one ABC transporter genes were selected for gene expression evaluation. *BjGSTF5* gene (AY299480) form phi group and *BnGSTZ-B* gene (AY208157) from zeta group were chosen for Real-Time PCR analysis. The chosen ABC transporter gene (DQ296184) encodes a protein which contains two transmembrane and two multidrog resistance domains.

According to our measurements, the GST activity in the shoots was induced by 50 µM Cu^{2+} treatment. Twenty five µM Cu^{2+} treatment enhanced the transcript levels of the phi group GST gene and the ABC transporter gene in roots. Zn^{2+} treatment induced the GST activity at all concentrations in shoots, but in roots only 50 µM Zn^{2+} treatment caused induction. In correlation with the activities, the gene expression of the phi group GST sequence was higher than controls both in shoots and roots. The transcript amount of the zeta group GST sequence increased in roots due to Zn^{2+} treatment, but no changes were detectable in the expression rate of the examined ABC transporter. Our results indicate differences in the response of phi and zeta group GST expression to Cu^{2+} and Zn^{2+} treatments. The transcript amount of *BjGSTF5* gene was enhanced in both cases in a different manner, while the level of *BnGSTZ-B* gene reacted only to Zn^{2+} treatment. The expression of the chosen ABC transporter gene increased similarly than the phi group GST gene.

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P11 - Silicon effect on cadmium distribution in wheat tissue

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Cadmium concentration in wheat grains is a problem for cultivation of wheat flour used for bread, pasta etc. Wheat accumulates Cd in the grains and those cultivars with high protein content usually also have high Cd content in the grains. Since 43 % of the Cd intake among Swedish people originates from wheat products it is necessary to decrease the Cd level in the grains. Since wheat is a grass it needs Si and we have found that Si decreases the Cd content in the wheat grains up to 50 % and also decreases the Cd content in the shoots. In addition Si decreases Cd toxicity in wheat. Our preliminary data show that Si increases the binding of Cd in the cell walls. The aim was to investigate the Si effect on Cd accumulation at the tissue level, due to when Si was added.

A high grain Cd accumulating variety of spring wheat of *Triticum aestivum* was investigated on uptake and distribution of Cd in cell walls, cytoplasm and vacuol as well as in the apoplast solution and xylem sap. Plants were treated for 4 weeks as follows: Two weeks with Si and/or Cd and the next two weeks with Si and/or Cd. Cadmium was added as 0.034µM ^{109}Cd and Si as 500µM K_2SiO_4. Five-week-old plants were harvested and analysed on ^{109}Cd and Si in cell wall, cytoplasm and vacuol as well as in apoplastic solution and xylem sap in both root and shoot tissue.

Cadmium concentration decreased in shoot to a higher extent if Si was added before Cd or when added simultaneously compared with if Cd was added 2 weeks before Si. There are indications that Si added after 2 weeks increased the translocation of Cd from shoot to root. Except when Si was not added, the Si content in the different compartments did not change due to treatment. However, the Cd content did. The share of Cd increased in the cell wall fraction and decreased in the vacuolar fraction, especially when Si was added prior to Cd. Silicon decreased the Cd content in the apoplastic solution while no effect was found in the xylem sap. We can conclude that binding to cell walls of Cd caused by Si seems to be one of the main reasons to the change in Cd accumulation and toxicity by Si.

P12 - Phytochelatin induction in Cd-treated cell suspension cultures of flax

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Flax (*Linum usitatissimum* L.) is an industrial crop with the ability to absorb heavy metals from polluted soils and contaminated biomass can be subsequently processed for various industrial products; the main portion of data on heavy metal tolerance/accumulation in flax was generated for Cd. There are differences among commercial flax cultivars in Cd tolerance/uptake/transport/accumulation, and thus it is possible to find contrasting genotypes for partial characters. In order to better understand the biochemical basis of metal accumulation/tolerance, changes in phytochelatin levels of two contrasting flax cultivars were studied in cell suspension culture as a suitable model system. Phytochelatins have been reported to exhibit a specific electrochemical behavior at the hanging mercury drop electrode (HMDE) in Brdička cobalt solution, producing a characteristic pattern of voltammetric or chronopotentiometric signals (Fojta et al. 2006).

Fiber flax cv. Jitka exhibits in in vitro conditions high Cd-tolerance and accumulation, while cv. Tábor shows low Cd-tolerance, but also high Cd-accumulation. Cell suspension was established from hypocotyl-derived callus on a liquid MS medium supplemented with 0.215 mg/L KIN and 0.0225 mg/L 2,4-D and incubated in the dark with shaking at 120 rpm and 23±2°C. Different concentrations of Cd (10, 50 and 100 μM $Cd(NO_3)_2$) were added to the stabilized cell suspension culture; samples were collected after 3, 12, 24 and 48 h after Cd-treatment and analyzed using the adsorptive transfer stripping (AdTS) linear sweep voltammetry at HMDE. Results show remarkable Cd concentration- and time of cultivation-dependent changes in the PCs levels. In both cultivars, 10 μM $Cd(NO_3)_2$ appeared to induce PC accumulation without affecting the cells viability. Lower PC levels observed for cv. Tábor cultivated in the presence of 50 μM $Cd(NO_3)_2$ for 3 to 24 h can be attributed to inhibition of cell growth and/or dying of certain population of the cells at this Cd level, while the intense signal observed after 48 h to accumulation of high PC levels in surviving cells. In the more tolerant cv. Jitka, much lower PC levels were observed during cultivation in 50 μM $Cd(NO_3)_2$, which may be in agreement with the speculation (Fojta et al. 2006) that extremely high amounts of the induced PCs may contribute to the heavy metal lethality. The prevailing lethal effect of 100 μM $Cd(NO_3)_2$, in agreement with no or only relative small increase of the induced PC amounts, was detected in both flax cvs. The results clearly indicate that the upper limit of Cd tolerance in *Linum usitatissimum* cell suspension is 50 μM (depending also on cultivar) and higher concentrations cause toxicity to the cells. PCs changes were significantly enhanced with increasing concentrations of Cd in the medium. This indicates the role of these proteins in Cd tolerance and accumulation in flax.

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P13 - Drought-like symptoms associated with growth of *Festuca arundinaceae* in soil contaminated with Naphthalene

