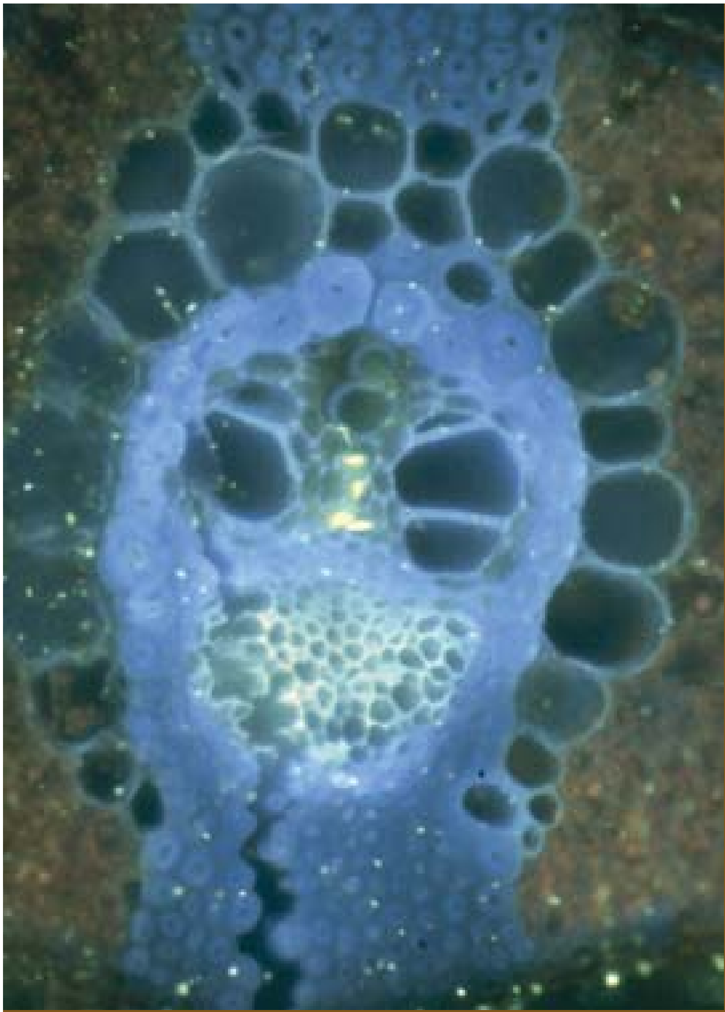

Metal fluxes and stresses in terrestrial ecosystems

Abstracts

Workshop
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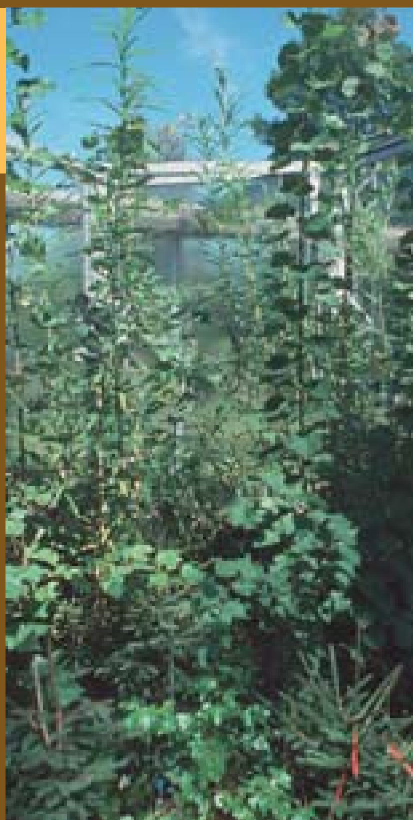

Centro Stefano Franscini Monte Verità
Ascona, Switzerland

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Vegetation analysis und copper uptake patterns of plants in a mine tailings dumpsite, Cebu (Toledo) Philippines

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Keywords: copper tolerance, phytoremediation, hyperaccumulation

Vegetation analyses were conducted on an abandoned copper mine tailings dumpsite in Toledo City, Cebu, Philippines as an initial step to screen for potential phytoremediation agents. The ten most dominant plants in the area were identified and analyzed for total copper content using AAS. Similarly, three soil samples from each of the three sampling points in the area were analyzed for total copper content. Results reveal that *Saccharum spontaneum* significantly dominated the area covering 44% of the total vegetation cover, followed by *Nephrolepis exaltata* with 19%, *Calopogonium mucunoides* with 10%, *Paspalum conjugatum* with 8%, *Pennisetum polystachyon, Mimosa pudica*, and *Fimbristylis ovata* with 5% each, *Nephrolepis cordifolia* with 2%, and *Chromolaena odorata* and *Mimosa invisa* both covering 1% each. However, based on the AAS analyses, only *Nephrolepis cordifolia* emerged as a potential hyperaccumulator of copper containing 1,100 mg/kg Cu making it the most promising phytoremediation agent in copper contaminated areas. In addition, four other plants have the potential for phytoremediation purposes because of their high copper uptake and biomass. These are *F. ovata* (900 mg/kg Cu), *C. odorata* (850 mg/kg Cu), *M. invisa* (700 mg/kg Cu), and *M. pudica* (680 mg/kg Cu). Also, total copper content in soils sampled from 3 different sites showed significantly toxic amounts of copper with 400 mg/kg Cu (site 1), 436 mg/kg Cu (site 2), and 425 mg/kg Cu (site 3). According to the USDA, the maximum tolerable Cu levels in both plant and soil should only be 30 mg/kg Cu, thus, indicating that the area is severely polluted with Cu and is highly bioavailable for plant uptake. Moreover, there appears to be a positive effect on the impact of vegetation on the copper polluted mine tailings dumpsite such that a correlation exists between amount of total copper in soils and vegetation density. Based on the findings on the 3 different sampling sites, as vegetation cover and/or density increases, total copper content of the area appears to decrease. This can be attributed to the high copper accumulating abilities of plants tolerating the area which has a high potential as a phytoremediation agents in other copper contaminated areas.

Bioavailability of mercury to plants thriving in a mine tailings dumpsite

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Keywords*:* mercury, phytoremediation, bioavailability

Soil contamination with Hg is not usually considered a serious problem but changes in soil conditions render mercury complexes to be bioavailable for plant uptake. In an abandoned mine tailings dumpsite in Itogon, Benguet, Philippines, total Hg levels in soils sampled within the area fluctuated from 0.493 to 0.805 ug/g. Soil properties such as pH, textural class, CEC, organic matter content, moisture content and total Hg content were determined to assess the interplay of Hg in the soil system and the type of flora tolerating such conditions. It appears that soils having lower pH and higher cationic exchange capacities contained higher Hg concentrations available for plant uptake. However, mine tailings soils gathered from the site generally lack clay particles and organic matter to trap the nutrients as well as the heavy metal. Moreover, vegetation thriving in the contaminated area were identified, quantified, and analyzed for total Hg content to assess if certain plant species, locally adapted with high biomass, can be explored for phytoremediation purposes. Among the ten most dominant plants thriving in the area, the three plants which concentrated the highest Hg levels were *Pteridium aquilinum* (0.136 ug/g), *Chromolaena odorata* (0.120 ug/g), and *Mimosa pudica* (0.119 ug/g) which can be explored further as potential phytoremediation agents in mercury polluted sites.

Heavy metal accumulation by some plant species from Azerbaijan flora

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Keywords: *Artemisia* sp., lichens, soil, heavy metals, phytoremediation

Wormwood (*Artemisia* L.) plants and foliaceous lichens are known to be capable to accumulate in their bodies several heavy metals from soil and aerial deposition (1, 2). These higher and lower plants widespread on polluted sites of Azerbaijan were not investigated in this respect. *Artemisia* species (*A.fragrans* Wiild., *A.scoparia* Waldst., *A.arenaria* DC Prodr., *A.szovitsiana* Grossh., *A. caucasica* Willd.) have been collected from Absheron region, but *A.fragrans* and lichens (*Xantoria parietina* L., *Physcia adscendens* Fr.) from Ali-bayramli – industrial city of Azerbaijan. Collected from different contaminated (by oil, chemical and metallurgic waste products) and non-contaminated sites plant and surface soil samples were measured for cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) concentrations by using ICP-AES. The results found indicated that all 5 species of *Artemisia* were effective to accumulate Zn in shoots at very high amounts (up to 20–280 times higher than in soils). Among the *Artemisia* species tested the *Artemisia scoparia* showed the highest capacity to accumulate all 5 heavy metals in shoot. Comparative studies with *Artemisia* species and lichens to measure their capacity in uptake and accumulation of heavy metals from the Ali-Bayramli location indicated that both lichen species were distinguished by very high amounts of 5 heavy metals in their bodies. In soil samples around of lichens, metal concentrations were remained in high contents (Cd by a factor of 400, Cu – 400, Ni – 200, Pb – 900 and Zn – 300) than in soils collected in adjacent only with *Artemisia* sites. Furthermore *A.fragrans* was shown to accumulate all tested metals, mainly Zn much more than the plants growing in the same place (e.g., *Salsola dendroides* and *S.grassa*). The results obtained indicate that *Artemisia* species and lichens accumulate metals in their organs when grown around the contaminated soils and air, representing a good source for heavy metal-remediation approach. By considering their larger biomass, the *Artemisia* species (especially *Artemisia scoparia*) possess a high genetical potential to be used for phytoremediation of heavy metals from polluted soils.

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Use of holly (*Ilex aquifolium* L.) as biomonitor of heavy metals

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Keywords: *Ilex aquifolium,* biomonitor, heavy metals, bioindicator

The distribution of Cd, Cu, Mn, Ni, Pb and Zn in *Ilex aquifolium* L. growing in beech forests soils was studied in order to check its capacity to accumulate heavy metals and to assess its usefulness as bioindicator of anthropogenic pollution.

In this work, foliar analysis of holly was used to make an indirect estimation of the heavy metal pollution in the beech forests of Navarra. Both, beech and holly have a similar distribution area in Navarra, so we could compare their behaviour with respect to the heavy metals and their capacity as bioindicators of anthropogenic pollution.

The study was conducted in Navarra (north of Spain), where the beech forests cover an area of 134.280 ha. which means about 30% of the all beech forests in Spain, being the region with a bigger area of beech forests in the Iberian Peninsula.

In order to carry out this work, 42 sample plots were chosen along the area of study. In each plot, a composite leaf sample coming from several trees was taken. Moreover, several parameters as altitude, orientation, slope, location and distance to the nearest road were studied.

In the laboratory, unwashed leaf samples were desiccated at 60 ºC for 48 hours and finely ground. A carefully weighted aliquot of about 1 g was subsequently mineralized using a mixture of concentrated HNO_3 and $HClO_4$ Suprapur grade (Merck). After mineralization, samples were determined by F-AAS (Cd, Cu, Mn, Ni and Zn) and GF-AAS (Pb).

Our results show a high levels of Zn and Cd. Zinc content in holly leaves was about four times higher than in beech leaves, whereas Cd level was in the order of five times (Table 1). Taking into account that *Fagus sylvatica* L. is considered as a very appropriate species as bioaccumulator of heavy metals, it can be stated that *Ilex aquifolium* L. show an excellent aptitude as bioindicator of potential pollution of Zn and Cd.

Table 1. Concentration values for Zn and Cd in leaf samples of holly and beech

	Zn (µg g-1)	Cd (µg g-1)
Ilex aquifolium (holly)	124 ± 67	1.6 ± 1.2
Fagus sylvatica (beech)	32 ± 12	0.3 ± 0.7

With regard to Cu, Mn, Ni and Pb, holly leaves didn't show any special capacity to accumulate these metals since their mean contents were similar to those found in beech leaves.

These results agreed with those obtained by Cellini Legitimo *et al*. (1995) and showed a special aptitude of holly in concentrating Zn and Cd with respect to the other metals that were verified.

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Soils of beech forest in the North of Spain (Navarra): assessment of metal pollution and pollution sources

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Keywords: soil, beech forest, metal pollution

In this work we present the results of the study of soils in 30 beech forest plots. This study is part of a global work that deals with the nutritional state of the beech forests in Navarre, North of Spain (Amores Olazaguirre, 2002). The main objective is to get the levels of soil trace elements to establish their relations with the values found in beech leaves. Forest plots are located in different lithological substrates where most of the plots show an udic soil moisture regime. However, there are also areas with xeric soil moisture regime. Soil typology is varied, predominating inceptisols (22), over entisols (4), mollisols (2), ultisols (1), and spodosols (1). In the 30 selected plots, topsoil samples (0-5 cm) are taken around the tree trunks and others in zones out of this influence. In the sampled soils we have performed the measurement of pH and organic matter content. In addition, total concentration and extractable fraction (using.NH_4-Acetate-EDTA pH=4,65, as extracting agent) have been analysed. Trace elements Cd, Cr, Cu, Mn, Ni, Pb and Zn have been measured by F-AAS in total fraction, and by F-AAS and GF-AAS in extractable fraction.

Statistical analyses performed, as well as the ratio observed between the total and bioavailable contents, show that all the plots receive atmospheric lead and copper pollution from medium-long distance. In half of them, a zinc pollution is also detected. As regards the sources of the detected pollution, anthropic contributions of lead and zinc comes from industrialized areas located in the west and northwest of Navarre, whereas in the case of the copper pollution a defined geographic pattern has not been observed, perhaps due to a diffuse pollution coming from northern regions of Europe.

In the case of lead, considering that the main source of its atmospheric levels on world-wide scale is the road traffic, we suggest that the contribution by diffuse pollution to the beech forests in Navarra is as important as the originated by the mentioned industrial activity. With regard to cadmium, chromium and nickel, none anthropic contribution has been detected, although this possibility cannot be discarded in a low magnitude. In spite of all the aforementioned, it has been found that whereas the total contents of lead, zinc and copper have not reached toxic concentrations for soil micro and mesofauna, such level has been reached in the case of cadmium and chromium, with the consequent injurious effects on the biogeochemistry cycles.

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Cadmium accumulation and toxicity in barley (*H. vulgare* L.)

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Keywords: cadmium, barley, productivity, growth, photosynthesis

A part of agricultural soils all over the world is slightly to moderately contaminated by cadmium (Cd) creating risk for human and environmental health. There are approximately 20 000 ha contaminated by heavy metals arable land in Bulgaria and Cd appears in about 3 000 of them. Therefore, a significant scientific effort has recently addressed many aspects of plant – Cd interactions, including mechanisms of Cd uptake, transport and accumulation as well as potential Cd phytotoxicity.

During the last decade such kind of experiments using barley as a model plant have been conducted in the Agricultural University of Plovdiv, Bulgaria. One part of them was aimed to study Cd accumulation and productivity of barley grown in soils differing in Cd contamination and soil properties. Another part of experiments was directed to Cd-induced physiological disorders in barley plants grown in hydroponics, sand and soil conditions. A summary of the main results obtained in these experiments as well as some initial results from the present studies on plant ^{109}Cd uptake will be presented. Briefly, the main results are:

- The minimum soil Cd concentration where seed Cd accumulation exceeds Cd limit for cereals (0.1 mg kg^{-1}) is about 5 mg Cd kg^{-1} soil. Barley productivity was not significantly affected at treatments with realistic concentrations (2–10 mg kg^{-1}) but decreased at higher contamination levels (25–45 mg Cd kg^{-1} soil). Slower development and mostly weaker tillering of barley plants expressed this chronic Cd toxicity. Some negative impact on leaf gas exchange in the earlier stages was detectable. The seedling characteristics of Cd-enriched seeds (1–2 mg Cd kg) were unaffected and barley plants grown on non-contaminated soils during the next year have grain Cd levels within the norm.
- The reductive analysis of factors limiting growth of Cd-exposed barley plants revealed photosynthesis retardation and water relations disturbances as the most important factors. Cd significantly decreased water potential and increased stomata limitation to CO_2 uptake. These responses were found to be very specific, as they were not expressed in the same manner in Cu-exposed plants having similar photosynthesis rate inhibition. Further, Cd induced weaker incorporation of 14C in the primary photosynthetic products and lowered the relative part of the labeled mono sugars in the total sugar fraction. In addition, Cd dramatically decreased in vitro measured activities of PS2 and PS1 due to mostly Cd-induced thylakoid lipid peroxidation and enhanced lipid degradation. Cd did not lower significantly the actual yield of photochemical energy conversion (Genty parameter) measured at steady-state photosynthesis, but its negative impact was easy for detection applying the so-called “fast fluorescence curves” by MINI-PAM.

Validation of visible leaf symptoms caused by heavy metal stress in sycamore maple

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Keywords: Heavy metal stress, visible symptoms, bioindication, microscopy, validation, cytochemistry, condensed tannins

Heavy metal (HM) stress can induce visible symptoms in leaves of different tree species. Once these symptoms have been validated, they can be used as bio-indications to follow up the uptake of HM or for monitoring purposes in a contaminated area. In this study, visual HM leaf symptoms were validated in sycamore maple (*Acer pseudoplatanus* L.) growing in experimental plots simulating young forest conditions. Dust particles collected from smelter filters were mixed into the topsoil by hand to increase soil levels of HM in the HM plot replicates at levels as found in moderately contaminated sites (Cd 10 mg/Kg, Cu 385 mg/kg, Pb 63 mg/kg and Zn 2700 mg/kg). Visible symptom development was followed up during the entire vegetation season. For validation purposes, 1) HM content of leaves was determined, 2) Zn was cytochemically revealed, 3) changes in the cell and tissue structure and the physiological responses to HM stress were characterised with microscopical analyses, and 4) the amounts in condensed tannins (a phenolic class of compounds involved in defence responses) were quantified biochemically. The first HM symptoms were observed during the third year of exposure to HM stress. They developed throughout the vegetation season as characteristic gradients starting from the annual shoot basis. They included necroses and brown stippling along the veins at the leaf basis, green stippling and chlorosis throughout the leaf limb. Zn was the main metal contaminant (in 2001: 182 vs. 70 ppm in HM vs. Control) imported in the leaf followed by Cu (7 vs. 5 ppm); Cd and Pb remained below detection levels. Zinc was cytochemically detected in vein tissues especially in the adaxial collenchyma. Stress reactions were observed in the conducting tissues of veins and in the assimilating tissues in the leaf blade especially near veins. They principally indicated an acceleration of senescence and an enhanced oxidative stress along HM traffic ways. Condensed tannins were slightly increased either where Zn was detected or in gradients in stressed tissue portions near veins. Validation analyses thus indicated that mainly Zn was moderately imported in leaves where it principally accumulated close to the transport tissues. HM caused cellular stress reactions, accelerated the leaf senescence and induced reliable visible symptoms, especially those in connection with the vein system. Condensed tannins were apparently implied in Zn detoxification or in quenching the oxidative stress resulting from HM toxicity. In conclusion, this study showed that leaf symptoms like basipetally-increasing gradients along the annual shoot of necrotic areas and brown stippling in the leaf blade next to the basis of leaf veins provide reliable bioindications of HM stress in sycamore maple.