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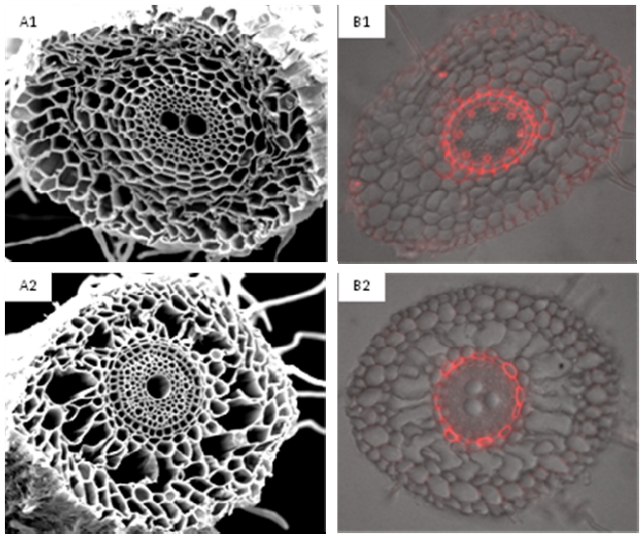
Tall fescue (*Festuca arundinaceae*) was grown in sand contaminated with Naphthalene (0.8g kg^{-1}) to investigate how contact with this low molecular weight PAH influences its root structure. Transverse root sections were fixed in 1% (w/v) OsO_4 and 2% (w/v) glutaraldehyde in a 0.1M cacodylate buffer (pH 7.2) solution. The root samples were dehydrated in ethanol and critically point dried using CO_2. Images were prepared using an S360 Cambridge model scanning electron microscope. This showed an extensive thickening of the cell walls in the Naphthalene-treated roots (A2) compared to those grown in 'clean' sand (A1)($p<0.001$). This effect was most pronounced around the endodermis. The root cortex was significantly larger ($p<0.001$) where plants were grown in contaminated sand. Its cells were no longer isodiametric resulting in a partial collapse of the cortex. The root's vascular bundles contained fewer, smaller xylem vessels with extensive wall thickening. These symptoms are similar to those described by Enstone *et al*., (2003) investigating water-stress in seminal wheat. This suggests that water-balance issues are the most likely cause to explain the adaptations observed when Tall fescue is grown in a hydrocarbon contaminated environment. The transfer of PAHs across the endodermis was investigated using the hydrophobic Nile red stain as a PAH homologue. Nile red was dissolved in a (1 : 3) phosphate buffered saline (PBS) : glycerol solution. Excess stain was removed by centrifugation. Living roots were exposed with a soft brush. A 1 cm^2 sheet of rockwool was placed in the cavity between the root and the sand. The Nile red solution was gently applied to the living root with a soft brush. Another rockwool sheet was used to protect and mark the stained root. The stained roots were sampled after 48 hours, fixed in 2% (w/v) paraformaldehyde and hand-sectioned in a few drops of PBS in parafilm on a dental wax surface. The distance travelled by the stain into the roots tissues was investigated using epifluorescence microscopy (Nikon Eclipse 90i). This showed that the Nile red applied to the roots grown in Naphthalene-contaminated sand (B2) was unable to penetrate the roots beyond the endodermis, whereas those grown in 'clean' sand (B1) showed evidence of uptake into the xylem vessels beyond the endodermis.



Enstone et al., (2003) J Plant Growth Regul. 21:335-351. Root endodermis and exodermis: structure, function and responses to the environment

P14 - Expressing a vacuolar metal transporter of the heavy metal hyperaccumulator *Thlaspi caerulescens* enhances metal tolerance and accumulation in Arabidopsis and tobacco

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Phytoremediation is the use of plants to remove pollutants from contaminated environments. Zinc, nickel and cadmium metals contaminate the soil when present in high concentrations thus causing soil and ultimately environmental pollution. Plants tightly regulate their internal metal concentrations in a process called metal homeostasis. *Thlaspi caerulescens* is a model metal hyperaccumulator species that can accumulate high amounts of zinc, nickel and cadmium. Metal ions are hydrophilic, so they do not cross the cell membranes or other membranes by passive diffusion. In order to allow transport of metals, different metal transporter proteins are active in plants. *TcZTP1* is a member of the CDF gene family in *T. caerulescens*, predicted to localize to the vacuolar membrane. It is very similar to *AtMTP1* of *Arabidopsis thaliana,* but showing constitutively high expression. We have expressed this gene into *A. thaliana* and *Nicotiana tabacum* under control of the constitutive CaMV 35S promoter. Transgenic plants with high expression of the transgene were grown hydroponically under high Zn, Cd or Ni supply or low Fe supply. The phenotype and mineral content data suggest that expression of this gene enhances metal tolerance and hyperaccumulation in model species Arabidopsis and tobacco. Transgenic plants are more tolerant to high Zn, Cd and Ni and low Fe, and accumulate more Zn and Cd, particularly in roots. We speculate that the high expression of *TcZTP1* acts to increase both tolerance and accumulation of toxic metals through enhanced Zn compartmentalization in the vacuole. This can be one step further towards the application of a viable GMO-based phytoremediation technology.

P15 - Metal Accumulation by Water Lettuce (*Pistia stratiotes* L.) in Stormwater Detention Ponds and Its Distribution in the Plant

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Water quality impairment by nutrient enrichment from agricultural activities has been a concern worldwide. Phytoremediation technology is increasingly applied to remediate eutrophic waters from agriculture and urban area. Besides N and P, aquatic plants are known to remove also metals from contaminated waters. In this study, a floating aquatic plant, water lettuce (*Pistia stratiotes* L.), was evaluated for its effectiveness in removing metals from two retention ponds (East and West Pond) in Fort Pierce, Florida. Water and plant samples were collected weekly and monthly, respectively from the control (without plant) and the remediation plots (with water lettuce) for metal concentration analysis. Aluminum, Ca, Fe, K, Mg, and Na were the main metals detected in the waters with a mean concentration of 0.25, 22.4, 0.15, 3.87, 2.82, 16.3 mg L^{-1} in the control plot of West Pond, respectively and 0.21, 43.8, 0.33, 7.83, 15.0, and 51.2 mg L^{-1} in the control plot of East Pond, respectively. Copper, Mn, Ni, and Zn were of very low concentrations (close to or below 0.01 mg L^{-1}) in both ponds. Cadmium, Co, Cr, and Pb were not detected. Aluminum, Fe, and Mn were significantly reduced (by more than 20%) in the remediation plots, as compared to the control plots. Potassium was reduced by more than 10% in the remediation plots. Calcium, Mg, and Na concentrations were also reduced, but to a less extent. Metals were observed to accumulate in the roots of water lettuce even when their concentrations in the water were below detection limits (DLs). All the 14 metals (Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) had a plant concentration factor (CF, the ratio of root metal to metal concentration in the surrounding water) higher than 10^2, with CF> 10^4 for Mn, Fe, Al, and Cr. All the metals except for Ca had a root/shoot (R/S) ratio > 1, with Fe having a R/S ratio > 15. Periodic harvest of water lettuce annually removed 10455 and 26005 kg dry matter per ha from East and West Pond, respectively, which translates the annual removal of Fe, Mn, Zn, Cu, Cr, and Pb at 55, 6.9, 1.6, 0.26, 0.22, and 0.11 kg ha^{-1}, respectively. A dithionite-citrate-bicarbonate (DCB) procedure was developed to differentiate metals deposited on the root surface from that absorbed into the root. A higher proportion of Ca, Cd, Co, Fe, K, Mg, Mn, or Zn was measured on the root surface, whereas more Al, Cr, Cu, Ni, and Pb were absorbed into the root system.

P16 - Potential use of *Vetiveria zizanioïdes* for the phytostabilisation of Cu-contaminated soils at a wood treatment site

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Native from South/Southwest Asia, *V. zizanioïdes* is cultivated for essential oil and as fodder. This perennial, herbaceous *Poaceae* has a rapid growth, a dense root system, and a high aerial biomass able to prevent eolian dispersion (Rotkittikhun et al 2007). It is tolerant to metals, extreme climatic and soil conditions (Chiu et al 2006), and able to phytodegrade some organics. According to its metal-excluder phenotype, physiological traits, and potential financial returns from its biomass it would be a candidate to phytostabilise Cu-contaminated soils at a wood treatment site. Questions arise whether Vetiver is sufficiently Cu-tolerant and whether its cropping previously needs a soil amendment.

Creosote, $Cu(SO_4)_2$, and CCA-type C have been successively used in soluble formulations as wood preservatives against insect and fungal attack at a treatment site (Bes and Mench, 2008). For the pot experiment, soils were collected in the 0-25 cm depth layer of field plots (P1-3 experiment established in 2005-2006, 1460 µg Cu g^{-1}) and an uncontaminated kitchen garden with a similar soil texture, to consist 6 treatments: CTRL (Control), UNT (untreated Soil P1-3), ORI: compost of pine bark chips and poultry manure (5%), ORIxZ: in spring 2005, two applications (1%) of zerovalent iron grit (GH120, Wheelabrator) and in spring 2006, 5% of compost, CaO: dolomitic limestone 0.2%; ORI x CaO : compost (5%) and dolomitic limestone. Transplants of a sterile Vetiver cultivar were cultivated in plastic pots (3L) placed in a greenhouse (triplicates). Shoot length and yield were measured after 1 year. Wet-digested shoots and soil solutions collected with Rhizon MOM were analysed by ICP-AES.

Vetiver was able to grow on the UNT soil but showed dwarf shoots and symptoms of Cu-toxicity on roots. Soil amendment enhanced shoot (FW, DW) yield and length. The most effective were ORIxCaO, ORIxZ, and ORI showing a major effect of compost addition. Root length remained lower in these treatments compared to control but the visible toxicity symptoms disappeared. Shoot Cu concentration varied from 2.2 to 7.4 µg Cu g^{-1} DW, remaining in frequent fodder range (1-10 µg g^{-1}, Kabata-Pendias and Pendias, 1992), and peaked in the UNT plants. Vetiver displayed a Cu-excluder phenotype. Shoot Cu decreased in ORIxCaO, ORIxZ, and ORI treatments. This was related to changes in total Cu concentration in the soil solution. The lowest Cu labile pool occurred in ORIxZ showing its interest for limiting Cu leaching but this amendment did not differ from ORI and ORIxCaO regarding Shoot Cu. The Cu bound by dissolved organic matter would be sorbed by newly formed Fe oxides. Shoot K and P decreased whereas shoot Ca, Fe, B, Al, and Zn increased with shoot Cu, likely in relation with shoot biomass and the root development. Changes in shoot Mn and soil pH were related. Cu sorption by organic matter, nutrient availability, and increased water holding capacity likely explained the promotion of Vetiver growth. Vetiver has a potential to be used for aided phytostabilisation on this Cu-contaminated Soil P1-P3, and to gain some financial return through essential oil. This will be further assessed in field plots on the site.

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P17 - Early responses to toxic elements: A transcriptomic and proteomic approach

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Exposure of plants to toxic elements may result in a rapid imbalance of cellular redox homeostasis. Part of these responses are activation of antioxidant systems or accumulation of biothiols (phytochelatins, PCs), which might bind to metals. Glutathione (GSH) and homoglutathione (hGSH) contribute to both defence levels, first as an important antioxidant metabolite (Asada-Halliwell Cycle), and secondly as PCs precursor. The early responses of Alfalfa (*Medicago sativa*) to Hg and As were investigated using holistic approaches. Seedlings grew in a *microscale* system (Ortega-Villasante et al. 2007 New Phytol. 176:69-107), where can be exposed from few minutes to 24 h. The harvesting time points and doses were chosen according to the inhibition of root growth, being selected those treatments not affected or just suffering inhibition. The idea was to search for cellular components involved in the first events of toxicity, rather than those related with general alterations of the metabolism. Another important parameter was the quality of RNA extracted, which was analysed by using an Agilent 2100 Bioanalyzer. RNA quality is essential to ensure a proper description of the transcriptional profile, so degraded RNA might interfere with those genes that are specifically repressed under element toxicity. Hg was applied at increasing doses (0, 3, 10 y 30 μM), and seedlings were sampled after 3, 6 or 24 h of exposure. On the other hand, 50, 100 and 200 μM As was supplied for 24 h. For the transcriptomic analysis, *Medicago truncatula* Mt16kOLI1 microarrays containing 16,086 70-mer oligonucleotide probes (Hohnjec et al. 2005 Plant Physiol. 137:1283-1301), was hybridized with RNA extracted from alfalfa seedlings challenged with Hg or As. Preliminary comparison of expression revealed some similarities between both toxic elements, in particular with stress proteins like *Heat Shock Proteins* (HSP). We have also started a proteomic analysis using two-dimensional electrophoresis techniques. Proteins extracted from roots of alfalfa seedlings exposed to 10 and 30 μM Hg for 3 and 6 h were labelled with CyDye fluorescent tags (GE-Healthcare), and subjected to two-differential gel electrophoresis (DIGE). Our data confirm that HSPs might be involved in the early steps of cellular responses to Hg, which might be important to protect altered or denatured proteins.

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P18 - Potential use of sunflower for the phytoremediation of Cu-contaminated soils at a wood treatment site

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$Cu(SO_4)_2$ and CCA-type C have been successively used in soluble formulations as wood preservatives against insect and fungal attack at a treatment site (Mench and Bes, 2009). Consequently topsoils were contaminated up to 2600 mg Cu kg^{-1}. At this large site, phytoremediation is an option to manage the pollutant linkages and achieve some remediation objectives. Ideal plants should offer a permanent, in situ, non-intrusive, self sustaining method of containment or removal of soil contaminant. The plant canopy should serve to minimize eolian dispersion whereas plant roots should prevent water erosion and store a fraction of contaminant labile pool. Sunflower is a candidate, providing financial return through oil production, that can be include in a sustainable cropping rotation promoting soil development processes, nutrient cycles, microbial community, and soil ecosystem functions with either no or acceptable residual pollutant linkages. In preliminary pot and field experiments, two sunflower cultivars did not grow on untreated Soil P1-P3 (1460 mg Cu kg^{-1}) at this wood treatment site. However, for the purpose of Cu *in situ* stabilisation in contaminated topsoils, soil amendments were tested (Bes and Mench, 2008). Compost, zerovalent iron grit and alkaline materials promote plant growth. Accordingly, field plots (10 m x 10 m) with and without compost (5% w/w) and dolomitic limestone (0.2%) were established on Soil P1-P3. In parallel, some sunflower mutants display a higher tolerance and accumulation for several metals (Nehnevajova et al 2007). Therefore, this study aimed at field testing several sunflower cultivars and mutants on Soil P1-P3 amended with compost and dolomitic limestone.

Material and Methods The field plot (amended Soil P1-P3) was split in 30 sub-plots (1 m x 3 m). Six sunflower varieties and two mutants were cultivated in a randomized system with three blocks and three rows per sub-plot. To avoid the edge effect, Tekny and Country varieties were cultivated on external sub-plots. Split-applications of NPK fertilizer were made. The 13th leaf starting from the flowerhead was collected, washed with distilled water and oven-dried for analysis. Shoot length and yield, and flowerhead and seed yields were determined. Ground leaf and shoot samples were wet-digested and analysed for trace elements by ICP-AES. Soils were collected in each sub-plot for physico-chemical analysis and soil solutions sampled with Rhizon MOM.

Results The poster will present the sunflower responses depending on cultivars and mutants. Changes in plant responses will account for micro-spatial variability of soil contamination and Cu labile pool in the soil solution. Changes in seed yield and trace element offtakes by shoots will be interpreted based on Cu exposure descriptors and plant material.

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P19 - Short- time effect of copper ion (Cu^{2+}) on nitric oxide (NO) production in *Sorghum sudanense* L. roots

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Nitric oxide (NO) is a multiface signal molecule, which acts in diverse physiological responses in plants such as heavy metal stress. Previous studies in our lab evidenced the activation of NO production in copper (Cu^{2+})- treated pea roots.