Heavy metal transport by AtHMA4, a P-type ATPase of *Arabidopsis thaliana*

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Keywords: AtHMA4, heavy metal, transport, *Arabidopsis thaliana,* shoot translocation

Zinc homeostasis implies both its acquisition, its transport among plant tissues and its appropriate subcellular compartmentation. These events involve the coordinated action of membrane transporters from various protein families including CDFs, ZIPs, NRAMPs and P_{1B}-ATPases. Among the 8 P_{1B}-ATPases present in the Arabidopsis genome, AtHMA2/3/4 form a cluster of closely related proteins which were predicted to transport the divalent cations Zn, Cd, Co and Pb. Many homologues of these pumps have been characterized in microorganisms where they participate mostly to the release of Cd or Zn into the extracellular medium. There is however no known homologue of Zn/Cd/Pb/Co P_{1B}-ATPase in the animal kingdom. How do participate AtHMA4 to metal homeostasis in multicellular organisms such as plants is then a challenging question.

A fusion with the GFP protein in C-ter allowed us to localize AtHMA4 at the plasma membrane through expression in protoplasts, suggesting an involvement in the transport of metals from the cytoplasm of cells to the apoplast. Histological studies were performed with the *iudA* gene as reporter. AtHMA4 expression was found in stellar root cells surrounding the conducting vessels, in stems at the level of leafstalks insertions and in stamens. Compared to the control, plants of a knock-out line do not present any obvious phenotype except they contain lower levels of Zn in rosette leaves whatever the concentrations of Zn in the nutritive solution (from limited to toxic), suggesting a defect in Zn transfer from roots to shoot. Similar results where found with Cd translocation when plants were exposed to this toxic element. AtHMA4-overexpressing lines displayed higher Zn and Cd transfer levels from roots to shoot. Plantlet root growth was found to be substantially less affected by the presence of toxic concentrations of Zn, Cd, Co and Ni for these overexpressing lines compared to the wild-type control. Taken together, our results suggest a role of AtHMA4 in the Zn/Cd xylem loading. The strong expression levels of AtHMA4 in anthers and in petiole insertion areas probably reflect other functions of this transporter *in planta*, which remain to be determined.

Can a contaminant be both a toxin and a resource for biological communities?

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Keywords: selenium, toxin, phytoproduct

Interest in selenium pollution and remediation technology has escalated during the past two decades. Although not known to be essential for plants, selenium is essential but could be toxic for humans and animals, depending on its concentrations. A major selenium controversy in the 1980's emerged in California when the general public and scientific community became aware of selenium's potential as an environmental contaminant on fish, waterfowl, agricultural-based industries, and in rare cases-humans!. After extensive research on several strategies to reduce loads of mobile Se for entering the agricultural ecosystem, a plant-based technology defined as 'phytoremediation' received increasing recognition, as a low-cost environmentally friendly approach for managing Se in the soil and water environment. Successful long-term field remediation of Se by plants is, however, dependent upon acceptance and widespread use by growers, who are also concerned about potential commercial value from using the plant-based technology. Obtaining products with economic value from plants used in the cleanup of soil would certainly be an additional benefit to phytoremediation, which could help sustain its long-term use. In this regard, new phyto-products were developed from plants used in the field phytoremediation of Se in central California. These included; Se-enriched broccoli, Se animal feedstuff, and organic fertilizer to enhance human and animal nutrition, and canola oil for use as a blend with diesel fuel in the production of biofuel. This presentation discusses the production of these alternative, potentially cost-rewarding phytoproducts from plants grown for managing toxic levels of Se under field conditions.

Heavy metal ion-induced gene and protein expression in barley (*Hordeum vulgare* L.)

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Keywords*:* cadmium, mercury, platinum, transcriptom, proteom

Heavy metal ion-induced gene expression was studied in barley. Plants were grown for 6 days in the presence of Hg2+, Cd2+ and Pt4+, respectively and gene expression was studied by suppression subtractive hybridisation. Analysis of differential expressed clones in root, as well as in leaf tissue, resulted in the identification of genes encoding proteins rich in SH groups, involved in detoxification processes, energy metabolism, metal ion uptake, reductive potential, water balance and pathogen defence. Most of the isolated clones were affected only by a specific metal ion application. However, also common induced genes were found. Proteom analysis of Pt4+-treated barley resulted in an increased amount of several heat shock proteins and a superoxide dismutase. Two genes that were induced at the transcript level were repressed at the protein level. In addition a comparison of differential expressed genes in cv. Barke versus cv. Scarlett upon heavy metal ion application will be presented.

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Phytoremediation potential and tolerance of *Salix* spp. – *Amanita muscaria* symbiosis to heavy metals

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Keywords: phytoremediation, heavy metals, *Amanita muscaria, Salix* spp.

Ectomycorrhizal (ECM) fungi play an important role in plant adaptation to contaminated soils. However, little is known about the role of ECM on the growth and metal uptake of specific plants utilized for phytoremediation such as willows (*Salix* spp.). Different *Salix* species showed excellent potential for phytoremediation of heavy metal contaminated soils. The present work evaluated the effects of the ECM fungus *Amanita muscaria* on the efficiency of phytoextraction of heavy metals (Cd, Cu, Pb, Zn) from contaminated soils using one *Salix* x *dasyclados* and one *S. viminalis* clone in a pot experiment.

Ectomycorrhizal colonisation after inoculation was significantly higher on *S.* x *dasyclados* than on *S. viminalis*. The inoculation with the ectomycorrhizal strain increased the shoot lengths of *S.* x *dasyclados* without changing the number of shoots per plant and decreased the shoot lengths of *S. viminalis*, but increased the number of shoots per plant. The concentrations of Cd and Zn in the shoots were increased after inoculation with *A. muscaria*. The inoculation with *A. muscaria* significantly changed growth and heavy metal uptake of the willow clones. The results demonstrate the importance of incorporation of ectomycorrhizal effects in phytoremediation planning.

Heavy metal micro-localisation and stress reactions in fine roots of *Populus tremula* (L.)

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Keywords: soils, roots, heavy metals, cytochemistry, microscopy, EDX-SEM

Heavy Metal (HM) contamination is widely spread and decreased soil fertility and restrictions in land use may result. Understanding HM fluxes and toxicity is required in view of soil decontamination and land management. In the interdisciplinary project 'From cell to tree' the effect of HM contamination on afforested 'model ecosystems' growing in large lysimeter facilities has been investigated.

The aims in this study were to analyze HM allocation and stress reactions in the different tissue types of fine roots of metal-sensitive *Populus tremula*. HM distribution and micro-localisation were investigated with several cytochemical and physical methods by transmission and fluorescence light and scanning electron (SEM) microscopy. For the cytochemical detection of Zn and Cu, 30 and 60 μm-thick hand sections were obtained either from 2.5% glutaraldehyde-fixed or fresh samples. They were coloured using two specific staining methods for Zn and one for Cu. Physical detection of Zn and Cu was carried out by means of Energy Dispersive X-ray analysis (EDX) using fresh samples immediately frozen in the field or critical-point dried after fixation with 2.5% glutaraldehyde. Structural changes and defence reactions were investigated in semi-thin sections (2 μm, embedded samples) after meta-chromatic and specific staining. Three root zones were analyzed: conducting roots with well developed secondary tissues (sometimes more than 1 year old), conducting roots with mostly primary tissues, new roots in the absorption zone. Both Zn specific cytochemical methods provided similar results with only minor micro-localisation differences. Zn signal amounts were only slightly reduced in the 2.5% glutaraldehyde-fixed in comparison to the fresh samples, especially in the conducting elements of xylem. One of the cytochemical methods for Zn with a strong and specific fluorescent signal allowed us to detect and compare this irregularly distributed element at tissue and cell level and on the entire cross section. Zn distribution maps were obtained merging detail pictures of the coloured hand sections. Each tissue allocated Zn in a characteristic way inside one or several of its apoplastic and symplastic compartments. Zn was especially frequent in conducting roots with well developed secondary tissues where it was mainly observed in periderm followed by xylem (II and I). A signal was also observed in cortex, phloem and cambium. Samples form the contaminated soil exhibited a slightly stronger Zn signal than the controls. Cu could not be detected cytochemically. Zn maps were used for EDX-SEM analyses. This technique was less sensitive than the cytochemical methods to detect Zn but could also reveal Cu. Cd signal could not be differentiated from K. Changes in the cell and tissue structure related to Zn allocation and apparent concentration.

Cytochemical detection of HM in plants is rare. One of the Zn methods in use here has never been applied to plant material yet. Zn maps allowed us a focused and more successful metal detection with EDX-SEM in contrast to the usual random and blind research for metal elements. Cytochemical detection thus complemented the EDX-SEM analyses by providing 1) a superior detection sensitivity 2) an overview of Zn distribution in all tissues and 3) an overview of Zn distribution between the different organelles at the cell level. For the efficient combination of cytochemical and physical techniques the cross identification of tissue elements requires good quality images.

Phytoextraction of Zn using *P. tremula* plants was demonstrated here with metal contaminants crossing apoplastic and symplastic barriers and finally uploading in conducting elements for transport to above-ground plant parts.

Evolution of a heavy metal ecotype in Silene

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Keywords: Silene, heavy metal, ecotype, genetics

The genetics underlying habitat adaptation and the evolution of ecotypes is of great interest to evolutionary biologists. Serpentine areas represent an ideal system for studying these processes. Using an F2 mapping population derived from a cross between a serpentine and a non-serpentine ecotype of Silene vulgaris, the genetic architecture of 7 morphological, physiological and life-history traits was explored. A QTL analysis identified 23 QTLs, 14 of which were classified as major QTLs. Five linkage groups harboured overlapping QTLs for different traits, suggesting either pleiotropy or linkage. This genetic architecture indicates that adaptation to serpentine can evolve rapidly and that traits potentially involved in adaptation have diverged as a consequence of directional selection.

Soil arthropods in metal polluted soils: is the taxonomic aggregation a valuable tool to detect changes in oribatid coenoses?

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Keywords: taxonomic aggregation; metal pollution; oribatid mites

Soil arthropod communities are known to be sensitive to heavy metal pollution because the enhanced bioavailability of metals may affect their physiology and/or ecological strategies. However, in a specific taxon, pollutant effects may be shared at taxonomic levels higher than the species rank, and, as shown by marine invertebrate communities under human disturbances, little multivariate information could be lost in the taxonomic aggregation of species data to higher taxa. This hypothesis was tested in oribatid mite assemblages from Zn, Cu and Pb polluted soils in a abandoned mining and smelting area in southern Tuscany, Italy. Results indicated that species data, when aggregated to genus or to family rank, lead to the same multivariate result with little loss of information. Considering the key-role of Oribatida in soil food webs this is a promising result in view of the possible use of the community level as an initial tool for monitoring metal polluted soils.

Fluxes of trace metals in agro-ecosystems in the North of France

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Keywords: atmospheric deposition, agricultural inputs, drainage water, crops

The objective of this work is to quantify the fluxes and the balances of trace metals (Cd, Cu, Zn) in the cultivated horizon of three agro-systems (situated in the North of France) which have been submitted to different sources of contamination.

The input fluxes (atmospheric deposition, agricultural inputs) and output fluxes (crops, draining water) are considered for calculating the balances; in order to draw conclusions on the sustainability of the agro-ecosystems.

The selected sites have more or less the same soil characteristics, but with different histories of agricultural practices and contamination. Table 1 presents the main characteristics of plough horizons. The first plots are located near Versailles and correspond to an experimental field where we considered 2 systems: organic and productive. The second is in the region of Vexin with a long history of urban sewage sludge application (30 years). The third one is near Mortagne-du-Nord (M-d-N) with soils contaminated for 60 years by industrial atmospheric fallout produced by a previous zinc-smelter complex.

Input fluxes: Atmospheric deposition: A collector was installed in Versailles' site, and a 7 days sampling was performed from 1 October 2001 to 22 November 2002. For the other sites, results were obtained by people working on them.

Agricultural inputs: Fertilizers applied in the studied plots were analyzed for trace metals contents using a flame (Zn) and furnace (Cd, Cu) atomic absorption spectrometer (AAS Varian SpectrAA 220), after being digested in a microwave oven (MarsX, CEM corp.). In the case of M-d-N, recent sewage sludge applications have been taken into account, from the statutory analysis and records.

Output fluxes: Drainage water: Tubes of PVC with a diameter of 10 cm and a length of 45 cm were used for sampling undisturbed soil columns from the surface to 25–30 cm depth. A Teflon end cap with 8 mm hole in the middle was fixed at the bottom. The water drains through the hole into the plastic bottle installed under the column. Three replicates of undisturbed soil cores, were obtained from the middle of each plot. The columns were fixed outdoor, in the shade and submitted to rain. Water samples were filtered through 0.20 μm Minisart membranes and acidified (1%) with HNO_3 (69% Prolabo Suprapur). The obtained solutions were stored at 4°C before analysis.

Crops: Wheat samples were digested (microwave oven MarsX, CEM corp.) and then analyzed by atomic absorption spectrometry and the fluxes were obtained using the yield of each plot, except for the crop at Vexin's (beet) where the results were obtained from the literature.

Fluxes and balances for Cd, Cu and Zn are calculated for each plot.

For Zn a positive balance is observed in the case of M-d-N, (and for Cd in Versailles PRO, where inorganic fertilizers are used), which should lead to a trace metal accumulation in the plough horizon of this plot. This is mostly due to atmospheric deposition and the use of sewage sludge in M-d-N. The fluxes from agricultural inputs are estimated to be high for the plot of Vexin, considering that sewage sludge were applied many years ago.

Trace metal accumulation or not is observed according to the fluxes of each plot. The higher fluxes were estimated to be the input ones in the case of M-d-N and Versailles PRO.

Copper bioavailability to plants as affected by root-induced rhizosphere processes

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Keywords: bioavailability, copper, phytosiderophore, pH, rhizosphere, root

Copper bioavailability to plants is expected to vary with root-induced changes in rhizosphere pH and exudation of C-compounds that can complex Cu. This was studied in vineyard soils that had been contaminated by a long history of spraying Cu-based fungicides. Such Cu-salts have been heavily used in vineyards since 1885 to control mildew, yielding total Cu contents in topsoils of 50–1000 mg kg^{-1} (10–100 fold the background level). Copper is one of the most phytotoxic metals, resulting essentially in rhizotoxicity, similarly to the better-documented Al rhizotoxicity (Chaignon and Hinsinger 2003). Cases of Cu rhizotoxicity have been seldom reported for vines. In the Languedoc region of Southern France, extensive areas of vineyards have been used for growing other crops, including plant species that might be more sensitive to Cu phytotoxicity than vines. This paper summarizes results obtained for various plant species grown in soils sampled in vineyard topsoils. Plants were grown in an experimental device designed to provide an easy access to roots and rhizosphere (Chaignon and Hinsinger, 2003). A first experiment conducted with 29 soil samples showed that shoot Cu varied little in tomato, while root Cu concentration was a more sensitive indicator of Cu bioavailability: in the 24 calcareous soil samples, it increased linearly with total soil Cu, while the most acidic soil samples resulted in much larger root Cu concentrations. Another experiment with rape and tomato grown in a very acidic soil and in calcareous soil exhibiting similar levels of total and EDTA-extractable Cu confirmed the greater bioavailability of Cu in the acidic topsoil. However, it was found surprisingly that Cu bioavailability increased with increasing rhizosphere pH, possibly due to enhanced adsorption of Cu on root cell walls (apoplasm). This was further confirmed when liming this acidic soil to yield pH ranging from 3.7 to 6. Apoplastic Cu in rape roots was found to increase with increasing pH. However, the bioavailability of soil Cu as assessed by either root (total) or shoot Cu was not influenced by liming. Remarkably, rape acidified its rhizosphere at pH above 4.8 and alkalinised its rhizosphere at lower pH. The absence of pH dependency of Cu bioavailability might be partly due to such root-induced pH changes which somewhat counterbalanced the effect of liming: a much smaller range of pH and consequently, of $CaCl_2$-extractable Cu was found in the rhizosphere, compared with uncropped soil. Another experiment with tomato confirmed that the decrease in $CaCl_2$-extractable Cu in the rhizosphere was rather related to root-induced pH changes (alkalinisation at low soil pH) than to plant uptake. This experiment comparing Fe-deficient and Fe-sufficient tomatos showed that the bioavailability of Cu significantly increased with Fe-deficiency. This could not be directly related to either rhizosphere acidification or reduction, as expected to occur in response to Fe-deficiency. In comparison, a considerable effect of Fe-deficiency was found to occur in wheat plants grown in a calcareous soil. Copper bioavailability increased 3–4 fold in Fe-deficient wheat, possibly due to the 3–4 fold increase in phytosiderophore secretion, as these exudates can strongly complex Cu.

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Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.). Macrolocalization and phytotoxic effects of cadmium

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Keywords: autoradiography, cadmium, metal allocation, *Salix viminalis*, tolerance, toxicity

Willows have been shown to be promising for Cd phytoextraction. Nevertheless, plant responses to Cd are still not clearly understood. We investigated the effects of Cd accumulation on morphological parameters, and Cd allocation in leaves by autoradiography in *Salix viminalis* grown in hydroponics with increasing concentrations of Cd (0–200 μM) to assess the effect of Cd localization on plant performance.

Willow was highly tolerant to Cd, with only an 18% shoot and no significant root biomass reduction at 20 μM Cd, although Cd concentration in shoots exceeded 100 mg kg^{-1}. At 50 μM significant reduction in root and shoot biomass and total root length, as well as change in root architecture was observed. At 100 μM, *S. viminalis* exhibited strong stress symptoms, whereas 200 μM impaired survival. Cadmium accumulation and the intensity of visible phytotoxicity symptoms on the leaves increased from 3 to 50 μM and then decreased at higher concentrations.

At all Cd concentration exposure Cd was localized mainly in the tips and edges of the younger leaves, whereas it was mainly located at the base of the older leaves. This localization coincided with visible necrotic spots and indicated that Cd tolerance had been exceeded.

Comparison of root morphological plasticity in metallicolous and non metallicolous ecotypes of *Thlaspi caerulescens*

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Keywords: ecotype, metallicolous, heterogeneous soil, root foraging, heavy metals

Thlaspi caerulescens is a plant species able to accumulate zinc, cadmium and nickel up to very high concentrations in the leaves. One of mechanisms that may contribue to hyperaccumulation is the proliferation of roots in patches of soil containing higher concentrations of heavy metals (Haines 2002; Whiting *et al.* 2000; Schwartz *et al.* 1999). This positive response of roots to heavy metals was only shown in metallicolous populations. Now, we know that populations from normal soil have higher zinc accumulation capacity compared to populations from metalliferous soil (Assunção *et al.* 2003; Escarré *et al.* 2000; Meerts and Van Isacker 1997). In this study, we compare root proliferation capacity in response to patchily distributed metals between the ecotypes of *Thlaspi caerulescens* (metallicolous and non metallicolous). More specifically, we test if the higher accumulation capacity of non metallicolous ecotype can be explained by a higher proliferation of roots in patches of metal.This behaviour was tested for a heterogeneous distribution of Zn, Cd and Ni.