In our present experiments short-time effects of copper sulphate ($CuSO_4$) treatment on NO production in *Sorghum sudanense* L. roots were investigated. Plants were treated with 2, 50 and 100 µM $CuSO_4$ with or without 200 µM ethylenediaminetetraacetic acid (EDTA). One, two and three hours after treatments, NO formation was visualized with the help of the fluorescent probe, 4,5 diaminofluorescein-diacetate (DAF-2DA) in 2 mm-long root segments. Additionally, we performed element analysis by atom absorption spectroscopy to follow the uptake and transport of copper ion. For these experiments plants were treated with 2 µM Cu^{2+} alone and together with 200, 250 or 300 µM EDTA.

The effect of „free" Cu^{2+} on NO generation proved to be concentration- and time dependent, since 2 µM Cu^{2+} had no effect on NO levels compared to control, and the highest NO levels were detected after 3 hours in 100 µM Cu^{2+} -treated roots. Application of Cu^{2+} chelating agent (EDTA) prevented Cu^{2+} induced- NO accumulation in root cells. Uptake of Cu^{2+} into roots and the transport of it into shoots were determined in copper-treated plants. Every applied EDTA concentration inhibited both the uptake and the root-to-shoot transport of copper ion.

We conclude that „free" $Cu^{2+,}$ - which was uptaken by the roots- resulted in a transient NO accumulation in *Sorghum sudanense* L. roots. Cu^{2+} chelating agent (EDTA) inhibited the uptake and the transport of this ion and prevented NO generation.

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P20 - Recombinant cyanobacterial reporter strains for bioluminescent detection of nickel, cobalt, and zinc

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Detection and assessment of environmental pullutions in surface water bodies and complex soil samples are in focus of research and development worldwide that resulted in various chemical and biological methods. As compared to chemical methods, bacterial biosensors present certain advantages, such as selectivity, sensitivity, simplicity, and low cost.

For this end we have chosen to develop bioluminescence-based cyanobacterial biosensors for Co^{2+}, Zn^{2+} and Ni^{2+} pollutants. We aimed to achieve this goal using genetically engineered cyanobacteria, constructed by fusing transcriptionally active components of metal resistance mechanisms to lux genes from *Vibrio fischeri*, a naturally bioluminescent bacterium for the assessment of metal toxicity and bioavailability in polluted soils.

Cyanobacterial cells have complex metal resistance mechanisms that include enzymatic detoxification, sequestration and exclusion via active transport with either CPx-ATPases or chemiosmotic efflux systems. In order to develop Co^{2+}, Zn^{2+} and Ni^{2+} sensors, concentration-dependent gene expression patterns of heavy metal stress inducible genes were assessed. These experiments revealed that the promoter of the nrsB gene had a strong response to Ni^{2+}, while coaA promoter responded highly to Co^{2+} and Zn^{2+} exposures. The promoters of these respective detoxifying genes, together with their regulatory gene sequences, respectively, were fused to promoterless luciferase (luxAB) reporter genes. These fusions were introduced into the genome of *Synechocystis* PCC6803, a cianobacterium. For developing a simple detection protocol, luxCDE genes, coding for the fatty acid reductase complex that produces the substrate of luciferase complex, were also introduced to the genome under a constitutively expressing promoter.

The obtained bioreporter strains coaLux and nrsLux as whole cell biosensors were assessed by investigating their responses to a range of various metal and metalloid salts.

Both strains showed selective and dose-dependent responses to the metal salts added into the culture medium. When incubated in light, the coaLux sensor responded to Co^{2+} and Zn^{2+} with a detection range of 0.3–6 μM and 1–3 μM, respectively, the maximal luminescence signal intensities being 70-fold and 25-fold higher than the background, respectively. In mixed samples Co^{2+} and Zn^{2+}, the coaLux reporter strain responded in an additive manner. The nrsLux sensor showed a detection range for Ni^{2+} of 0.2–6 μM. The maximum response was about 50-fold induction of luminescence intensity at 6.4 μM Ni^{2+}.

We found that Zn^{2+} contamination interferes with the detection of Ni^{2+} detection by the nrsB promoter system. Moreover, light availability has significant influence on the bioluminescent responses to heavy metal exposures, with respect to both the sensitivity ranges and the maximal induction levels. These phenomena indicate a rather complex nature of the heavy metal responses in cyanobacteria.

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P21 - Simulating the influence of plants on the treatment performance of constructed wetlands

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Plants in constructed wetlands (CWs) have several functions related to the treatment processes. It is generally agreed that nutrient uptake is a minor factor in constructed wetlands treating wastewater compared to the loadings applied. However, for low loaded systems plant uptake can contribute a significant amount to nutrient removal (e.g. Brix, 1997; Tanner, 2001). The effects of plants on the treatment performance of subsurface flow CWs have been simulated using the multi-component reactive transport module CW2D (Langergraber, 2001; Langergraber and Šimůnek, 2005) which is incorporated in the HYDRUS variably-saturated water flow and solute transport program (Šimůnek et al., 2006). CW2D is able to describe the biochemical elimination and transformation processes for organic matter, nitrogen and phosphorus in subsurface flow CWs. The implemented model for plant uptake describes nutrient uptake coupled to water uptake and can be also used to model oxygen release from the roots into the subsurface. In the presentation the effects of the plants is shown for two case studies: i) a pilot-scale two-stage CW from Sicily, Italy, used for secondary/tertiary treatment of urban wastewater (Toscano et al., 2008) and ii) a horizontal flow CW from Australia (Headley et al., 2005). It can be shown that the treatment performance of the described systems can only be simulated when the plant effects are considered in the model. This strengthens the statement by Langergraber et al. (2008) that the influence of plants is important and one of the processes to be considered for the formulation of a full model for CWs.

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P22 - Tobacco mutants as example for successful detoxification caused by multiple pollution

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Tobacco (*Nicotiana tabacum*) is an important crop worldwide. Its cultivation in many countries, as well as its specific characteristics, such as high biomass production, extraordinary drought and metal resistance make tobacco interesting for model investigations.
Tobacco smoke leads to higher heavy metal exposure. The main route of the heavy metals in *Nicotiana* happens via the roots into the shoot. After heavy metal uptake the plants have to cope with elevated stress influencing antioxidant systems including different enzymes and glutathione metabolites.

In the present study the performance of *N. tabacum* cultivars cv. Badisch Geudertheimer (BAG) and Forchheim Pereg (FOP) dependent on nitrogen fertilization was investigated. We aimed to show responses of *N. tabacum* plants against oxidative stress caused by cadmium from the soil in which the plants were grown. We sought for effects on biomass, photosynthetic pigments, antioxidant enzymes, on GST´s and antioxidants.
Selection of heavy metal tolerant callus cultures had been carried out in four selection media containing sublethal concentrations of Cd, Zn and Cu; over a period of 18 months *in vitro* plant generation was stimulated. Regenerated plants were well established and micropropagated.

Selected somaclonal populations of self-pollinated F1-tobacco variants and their mother-clones BAG and FOP, which were previously identified as metal efficient in lab and free land conditions, were grown on Zn, Cd and Pb contaminated site in Bettwiesen (Switzerland). At harvest plants were separated into shoots and roots. All samples were immediately frozen in liquid nitrogen. Metals were determined by either flame or graphite furnace depending on concentrations. Plant samples were transferred to the laboratory in Germany on the same day, where enzyme extraction was done.

Different treatments of tobacco clones of BAG exerted highest effects on SOD activity. After fertilization with 2AN and 1AN + 1AS BAG clones show 50 to 70% higher SOD activity. Other fertilization regimes with different concentrations did not induce any changes in SOD. In the clones with increased SOD activity the catalase activity has also increased. Probably higher sulphate and nitrate concentrations govern heavy metal uptake, which cause the production of ROS. The APOX was generally higher than POX. The difference between the highest POX and APOX activity was 5fold. In the FoP tobacco samples increase of the DHAR activity was detected after ammonium nitrate fertilization.

In analogy to the GPOX the highest GR activities were measured in the BAG and the FoP tobacco samples treated with mixed fertilizer (GR 10 times lower than the GPOX). None of the treated samples showed higher GR activity than GPOX activity. GSH and GSSG contents depend not on fertilization. Doubling ammonium nitrate and ammonium sulphate led to same biomass development and metal uptake, albeit at same GSH and GSSG concentrations.GST activities were highest for the model substrate NBoC. The other activities were reduced in the order: NBoC, NBC, CDNB, Fluorodifen, DCNB. Mixed fertilization again induced essential increase of activities, especially for NBoC and NBC GST. This points at differential induction of GST isoforms in tobacco under heavy metal stress and fertilizer amendment.

P23 - Worldwide presence of naturally occuring elements in plant organisms

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Inorganic environmental chemistry is far from having a detailed understanding of interdependence and possible synergistic interactions. Two thirds of naturally occurring chemical elements in ecosystems are normally not investigated although there is no proof of their inessentiality or nontoxicology. More effort must be undertaken to remedy this lack of knowledge. In view of the important role played by plants in most ecological systems an inorganic chemical characterization using different instrumental multi-element techniques in combination with speciation studies seems of the highest interest. For this the establishment of a "Reference plant"can be a successful tool for inorganic chemical fingerprinting. Advances in geobotanical prospecting have led to a better understanding of plant substrate relationships which in turn provide a firm basis for the study of global and regional patterns. In this paper we are presenting a rough estimation of the total element content in the world plant biomass in tons. Beyond the value of establishing the parameters of elemental abundance in ecosystems and species, the study can provide the basis to determine the influence of the abundance of one element to another, and on the chemical interrelationships among the biota and its substrate and of plants and consumers. A strong support by genetically and microbiologically orientated scientists would be very helpful for the future activities.

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P24 - Transcriptome analysis of Cu responses in metal accumulator plant *Brassica nigra* Diyarbakir ecotype

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Toxic effects of both essential and non-essential heavy metals are well documented in plants. Some plant species have been reported not only tolerate heavy metals but accumulate huge amounts of metals in their roots and shoots. For this reason these accumulator plants are valuable source for use in phytoremediation of contaminated soils. The use of metal-accumulating plants to clean soil contaminated with toxic metals is the most rapidly developing component of this environmentally friendly and cost-effective technology. The Brassicaceae is a family containing many metal accumulating species. The heavy metal accumulating species *Brassica nigra* has received attention due to its possible use for phytoremediation of heavy metal-polluted soils. The objective of the current study is to evaluate the potential of *B. nigra* for phytoremediation of Cu from contaminated soils and to find out the genes highly expressed in roots and shoots of the plants when exposed with high amount of Cu in the media. Comparative transcriptome analysis was carried in order to find out the expression level of metal induced genes and transcriptome changes both in low and high Cu treated plants. Microarray analysis showed that some of the genes were highly expressed (several hundred fold) with Cu treated plants compared to control. The most important expressed genes were as follows:

1. ABC transporter proteins
2. Leucine rich repeat protein kinase
3. Metal transporting P-type ATPase
4. Zinc- Finger homeobox protein
5. Glutathione S-transferase
6. Sucrose transporter
7. Auxin responsive GH3 family protein
8. Myb family transcription factor
10. Abscisic acid responsive HVA22 family protein
11. CDPK family protein
12. RHD3 family protein

The Cu accumulation capacity of Diyarbakır ecotype was determined and compared with other *Brassica nigra* ecotypes 6619, 6620 and 6630 obtained from different sites of Western Europe. The metal uptake capacity of *Brassica nigra* Diyarbakır Ecotype was 4-5 times higher than that of other ecotypes. The genomic DNA from the tissues of *B. nigra* was isolated and digested with 5 restriction enzymes (*Eco*RI, *Hind* III, *Bam*HI, *Pst*I, SalI). Southern blot was carried out with cDNA probes of γ-ECS and phytochelatin synthase (PC). Real time PCR was carried out with total RNAs isolated from *B. nigra* treated with different concentrations of Cu. The expression of both γ-ECS and PC was increased in the roots and shoots of plants when exposed with increasing concentrations of Cu in the solution. Our data show that *B. nigra* accumulate Cu both in roots and shoots and could be used for cleaning up Cu from contaminated soils.

Key words: *Brassica nigra* L., gene expression, γ-EC, Phytocheletin synthase.

P25 - Preparation of transgenic plants for heavy metal remediation

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Heavy-metal pollution of soils and waters, mainly caused by mining and the burning of fossil fuels, is a major environmental problem. Phytoremediation is an emerging, cost-effective and noninvasive remediation method. Heavy metals or metalloids can be removed from polluted sites by phytoextraction, which is the accumulation of the pollutants in the plant biomass. Plants have several defence mechanisms allowing them to deal with heavy metal stress. One of these mechanisms involves the production of cysteine-rich non-protein thiols (NPTs), that bind many toxic metals and metalloids. Glutathione (GSH) plays several important roles in the defense of plants against environmental threats. Glutathione is not only a substrate for glutathione S-transferases, enabling neutralization of potentially toxic xenobiotics such as arsenic, mercury, and cadmium, but it is also reductant of dehydroascorbate. Moreover, GSH is the precursor for phytochelatins (PCs), heavy-metal-binding peptides involved in heavy metal tolerance mechanism and sequestration. Glutathione is synthesized from its constituent amino acids in two sequential, ATP-dependent enzymatic reactions catalyzed by γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS), respectively. From previous studies it seems that both γ-ECS and GS co-limit GSH production under stress conditions. Thus overexpression of both enzymes together may result in increased GSH production under stress conditions where great GSH depletion is caused by phytochelatins synthesis leading to increased heavy metal tolerance. The aim of the present study is to overexpress *Saccharomyces cerevisiae gsh1* gene for γ-ECS and *gsh2* gene for GS in *Nicotiana tabacum*, as a model plant, and a species of technological use, *Linum usitatissimum*, which is an annual plant species widely cultivated in temperate climates, to obtain plants with enhanced Cd accumulation and tolerance.

Several vectors were designed for *Agrobacterium* mediated transformation, pNOV1 and pNOV2 contain *gsh1* gene and *gsh2* gene, pNOV12 contains both *gsh1* and *gsh2* genes. Each gene was flanked by Rubisco small subunit light-inducible promoter RbcS from *Chrysanthemum sp.* and at 3′ end by RbcS transcriptional terminator. Special vector was constructed for promoter expression study harboring *gus* gene for β-glucuronidase. Transient expression with tobacco leaves was carried out applying this vector and proving promoter activity.

A method was designed for determination of glutathion and phytochelatins using RP-HPLC. For this purpose aseptically grown flax was stressed in 14 days' period by different concentrations of $CdCl_2$. Analysis of leaves extracts indicated the presence of GSH, PC2, 3, 4 and 5.

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P26 - Enhanced Copper Tolerance Due to Antioxidant Defence in Maize (*Zea mays* L.) Root Tips.