As Whiting *et al*. (2000), we used a rhizobox system to estimate the effects of localized zinc (ZnO), cadmium (CdS) and Nickel ($Ni(OH)_2$) on the allocation of root biomass, root length, accumulation of metals and other nutrients.

For Zn heterogeneous treatment, NMET ecotype developed higher root allocation into Zn enriched compartment than the MET one. No response to heterogeneity of Cd was noticed for the two ecotypes. For Ni, only the MET ecotype exhibited «nickelophilic» root foraging in response to heterogeneously distributed Ni. For both ecotypes, an increase of biomass production (roots and leaves) was observed in the Zn heterogeneous treatment. The significance of the results to the understanding of the contrasting metal accumulation patterns of the two ecotypes will be discussed.

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Phenolic substances as Cr(VI) decontaminating agents

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Keywords: caffeic acid, chromium, detoxification

The most diffuse species of chromium in the soil-plant system are Cr(III) and Cr(VI) which differ in their chemical and biological behaviour. Compared to Cr(III) species, anionic compounds of Cr(VI) (CrO_4^{2-}, $Cr_2O_7^{2-}$) are more toxic environmental pollutants due to their high mobility. In natural systems the Cr(VI) toxicity can be buffered through redox reactions. Phenolic substances, such as caffeic acid (CAF), may play an important role at this regard. In this communication we report the reducing activity of CAF towards Cr(VI) in aqueous phase. The reactivity of *o-, m-,* and *p*-coumaric acids was also investigated.

The redox reaction between Cr(VI) and CAF was studied under air as a function of both time and pH at different initial metal concentrations (from 24.8 to 300 μM). The reaction was particularly effective at pH 2.5 and a linear increase in the yield of Cr(III) was found with increasing Cr(VI) concentration until constant values were reached at Cr(VI)/CAF molar ratios higher than 3.4. On the basis of the results the following stoichiometry was established:

CAF + 3 Cr(VI) $\rightarrow$ 3 Cr(III) + degradation products

The kinetic data indicate that the reaction proceeds through two steps: the first is faster and involves 4 electrons, the latter 5 electrons. The chromatograms are characterized by the presence of intermediates with different redox activity and a retention time lower than that of CAF. These products can be regarded as more polar or with a molecular weight lower than that of CAF.

A yield of Cr(III) (180 μM) equal to that obtained at pH 2.5 and pH 3.1 in about 7 and 25 hours, respectively, was reached at pH 4.2 only after a much longer reaction time (150 hours). At pH > 4.2 the reaction occurred even more slowly, and its kinetic trend was more and more difficult to study with increasing pH from 5 to 6 because of the formation of chromium hydroxide or Cr(III)-CAF precipitates, the latter suggested by HPLC tests. Systems at pH > 6 could not be studied due to the CAF instability.

The other phenolic acids investigated (*o-, m-, p*-coumaric acids) show a reducing activity negligible compared to that of caffeic acid: about 30% of *p*-coumaric acid was reduced at pH 2 after two months of reaction.

The results indicate that caffeic acid reduces Cr(VI) through a mechanism which differs from that previously observed for Fe(III) and within a wider pH range. This allow to consider both CAF and other structures with o-phenolic groups as decontaminant agent towards this dangerous metal ion in the environment. Studies about the nature of the CAF degradation products as well as the influence on the redox reaction of several organic acids released by the root plants are in progress.

Manganese toxicity and tolerance in two races of Douglas fir seedlings *(Pseudotsuga menzesii)* var. *viridis* and var. *glauca*

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Keywords: manganese, toxicity, tolerance, Douglas fir

Excess Mn, which may occur for example under acid soil conditions, causes injury to plants (Ducic and Polle, 2005). In some sites in Germany decline of Douglas fir has been related to Mn-overaccumulation and appeared to affect especially the race *glauca*. Little is known about the uptake and translocation of this element in conifers and the physiological mechanisms resulting in differences in Mn-sensitivity between genotypes (Rengel 2000). In the xylem sap Mn moves freely in the transpiration stream and accumulates in roots, stem and leaves in a pattern described as “phloem immobile” (Loneragan 1988). The aim of our work was to investigate manganese uptake, its subcellular localisation under normal conditions and excess Mn-exposure to characterise Mn-toxicity in two races of Douglas fir, *viridis* (tolerant) and *glauca* (sensitive).

The effect of Mn was studied for two weeks in six-weeks-old Douglas fir seedlings grown in hydroponics. We measured root and shoot growth and biomass during the treatment with 5 mM Mn. Element analyses on the whole plant level was carried out using ICP-OES method and element localisation on cellular level was done using EDAX-EM method. Mn transport was determined by radioactive labelling.

Mn stress had negative effects on biomass production and affected growth of roots especially in *glauca* and shoot biomass more in *viridis*. On the whole plant level Mn stress led to increases in Mn in roots by factors of 68- and 14-fold in *viridis* and *glauca,* respectively. The Mn-accumulation in needles was significantly stronger in *glauca* than in *viridis*, which was also evident under normal conditions since radioactive labelling revealed higher uptake rates and transport to above-ground compartments in *glauca* than in *viridis*. Measurements at the subcellular level showed that *glauca* displayed higher Mn concentrations in every compartment of needles than *viridis*. After treatment, phloem in needles content 10–100 folds higher Mn-concentration. In root cells Mn increased 100–1000 folds after exposure to Mn-stress. In epidermis and cortex cell the appearance of “black bodies” – points with approximatively 1000 mM Mn concentration was observed. Mn was correlated in these points together with Ca and P. Density of black bodies in roots was much higher in case of *viridis* (tolerant spices) than in glauca, possibly pointing to a detoxification mechanism.

Our data show different distribution of Mn across the whole plant in the two genotypes of Douglas fir *viridis* and *glauca*. Higher Mn-translocation to *glauca* needles could explain higher its sensitivity. The retention of Mn in the root of *viridis* could be a protecting effect for the shoot.

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Local adaptation of mountain birch to heavy metals in a subarctic industrial barren, and the search for mechanisms behind facilitation

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Keywords: mountain birch, heavy metals, trade-off, facilitation, stress, local adaptation

Local adaptation to stress has often been found to result in a trade-off with performance in pristine conditions; stress-tolerant individuals often express slow growth with poor competitive ability. Another stress-related theory is the increase of positive interactions (facilitation) with increasing stress. In low-stress environments where competition dominates, individuals should do better alone, whereas in high-stress environments positive interactions should become more important and individuals should do better grouped. The subarctic industrial barrens of Kola Peninsula, NW Russia, are characterized by anthropogenic stressors such as sulphur dioxide and heavy metals, and due to forest decline also natural stressors such as high wind stress and winter freezing due to thin snow cover. In past studies in industrial barrens we have found that mature trees facilitate the growth of dwarf shrubs and mountain birch seedlings, and in ongoing experiments we are studying seedling-seedling interactions in stress gradients. Possible mechanisms for facilitation include protection from wind and, due to snow accumulation, protection from freezing. In the current greenhouse experiment we addressed the questions 1) has there been local adaptation specifically to toxicity stress in the 60–70 years of smelter operation, 2) what are the important stressors (and relatedly, possible mechanisms of facilitation) for mountain birch and 3) does adaptation to natural stressors (wind, freezing) also help cope with toxicity of pollutants. To check this, seedlings from the extreme ends of three kinds of stress gradients (each in two replicates) were used; pollution gradients around two nickel-copper smelters, two altitudinal and two seashore gradients. Seedlings were planted in groups of four to check the competitive ability of seedlings from different stress origins. Half of the seedlings had heavy metals added to the soil (170mg/kg Ni, 80 mg/kg Cu) over time to see how it affected the competition balance, and half of the pots had the mycorrhiza *Paxillus involutus* added, as the role of mycorrhiza in polluted habitats is still largely unclear. During winter half of the seedlings will be lightly frozen to simulate the thin snow cover. Seedlings were also planted alone to verify competition. Local adaptation to heavy metals was detected for seedling from the pollution gradients; stress origin×pollution interaction was significant for both leaf length ($p=0.0048$) and seedling height ($p<0.0001$). Seedlings from polluted environments did better than control seedlings in the pollution treatment and, vice versa, control seedlings were stronger in control treatment. No such interaction was found for seedlings from the natural stress gradients. In greenhouse conditions heavy metal contents of roughly one third of what occur in industrial barrens were enough to significantly suppress growth and cause heavy mortality. The effects of mycorrhiza were not straightforward. Our results indicate that heavy metals are a major factor affecting seedling establishment in the subarctic industrial barrens, and that the survival selection caused by pollutants has been strong enough to cause local adaptation in 60 to 70 years. This finding implies that, at least in our study environment, the success of restoration efforts could be increased by using seeds from local mother trees.

Evolution of metal tolerances and the implication on processes from the cellular to the whole-plant level

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Keywords: compartmentation, metallothionein, metal transporter, phytate, phytochelatin

A limited number of plant species have evolved mechanisms at the (sub) cellular up to the whole-plant level that allow them to establish and maintain populations on highly metal-enriched soils. High-level tolerances are confined to those metals present at high concentrations in the soil. Three model plant species, all without any significant mycorrhizal association, i.e. *Arabidopsis halleri, Silene vulgaris* and *Thlaspi caerulescens,* are currently in the focus of investigating metal tolerances because these species have ecotypes on soils with a huge range and combination of metal concentrations allowing intraspecies comparison.

Genetically the metal-specific tolerances are obviously controlled by one or a few genes and some modifyers, the best elaborated for tolerance to As. Cu, Zn, and partially for Cd, but not for the same degree. Physiologically the ecotypes have evolved metal-specific adaptations, however not metal-tolerant enzymes in the cytosol. Due to a lack of metal exclusion by metal tolerant ecotypes, metal uptake by root cells from a metal-enriched soil solution is controlled by up- resp. down-regulation of transmembrane metal transporters from the ZIP en CDF-families, followed by intra- and intercellular compartmentation and by binding to and/or precipitating metals in cell walls. Chaperones are candidates for the intracellular metal transfer to organelles and general metabolic processes whereas cytosolic metal surplus has to be avoided with help of rapidly metal-binding compounds such as phytochelatins, metallothioneins and organic acids, primarily in roots and secondarily in vegetative and generative aboveground plant parts. Due to the restricted capacity of root cells to store metals, the transfer across the endodermis is a not well understood critical step, followed by xylem loading and transfer into the various shoot parts with a high metal accumulation in the leaves. Although the degree of tissue-specific metal distribution in leaves is well-known, the molecular processes have still to be elucidated. The most protected plant part in metal-tolerant plants is the embryo in the seed by avoiding an overflow of metals which seems to be controlled by a switch of the metal transfer from xylem to phloem. The seed as a metal-poor niche in metal-enriched plants is a welcome target to seed predating insects which severely can decrease seed production and thus population rejuvenation.

Up to now there is just a glimpse of the complex processes of metal tolerances, still far away from understanding the adaption of plants to multi-metal enriched environments with additional selection pressure for adaptation to shortage in water supply and major nutrients. Despite evolution of metal tolerances is lasting for at least some hundred thousands of years, there are still extremely multi-metal-enriched soils without any plant growth demonstrating the limits of tolerance mechanisms.

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Ascorbate promotes emission of mercury vapor from plants

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Keywords: antioxidative defense, ascorbic acid, mercuric chloride, mercury vapour, phytoreductants

Mercury vapor (Hg°) emission from plants contributes to the atmospheric mercury cycle. Although a part of this Hg° emission originates from Hg(II) uptake by the roots, the question how terrestrial plants reduce Hg(II) has not been addressed so far. Young barley plants grown on a hydroponic cultivation containing Hg(II) increased the Hg° emission significantly. Homogenates of barley leaves added to dissolved Hg(II) induced a powerful volatilization at alkaline but not at acidic pH. The same pH dependence and emission kinetic together with the highest reduction capacity was observed for ascorbic acid as compared to other phytoreductants. The electrochemical potentials of the reactions involved suggest an electron transfer from NADPH via GSH and ascorbate to Hg(II). The results support the assumption of a novel mechanism how plants transfer reduction equivalents from the antioxidative defense system via ascorbate to reduce Hg(II) ions, thus counteracting mercury toxicity by volatilizing the metal. This effect appears to be assisted by other light-dependent processes such as transpiration and ascorbate synthesis. In addition to Hg^o emission in barley also results from tree saplings and branches will be presented

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Assessment HM critical loads for agricultural areas in the Crimea

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Keywords: cadmium, lead, critical loads, terrestrial ecosystem

The calculation and mapping of critical loads for Pb and Cd were carried out for drinking water protection in agricultural areas (effect No. 1) in the Crimea by initiative group. In accordance to ICP Modelling and Mapping (ed.) 2004, the calculation of critical loads of lead and cadmium was based on one of the suggested approaches, namely effect-based approach, accounting the possible negative HM influence on biota under definite (critical) concentrations of these metals in the environmental media (soil, soil solution, vegetation, etc). The calculations were based on the simplified mass balance of metals in 30 cm soil layer suggesting that the airborne input of HM into the agricultural ecosystem must not exceed the total fluxes of these metals from the system. As the most part of agricultural areas in the Crimea is the north territories, where wheat and other cereals take place. At first step only these cultures have been taken in account and the critical loads of metals (g/ha per year) were calculated:

CL(M) =Mu + Mle(crit), where: Mu is the metal net uptake in the harvestable parts (g/ha per year); Mle(crit) is critical leaching flux of heavy metal from the considered soil layer (g/ha per year). The metal plant uptake by wheat biomass was estimated as follows:

Mu = fMu*Yha*[M]ha, where: Mu is metal net uptake in harvestable parts of wheat under critical load conditions (g /ha/year); fMu is fraction of metal net uptake within the considered soil depth (0,3m), Yha is the yield of harvestable biomass (dry weigth) (kg/ha per year); [M]ha is the metal content in the harvestable part of wheat (g/kg dw);

Mle(crit)= 10*Qle*[M]ss(crit).

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Availability of heavy metals and arsenic in tailings and soils contaminated by mining activity (Northern Portugal)

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Keywords: bioavailability, heavy metals and arsenic, mine tailings, sequential extraction, soils

A chemical sequential speciation of heavy metals (Fe, Mn, Cu, Zn, Pb, Cd, Sn, W, Bi, Ni, Cr, Mo, Co) Al, Ti and As in tailings and soils samples around a five W/Sn mines (Adoria Mine, Ervedosa Mine, Regoufe Mine, Rio de Frades Mine and Tarouca Mine – Northern Portugal) was realized using a 7-steps fractionation procedure.

Each of the chemical fractions was operationally defined as (Dold and Fontboté 2001): (1) water-soluble; (2) exchangeable; (3) bound to Fe oxyhydroxides (or easily reducible); (4) bound to Fe oxides (or moderately reducible); (5) bound to organic matter and secondary Cu-sulfides; (6) bound to primary sulfides; (7) residual.

The extracted element contents at each step were measured by ICP-MS. Samples were also analysed for pH, electrical conductivity and organic matter.

The results allowed us to notice that: 1. The pH was the main factor for controlling the geochemical distribution of the studied elements. The tailing and soil samples were very acid, with an average pH of approximately 4,37. Some metal cations (Mn, Cd, Cu, Zn, Pb, Co, Cr, Ni) behave in a similar way, revealed important enrichments in the most bioavailable fractions (water-soluble and exchangeable fractions). In contrast, oxyanions as Mo and As show low mobility through adsorption to Fe(III) oxyhydroxides dissolved in the two reducible fractions. These results reflect the pH dependent adsorption on the clay minerals, Fe and Mn oxyhydroxides and the co-precipitation with these secondary minerals; 2. Residual fraction was the most important fraction for Sn, Ti, Al, Mn, V, Cr and Zn. These results suggest that in these soils these elements are in a detrital, non available form; 3. Large amounts of Fe, Mn, Cu, Zn, Pb, Cd, Bi, Cr, Ni, Co and As were present in the most bioavailable fractions in all sampling sites as consequence of the oxidation and acidification processes in sulfidic mine wastes; 4. Scavenging of mobilized elements (mainly Fe, Mn, Cu, Zn, Cd, Pb, W, Bi, Mo, V, Cr, Ni, Co and As) in secondary (oxihydroxides) mineral phases and sulfides could be considered a temporary mechanism of metal retention. These metal fractions are susceptible of being set free depending on some changes of physical-chemical environmental conditions.

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Soil ecological impact of a hotspot Cu contamination

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Keywords: soil ecotoxicology, copper, field assessment

The ecotoxicity of copper is well established. A multitude of test results in the literature for earthworms (mortality, reproduction, avoidance), other soil organisms, plants, and soil respiration supports the assumption that there should be a severe impairment of ecological soil functions at copper contaminations above 500 mg kg^{-1}. Here observations from a spot (15 m x 20 m) with a copper contamination up to more than 20 000 mg kg^{-1} are reported: How did the ecosystem adapt?

The available Cu (Ammonium Nitrate extraction) amounted to more than 75 mg kg^{-1}.The area containing the contaminated spot was under pasture use for 15 years. In 2000 it was fenced and planted with trees and shrubs.

In the field the extremely contaminated place was well vegetated but recognisable by its spontaneous vegetation (high dominance of Urtica dioica) and a mor / moder like humus form with plant residues and a dense root mat of nettles overlaying the mineral soil. Leaf chlorosis (indicating copper intoxication) occured only in some of the trees that were planted at the contaminated hot spot. Soil microbial parameters (field respiration, basal respiration, microbial biomass) and decomposition of plant residues in litter bags differed not significantly between the hot spot and an adjacent reference plot with low Cu contamination. In contrast the feeding activity of soil animals (assessed with the bait lamina test) was extremely reduced as compared to the reference plot.

Tests in the laboratory using dilutions with uncontaminated soil showed that the soil substrate was most toxic to earthworms (avoidance reaction of Eisenia fetida) less toxic for plants (growth depression of Lepidium sativum) and least toxic for microbial respiration (determined according to ISO 17155). MPN-assessments showed a higher share of Cu-resistant microorganisms in the extremely contaminated soil as compared to the reference soil with a Cu concentration around 100 mg kg^{-1}.

It is concluded that the contamination disrupted the soil ecological system. A decomposer refuge and shortcut nutrient cycle developed in the litter layer on top of the contamination. Bioturbation is the most affected soil biological process, therefore the spread of the contaminated substrate into the surrounding soil is minimized. Trees are probably able to circumvent the contaminated zone with their root system.

Cadmium affects tobacco cells by a series of three waves of reactive oxygen species that contribute to cytotoxicity

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Keywords: heavy metals, oxidative burst, NADPH oxidase, calcium signalling, lipid peroxidation, hydroxy radical, hydrogen peroxide, superoxide anion, cell death

Cadmium is suspected to exert its toxic action on cells through oxidative damages. Nevertheless, in a biological environment, the transition metal is unable to directly generate reactive oxygen species (ROS) *via* redox reactions with molecular oxygen. Therefore, mechanisms by which oxidative stresses may arise from cadmium action are still unclear. Here we show that BY-2 tobacco cells challenged with millimolar concentrations of $CdCl_2$ developed cell death within 2–3 h. The death process was preceded by two successive waves of ROS differing in their nature and subcellular localization. These consisted, first, in the transient NADPH oxidase-mediated production of H_2O_2 (starting 5 min post treatment) followed by the mitochondrial formation of $O_2^{-\cdot}$ (from 15 min post treatment). Finally, a third wave of ROS consisting in fatty acid hydroperoxide accumulation was concomitant with death. Production of H_2O_2 was preceded by an increase in cytosolic free calcium from internal pools that was essential for NADPH oxidase activation. The initial production of H_2O_2 was not sufficient to induce most of the $CdCl_2$ effects in that the gp3 cell line, impaired in NADPH oxidase activity, went through cell death. Rather, our data indicated that the mitochondrial $O_2^{-\cdot}$ production along with membrane peroxidation were both deciding factors leading to Cd^{2+}-induced cell death.

From Cell to Tree

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Keywords*:* heavy metal allocation, trees, ecosystem, acid rain, soil acidity

The project 'From Cell to Tree' http://www.waldschutz.ch/bioindic/monte_verita/ was designed to investigate in near-natural model forest ecosystems the fate of mixed heavy metal contamination in the topsoil and its effects on plants and organisms with the following modifying factors: subsoil acidity, precipitation acidity, root pathogen, chambered lysimeters *vs*. field conditions, different climate during 4 years, deciduous trees *vs.* spruce and understorey plants, competition and provenance. A controlled experiment in large hypsometers and a field experiment with similar treatments together with experiments in the lab and sampling at contaminated field sites were established. The participation of 30 projects (13 institutes) from different disciplines was organised in a matrix structure. The projects were independent, with different size, starting time and length, but working together when appropriate. The large experiments were started in the year 1999 and run from 2000 to 2004 and 2005 respectively, but many analyses, calculations and interpretations are still at work for scientific and public output.

The 16 chambered lysimeters arranged in a Latin square with 6 m^2 area, 3 m height, 1.5 m depth (15 cm topsoil, 80 cm subsoil and 50 cm drainage layer of increasing diameter of pure quartz gravel) were split each in a (otherwise similar) calcareous and a acidic subsoil compartment. Two additional lysimeters were not chambered. Four treatments were applied: 1) control; 2) heavy metal contaminated topsoil (dust from a smelter: 2700 Zn, 385 Cu, 83 Pb and 10 Cd mg/kg); 3) acidic irrigation established with artificial rain acidified by HCl and with exclusion of natural precipitation by automatically closing roofs; 4) metal contamination and acid rain. In the plots 6 spruces (3 provenances), 8 deciduous trees and understorey plants at 20 places were planted per soil compartment. In the controlled field experiment (acidic subsoil only) 14 trees and 25 understorey places were assigned per each of the 20 plots (each 3 m^2 area), resulting in a total of 2064 trees and understorey assigned places.

The results include the metal availability, soil chemistry and water household, the different specific tolerance and heavy metal uptake in foliage and wood, the interorganismic effects (insects, fungi including pathogens, enchytraeidae and bacteria) and the cellular heavy metal detection and plant defence strategies. Overall the plants developed well with minor, specific growth decrease by the acidic subsoil and the metal contamination, with specific metal uptake (mainly Zn and favoured by the acidic subsoil) during four years (only Cd uptake decreased), but with only a few effects by acid rain. Cu was more allocated in the understorey plants than in trees, spruce was most tolerant with the least metal uptake in aboveground parts. Phytostabilisation was good with no metal dislocation in the subsoil or runoff in the drainage water.

Responses of young deciduous and coniferous trees to metal stress

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Keywords: heavy metal allocation, growth, gas exchange, acid rain

The project "From Cell to Tree" is designed to clarify responses of autochthonous, competing plants to heavy metal (HM) stress in the soil and to examine their potential of energy production, afforestation and landscape improvement through decontamination or long-term stabilisation. This part of the project focuses on the HM uptake and allocation as well as on the gas exchange and growth of young deciduous and coniferous trees under different environmental conditions.

In 16 large open-top chambers with 4 replicates per treatment, model ecosystems with *Salix viminalis* L., *Populus tremula* L., *Betula pendula,* and *Picea abies* (L.) Karst. were studied during 4 years on acidic (pH 4.2) or calcareous subsoil (pH 7.4). The treatments were: 1) uncontaminated topsoil plus rainwater of pH 5.5 (control); 2) uncontaminated topsoil plus rainwater of pH 3.5; 3) HM-contaminated topsoil plus rainwater of pH 5.5; 4) HM-contaminated topsoil plus rainwater of pH 3.5.

Filter dust from a non-ferrous metal smelter was mixed into the topsoil to ensure HM contamination that resulted in HNO_3-extractable concentrations of Cd = 10 mg kg^{-1}, Cu = 385 mg kg^{-1}, Pb = 63 mg kg^{-1} and Zn = 2700 mg kg^{-1}; concentrations of Cd, Cu, Pb and Zn were 0.1 mg kg^{-1}, 8.7 mg kg^{-1}, 7.9 mg kg^{-1} and 18 mg kg^{-1} in the control, respectively.

HM allocation in above-ground plant parts showed species and element dependency, with the highest uptake occurring in *P. tremula* (Cd) and *B. pendula* (Zn), and the lowest in *P. abies*. Cd and Zn where mostly incorporated into the foliage, whereas Cu remained in the wood. Furthermore, acidic subsoil favoured Cd and Zn accumulation in *P. tremula* and *S. viminalis*. Deciduous tree foliage and wood mass, height and stem diameter were on average reduced by HM and modified specifically by the subsoil type. Photosynthesis and transpiration were significantly reduced in *P. tremula* under HM treatment. The subsoil type had no influence on the gas exchange of either tree species. Year-by-year changes in growth dynamics as well as shifts in competitiveness of the various species modified treatment effects on water consumption and HM extraction patterns.

Amongst the deciduous tree species, *S. viminalis* had a lower HM uptake rate than *P. tremula* resulting in no negative effect of the HM on photosynthesis and transpiration. Both tree species had a higher Cd and Zn uptake on acidic rather than calcareous subsoil. As an early-succession species, *S. viminalis* invested more carbon into the wood than foliage production as compared with *P. tremula*. This allocation pattern of *S. viminalis* represents an advantage in terms of competition for space. *B. pendula* displayed a behaviour in carbon allocation similar to that in *P. tremula* with limiting effects on foliage development and growth. Only *B. pendula* responded significantly to the rain treatment of pH 3.5 with increased incorporation of Cd, Cu and Zn into the foliage.

The examined, autochthonous tree species can be used for wood production on sites of moderate HM-contamination, allowing landscape amelioration and stabilisation. *S. viminalis* as a vigorously growing pioneer species was found to be most HM-tolerant.

Effect of incubation time on Zn and Cu uptake by sunflower, barley and pea from soil contaminated with filter dust

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Keywords: heavy metals, plants, uptake, incubation time, speciation, metal immobilization

The bioavailability of heavy metals in contaminated soils depends on the kinetics of their release from contaminant phases and on the relevance of immobilizing processes such as the formation of metal precipitates or specific adsorption complexes. It may therefore change with time. In a project addressing the impact of heavy metal contamination on ecosystem functioning from the level of cells to trees (Cell-Tree project), non-calcareous soil (pH 6.5) in field lysimeters was contaminated with filter dust from a brass foundry (~85% ZnO, 10% $Cu_{0.6}Zn_{0.4}$). Spectroscopic data have shown that the ZnO completely dissolved within 9 months and that half of the Zn precipitated as layered double hydroxide (Voegelin *et al.* 2005). Brass particles ($Cu_{0.6}Zn_{0.4}$) are expected to dissolve more slowly. In this context, the objective of the present study was to investigate the effect of incubation time and concomitant changes in metal speciation on the bioavailability of Zn and Cu to plants.

Sunflower *(Helianthus annuus),* barley *(Hordeum vulgare),* and pea *(Pisum sativum)* were grown for 30 days in the climate chamber in soil that had been contaminated for 4 years (1770 mg/kg Zn, 380 mg/kg Cu), in freshly contaminated soil (2290 mg/kg Zn, 515 mg/kg Cu), and in uncontaminated soil (137 mg/kg Zn, 32 mg/kg Cu). Each experiment (soil×plant) was run in 4 replicates (pots).

While sunflower did not grow at all in the contaminated soil, pea and barley showed various symptoms of heavy metal toxicity: reduced height and dry weight (both plants), reduced evapotranspiration (pea), increase of fungal infection (barley), and strong reduction in the number of root nodules (pea). Comparing plants grown in freshly contaminated and 4 years contaminated soil, no systematic differences in the degree of toxicity symptoms could be observed. Plants grown in contaminated soil contained significantly higher levels of Zn and Cu both in roots and shoots than plants grown in the uncontaminated soil. The bioconcentration factors (= metal content in tissue/metal content in soil) of Zn and Cu in the roots and shoots of barley and pea were higher in the soil which had been contaminated 4 years ago than in the freshly contaminated soil, suggesting an increase in Zn and Cu availability to plants within that period of time. While barley contained higher concentrations of Zn and Cu in the shoots than in the roots, the opposite trend was observed for pea.

The increase in Zn and Cu bioavailability from the freshly contaminated to the 4 years incubated soil indicates that over that period of time, metal release from contaminant phases dominated over metal immobilization by precipitation and specific adsorption processes. With respect to Zn, the results of this study show that the relevance of metal precipitate formation as a mechanism for reducing metal bioavailability in soils may be limited and needs to be critically assessed.

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Parametrization of multi-scale root branching system uptake model

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Keywords: root uptake model, root input parameter, pot experiment, willow

Root uptake models operate on different spatial scales and therefore various complexity of information is introduced. As a result, different kinds of root and root system characterizations are required for the model parametrization. For root system uptake simulation a comprehensive knowledge of root branching structure and functioning and its alteration with time and space is required.

So far, the efforts have concentrated on modeling the nutrient uptake at a single root level (Schnepf *et al.* 2005). The aim of the current project is to generate realistic data for parametrization and corroboration of root system nutrient and water uptake model based on the mathematical model of Roose *et al.* (2001), Roose und Fowler (2004a, b). For this purpose, the model will be adapted to specific research problems related to the uptake of heavy metals by hyperaccumulating plants. The inclusion of mycorrhiza associations will also be assessed.

Pot experiments using Salix caprea were carried out to investigate whether the soil microbes and mycorrhiza fungi affect the metal uptake as part of a screening study for phytoremediation purposes (Dos Santos und Wenzel 2005). To provide with necessary model input parameter, water and solute depletion as well as root and plant development was observed. Time-Domain Reflectrometry (TDR) technique was applied for monitoring soil water changes. Root morphology was evaluated using image analyses approach according to Himmelbauer *et al.* (2005).

This paper discusses established difficulties in model parameterization in search of the essential root parameters as well as the way of deriving them from the existing experimental data. The results will serve as a basis for a future parameterization studies in order to improve rhizosphere model predictions and contaminated land management practices.

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Ectomycorrhiza's impact to change of speciations of Cu and Cd in rizhosphere of pine growing under excessive heavy metal soil

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Keywords: ectomycorrhizal fungi, *Pinus tabulaeformis,* heavy metal speciation

Ectomycorrhiza is a symbiont between ectomycorrhizal fungi and nutritive root of high plant, which can enhance the plants' growth in heavy metal stressed environment, and increase the host plants' tolerance to heavy metal pollution. With the development of research, it was found that ectomycorrhizal plants' resistant abilities to heavy metals were not only from the metabolically regulation by inoculation, but also from the great changes in ectomycorrhizosphere influenced by ectomycorrhizal roots. So far, it had been demonstrated by many studies that the speciation of heavy metal in rhizosphere and non-rhizosphere soil was remarkably different, but there are not much researches relevant to ectomycorrhizosphere.

This paper, which focused on two ectomycorrhizal fungi *Boletus edulis* and *Xerocomus chrysenteron*, mainly aimed to study heavy metal accumulation and micro-environment changes of mycelia *in vitro*, and the effects of single or co-inoculation on the growth of *Pinus tabulaeformis* seedlings. Moreover, through analysing heavy metal accumulation and distribution in the ectomycorrhizal *Pinus tabulaeformis* seedlings, the speciation of heavy metal in soil, and the changes of mycorrhizosphere, we further discussed possible tolerant mechanism of ectomycorrhizal fungi, which may gain through regulating the micro-environment.

Studies found that *Boletus edulis in viro* had a strong ability to absorb Cu and Cd, but the accumulation models of two heavy metals were significantly different. Mycelia could alleviate heavy metal damage by changing cultivation environment. In symbiosis experiments, compared with non-ectomycorrhizal ones, ectomycorrhizal inoculation not only enhanced the growth, but also reduced the concentration of heavy metals and their transportation from root to shoot of its host plants. Seedlings inoculated with dual-ectomycorrhizal fungi strains had higher heavy metal tolerance than those inoculated with single-ectomycorrhizal fungi strain.

The results of speciation analyses and micro-environment showed that compared with rhizosphere, there was a apparent tendency in heavy metal speciation of ectomycorrhizosphere, which changed from loose associated speciation to compact associated speciation. The content of exchangeable heavy metal reduced, and the content of organic bound increased in rhzospheral soil than in bulk soil. The changes of micro-environment in soil contaminated by Cu and Cd were significantly different. The transformation of speciation of heavy metals in ectomycorrhizosphere might be due to the changes of micro-environment caused by exudates of mycelium of ectomycorrhizal fungi, which could be one of the mechanisms in heavy metal tolerance of ectomycorrhizal plants.

Sequestration of cadmium in crystals excreted by tobacco

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Keywords: cadmium, tobacco, crystal excretion, SEM-EDX, µXANES, µXRD

Plants developed a number of strategies to resist the toxicity of heavy metals. Compartmentalization and sequestration in trichomes were found to be an important way for cadmium detoxification (Salt *et al.* 1995; Küpper *et al.* 2000). Exclusion of Cd by forming Ca/Cd -containing crystals, which are excreted through glandular trichomes, was also shown as a detoxification mechanism in Tobacco (Choi *et al.* 2001). Choi *et al.* demonstrated that increased levels of cadmium promote the formation of trichomes and the number of excreted crystals.

In this work, we intend to clarify the mechanism of Cd sequestration in the Ca-containing crystals excreted by Tobacco (*Nicotiana tabacum* L.). Scanning electron microscopy (SEM-EDX), microfocused X-ray diffraction (µXRD), and microfocused X-ray absorption near edge structure (µXANES) were used to determine the morphology of the grains and the chemical associations, identify the crystallized mineral phases, and specify the cadmium species, respectively.

Extracellular crystals were isolated from Tobacco plants grown during four weeks on medium containing 25µM Cd. Among various sizes and shapes of crystals, spherular grains were clearly distinguished, and were highly Cd-enriched compared to other crystals. µXRD identified three main mineralogical compositions on the various investigated grains: magnesian calcite in some grains, vaterite (a $CaCO_3$ polymorph, generally observed as spherular crystals (Sondi *et al.* 2001)) in others, and a mixture of both phases in the remaining crystals. Cadmium was detected in each type of mineral composition. µXANES spectra recorded at Cd L_{III}-edge on the different grains also indicated three different Cd species. Preliminary results suggested that in crystals composed of magnesian calcite, Cd could be either substituted to Ca or present as $CdCO_3$ clusters. In vaterite grains, Cd could be substituted to calcium. Experimental syntheses of calcite and vaterite containing cadmium are in progress to clarify these mechanisms and the crystal morphology.

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Localization and speciation of cadmium in *Arabidopsis thaliana* plants

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Keywords: cadmium, *Arabidopsis thaliana,* trichome, SEM-EDX, µSXRF, µXANES

Hyperaccumulating plants are able to extract metals from soils and to actively transfer them to their aerial parts. Using such plants for extracting metals, called phytoextraction, can represent a green and low cost strategy to reduce the metal contents in soils. Although *Arabidopsis thaliana* is not a metal hyperaccumulating species, its genome is completely known and is very close to the genome of the Cd hyperaccumulator *Arabidopsis halleri*. Consequently, information obtained on the mechanisms of Cd accumulation in the plant model *A. thaliana* can provide keys to understand the mechanisms occurring in the hyperaccumulating species.

In this study, we investigated *A. thaliana* plants exposed to 200µM Cd during four days. Our objectives were determine the distribution of Cd in the different plant tissues, and to identify the chemical forms of the metal in the different identified compartments of accumulation. Particular interest was focused on trichomes, epidermal hairs at the surface of the leaves, which may play a role in the sequestration of Cd (Salt *et al*. 1995; Küpper *et al*. 2000). To achieve this, scanning electron microscopy (SEM-EDX) and microfocused synchrotron-based X-ray fluorescence (µSXRF) were applied to determine the distribution of Cd and its chemical associations. Then, microfocused X-ray absorption near edge structure (µXANES) at the Cd L_{III}-edge was used to identify the Cd ligands in the different locations evidenced by µSXRF.

Results showed that cadmium was mainly accumulated in trichomes in leaves. µXANES spectra indicated Cd-O/N interaction bonds, probably occurring as organic species in these trichomes. In roots, Cd was mainly located in vascular bundles in association with sulfur, and µXANES evidenced S-containing ligands, likely provided by phytochelatins (Kupper *et al.* 2004). This work highlighted the role of trichomes in cadmium detoxification in *A. thaliana* and the trafficking of the metal from root to the compartments of accumulation.

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Complexation can decrease or increase the bioavailability of heavy metals in a multi-component environment

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Keywords: bioavailability, humic acid, speciation, donnan membrane technique, diffusive gradients in thin films

According to the Free Ionic Activity Model (FIAM) and the Biotic Ligand Model (BLM), metal uptake by organisms is mainly determined by the free metal concentration rather than the total metal concentration (Campbell 1995). However, most studies concerning this issue dealt with algae uptake in single-metal systems. Therefore, the objective of our study was to relate metal uptake by Ryegrass with different total, labile and free metal concentrations in a multi-component system. The hypothesis tested was that humic acid complexes part of the metals present in the system and therefore lowers their free and labile metal concentrations, which would lead to a lower metal uptake. Cd, Ni, Pb, Cu and Zn concentration of 0.1 and 1 μM were used, in the presence or absence of humic acid. Plants were in a nutrient solution that included Fe and Mn. The pH was kept constant at pH 6.