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High soil copper concentration originated by mining activities, industrial fallout, or phytosanitary treatments (e.g. long-term use of Burgundy mixture in vineyards) can cause adverse effects in sensitive plants. Direct and indirect toxicity mechanisms have been described. Copper, as a Fenton metal, can directly damage roots by causing oxidative damage in cell membranes. Indirectly injury can be caused by Cu-induced Fe deficiency.

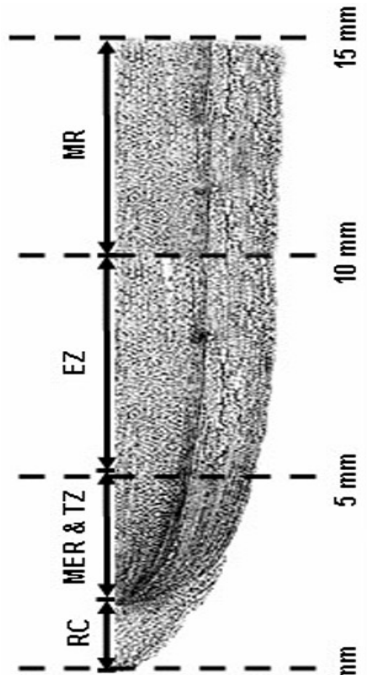

Fig 1: *Maize root tip showing the three different zones of analysis. RC, root cap; MER, meristem; TZ, transition zone; EZ, elongation zone; MR, mature root.*

Avoidance of excess Cu accumulation in plant tissues, either or both by reduced uptake and enhanced efflux seems mainly responsible for differences in the performance of plants under excess Cu. The role of antioxidant defences in the varietal differences in Cu tolerance is unclear.

Here we analysed the initial responses of root tips of two maize cultivars (*Zea mays* L. cv Oropesa and cv Orense) to excess Cu under controlled conditions (Hoagland solution without EDTA; Cu at 0.25, 2, 5, and 10 μM for 24 h). Three different root tip zones were considered (see Fig 1) : 0 – 5 mm (root cap, meristem and transition zone), 5- 10 mm (elongation zone) and 10 -15 mm (mature root zone) Relative root elongation of plants growing in 5 μM Cu solution revealed clear differences in Cu tolerance between the cultivars. Oropesa was significantly less affected than Orense. In the 5 μM Cu solution Orense, but not Oropesa, exhibited severe damage of cell integrity, especially in the transition zone. After desorption with 5 mM ice-cold $Pb(NO_3)_2$, analysis of Cu in the root tips, did not reveal better Cu exclusion in Oropesa than in Orense (Table 1). A higher rate of root tip cell turnover and more efficient detoxification of superoxide anions by a local (0-5 mm root tips) enhancement of SOD in the root tips seemed to be essential for the better Cu tolerance in the cv Oropesa.

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P27 - Iodine deficiency and fortification strategies in Bhutan

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Himalayan regions are reported to be deficient in Iodine (I) and is a public health problem in that region. It is most essential for health and promoting mental development in children. Iodine is also required for thyroid gland function and metabolizing fats. Iodine is essential to make thyroid hormone, which has a variety of functions in human embryo development. Soils in himalayan ranges area subjected either to flooding, or to intense and prolonged glaciations are attributed to iodine deficiency. Evidence for the lack of Iodine in Bhutan is supported circumstantially by the occurrence of goiter and cretinism, which were common in the people of the region (Figure 1). Diffuse goiter and mutinodular toxic goiter neurocognitive impairment are the prevalent symptoms of IDD (Zimmermann 2008). Iodine deficiency disorder (IDD) in Bhutan was addressed by iodine fortification and distribution of iodized salt programmes which were initiated aggressively in 1985 (Lhendup 2008). This paper reviews the extent of IDD prevalence in Bhutanese population and coordinated multi-sectoral iodine deficiency disorder control programmes undertaken for total elimination of IDD. IDD are also prevalent in countries of Africa, America, Eastern Mediterranean, Europe, South east Asia and western Pacific. Therefore, IDD is a subject of public health concern which can be dealtwith fortification of salt and oil with iodine.

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Figure 1. Goiter formation in women prevalent Iodine deficiency disorder in Bhutan.

P28 - Modification of a plant response to Zn/Cd by heterologous expression of *AhHMA4*

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Although the Zn/Cd hyperaccumulation of *Arabidospis halleri* is associated with many genes, recently it has been demonstarted that *AhHMA4 (P_{1B}*ATPase) plays a key role in Zn hyperaccumulation and full hypertolerance to both metals. Associated with this phenotype very specific pattern of *AhHMA4* expression was detected. Contrary to non-accumulating *A.thaliana,* its expression in roots was constitutively very high whereas in shoots the transcript level was moderately induced under Zn deficiency [1,2].

It was shown that transfer of *AhHMA4* under its own promoter into non-accumulating *A. thaliana* resulted in a Zn distribution typical of *A.halleri* in *A.thaliana* [2]. The aim of the current research was to check whether the introduction of *AhHMA4* under its own promoter into tobacco genome would generate a phenotype similar to that one described for *A.thaliana*. Secondly, a comparison of expression of chosen metal hyperaccumulation genes in tobacco between transgenic lines and the wild type under conditions of zinc deficiency and Zn sufficient supply and in the presence of Cd, would help to assess its involvement in the generation of the detected phenotype.

The Ah*HMA4* under endogenous promoter is strongly expressed in roots, stems and leaves of tobacco and the presence of cadmium does not modulate the level of its expression. It was demonstrated that the expression of *AhHMA4* was not associated with the alteration of Zn accumulation. Its concentration in transgenic plants exposed to 0,5uM to 500uM Zn^{2+} was within the range of values determined for the wild-type lines. Similar to *A.thaliana* expressing *AhHMA4*, transgenic tobacco was also more sensitive to the excess of zinc and cadmium. More necrotic spots on leaves and enhanced wilting under the highest zinc concentration indicate the impairments of the detoxification processes. It is not known whether the immobilization or compartmentalization of zinc was affected.

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P29 - Transport and fate of xenobiotic glutathione conjugates in extraradical hyphae and spores of mycorrhizal roots of barley

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Arbuscular mycorrhizal fungi (AMF) like *Glomus hoi* are ecologically important for the growth and survival of most vascular plants. AMF also improve rhizosphere health and defense against pollutants by modulation of the activity of their host´s enzymes. Numerous agrochemicals as well as xenobiotic organic pollutants are detoxified in plants to glutathione conjugates. These xenobiotic GS-conjugates will be sequestered in plant vacuoles, but a proportion is also thought to be distributed in the apoplast, and hence, in root cells of plants. From there, it might be possible that xenobiotic conjugates will also reach AMF mycelia, and the rhizosphere.

The objectives of this study were to: i) study the possible distribution of xenobiotic conjugates in plant roots and AMF hyphae and to: ii) understand the continuum for the translocation of xenobiotic metabolites between the root and *Glomus*. We carried out a greenhouse experiment with *Glomus hoi* and barley (*Hordeum vulgare* L.) as host plant. Barley plants were grown in pots and irrigated with tap water in the greenhouse. *Glomus* was inoculated in the soil and allowed to form a symbiotic network with barley roots over time. Averagely, 55% of barely roots were mycorrhized by *Glomus hoi* at the end of four weeks of plant growth. Whole plants were carefully harvested from the pots and adhering soil was gently removed from the mycorrhized roots by rinsing them with tap water in a sieve.

In order to study xenobiotic conjugation and movement, monochlorobimane (MCB) was utilized as model xenobiotic that is conjugated rapidly in root tips with glutathione to yield a fluorescent metabolite. Using an inverse fluorescence microscope (Zeiss Axiovert 100), it is possible to visualize the fluorescent metabolite in vivo. To follow the conjugation reaction and the distribution in the root, roots of barley seedlings were cut under water, and the end was fixed in an aperture with a thin latex foil and transferred into a drop of water on a cover slide. The cover slide was fixed in a measuring chamber on the stage of the fluorescent microscope. The MCB was now applied to the root tip with a pipette, and fluorescence was monitored for 2000 sec at a resolution of 10 Hz.

In the present study, we are able to present evidence that MCB is conjugated in the tips of mycorrhizal roots of barley. The conjugate moves basipetally from the root tip towards the root base, and part of the conjugates distributes in the epidermal root cells, from where it undergoes long range transport into the extraradical hyphae and spores of the mycorrhizal fungi. Strong accumulation of conjugate was recorded in the sporangia of *Glomus*, ball-shaped, large structures. Our study demonstrates that plant borne xenobiotic metabolites will distribute in the rhizosphere continuum, including mycorrhizal fungi. The final fate of the conjugate is unknown, but the presence of peptidases indicates that transport may well be followed by individual metabolism. We expect to extract and qualitatively describe such metabolites in hyphae and spores.

P30 - Role of bacteria *Pseudomonas fluorescens* and chelating agent EDDS on metal accumulation in autochthonous Bulgarian *Brassica juncea* plants

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This study was made in the Dept. of Microbiology at University of Córdoba, Spain. In it we investigated the influence of PGPR *Pseudomonas fluorescens* and the chelator EDDS on the growth and accumulation of Pb, Cd and Zn in autochthonous Bulgarian plants of species *Brassica juncea* L., as well as, changes in availability of studied metals. Furthermore, we assessed the abilities of these bacteria to utilize aminocyclopropane carboxylic acid (ACC) as sole source of nitrogen, the production of indoleacetic acid (IAA) and siderophores, as main parameters of characterization of plant-growth promoters.

We found that addition of the chelating agent EDDS had a strong effect on the soluble fractions of Zn, Pb and Cd. As consequence, their accumulation in shoot biomass was increased significantly. On the other hand, the inoculation of bacteria *P. fluorescens* improved the plant growth and also the metal accumulation. This bacteria was found to produce important quantities of indoleacetic acid (IAA) in presence of tryptophan, and siderophores when was grown on nutrient agar medium. This means that the bacteria stimulate plant-root-elongation through production of phytohormone auxin and improving Fe-nutrition by secretion of siderophores.

Further investigations are directed to large scale studies.

KEYWORDS**:** *Brassica juncea*, plant-growth promotion, IAA, siderophores, *Pseudomonas fluorescens*, heavy metals, accumulation.

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P31 - Phytoremediation and pharmaceuticals in the environment

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Pharmaceutically active compounds are a new class of contaminants that have raised the great concern in the last 10 years. The continuous release of drugs into environment may cause a lot of health problems in wildlife and humans. At most of these compouds-drugs they are health risks at long-time low-level exposition because these substances often exhibit so called endocrine disrupting effect. The used pharmaceuticals and their metabolites are excreted into domestic wastewaters and cleaning efficiency in the sewage treatment plants is often low. The agricultural use of the activated sludge and input of the water into surface waters are permanent sources of contamination.

The most interesting pharmaceuticals are wide-used and off-prescription drugs. The big amounts of painkillers (ibuprofen, diclofenac, naproxen) are consumed in the european countries and all these compounds were found in various concentrations in european surface waters. The big consumption is also in other drug groups as antibiotics (penicillins, sulfonamides), beta blockers (propranolol, metoprolol), long-term prescripted antiepileptics (carbamazepine), lipid lowering agents (statins, clofibrate), antidepressants (fluoxetine, risperidone), contraceptive hormones (ethynylestradiol), and antihistaminics (loratidine, cetirizine).

The input of pharmaceuticals into environment is a reason for the investigation of methods for the removal of these compounds from ecosystem and for testing of plant ability to incorporate these compounds in their tissues (possible input into food-chains). Phytextraction methods seem to be from this point of view very attractive because the construction of hydroponic bioreactors/wetlands could result in the promising technology for decreasing of drug amounts in the sewage treatment plant effluents.

The phytoextraction of common used non-steroidal anti-inflammatory drugs diclofenac and naproxene was carried out using various terrestrial and aquatic plants, such as *Zea mays*, *Linum usitatissimum*, *Brassica napus* or *Ceratophyllum demersum*, *Egeria najas*, and *Elodea canadensis*.

Although the decrease in concentration of diclofenac by *Zea mays* plants was relative slow and final concentration below 1 % was reached at 7 day cultivation, naproxen was extracted faster and concentration about 1,5 % was obtained after 3-days cultivation time.

Generally, the extraction was found very efficient at *Zea mays* plants and its rate is higher for naproxen than for diclofenac (at naproxen after 3 days cultivation 1 – 1,5 % of starting concentration vs. 26 % for diclofenac). Other tested species *Linum usitatissimum* and *Brassica napus* decreased starting concentration of naproxen to 24 and 11 %, respectively by 3-days cultivation.

The use of above mentioned water plants did not reach the expected efficiency for diclofenac. During 17-days cultivation the starting concentrations are decreased to 29 and 15 % for *Elodea canadensis* and *Ceratophyllum demersum* respectively.

In this time the use of hydroponically cultivated terrestrial plants seems to be the best way for phytoextraction of pharmaceuticals from water solutions. The finding of other aquatic plants with higher extraction ability will be in the centre of further experiments.

P32 - Potential of selected plants for pharmaceuticals phytoremediation

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Today more than 100 000 different chemicals are distributed on the global market, and one third of them exceeds quantities of one tonne per annum. Most of them have been introduced for the benefit of daily life, medicine, food production and industrial purposes, and a good proportion of these compounds lacks natural counterparts. Amongst them are well known pesticides, plasticizers, fuel additives, flame retardants, medicaments and fragrances.

Industrial activities are a second source of pollution. Industrial wastewaters discharged into aquatic ecosystems either directly or because of inadequate treatment can lower water quality by increasing concentrations of pollutants such as organic matter, suspended particulates, micropollutants, phosphorus, ammonium or heavy metals, thereby causing adverse effects on human health and undesirable changes in the composition of aquatic biota. Utilization of such type of water in agriculture and generally for irrigation led to contamination of soil and finally food chain.

This contribution will be focused to the fast growing problem of environment contamination by so called "new contaminants" – pharmaceuticals, house-hold products and others.

According to USGS study (http://toxics.usgs.gov/pubs/FS-027-02/), the most frequently detected chemicals of such type (found in more than half of the streams) were coprostanol (fecal steroid), cholesterol (plant and animal steroid), N-N-diethyltoluamide (insect repellent), caffeine (stimulant), triclosan (antimicrobial disinfectant), tri (2-chloroethyl) phosphate (fire retardant), and 4-nonylphenol (nonionic detergent metabolite). Steroids, nonprescription drugs, and insect repellent were the chemical groups most frequently detected. Detergent metabolites, steroids, and plasticizers generally were measured at the highest concentrations.

Main problem with these compounds is connected with fact, that conventional treatment systems are not sufficient for cleaning such type of contamination.