Free metal concentrations were measured by the Donnan Membrane Technique (DMT) (Temminghoff *et al*. 2000). The DMT can be used *in situ* to determine 'free' as well as complexed metal concentration by making use of a negatively charged cation exchange membrane. Labile metal concentrations were measured by Diffusive Gradients in Thin Films (DGT) (Davison and Zhang 1994). The DGT is an *in situ* speciation technique that accumulates free metal ions and labile metal complexes in a resin gel. Metal uptake in the roots and shoots of the plants was analysed by microwave destruction, whereas metal adsorption on the roots was measured by an EDTA-wash. Results of these experiments will be shown and discussed. In most cases, metal uptake by the roots was related to metal adsorption to the root surface. The metal uptake in the shoots depended on the uptake in the roots. Essential metals had a maximum concentration in the shoots, whereas the uptake of non-essential metals increased linearly with the uptake by the roots. The Cu and Pb uptake in the roots was related to both the free and labile metal concentrations by means of a Langmuir-Freundlich isotherm. However, while humic acid decreased Cu, Pb and Fe uptake, it increased Cd, Zn and Mn uptake. Cu and Mn, as well as Cd and Fe, competed for metal uptake by the roots. The results of this study will show that competition plays a major role in multi-component metal uptake and that a complexing agent like humic acid can both decrease and increase the bioavailability of heavy metals in a multi-component system.

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Spatio-temporal patterns of element accumulation by *Moehringia trinervia* in the medium-polluted forest ecosystem (Niepolomice Forest, S Poland)

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Keywords: industrial pollution, heavy metals, spatial patterns, *Moehringia trinervia*

The aim of the study was to determine the recent (1999) levels and the spatial patterns of heavy metal accumulation in common plant species *Moehringia trinervia* (L.) Clairv. in the forest ecosystem and to compare them with the situation described in the past (1984). In 1999 the concentrations of both essential and non-essential elements in plant tissues were examined and some environmental variables were used to reveal the potential factors affecting the chemical composition of plants.

The study was carried out in the Niepołomice Forest (S Poland, 50°05'N 20°21'E), which represents a variety of habitats with two distinct, decoupled gradients: a latitudinal (approximately N-S) gradient of soil moisture/fertility conditions, and a longitudinal (approximately W-E) gradient of contamination. The latter resulted from 50 year-long impact of industrial emission (steelworks in the vicinity of Kraków city). In the last period this impact substantially decreased. In 1999, shoots of *M. trinervia* were collected in 44 sample plots (27 overlapping with the sampling in 1984) which cover the entire area of forest and the concentrations of 10 elements (N, S, Ca, K, Mg, Cd, Cu, Fe, Pb, Zn) were determined. The *t*-tests for dependent samples were used to compare the concentrations of Cd, Cu, Pb and Zn between years. Factor analysis on the chemical composition of *M. trinervia*, soil properties and atmospheric precipitation chemistry with principal component method (PCA) for factor extraction was carried out. It allows us to obtain a few independent variables, which were used in the simple correlation tests. Environmental variables had to be interpolated (using kriging algorithm) because of different sampling schemes. Contents of some heavy metals in *M. trinervia* were high and in both years frequently reached potentially phytotoxic level. In 1999 the concentrations of heavy metals (Cd 6.44±2.52 $\mu g\ g^{-1}$, Cu 7.20±1.56 $\mu g\ g^{-1}$, Pb 5.64±1.56 $\mu g\ g^{-1}$, Zn 304±62 $\mu g\ g^{-1}$) were significantly lower than in 1984 (Cd 9.27±2.47 $\mu g\ g^{-1}$, Cu 9.04±3.33 $\mu g\ g^{-1}$, Pb 7.15±3.46 $\mu g\ g^{-1}$, Zn 339±51 $\mu g\ g^{-1}$) due to decreased industrial emission. In the Niepołomice Forest Cu and Pb concentrations in plant showed similar large-scale spatial pattern, which was characterized by continuous decline of values with distance to the polluters. The spatial pattern of Fe in plant was explained by soil contents of N, K and Ca, which reflected the habitat gradient. Distributions of Cd and Zn in plant did not correlate with any of variables, which we controlled. Among the macronutrients measured in *M. trinervia* only Mg concentration was affected by soil properties; it was lower in situation of high concentration of soil Cd and Zn. This phenomenon may be an effect of the competition for uptake of these elements. This research was supported by the State Committee for Scientific Research, grant no. 3 P04G 107 25 and grant no. 6 P04F 023 15/p02.

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Background research of the tailings area of Ni-Cu mine for determination of an optimal phytoremedeation method

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Keywords: sulfide tailings, nickel, copper, phytoremedeation

The aim of the study was to examine (1) the potential of the tailings as a substrate for plants, (2) the state of the plants colonising the tailings surface, and (3) to choose the most appropriate strategy for phytoremediation of exposed area of the tailings. Thompson (Manitoba, Canada) is in the Boreal Forest climatic zone characterised by a short frost-free season (about 85 days). Nickel and Cu have been extracted by INCO Ltd. from 1960 to the present producing about 40 million tonnes of tailings, which contain high levels of rapidly oxidising phyrotite. Control samples of plants and soils were taken from a natural uncontaminated area (Control Area) located about 3 km NE from the tailings.

Tailings were classified as fine sand according to the International Society of Soil Sciences (ISSS) classification. Organic matter content in tailings was 2% on average, which was 3 times lower than control. Tailing profiles up to 1 m depth consisted of alternating layers of oxidised and reduced tailings. This is the result of discharging fresh tailings on top of older ones during ore processing. Along the tailing profiles, depending on the age of tailing layers, pH values varied from acidic to neutral, salinity (saturated paste method) from nonsaline to moderately saline, sulphide (as phyrotite) content from 1.9 to 17%. Concentrations of Ni (816 ppm) in the tailings were up to 13 times greater than for natural soils.

Plant species composition was much poor than in Control Area. Plants were mainly growing along the perimeter close to the natural forest, where the depth of tailings was less than 1 m. Concentrations of Ni in all parts of water sedge, dominant species, were tens times higher than control. The highest Ni content was in dead shoots forming the top soil horizon. Seeds of water sedge had very low viability (16% germination) compared to 54% germination in the Control Area.

Greenhouse experiments, consisting of growing plants in tailings-limestone-organic soil mixtures, were conducted to examine one of the most common techniques of phytoremediation of mine sites. Survival of Water Sedge and Fowl Bluegrass but Foxtail Barley was 100%. However, plant growth was inhibited compared to the control plants grown in organic soil and only Fowl Bluegrass formed seeds. Ni content in shoots was several times higher than the control. In the field scale neutralisation of sulfides (phyrotite) with limestone can increase salinity of tailings and technically can not be applied to all depth of the root zone (about 1 m). Organic soils could be incorporated only to the top 20 cm of tailings. Concentration of Ni in shoots is expected to be higher than control.

Based on the results of the field and laboratory studies and the greenhouse experiments it was decided to cover tailings surface with protective layer such as hard rocks (waste rocks), consist of cobbles (7–20 cm) to coarse sand, have neutral pH and low sulfide content (2.5%) and are hardly permeable for plant roots. Soil layer should be created on top of the protective layer.

Sorption behaviour of Cu, Cd and Zn in soils influenced by longterm management

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Keywords: adsorption-desorption, heavy metals, long-term field experiment, soil management, soil organic matter, dissolved organic carbon

One possibility to investigate the impact of soil organic matter on heavy metal retention is the use of soils from long-term field experiments. Different farming practices over a long period of time change SOM quantity and quality with a minor impact on the soil minerals. Following this observation, we examined the adsorption-desorption behaviour of selected heavy metals (Cu, Cd, Zn) in soils obtained from two long-term field experiments, located in Ultuna (Sweden, set up in 1956) and Gumpenstein (Austria, set up in 1962). Different farming practices over 44 years and 38 years have changed major characteristics, e.g. soil pH, SOM, CEC (Gerzabek *et al.* 2005; Antil *et al.* 2005), of top soil in both experiments. Experimental batch sorption experiments were conducted, using a standard procedure following an OECD guideline. Initial heavy metal concentrations ranged from 40 to 200 mg/l. Freundlich equations (K_F, 1/n) were suitable to describe the adsorption and desorption isotherms of the metals with $R^2 < 0.92$. Comparison of the distribution coefficients revealed significant differences in the sorption behaviour of heavy metals between the investigated soils. In the Gumpenstein experiment, for instance, the amount of adsorbed heavy metals was more than 4 times higher in the topsoil amended with animal manure than in the plots treated with mineral NPK fertilizer. In the Ultuna experiment, adsorption generally increased in the following order: sewage sludge < fallow < inorganic fertiliser without N < green manure < peat < $Ca(NO_3)_2$ < animal manure < permanent grassland. In all plots, Cu was adsorbed most and strongest, followed by Zn and Cd. Simple equations obtained from linear regressions were used to estimate K_F and n values of the sorption isotherms of the Ultuna soils. They exhibited the important role of SOM, especially of the carboxyl+nyl carbon and DOC. Both, carboxyl+nyl carbon and DOC, as well as soil pH significantly dominated the sorption behaviour of heavy metals in soils with similar mineral soil constituents. Sequential extractions after batch sorption experiments (Zeien and Brümmer 1989) as well as sorption data gained from experiments with particle size fractions increased our knowledge about different sorption mechanisms and sorption surfaces in soils. Results allow us to quantify the influence of different farming practices on the sorption properties of soils for heavy metals and provide a data set to gain more information about active sorption sites in soils. Further, they support theoretical sorption models on soil matrices.

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Metal content in epiphytic lichens and metal soil relation in a natural mountain valley (Aspe Valley, French Pyrenees): first results

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Keywords: lichens, soil, metal pollution, enrichment factor

In these last years an environmental study has been developed in the Aspe valley (French Pyrenean Mountains) in order to assess the traffic contribution to the pollution of the ecosystem. The traffic in the valley shows an increase of the number of vehicles since the opening (January 2003) of the Somport Tunnel. The study that has been carried out until now has been focused in the metal content in epiphytic lichens, as well as in the snow, wet depositions and air particles[1] to establish the air quality of the mountain ecosystem. In this work we present the first results of the relations of the metal content in epiphytic lichens and the metal content in the soils. For this purpose we sampled lichens before and after the opening of the Somport Tunnel, in order to estimate the impact of road traffic. Several species of epiphytic lichens have been identified and collected at different altitudes and distances from the road. The soils are sampled on perpendicular transects to the main road (N-134); these transects have been situated each five kilometres and in each transects four samples have been taken in increasing distance from the road (0, 5, 20 and 50 meters). To evaluate the variability of the lithological and topographical features of the valley we take representatives soil samples of the substrates from the Aspe valley. In all samples total digestions have been performed, and their content in Al, Ca, Mg, V, Cr, Mn, Cu, Zn, Rb, Cd, Sn, Sb, Ba, Ce, U and Pb have been measured by ICP-MS and/or ICP-OES. The Aspe valley presents geographical characteristics that involve little dispersion of the pollutants. The transfer of the soil metal content to the lichens may come from wind blow soil dust via wet and dry depositions[2]. We have calculated the enrichment factors[3] between the lichens and the soils referred to local background in order to evaluate the anthropogenic contribution of metals and to assess the traffic influence on these contents.

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Bioavailability of trace metals from mine tailing contaminated substrates to *Vicia faba* L.

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Keywords: trace metals, Vicia faba, sequential extraction, mine tailings, heavy metals

The bioavailability and influenced factors of Cu, Zn, Cd and Pb from soil and mine tailing to *Vicia Faba* L. were studied based on a pot experiment and sequential extraction methods. Metal concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that the mine tailing contained extremely high concentrations of trace metals by concentrations of Cu 212, Zn 15450, Cd 47 and Pb 7250 mg kg^{-1}. The metal concentrations in the plant tissues decreased in an order of root >> leaf > stem. The bioaccumulation factor (BAF) values of these four elements were decreased in an order of Cd >> Zn > Cu > Pb, and an order of root >> leaf > stem was observed for the different plant tissues. The exception was for Pb, which was root > stem > leaf. The major influenced factors which affect the bio-availability of trace metals to plants were the exchangeable portion of trace metals in the substrates and the physiological properties of the plant. In the meantime, the acid-soluble fraction and the total concentration of the trace metals in the substrates could affect trace metals uptake by plant in a certain extent. For Zn and Pb, the fraction of exchangeable content significantly positive correlated with the BAFs of metals to plant tissues; For Cd, the concentration factor could affect the BAF values besides exchangeable portion. But for Cu, the major influenced factor was the physiological properties of plant. On the other hand, the environmental factors could affect the bio-availability of trace metals in the substrates, too. High pH value could fix the cation contents of Zn, Cd and Pb in the substrates, this process could decrease the proportion of exchangeable content in the substrates, and thus decrease the uptake speeds by plant. However, Cu uptake by plant was regulated by N. The deficiency of N in the substrates can facilitate Cu uptake by plant roots.

Initial soil chemical changes and heavy metals in the soil solution of a model forest ecosystem

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Keywords: heavy metals, soil, soil solution, speciation, plant influence, mineralisation flush

In order to allow for controlled soil conditions in the model ecosystem experiment "From Cell to Tree", the soils were "built" with layers of homogenized soils. In half of the experimental treatments, the top soil was contaminated with heavy metals (HM). This was achieved by mixing with the filter dust of a secondary metal smelter, which was composed mainly of Zincite (ZnO), Gahnite ($ZnAl_2O_4$) and a copper-zinc alloy. Solid phase speciation of the contaminated soil shortly after mixing revealed that most of the Zn and about 50% of the Cu were mobile or easily mobilizable. After half a year of irrigation, the mobile fraction had become smaller. During the following 3 years of the experiment, the speciation did not change much any more. The dissolution of the Zincite was responsible for an initially much higher pH-value of the contaminated topsoil compared to the uncontaminated soil. The pH-value of both the contaminated and uncontaminated topsoil dropped to a constant and equal value within 1.5 years. Monitoring of the percolation water revealed two phases of system equilibration. The first 9 months of the experiment were characterized by chemical conditions that were very different from the second phase, in particular in the experimental variations with calcareous subsoils. They included a mineralisation flush leading to excessively high nitrate and sulfate concentrations. Also soil organic matter (SOM) degradation was somewhat accelerated and led to higher dissolved organic carbon (DOC) concentrations. The second phase of the experiment was characterized by small gradual changes in DOC and sulfate concentrations overlaid by seasonal variations. The soil solution in top and subsoil was monitored during the last 1.5 years of the experiment and compared to the one in plant free soil columns. In both the planted and the unplanted system, dissolved Cu, Zn and Cd were significantly higher in the contaminated top soil than in the control. Furthermore, Zn and Cd concentrations in the HM treatments were higher in the presence than in the absence of plants. Acid irrigation during the vegetation period led instantly to increased Zn and Cd concentrations, while Cu increased during winter after acid irrigation was stopped. Temporal variations in Zn and Cd concentrations in the contaminated top soils and respective acid rain effects can be explained mainly by variations in the Ca concentrations. On the other hand, Cu concentrations appeared to be governed by DOC and Ca. The fraction of free Cu^{2+} in the contaminated top soil solution exhibited some seasonal variation. In addition, it was larger in the presence than the absence of plants most of the times suggesting differences in DOC and SOM quality between the planted and unplanted systems. Soluble and exchangeable Zn concentrations indicate some translocation from the contaminated top soil to the acid subsoil but not to the calcareous subsoil, both in presence and absence of plants. Overall, the soil solution results suggest, that the planting of trees in our model ecosystems had led rather to an increase than a decrease in mobility and bioavailability of HM.

The mobility of heavy metals in Swiss forest soils as affected by soil forming processes and nutrient cycling

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Keywords: heavy metals, forest soils, mobility, dissolved organic matter, nutrient cycling

Important groundwater sources are situtated below forests. Therefore, the contents and mobility of potential contaminants in forest soils need to be known. This study aims at an overview of the status of heavy metal (HM) contamination of forest soils in Switzerland and is part of a broad pedological documentation (Walthert *et al.* 2004). We investigated soils from forest sites all over Switzerland with respect to total and HNO_3 extractable contents of Cr, Ni, Cu, Zn and Pb at different depths. The soils were selected to represent important soil types, bedrocks, plant communities and climatic regions. Using Zr as a tracer, which is only little affected by translocation processes and is practically not taken up by plants, we calculated enrichment factors (EF; Blaser *et al.* 2000). These factors relate the total metal content at a given depth to a calculated original lithogenic content. Depth profiles of HM contents, as well as enrichments and depletions were interpreted in terms of origin (lithogenic vs. anthropogenic) as well as influence of pedogenetic processes and nutrient cycling on metal mobility. In most of the top soils Pb from the former use as gasoline additive is accumulated. In some top soils also Cr is enriched. An anthropogenic origin was suspected but a source could be localized only in few cases. Enrichments of mostly lithogenic Ni, Cu and Zn in the top soil and corresponding depletions in the subsoil were often observed and explained as result of nutrient cycling. In particular the mobility of Zn, which otherwise can be considered one of the most mobile heavy metals in acidic soils, and the potential risk for groundwater contamination can be reduced effectively by this process. On the other hand, it can lead to potentially toxic conditions for microorganisms in the top soil. Translocation as complexes with dissolved organic matter (DOM) leads in most soils on acidic bedrock to a strong depletion of lithogenic Cu and Ni in large parts of the mineral soil. Often these metals are translocated to significantly greater depth than Fe and leached to the underground. Therefore, the importance of this process is not restricted to podsols. Copper can be strongly translocated with DOM even in weakly acidic soils on carbonate rock under the dry conditions in the central Rhone valley. Lead is another HM with a high affinity to DOM. In several soils, translocation of anthropogenic Pb that had accumulated in the top soil could be detected. This is remarkable given the fact that, compared to the rate of soil formation, this top soil contamination can be considered recent.