To solve this problem, phytotechnologies can be used for cleaning both soil and waters by accumulation and/or degradation of above mentioned compounds using different plant species.

This approach and its efficiency will be demonstrated using most frequently used pharmaceuticals (paracetamol, ibuprofen and diclophenac).

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P33 - Effect of ectomycorrhizal fungi on aspen (*Populus*) salt stress tolerance

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Introduction

The genus *Populus*, poplars, cottonwoods and aspens, contains about 30 species of woody plant all found in the northern hemisphere exhibiting one of the fastest growth rates observed in temperate trees (Taylor, 2002). A rapid growth gives high biomass production and the high transpiration rate and a wide-spreading root system is advantageous in remediation.
The aim of the work was to know whether there is genetic variation in *Populus tremula* saline stress tolerance and if this is affected by mycorrhiza.

Experimental

A controlled greenhouse experiment was set up with twelve *Populus tremula* families growing in lower organic matter (OM 3%) sandy soil and higher organic matter (OM 15%) forest soil. The soils were irrigated with NaCl solution, 80 mM and 160 mM and controls with tap water. The growth responses of below-ground plant parts in terms of root dry weight, root tip frequency were measured. Ectomycorrhiza (ECM) fungal colonization intensity was determined for four selected families. The foliar saline symptoms were recorded visually. Fresh ECM roots were sorted into morphotypes according to the criteria and terminology of Agerer (Agerer, 2001). The morphotyped mycorrhiza was further analyzed by PCR amplification of the ITS region (Bonello *et al.*, 1998).

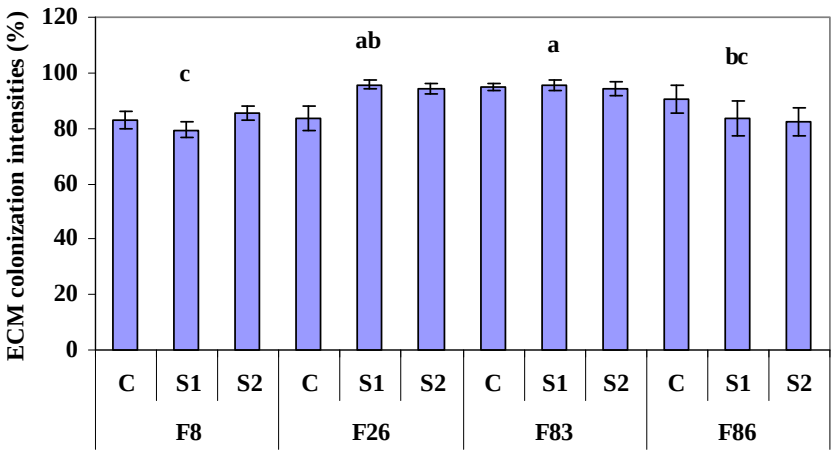

Fig. 1. Ectomycorrhizal colonisation intensity of four European aspen families, F8, F26, F83 and F86. Control soil is marked by C, lower saline soil by S1 and higher saline by S2.

Results

In below ground examination, root dry weight correlated mostly positive with seedling saline tolerance showing less visual foliar saline symptom. The ECM fungal colonization intensity varied between aspen families, and correlated negatively with early stage seedling growth rate (1-2 month). ECM morphotypes were significantly affected by saline treatments and families. Lower saline treatment (80 mM NaCl) clearly increased morphotype numbers of ECM from four tested families, but this effect was not observed with higher saline treatment (160 mM NaCl). The ECM roots were classified into twelve different morphotypes. The PCR-amplification of DNA from ECM roots showed a greater diversity in that some of morphotypes consisted of several ECM fungi.

Conclusion

This study indicated that there were variations in saline tolerance and in mycorrhiza among tested *P. tremula* families. The below-ground characteristics of seedling could be good indicators for screening aspen genotypes suitable for re-plantation in saline area.

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P34 - Activity of *AtMRP3* promoter in transgenic *Arabidopsis thaliana* and *Nicotiana tabacum* plants is increased by cadmium, nickel, arsenic, cobalt and lead but not by zinc and iron

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Heavy metal ions at low concentration play significant role in living cells, for example acting as enzyme co-factors or catalyzing electron transfer in mitochondria and chloroplasts. However, an excessive accumulation of heavy metals is toxic and due to this risk the living organisms must regulate their intracellular metal concentrations tightly. It can be achieved by maintaining the specificity of metal transporters which play central role in metal homeostasis. Therefore, metal-specific regulation of the transporters expression is one of the basic steps to understand transport and accumulation of toxic metals and metalloids by plants.

We investigated the response of the promoter of *AtMRP3* gene (At3g13080) from *Arabidopsis thaliana* to various metals. This gene has been previously demonstrated to be up-regulated by cadmium treatment and possibly involved in heavy metals accumulation in plant cells [1,2]. In this work, by using two model plants, Arabidopsis and tobacco, we monitored the activity of the *AtMRP3* promoter using *uidA* (*gusA*) as a reporter gene [3]. In both transgenic species we observed high GUS activity after cadmium, nickel, arsenic, cobalt and lead exposure but not by zinc and iron treatment. Our results indicate that the used DNA fragment contained the *cis*-regulatory elements necessary for metal-regulated expression of the *AtMRP3* gene. The transgenic plants constructed by us could be potentially used as biomarkers on metal contaminated soils. In the future experiments, this sequence can be applied for other potential phytoremediation-related purposes, for example, to drive of the cadmium-induced expression of the particular transgenes.

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P35 - Genetic and Physiological determinants controlling cadmium accumulation in lettuce (*Lactuca sativa*)

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Cadmium is an extremely toxic pollutant causing a great number of diseases. It is a widespread metal which concentration rises in cultivated soils, thus exposing food or feed crops to it and ultimately causing potential major sanitary problems as a consequence of its entry in the food chain. Among the vegetable species used for food, lettuce shows one of the highest capacities of accumulating cadmium. Our objectives are to characterize mechanisms controlling cadmium accumulation in this species and identify the corresponding genetic determinants, ultimately aiming at breeding lettuce for cadmium under-accumulation.

A phenotypical analysis of cadmium accumulation was carried out on 18 lettuce genotypes representing the genetic diversity of the species, revealing a great variability of response, both with respect to cadmium accumulation and cadmium translocation from roots to shoot. The lettuce genotypes displayed independent variations for both traits, and also between these traits and cadmium tolerance. In contrast, a very strong positive correlation linked cadmium and zinc accumulation. Increasing calcium and iron concentration in the culture medium had a protective effect against cadmium accumulation, however this characteristic did not discriminate the lettuce genotypes presenting extreme cadmium accumulation phenotypes. Interestingly, experiments measuring ^{109}Cd influx in roots, ^{109}Cd efflux from roots and ^{109}Cd translocation from roots to shoots revealed that the genotype displaying the least cadmium accumulation could be discriminated from the genotype displaying the highest one by its markedly increased ability to efflux cadmium from the roots to the culture medium. Progeny analysis from crosses between the genotypes displaying extreme performances for cadmium accumulation, cadmium translocation from roots to shoots and cadmium tolerance revealed that none of these traits was supported by a single genetic determinism. QTL mapping is under development. Surprisingly, the genetic analysis demonstrated that the ability to limit cadmium accumulation (and thus probably to increase cadmium efflux from the roots) was recessive.

In conclusion, phenotypic analysis of cadmium accumulation in a large set of lettuce genotypes revealed that a major determinant limiting cadmium accumulation in these species is the ability to efflux cadmium from the root to the culture medium.

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