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Effects of poplar and willow on trace metal fluxes in silvopastoral ecosystems

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Keywords: poplar, willow, dissolved organic matter, dairy effluent, phytoremediation

Poplars and willows are used extensively in pastoral production systems for soil conservation and supplementary stock fodder during times of drought. Both foliage and small twigs can be browsed by sheep and cattle. In addition to providing an emergency food source, poplars and willows as stock feed have proven health benefits such as increased fecundity. These benefits may be derived from high protein, tannin or essential trace element concentrations. Trace-element accumulation by trees used for stock fodder may, in addition to providing a source of essential elements, also introduce toxic elements into the animals' diet. Many pasture lands have elevated Cd concentrations due to repeated applications of Cd-rich superphosphate fertiliser. For example, in New Zealand, the average concentration of Cd has increased from 0.13 $\mu g\ g^{-1}$ in unfertilized soils to 0.44 $\mu g\ g^{-1}$ in fertilized soils. We investigated trace metal accumulation by poplar and willow as well as the effect of these trees on metal leaching. Lysimiter experiments, field sampling, and experimental plots were used to determine the feasibility of using densely planted stands of poplars and willows as a recepticle (sink) for the disposal of dairy shed effluent. The objective of such systems is to reduce contaminant (e.g. nitrate) leaching, while providing a nutritious dietary supplement for stock containing elevated concentrations of essential trace elements. Poplars and willows accumulated high concentrations of the trace elements tested, relative to pasture. In poplars, elevated foliar Co concentrations may alleviate Co deficiency, or so-called 'bush sickness', on farms deficient in this element. Similarly, elevated Zn concentrations may be beneficial for the treatment of facial eczema. Leaf trace-element accumulation was a function of leaf age and the variety/species of tree. B, Cd, Mn and Zn concentrations increased throughout the season, while Cu decreased and Fe remained unchanged. The accumulation of Cd is of concern, while the ingestion of other trace elements may be beneficial. Analyses of willow clones grown under identical conditions revealed a six-fold difference in the leaf Cd concentration. Stock exposure to Cd could be managed by judicious clone selection, harvesting young shoots, or harvesting early in the season. Following the addition of dairy-shed effluent to soil, poplars and willows decreased the flux of trace elements through the rhizosphere. This effect was attributed to a reduced water flux due to the trees' evapotranspiration. The dissolved organic matter in the effluent and root exudates may mobilise trace elements, while rendering them unavailable for plant uptake. The results of these experiments were used to parameterise a semi-mechanistic model that calculates trace-metal fluxes as affected by trees in silvopastoral systems. Such models can be used to determine the clone variety as well as the density of trees needed to provide stock with sufficient essential trace-elements such as Cu and Zn while reducing exposure to toxic heavy metals such as Cd.

Bioindication of heavy metal contamination in vegetable gardens

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Keywords: heavy metal, soil, bioindicator, plant response, tolerance, visible symptoms

In Switzerland, private vegetable gardens are cultivated on around 50'000 hectares and are quite often polluted with heavy metals (HM). Gardens are characterised by their small size and a variable contamination history following differences in gardening practices. In this situation, simple and cost-effective “soil screening” methods for the identification of polluted areas are needed. Bioindication techniques based on the recognition of the visible symptoms in the leaves can be easily implemented once the symptoms have been validated. Through vulgarization media, they provide a way to reach individual gardeners for self-monitoring purposes. In this study, the reactions of vegetable gardens to moderate HM pollution (Cd 10 mg/Kg, Cu 385 mg/kg, Pb 63 mg/kg and Zn 2700 mg/kg) were investigated using seven vegetables from six different species (*Nicotiana sanderae* L.; *Spinacia oleracea* L.; *Lactuca sativa* L.; *Allium schoenoprasum* L.; *Phaseolus vulgaris* L.; *Raphanus sativus* L. with two cultivars) and six families planted as seeds in 0.9 L pots filled with an agricultural soil from the Swiss midland region. Visible symptom development was followed up from the onset of the first leaves (around three or four weeks after planting the seeds) till the final harvest (12^{th} week). The biomass of roots, shoots and edible parts was determined. For validation purposes, 1) HM content of roots, shoots and fruits, if consumed, was determined, 2) Zn was cytochemically revealed and 3) changes in the cell and tissue structure and the physiological responses to HM stress were characterised with microscopical analyses. Each species reacted in a species-specific way at each analytical level; the two cultivars showed differences in symptom intensity only. Two species (*Phaseolus* and *Raphanus*) showed characteristic HM leaf symptoms with gradients of vein browning culminating at the vein basis or hypersensitive-like leaf blade necroses, respectively. The other species showed either unspecific (chlorosis) or no symptoms. All tested species showed reduction of their biomass amounting to 45–90% of that reached by control plants. Each species absorbed and translocated each HM in a species-specific way and by the final harvest, differences up to 6 times according to the plant part were measured between the different tested species. Microscopical investigations focused on the two species showing HM-characteristic symptoms. Zn was cytochemically detected in the vein and leaf blade without apparent detoxification strategies in *Raphanus* and especially accumulated in secretory hairs at the leaf surface of *Phaseolus*. Few defence reactions were detected in *Raphanus* roots but heavy cellular injury occurred in its leaves, whereas *Phaseolus* thickened its root cell walls and showed only a slightly increased cell senescence probably mediated by an increased oxidative stress. The quantitative analyses confirmed that *Raphanus* imported more HM, especially Zn, in its shoots and thus showed more severe stress reactions than *Phaseolus*. These two species showed a good potential as bioindicators for HM detection in vegetable gardens. In conclusion, this study shows that developing a few well characterised bioindicator species can better fulfil bioindication purposes than an overall vegetable garden monitoring as a consequence of the variability in the plant reactions. Still, the practical application of this method must be developed.

Availability of heavy metals in soils

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Keywords: heavy metals, soils, speciation, activity, complexation, toxicity

The forms and lability of heavy metals in the solid phase of soils exert strong control over the solubility, mobility and toxicity of these metals. Characterization of metals in the solid phase remains problematic however, because of the the very complex nature of the soil solids and the relatively low concentrations of most trace heavy metals in soils. Sequential extraction, a commonly used chemical method intended to gauge lability of heavy metals in soils, has been show to be fraught with interpretational pitfalls arising largely from the chemically aggressive and non-discriminatory nature of the reagents employed. More direct and reliable identification of metal forms in the soil solids may be possible by spectroscopic methods such as EXAFS,

ESR and XPS. However, these methods have only been applied selectively to a few heavy metals because of limitations of the techniques. Spatial correlations at the microscale and macroscale between the concentrations of some heavy metals and other elements in soils (e.g., Mn, Fe, C, S) suggest that particular mineral or organic fractions in the soil have important roles in retaining and limiting the solubility of specific metals. Increasingly, the importance of reduced sulfur in heavy metal bonding by soils, both aerobic and anaerobic, has been recognized.

Although knowledge of the nature of solid phases to which metals are bonded may give indirect insights into metal reactivity in soils, metal toxicity is generally more directly estimated from the dissolved concentration of free or total metal in soil solution. The free metal cation is generally, but not always, found to be more toxic than complexed forms of the metal in soil solution. Nevertheless, the presence of metal-complexing ligands in soils such as dissolved organic matter (DOM) has the effect of increasing total dissolved metal concentration, thereby potentially increasing the rate of metal transport to biological surfaces such as plant roots. Thus, the free metal ion concentration in soil solution may not always be the best predictor of metal uptake and toxicity in plants and other biota.

Semi-empirical equations have been developed to predict free metal cation activity or metal solubility from basic soil properties such as pH, total metal concentration and organic matter content. Although not accurate predictors for any given soil, these equations describe soil conditions most likely to lead to excessive plant uptake or phytotoxicity of trace metals.

Comparison of Cd and Zn phytoextraction potential of *Salix dasyclados* Loden on different soil types

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Keywords: heavy metals, cadmium, zinc, phytoextraction, Salix, phytoremediation

Phytoextraction has been proposed as an economic alternative for some of the environmental issues involved in heavy metal pollution. The use of *Salix* spp. allows for gradual contaminant removal, while the produced biomass can be used for production of renewable energy. This in turn allows for economic valorization of contaminated soils during the remediation process. The potential of *Salix dasyclados* Loden for phytoextraction of heavy metals was evaluated in two field experiments and in a comparative outdoors pot experiment. In the pot experiment, accumulation in stem and leaves was evaluated in three types of soil: (i) a moderately contaminated calcareous and clayey dredged sediment derived (DSD) surface soil, (ii) a heavily contaminated calcareous and clayey DSD surface soil and (iii) a sandy slightly acidic (pH 6.6) soil which was moderately contaminated with heavy metals activities (mainly Cd and Zn) due to atmospheric deposition of historic smelter. The two field experiments were conducted on sites with soil characteristics comparable to soils (i) and (iii) of the pot experiment. In the comparative pot screening distinct differences could be observed between the three soil types with observed plant uptake of the various metals in three soils in the order (iii) > (ii) > (i). Particularly Zn uptake in the sandy soil was considerable, with foliar concentrations of 2000–2500 $mg.kg^{-1}$ whereas total content in the soil was merely 275 $mg.kg^{-1}$. Zn content in the stems was lower at about 500 $mg.kg^{-1}$. Analogously, foliar Cd concentrations on the sandy soil attained levels of 40 $mg.kg^{-1}$ although total soil content was only 5.5 $mg.kg^{-1}$. Corresponding levels in the DSD soil (i) were substantially lower with foliar concentrations of approximately 500 $mg.kg^{-1}$ Zn and 15 $mg.kg^{-1}$ Cd and stem concentrations of approximately 220 $mg.kg^{-1}$ Zn and 12 $mg.kg^{-1}$ Cd. In the first field experiment, *Salix dasyclados* Loden was planted in small plots on a test site historically polluted by smelter activities. Soil composition and pollution level corresponded with those of soil (iii) in the comparative pot experiment. Cd and Zn accumulation levels also corresponded perfectly with the findings of the pot experiment with foliar accumulation of respectively 36 $mg.kg^{-1}$ Cd and 2660 $mg.kg^{-1}$ Zn. Stem concentrations for this field experiment still require to be processed and compared to the pot experiment findings at this point. The second field experiment was performed on a DSD soil comparable to soil (i) of the pot screening. Stem analyses in the field experiment were subdivided in bark and wood tissue and are therefore difficult to compare directly to the pot findings. Bark concentrations were in the order of 14 $mg.kg^{-1}$ Cd and 319 $mg.kg^{-1}$ Zn. Wood concentrations were around 66 $mg.kg^{-1}$ Zn and 3.4 $mg.kg^{-1}$ Cd. Foliar concentrations in the field experiment were 686 mg.kg^{-1} Zn and 7.4 mg.kg^{-1} Cd. Accumulation levels in both field experiments proved to be very comparable to the observations made in the comparative pot screening. This demonstrates the importance and potential predictive use of pot experiments for phytoextraction research. Based on the various experiments conducted, the estimated potential annual removal rates from the soil by *Salix dasyclados* Loden are situated between 2.4–8.6 mg.kg^{-1} Zn and 0.04–0.2 mg.kg^{-1} Cd.

Distribution of Cu and Zn in a constructed wetland for tertiary treatment of the liquid fraction of pig manure

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Keywords: copper, zinc, constructed wetland, pig manure

Agricultural effluents derived from animal production can contain elevated levels of Cu and Zn. The agricultural wastewater used in the current experiments was derived from the liquid fraction of pig manure after centrifugation and biological treatment. This pre-treated wastewater contains Cu and Zn in the range of 1–5 mg.l^{-1}. These levels are generally too low to consider expensive physico-chemical removal systems, yet are still overly high and ecologically relevant when discharged into the environment. Alternative treatment of agricultural effluents by means of constructed wetlands (CWs) was investigated in a pilot-scale experiment. The removal efficiencies of Cu and Zn from the CW and distribution of metals over plant, wastewater and filter materials were assessed.

Mean concentrations in the (pretreated) influent were 150 μg.l^{-1} Cu and 300 μg.l^{-1} Zn. Based on concentration levels in the wastewater, removal efficiencies of the system were high, at 84.5–99.6% for Cu and 90.4–98.5% for Zn. Plant Cu concentrations ranged between 18–33 mg.kg^{-1} for duckweed, between 1.1–4.0 mg.kg^{-1} for stems and leaves of reed and between 6.8–9.6 mg.kg^{-1} for the roots of reed. Plant Zn concentrations varied between 268–440 mg.kg^{-1} for duckweed, between 22–48 mg.kg^{-1} for stems and leaves of reed and between 52–115 mg.kg^{-1} for the roots of reed.

The relative fraction of the overall Cu removal attributed to plant uptake amounted to merely 0.14% by reed and 2.5% by duckweed. For Zn these fractions were respectively 0.81% by reed and 14% by duckweed. These results are in line with greenhouse observations for Cu and Zn removal by surface flow CWs with reed for the treatment of agricultural effluents (Meers *et al*. 2005). In that study 1–2% of the removed Cu and around 30% of the removed Zn were recovered in aboveground plant parts of reed. The major part of Cu and Zn was recovered in the argex substrate and the percolation filter materials. Adsorption onto the filter materials appeared to be the main removal process of heavy metals in the pilot-scale CW, a result also reported by Lesage *et al*. (2005) in a study of heavy metal accumulation in an operational CW in Flanders, Belgium. Although good removal efficiencies were observed, accumulation in the filter materials and reed bed substrate cannot be considered to be a sustainable removal technique, as it implies the transfer of the pollutant load from wastewater to the filter matrix. Metal removal by substrate-less treatment wetlands, such as duckweed ponds, are therefore to be preferred. Based on the uptake performance of duckweed in the current system, approximately 400 g Cu and 5.400 g Zn could be removed per ha per year.

Mercury leaching from boreal forest and peat soils: links between spatial and seasonal patterns?

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Keywords: mercury, organic carbon, fractionation, soil profile, runoff pathways, watershed budget

Hg concentrations in forest soils are approaching ecotoxicologically critical levels, even in background areas, which actualizes the issue of how the atmospheric input of Hg is balanced by leaching. High-resolution sampling in a network of 15 streams draining a forested catchment in northern Sweden (Svartberget-Krycklan) showed that Hg concentrations in runoff water can vary locally by almost an order of magnitude. As expected, there was a strong correlation with organic matter (DOC). However, Hg-DOC relationships showed systematic variations, both seasonally and spatially, and covaried with catchment properties. Apparently, the export of mercury from boreal watersheds is linked not only to the quantity but also the quality and age of the exported organic matter. Since a dependence on hydrological flow paths can be suspected, the Hg and C concentrations in runoff waters were compared to those in groundwaters and soil solids along vertical profiles in the same catchment and elsewhere.

Influence of heavy metal pollution on soil-plant water regime

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Keywords: evapotranspiration, forest ecosystem, heavy metals, lysimeters, soil pollution, soil water regime

In a three-year factorial lysimeter study in Open Top Chambers (OTCs), we investigated the effect of topsoil pollution by the heavy metals Zn, Cu, and Cd on the water regime of newly established forest ecosystems (under “cell to tree” frame work). Furthermore, we studied the influence of two types of uncontaminated subsoils (acidic vs. calcareous) and two types of irrigation water acidity (ambient rainfall chemistry vs. acidified chemistry) on the response of the vegetation. Each of the 8 treatment combinations was replicated 4 times. The contamination (2700 $mg.kg^{-1}$ Zn, 385 $mg.kg^{-1}$ Cu and 10 $mg.kg^{-1}$ Cd) was applied by mixing filter dust from a non-ferrous metal smelter into the upper 15 cm of the soil profile, consisting of silty loam (pH 6.5). The same vegetation was established in all 32 lysimeters. The model forest ecosystem consisted of seedlings of Norway spruce (*Picea abies*), willow *(Salix viminalis)*, poplar *(Populus tremula)* and birch *(Betula pendula)* trees and a variety of herbaceous understorey plants. Systematic and significant effects showed up in the second and third growing season after canopies had closed. Evapotranspiration was reduced in metal contaminated treatments, independent of the subsoil type and acidity of the irrigation water. This effect corresponded to an even stronger reduction in root growth in the metal treatments. In the first two growing seasons, evapotranspiration was higher on the calcareous than on the acidic subsoil. In the third year the difference disappeared. Acidification of the irrigation water had no significant effect on water consumption, although a tendency to enhance evapotranspiration became increasingly manifest in the second and third year. Soil water potentials indicated that the increasing water consumption over the years was fed primarily by intensified extraction of water from the topsoil in the lysimeters with acidic subsoil, whereas also lower depths became strongly exploited in the lysimeters with calcareous subsoil. These patterns agreed well with the vertical profiles of fine root density related with the two types of subsoil.

Does the degree of esterification of Ca-pectates influence the accumulation of metals at the soil-root interface?

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Keywords: pectate, soil-root interface, degree of esterification, root exudates

Pectates are polysaccharides largely found at the soil-root interface based on a L-1,4-α-galacturonan core with rhamnose residues interspersed and neutral side chains attached. They are secreted as highly esterified pectates from the symplast into the apoplast where demethylation takes place by pectin methylesterase (Gaffe *et al.* 1992). These demethylation reactions in the apoplast lead to pectates with free carboxylic groups playing thereby an important role in affecting the cation exchange capacity, diffusion and indirectly thus the plant nutrition. In addition, the pectates forming a porous network of fibrils could act as direct channels for the movement of soil-bound cations to the surface of root cells (Leppard and Ramamoorthy 1975). Previous studies already showed how the sorption of toxic metals as aluminium altered the functionality of the soil-root interface reducing the phosphate diffusion and indicating that the root apoplast could play an important role in the expression of a possible mechanism of apoplastic Al toxicity (Gessa *et al.* 2005).

Calcium-pectates with different degrees of esterification (0, 26 and 65%) were formed in custom-made cells and used as a model of the soil-root interface. They were consequently treated with solutions of aluminium, cadmium and chromium at pH 4.50 at a concentration of 100 μM. The aim was to evaluate if the different degrees of esterification (DE) could influence the immobilization of the toxic metals at the soil-root interface. The results showed though no significant differences between the three DE's but showed different interactions of divalent and trivalent cations with the pectate matrix: Al > Cr > Cd. The metals would thus interact with different coordination sites as the free carboxylic groups present on the pectate chains decrease with increasing DE. The DE could though influence the affinity of the metals with the pectate matrix. To evaluate this effect the different metal-pectates were treated with organic acids (oxalic, citric and malic) at different concentrations (5, 50, 500 μM). Organic acids seem to mobilize more easily the metals coordinated to pectates with low degrees of esterification. This could indicate that the hydrophobic groups ($-CH_3$) present on highly esterified pectate chains hinder the mobilization of the metals enclosing the metals like in a shell. The DE would thus play a fundamental role in the mobilization/immobilization of toxic metals at the soil-root interface.

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Response of Scots pine to a long-term Cu and Ni exposure

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Keywords: heavy-metal deposition, lethal thresholds, needle analysis, peat records

Scots pine (*Pinus sylvestris* L.) stands growing close to Cu-Ni smelters are suffering from serious defoliation and growth retardation, fine root mortality is high and the understorey vegetation in the stands has been almost completely destroyed. Undecomposed needles and plant litter have accumulated on the forest floor due to retarded microbial activity and mineralization. Metal accumulation in the soil has resulted in a deficit of exchangeable Ca, Mg and K in the organic layer, caused by partial inhibition of the mineralization of these nutrients, and the displacement of base cations from exchange sites.

In this study the rate of Cu and Ni deposition on the forests was estimated by monitoring the current Cu and Ni concentrations in bulk precipitation and stand throughfall, and by estimating the past pollution loads on the basis of the amounts of Cu and Ni accumulated in the surface peat of an adjacent ombrotrophic bog. The measured Cu and Ni deposition at the forest site nearest to the smelters did not appear to be a reliable estimate of current metal input into the ecosystem, because of the high level of internal cycling via soil dust. The Cu and Ni concentrations in the surface peat of the adjacent bog were highly elevated compared to a reference bog at a background area. The extremely high metal concentrations in the peat of the polluted bog suggested also that the deposition level in the past has been higher than the current deposition in the immediate vicinity of the smelters.

The performance of experimental pine seedlings cultivated in smelter-polluted soil was similar to that of a mature pine stands growing along the study gradient. The variation in the biomass of the seedlings appeared to be related both to the toxicity of Cu and Ni and to differences in the nutrient status of the experimental soils. However, smelter induced pollution may affect pines also indirectly through changes in soil nutrient status, which are difficult to distinguish from the natural variation in fertility. Although Ni appeared to be more mobile than Cu in the polluted forest and peat soils, this was not reflected as relatively higher uptake by roots of the experimental pine seedlings. According to results of an exposure treatment with metal spiked sand, the lethal Cu concentration in pine roots would be approx. 1000 mg kg^{-1}, while the corresponding value for Ni would be 100 mg kg^{-1}, thus indicating higher toxicity of Ni.

The pine needle concentrations did not appear to be reliable indicators of Cu and/or Ni toxicity. This was especially the case in the field, where the surface contamination of needles by metal-containing particles in the heavily polluted environment further complicated the interpretation of the measured Cu and Ni concentrations. The chloroform washing did not remove all of the metal-containing material deposited on the needle surfaces.

Herbivory on the Zn hyperaccumulator *Thlaspi caerulescens* (Brassicaceae) in the field

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Keywords: *Thlaspi caerulescens,* zinc, hyperaccumulation, herbivory, defence hypothesis, field experiment

The adaptive and evolutionary aspects of heavy metal hyperaccumulation by plants have attracted much attention the last decade. In particular, the hypothesis that metals might protect plants against herbivores and pathogens, commonly called the defence hypothesis, has received much attention (Boyd 1998, 2004). However, most of the experiments aiming to test this hypothesis have been conducted in laboratory conditions and there is a lack of studies testing the defence hypothesis *in natura*.

Here we present the first test of the deterrent effect of Zn accumulation on herbivores in the field. The experiment was conducted in 8 natural populations of the Zn/Cd hyperaccumulator *Thlaspi caerulescens* J. und C. Presl (Brassicaceae) at a large geographical scale (Belgium, Luxembourg and Southern France) and in both metalliferous and nonmetalliferous environments where *T. caerulescens* naturally occurs.

A very interesting and unexpected result was that the intensity of herbivory was much lower on metalliferous soils, both in Belgium-Luxembourg and in Southern France. Moreover, metallicolous populations were consistently more consumed than nonmetallicolous ones in both geographical regions. This can be related to the lower concentration in glucosinolates of metallicolous populations and confirms earlier results obtained in laboratory experiments (Noret *et al*. 2005). Zn concentration of leaves did not affect the feeding preferences of herbivores in Southern France. However, Zn protected the Belgian metallicolous populations when transplanted in the nonmetalliferous sites of Luxembourg.

To better understand the respective roles of Zn and glucosinolates in the protection of plants in the nonmetalliferous sites of Luxembourg, we then studied 20 nonmetallicolous populations of *T. caerulescens* that show a wide range of foliar Zn concentration in their natural habitat (from ~2000 to 12000 $\mu g\ g^{-1}$). In that material, we investigated the consumption level of plants in relation to their Zn and glucosinolate concentrations. Results show that i) the consumption of leaves was not related to their Zn concentration and ii) foliar Zn concentration reflects the Zn concentration of the soil.

An evolutionary scenario integrating all these results will be proposed.

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Bioavailability of metals and influence of organic compounds on metal speciation in a model forest ecosystem

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Keywords: bioavailability, DGT, leaf litter, copper, zinc, complexation

Bioavailability and mobility of heavy metals depends to a great extent on speciation in solid phase and solution. Complexation of metals by organic compounds derived from soil organic matter or leaf litter is therefore important for understanding the behaviior and the effects of metals in polluted and non-polluted soils. In this work we have used different approaches to investigate these processes in the soils of a model forest ecosystem. We have used DGT (diffusive gradients in thin-films) to determine the plant available pool of Zn and Cu in the solid phase. To characterize the dissolved metal complexes we have developed and validated a method for the isolation of copper-complexing ligands from soil solution by immobilized metal ion affinity chromatography (IMAC). We have also investigated metal behavior during leaf litter degradation and the influence of the leaf litter leachate on metal solubilization.

The kinetics of Cu and Zn resupply from the solid phase and the plant availability were determined by DGT in the field, and in undisturbed and homogenized soil samples in the climate chamber. The results of the field samples were similar to those of the undisturbed lab samples and showed that DGT can be used in the field if the heterogeneity of the soil and the changing water content is taken into account. Both Cu and Zn were characterized in the field by a small pool of rapidly available metal that was depleted within 24 hours. Slow desorption from the solid phase and diffusion to the gel resulted in a steady resupply to the gel at a concentration of about 3% of the dissolved Zn and about 25% of the dissolved Cu concentration in soil solution. The rapidly available Zn pool was absent in the homogenized soil but the slow desorption part was very similar.

IMAC chromatograms of the soil solution samples contained two major resolved fractions indicative for Cu-binding ligands. The results show a strong effect of vegetation, soil depth and soil type on the percentages of retained ligands, ranging from 5% to 30%. IMAC separated fractions were further characterized by excitation/ emission spectroscopy, revealing common peak for all fractions and additional peak corresponding to retained fraction with strong fluorescence maxima pattern.

The heavy metal concentrations in leaf litter exposed to polluted soil increased considerably (factor up to 57) during decomposition. During the decomposition the solubility of metals in the decomposed leaves in non-polluted soil remained about the same compared to fresh leaves. In contrast the solubility of the metals in leaves placed on polluted soil was markedly reduced. The results show that on bare polluted soil a net uptake of metals by the decaying leaf litter is observed but that the relative solubility of the metals is reduced during that time.

Critical loads of mercury related to human health for agricultural areas in the Crimea

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Keywords: mercury, critical loads, human health

The calculation and mapping of critical loads of mercury addressing human health in view of ground water protection (keeping quality criteria for drinking water of WHO 2004) was carried out in agricultural areas in the Crimea by initiative group. In accordance to ICP Modelling and Mapping (ed.) 2004, the calculation of critical loads of mercury was based on the effect-based approach, accounting the possible negative mercury influence on biota under definite (critical) concentrations of the metal in the soil, soil solution, vegetation, etc. For calculations of critical loads of mercury with respect to protection of ground water quality the entire soil column was included. As the most part of agricultural areas in the Crimea is the north and central territories, where wheat and other cereals take place. At first step only these cultures have been taken in account and the critical loads of the metal CL(M) (g/ha per year) was calculated according:

CL(M) =Mu + Mle(crit), where: Mu is the metal net uptake in the harvestable parts (g/ha per year); Mle(crit) is critical leaching flux of heavy metal from the considered soil layer (g/ha per year).

Data sources:

1. ICP Modelling and Mapping (ed.) 2004: Manual on Methodologies and Criteria for Modelling and Mapping Critical Loads und Levels and Air Pollution Effects, Risks and Trends, Federal Environmental Agency (Umweltbundesamt) Berlin, UBA-Texte 52/04.
2. Atlas “The Autonomous Republic of Crimea”, 2002.
3. Annual statistic bulletin of the Republic Statistic Committee, 2002.

Changes in heavy metals concentrations in forest ecosystem after wood ash application

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Keywords: Raw wood ash, heavy metals, Scots pine, Arenosol, Pleurozium schreberi, needles.

The export of plant nutrients from forest ecosystems recently increases due to the consumption of primary biomass for the bioenergy in the European Union. The sustainable forest management requires recycling wood ash containing the main nutrients (Ca, K, Mg, Na, P) back to the forests. Despite the nutrients, wood ash contains the diverse amounts of heavy metals, which being ecotoxical elements can cause the changes in the soil, soil solution, ground vegetation, or even groundwater chemistry.

The integrated field experiment was established in a 38-year-old Scots pine (*Pinus sylvestris* L.) stand on Arenosols in Central Lithuania in 2002. The experiment was laid on the plot area of 1.2 ha, and totally consisted of 24 plots grouped into 4 blocks. The doses of 1.25–5.0 t ha^{-1} of pure ash, 2.5 t ha^{-1} of ash together with 180 kg N ha^{-1}, and 180 kg N ha^{-1} were applied. The effects of maximum dose (5 t ha^{-1}) of wood ash were evaluated in purpose to clarify the possible risk of raw wood ash recycling on the changes of heavy metals concentrations in the forest floor, mineral topsoil, soil solution, vegetation, and Scots pine needles. The following doses of heavy metals were spreaded with 5 t ha^{-1} of wood ash: Cr – 47.6 g ha^{-1}, Ni – 40.3 g ha^{-1}, Cu – 65.4 g ha^{-1}, Cd – 3.1 g ha^{-1}, Zn – 385.4 g ha^{-1}, and Pb – 22.7 g ha^{-1}. The concentrations of heavy metals in topsoil, soil solution, mosses, and needles were analysed 5 months–2 years after the application of ash.

The applied relatively small contents of metals with ash significantly affected only O horizon, where the increased concentrations of Cr by 1.9–2.2 fold, Ni by 1.4 fold, Cu by 2–3 fold, and Zn by 1.5–1.6 fold were determined. As the most of metals were bound in the forest floor, significant downward transport was determined only for Zn at 20 cm, and Ni at 50 cm depth after 2 years. No ash influence on the leaching of Cr, Cu, Cd and Pb was found. The chemical composition of *Pleurozium schreberi* was not affected by ash either. The wood ash increased the concentrations of Cd, Pb and Zn in the current year needles, meanwhile the concentrations of Ni and Cu in the needles showed even decrease tendency.

Comparison of different methods for copper free ion activity determination in soil solutions of contaminated and background soils

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Keywords: heavy metals, free ion activity, bioavailability

It is believed that free metal ion in soil solution is the most bioavailable and toxic form of Cu for plants and microorganisms. Recently soil critical limits for a number of metals have been derived on the base of free metal ion in soil solution and pH (Lofts *et al*. 2004). However determination of free metal ion activity in soil solution is a difficult and time consuming task which cannot be done in a routine way. Therefore comparison and verification of different methods is needed to get reliable data.

Here we present the result of Cu activity determination using different chemical methods (measurements with ion selective electrode (ISE, Avdeef *et al*.1983), and Soil Column – Donnan Membrane Technique (Temminghof *et al*. 2000)), as well as speciation calculations using WHAM IV (Tipping *et al*. 1998). Soil samples were collected from O_h and B_{hf} horizons of Al-Fe Humus Podzol along pollution gradient of Monchegorsk Cu - Ni smelter (7, 20, 28, 100, 200 km from smelter), Kola Peninsula, Russia. Activity of Cu was determined with DMT as described in Temminghof *et al*., 2000, using Na for background correction. Soil solution ratios were 1:2 and 1:7 for B_{hf} and O_h horizon respectively. Activity determination with Cu-selective electrode was based on a procedure of electrode calibration with Cu-ethylenediamine buffers. This method allows measuring of Cu activity in the range pCu 3,5–10. Deviations between measured values and calculated ones were within one order of magnitude and agreement was better for the case of DMT. For O_h horizon ISE method overestimated (up to one order of magnitude) Cu activities in the range of low concentrations in comparison with DMT. No agreement was observed between ISE and DMT for B horizon. The reason may be very low total Cu concentration in soil solution in B_{hf} horizon, which makes measurement with ISE unreliable. This conclusion can be also illustrated by the fact that at total Cu concentration below 10^{-6} – 10^{-7} mol.L^{-1} Cu activities were higher then total Cu concentrations (all data for B_{hf} horizon along pollution gradient except the most contaminated site, 7 km from smelter), which can not be correct. For DMT activity measurements ratio activity/total concentration was higher then 1 only for one sample (B_{hf} horizon, background soil, 200 km from smelter) with lowest Cu concentration (below 10^{-8} mol.L^{-1} in soil solution).

Conclusions: 1. ISE method overestimates Cu activities in the range of low concentrations in comparison with DMT and looks not reliable for the range of total Cu concentrations below 10^{-6} – 10^{-7} mol.L^{-1}. 2. DMT method of activity measurements demonstrated better reliability and agreement with speciation calculation (WHAM IV) then ISE method. Another advantage of DMT technique is possibility of activity determination for many elements simultaneously. Limitation is a value of detection limit of ICP-MS, used for metal concentration determination in acceptor solution (about 10^{-8} mol.L^{-1}).

Heavy metal content and effects on *Quercus pubescens* seedlings under acidic deposition and heavy metal contamination in the soil

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Keywords: acid deposition, ectomycorrhizal fungi, fine roots, heavy metals, pubescent oak

Quercus pubescens Willd. is a deciduous tree, whose natural distribution area is from Spain to Turkey, and from European Mediterranean coastlines to central France and Slovakia. It adapts to any kind of soils and is heliophilous, termophilous and xerophilous, so that it prefers the warm and dry calcareous soils when living at its latitudinal and altitudinal limits. *Q. pubescens* responses to acidity or heavy metal pollution have been little investigated. Our aims were to assess *Q. pubescens* tolerance to acidity (AR) and heavy metals (HM), and to investigate the use of this species in bioindication and phytoremediation. Attention focused on the root-to-soil (ectomycorrhizas and fine roots) interface. Leaf and root dry weight and element content were also measured. Leaf and root samples were collected from an open-top chamber experiment, where the treatments in four chambers each were: control (irrigation pH 5.5), acidic irrigation (pH 3.5), heavy metals (Zn, Cu, Cd, Pb) in the topsoil, the combination of acidic irrigation and heavy metals. Each chamber was split into two soil compartments, filled with an acidic (pH 4.5) or a neutral (pH 7.5) subsoil. At the leaf level, a three way (HM, AR, and subsoil) MANOVA on element content showed significant effects just for HM and the type of subsoil. The acid subsoil increased Mg and P, and decreased Mn contents, as compared to the calcareous subsoil. The HM treatment increased several micronutrients (Cu, Zn, B, Fe) and not essential elements (like Al, Cr, Na, Ni) and decreased important nutrients like N, S and Mg. Copper accumulation in the leaves was confirmed by a SEM microanalysis. Among the elements administered, Cu was the only one to show peaks above the background levels, mainly at the trichome tips and at the leaf edges. A significant AR x HM interaction was due to Mn behaviour: AR increased it with no HM and decreased it when HMs were present. At the fine root level, only weak effects were recorded, consisting in a tendency to increased biomass, length, and total number of tips in acid subsoil, and decreased length in the HM treatments. Element content analysis is still in progress. Despite *Q. pubescens* is well adapted to live in calcareous soils, our results show a slight beneficial effect on fine roots by the acidity of the subsoil. Such a stimulation may be interpreted like a counter-reaction to a slight or initial acidic stress. It is known that many stress factors induce an alarm phase, during which the stress is offset by counter-reactions, that may lead to overcompensation. The slight HM effects we observed both at the leaf and at the fine root level suggest that *Q. pubescens* may tolerate the realistic contamination applied. In conclusion, pubescent oak has the potential to be used for afforestation in contaminated areas, in the range of tested concentrations. As no significant accumulation of HM was recorded in the leaves, this species demonstrated to be a not suitable species for bioindication of HM contamination and for phytoremediation of HM contaminated soils.

Copper and lead tolerance of *Buddleia davidii* and *Chamaenerion angustifolium* populations on industrially contaminated land

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Keywords: Buddleia davidii, Chamaenerion angustifolium, inter-populations, copper-lead-tolerance

Vegetation on derelict, urban metal contaminated sites is often sparse or even absent. Metal tolerant species facilitate the revegetation of these sites and provide an aesthetically pleasing landscape that with specific management helps to stabilise, render harmless or even remove heavy metals from soils. *Buddleia davidii* (Loganiaceae), a shrub species, and *Chamaenerion angustifolium* (Onagraceae), a tall clonal forb species, both naturalised in the UK, are often found as colonists of lightly contaminated industrial land in Britain. The massive seed production and ubiquitous nature of these species enable them to rapidly colonise derelict post-industrial sites and, suggest that these species may have had the opportunity to evolve metal tolerance. This research investigates the tolerance between seedlings from three populations of *B. davidii* and C. *angustifolium* collected across the industrial conurbation of Northern Britain. A population collected from an uncontaminated site was also used as a control. Seedlings of both species were treated with lead or copper in 10% Rorison solution. *B. davidii* and C. *angustifolium* seedlings showed a degree of copper and lead tolerance typical of excluder species. Inter-population differences of young seedlings of *B. davidii* and C. *angustifolium* were relatively inconsistent, however, there was some evidence that tolerance was related to the degree of copper and lead contamination of the sites from which they were collected. It appears that these species have been overlooked and possibly may play a significant role in reducing the effect of heavy metal contamination on post industrial sites in Britain

Bioavailability of Ni and Cr in hay meadow on different soils from Auvergne

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Keywords: bioavailability, grass land, nickel, chromium

Introduction: The existence of volcanic geological matter in Auvergne, center of France, induce the agricole soils naturally rich in Ni and Cr. The French regulations for organic waste spreading (JO: decretn°97-1133 and arrete 8/1/98) permit derogation on anomalique area if MTE bioavailability isn't increase.Our project register in derogatoire context. The aim of our work presented here is: -to caracterize Ni and Cr bioavailability to quantify element flow at different consummation stage of grassland. Here, we are deal with hay weadow; -and to definie the effect of soil and plant to element transfer.

Methods: Five sites (S1-S5) of grassland were studied on different geological matter, Ni and Cr contents and pH values. The S4 and S5 soils are used as soils exempt from Ni and Cr anomaly. The sampling of soils and plants are carried out at two times of cutting (cutting or grazing). They are always achieved on a same geographic point. The Ni and Cr contents are analyzed by ICP-AES.

Soil discription	S1	S2	S3	S4	S5
Geological matter	volcanic	volcanic	volcanic	crystalline	crystalline
Clay and limon %	75.5	71	58.5	51	43
Total Ni soil (ppm)	180	230	290	16.5	7.6
Total Cr soil (ppm)	240	360	450	28.6	24
pH	5.1	5.3	6.5	5.4	6.5
OM %	11	13.6	4.5	13	2.9

Results and Discussion: The volcanic soils have more Ni and Cr contents than crystalline soils (S4, S5).These elements result of degradation geological matter. The texture of these soils is fine (2). S1, S2 et S4 soils are caracterized by: – acid pH well known to facilitate element (7) – high OM content, factor of MTE immobilization (4). Thus, the favorable conditions at a tranfer isn't join within a same soil.

The Ni content in different grassland are low from 0.5 ppm (S4 et S5) to 2.5 ppm (S1, S2). For Cr, the contents are from 0.5 ppm (S1, S4, S5) to 1 ppm (S2, S3). These values are normal (3,5,6). Ni and Cr aren't correlate in aerial parts contrary to in soil. A correlation soil/plant (0.6) for two elements is observed. The amount exported by aerial parts varie to 1 from 10 g Ni / ha and to 1 from 4 g Cr / ha. The aerial biomass is to 3.4 from 4.4 t MS / ha (S5 < S4etS1 < S3 < S2). Ni is more available than Cr except for S4 (1).

The transfer cofficient that is the ratio of the concentration of a given element in the plants to that in soil, is more high for soils S4 (Cr) and S5 (Ni and Cr). The roots may play a part on anomalics soils: in effect, the aerial parts contain only 1.6 to 3.5% roots content in Ni and 0.3 to 1.2% roots content in Cr.

Conclusions: The roots may play a part in element exportation into aerial parts.These results permit now to calculate Ni and Cr flow and affirm that the Ni and Cr content in meadows at hay stage are normal. They can also contribute to enrich knowledge in field of phytoremediation where the intern allocation mechanism in plant observed at some stage of growth are to take into account.

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Transcriptional profiling of the metal hyperaccumulator *Thlaspi caerulescens* (J. und C. Presl)

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Keywords: *Thlaspi caerulescens*, zinc hyperaccumulation, microarray

Thlaspi caerulescens is a well studied metal hyperaccumulator of Zinc, Cadmium and Nickel, belonging to the Brassicaceae family. Moreover it is one of the few hyperaccumulators that occur on different metalliferous soil types, as well as on nonmetalliferous soils. We are interested in the development of systems to improve phytoremediation of metal contaminated soils through advanced metal-accumulation. About 1900 isolated ESTs from *Thlaspi* roots and 2700 ESTs from *Thlaspi* shoots were hybridized with RNA from Zinc-treated *T. caerulescens* plants derived from two different soil types. This comparative transcript profiling of *T. caerulescens* plants resulted in the identification of genes by the means of hierarchical clustering that are involved in the molecular mechanisms of plant heavy metal tolerance, accumulation and transport. The developed microarray chip proofed to be an appropriate tool for this challenge. Additionally array data of selected genes were confirmed by quantitative RT-PCR.

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Interactions between metal toxicity and defences against biotic stress: glucosinolates and benzoxazinoids as case studies

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Keywords: biotic stress, chemical defence, DIMBOA, glucosinolate, metal toxicity, resistance

Metal ion toxicity has a deep impact on plant's metabolism. Primary processes like photosynthesis have deserved much attention. However, linked either to general stress effects (e.g. ROS production) or to ion specific responses (toxicity or resistance related), metabolic pathways implied in the defence against biotic stress may also be considerably altered under metal ion exposure. Fast acting chemical defences against herbivores and infective microorganisms comprise preformed substances and elicitor-induced phytoalexins. Among others, phenolic compounds, sulphur-containing molecules and terpenoids can be active against biological stress agents. Metal ions themselves can act as elicitors. Sulphur metabolism and secondary pathways like the shikimate pathway produce potential metal-ligands like phytochelatins and flavonoid-type phenolics, respectively. Antibiotics produced by microorganisms against microorganisms often are extremely strong metal ligands. Also certain plant-derived chemical defences can bind metals *in vitro*. However, relatively little is known about the possible interactions between defence related compounds induced by biotic and abiotic stressors inside plants and in the rhizosphere. After a short, comprehensive, overview about the different cross-points of metabolic interactions between metal ion toxicity and biotic stress factors, this presentation focuses on two specific case studies, concerning glucosinolate-Zn interactions in the metal hyperaccumulator *Thlaspi caerulescens* and aluminium-induced alterations on benzoxazinoids in maize (*Zea mays* L.) root tips. Glucosinolates are well-known, sulphur-containing anti-feedant agents that are effective agent insects, but also against fungal infections. Excess Zn has been found to specifically decrease indolyl glucosinolates in roots of *T. caerulescens* (Tolrà *et al*. 2001, New Phytol. 151: 621–626). Recent investigations in our laboratory have found fast Al-induced decrease of benzoxazinoids in root tips of Al sensitive but not in Al resistant maize. Benzoxazinoids (bxs) like DIMBOA or DIM2BOA are indolyl-derived phenolic compounds with an extraordinarily high affinity for iron. Bxs have been implied in defence against corn borer attacks and in alellopathyc interactions of Poaceae and of other plant families. Related literature data and our lab's work in this context will be critically discussed. Based on current knowledge a working hypothesis and further research requirements are highlighted for revealing possible links between stress-induced alterations in root growth and architecture, auxin metabolism and transport, and indolyl-derived biotic and abiotic stress defence substances.

Implication of oxidative stress in lead-induced genotoxicity in *Vicia faba* roots

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Keywords: lead, *Vicia faba,* reactive oxygen species, NADPH oxidase, antioxidative enzyme, genotoxicity

Lead (Pb), similar to other heavy metals and abiotic factors, causes many unfavorable changes at the subcellular and molecular levels in plant cells. Even if Pb is reported to produce reactive oxygen species (ROS) and enhance activities of enzymes involved in antioxidant defense (Rucinska *et al*. 1999; Malecka *et al*. 2001) few is kown about the genotoxicity of this heavy metal. The aim of this study is to determinate the possible relationship between lead-induced oxidative stress and genotoxicity in broad bean *(Vicia faba, Vf)* cells was studied in our work. Plants were cultivated in aerated nutrient solution in growth chamber. Two-weeks-old seedlings were exposed to an environmentally realistic concentration of lead (2ppm). In the first part of the present study, the mechanism of ROS production induced by lead in *Vf* excised roots was investigated by luminol-dependent-chemiluminescence (CL). Results showed that lead triggers a rapid and dose-dependent increase in ROS production. The rate of ROS generation presented a fast and brief increase, following by a deceleration phase until it reached a constant level, which persisted for 15–20 seconds. Then, ROS production decreased gradually to the basal line. The production of ROS induced by lead has been shown to be very sensitive to micromolar concentration of diphenyleneiodonium (DPI), an inhibitor of the NADPH oxidase (Olmos *et al*. 2003). Our results also demonstrated that in *Vf* whole plants, lead activates some of the key enzymes of the antioxidant defence system. In roots, lead enhances a sequential activation of ascorbate peroxidase (APX) and catalase that functions complementarily to remove H_2O_2. In leaves, catalase is the major enzyme involved in H_2O_2 detoxification. In addition, lead induces an enhancement of the glutathione reductase activity in *Vf* roots and leaves. In whole plants, DPI inhibition of antioxidant enzyme activations in roots and leaves, gives the evidence of the major role of NADPH-oxidase activation in oxidative stress induced by lead. In the second part of the study, the potential use of micronucleus assay (MN) for the detection of genotoxic effects of lead was investigated on *Vf* root tips. Datas showed that 2ppm of lead produced a 5.11-fold increase of MN frequencies without significant modification of mitosis. On the other side, a treatment with DPI at 0.1 and 1μM totally prevents lead from generating MN. Same results were obtained with 100 μM of α-tocopherol (vitamine E, Vit E), a powerfull antioxidant. In this condition, MN frequencies remained at control level. These results indicate that lead strongly induces ROS production and genotoxicity in *Vf* roots. The protective effect of DPI and α-tocopherol against Pb ions indicates that lead genotoxicity may be mediated by ROS generation via NADPH-oxidase. Moreover, activation of some antioxidant enzymes in response to oxidative stress induced by lead is not enough to prevent metal genotoxic effects.

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Enzymatic activities of absorbing roots of seven tree species exposed to heavy metals and acid rain

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Keywords: mycorrhiza, stress response, forest trees, extracellular enzymes

Extracellular enzymes of soil organisms are involved in soil nutrient cycles and organismic interactions in soils. The influence of single and combined heavy metal and acid rain treatment on extracellular enzymatic activities in the mycorrhizosphere of 7 different tree species was studied addressing below-ground effects on fine root functions of important forest tree species due to the stress imposed.

Root samples were taken in May 2005, at the final harvest of the Cell-to-Tree-Experiment at WSL, Birmensdorf, Switzerland. 14 fully vital individual fine roots of every individual plant species at each of 20 plots (control, acid rain, heavy metal, combined treatment, each in 5 replicates) were used for multiple enzyme assays designed for measurements on single root tips (Pritsch *et al.* 2004; Courty *et al*. 2005). A total of 1960 roots analysed were subjected to 6 different enzyme assays.

Ectomycorrhizae were present at the root systems of the known ectomycorrhizal (EM) species *Betula pendula*, *Fagus sylvatica*, *Populus nigra*, and *Picea abies*. *Salix viminalis* and *Alnus incana* which can have both, ecto- and arbuscular mycorrhizae formed EM only exceptionally on less than 5% of their absorbing roots. *Acer pseudoplatanus*, an arbuscular mycorrhizal (AM) species never formed ectomycorrhizae.

First evaluations of the enzyme data indicate that the activity levels of the six studied enzymes phosphomonoesterase, N-acetyl glucosaminidase, ß-glucosidase, cellobiohydrolase, xylosidase and laccase were usually higher in the EM than in AM plant roots.

Compared to the control, both heavy metal treatments (with or without acid irrigation) affected enzyme activities more strongly than the acid rain treatment alone. However, not all species appeared to react the same way. Species specific effects and the main effects influencing enzyme activities of absorbing roots will be presented.

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Effects of heavy metals on dissolved organic matter, sulphate, phosphate and nitrate in the soil solution of a forest model ecosystem

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Keywords: heavy metal contamination, mineralisation, soil microbial assays, soil solution

The mineralisation of leaf litter and soil organic matter leads to the release of nutrients such as nitrate, sulphate, and phosphate that can be utilized by plants and other soil organisms. The main actors of mineralisation processes are microorganisms. Thus, any contaminant that poisons soil microorganisms may imperil the functioning of the soil ecosystem and the sustainability of the whole terrestrial ecosystem. Since soil solution composition is the net result of all relevant processes in the soil ecosystem at natural conditions and reacts quickly to pollution effects, the *in situ* soil solution monitoring might be a complementary tool to microbial assays to determine whether a natural soil ecosystem is being altered by pollutants. The effects of heavy metals (HM) on dissolved organic matter (DOM), sulphate, phosphate and nitrate in the soil solution were investigated over 1 year in young forest model ecosystems and plant-free references. The results were interpreted in terms of effects of HM contamination and plant influence on the mineralisation potential of a whole soil compartment. In addition, different biological parameters were investigated with microbial assays. In the factorial lysimeter facility of the "Cell to Tree" experiments, the factors topsoil contamination (with/without HM), subsoil type (acidic/calcareous) and plant cover (with/without plants) were considered. Biological parameters of topsoil samples from the planted lysimeters revealed a significant reduction of the microbial activity by HM contamination. In good agreement with these results, DOC and SO_4^{2-} concentrations were lower in the contaminated planted topsoil than in the uncontaminated planted topsoil. No HM effects were observed in absence of plants with DOC and SO_4^{2-} concentrations remaining at a similar level as in the planted contaminated topsoil. These results suggest on one hand a base level of mineralisation in the absence of plants that was not affected by HM contamination. On the other hand the stimulation of the microbial activity by root exudates was very limited under HM stress probably partly because of the negative effect of HM on root growth as suggested by root density measurements (Menon *et al.* 2004). In contrast to DOC and SO_4^{2-}, PO_4^{3-} concentrations in the topsoil were reduced by HM not only in presence but also in absence of plants. This was interpreted as an additional chemical effect of reduced PO_4^{3-} solubility in the contaminated topsoil. Higher NO_3^- concentrations in the HM treatment than in the control in the planted topsoil were inconsistent with the HM effects on nitrification potential. The much higher concentrations in absence than in presence of plants suggest that NO_3^- concentrations were controlled mainly by plant uptake.

Reference:
Menon, M., Hermle, S., Abbaspour, K.C., Günthardt-Goerg, M.S., Oswald, S.E., Schulin, R. (2004). Water regime of metal-contaminated soil under juvenile forest vegetation. Plant Soil. (in press).

Armillaria root disease in model ecosystems exposed to heavy metals and acid rain

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Keywords: tree-fungus interactions, soil conditions, Armillaria rhizomorphs, biosorption

In April 2000, model ecosystems were established in 16 open top chambers in the frame of the "cell-to-tree" project. Each chamber was split into two ecosystem compartments one containing an acidic (pH 4.2) and one a calcareous subsoil (pH 7.4). Each compartment was again split into two quarter-chambers of which one was used for the *Armillaria* inoculations and one served as control. The ecosystem in each quarter chamber consisted of three Norway spruce *(Picea abies)*, two poplar *(Populus tremula)*, one willow *(Salix viminalis)*, and one birch *(Betula pendula)* trees and various understorey plants. The treatments applied in each of four open top chambers were a) control without heavy metals, irrigation pH 5.5. b) heavy metals dust resulting in: Zn, 2700 $\mu g\ g^{-1}$; Cu, 385 $\mu g\ g^{-1}$; Pb, 63 $\mu g\ g^{-1}$; Cd; 10 $\mu g\ g^{-1}$ in the top soil, irrigation pH 5.5. c) acidic irrigation pH 3.5 imitating acid rain. d) the combination of acidic irrigation and heavy metals. In Mai 2000, a pathogen-saprophyt system using *Armillaria ostoyae* and *Armillaria cepistipes* was inoculated by inserting colonised hazelnut segments into the soil near each of the spruce trees. *A. ostoyae* is a pathogenic soil-borne fungus causing root rot in many tree species, especially conifers. *A. cepistipes* preferentially behaves as a saprotrophic wood decomposer.

Mortality of spruce caused by Armillaria root disease was first observed in 2001. By the end of 2003, *A. ostoyae* had killed 16 out of 96 spruce trees. In addition, 25 surviving spruce trees showed root infections, mostly also caused by *A. ostoyae*. There were significantly less tree mortality and root infections in the chambers with heavy metal contaminated soils (with and without acidic rain). The acid rain treatment and the subsoil type had no effect on mortality or infections. Examinations of the roots in the end of the experiment suggest that (1) heavy metals reduced the ability of the rhizomorphs (root-like fungal structures, 1–2 mm in diameter, that spread *Armillaria* in the soil) to attach to the roots and (2) heavy metals reduced the expansion of lesions formed on the roots. *A. ostoyae* and to a lesser extent *A. cepistipes* also caused root infections on poplar and willow, but without significant influence of the treatments. No root infections were observed on birch.

In the rhizomorphs produced by the *Armillaria* species, the concentration of Zn, Cu and Cd, but not Pb was significantly higher in soils with heavy metals than without heavy metals. In the contaminated soils, the mean concentrations of heavy metals in the rhizomorphs were 6300 $\mu g\ g^{-1}$ Zn, 228 $\mu g\ g^{-1}$ Cu, 10.6 $\mu g\ g^{-1}$ Cd, and 12.1 $\mu g\ g^{-1}$ Pb. EDX-analysis showed that Zn was preferentially absorbed in the melanized cortex of the rhizomorphs.

Phytoremediation for the management of metal fluxes in contaminated sites

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Keywords: hydraulic control, phytostabilisation, phytoextraction

Phytoremediation describes the use of plants to improve degraded environments. On metal polluted sites, phytoremediation affects metal fluxes via the extraction of contaminating metals (phytoextraction), or their immobilisation (phytostabilisation). Phytoextraction removes metals from the soil by repeated crops of plants that accumulate large amounts of one or more target metals in their above-ground biomass. The harvested plant material is removed from the site. Despite more than ten years of research, there are few examples of successful phytoextraction. This technology is limited by the long period required for cleanup, the restricted number of target metals that can be extracted, the limited depth that can be accessed by roots, and the difficulty producing a high-biomass crop of the desired species. There is also concern about metal-accumulating plants providing an exposure pathway for toxic elements to enter the food chain. The addition of chelates, to enhance plant-metal uptake, invariably leads to metal leaching due to preferential flow processes. Phytostabilisation exploits transpiration and root-growth to immobilise contaminants by reducing leaching, controlling erosion, creating an aerobic environment in the root-zone, and adding organic matter to the substrate that binds metals. Soil amendments can promote plant growth and further decrease metal mobility. Phytostabilisation requires the establishment of tailored vegetation on the site that is left there in perpetuity. A succession of plant species may be used to establish the desired climax vegetation. Unlike phytoextraction, there are numerous examples of successful phytostabilisation on metal-contaminated sites. Vegetation profoundly affects metal fluxes in contaminated sites. Clothier *et al*. (1997) described roots as “the big movers of water and chemicals in soil”. Of the global average of 720 mm of rainfall per year that falls on land, some 410 mm are returned to the atmosphere via evapotranspiration. The rate of evapotranspiration is species dependent. In many cases, fast-growing deep-rooted tree species return more rainfall to the atmosphere than shallow-rooted herbs or grasses. During periods of drought, deep-rooted species have greater access to water and continue to transpire long after shallow-rooted species have become inactive through drought. Tree canopies act as umbrellas where 15–30% of rainfall may be re-evaporated before it reaches the ground. The success of phytoremediation is site specific due to the plethora of environmental variables that affect plant growth and metal mobility. Most contaminated sites contain a heterogeneous mixture of several elemental and organic contaminants. Plant-growth may be limited by other environmental variables, such as low pH, low nutrient availability, salinity, anaerobic conditions or low water availability. The commercial success of phytoremediation is dependent on convincing decision makers that phytoremediation can satisfy environmental regulation. Obviously, field demonstrations at each site are not practical; therefore validated mechanistic models are required to calculate the effect of phytoremediation on metal fluxes. Central to such models is an understanding of root-metal interactions in these typically heterogeneous media. This paper describes the effects of plants on metal fluxes and the implementation of phytoremediation in the field.

Reference: Clothier *et al.* Soil Sci. 1997, 162(8), 534–543.

Evaluation of copper and zinc concentration in topsoil of the Ebro Basin by means of teledetecion

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Keywords: topsoil, geostatistical, Landsat-7 ETM+, copper and zinc, spatial distribution

This work seeks to evaluate the copper and zinc concentration in topsoil using spectral radiance data from a Landsat-7 ETM+ image. For this purpose, the topsoil content of these metals have been determined analytically in 624 plots distributed in a preliminary mesh of 8 x 8 km, in agricultural areas or grasslands. Showing values that oscillate between 5 mg/kg and 207 mg/kg with a mean of 17.33 mg/kg for copper and between 11 mg/kg and 175 mg/kg with a mean of 57.54 mg/kg for zinc.

The remote sensor information used in this work was derived from Landsat-7 ETM+ satellite image. For each sample, inside the image area, the values of spectral radiance of six bands were obtained. These values were equated by means of multiple regression of the copper and zinc concentration in the topsoil. The resulting equation in both cases presented a high r^2 (more than 90%).

The spatial distribution of the Cu and Zn concentration were studied using geostatistical interpolation methods (kriging). A “raster” with approximate values of the concentrations of these heavy metals was obtained based on the spatial variability of the values. The comparison between the two estimation methods of the copper and zinc concentration in topsoil layer has been evaluated by means of a 15 value control. It is observed that the best relationship is obtained using the spectral radiate data with a r^2 of 0.74.

Metal fluxes from soil to plant shoots via rhizosphere and roots: implication for food safety and phytoextraction strategies

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Keywords: metal fluxes, rhizosphere, roots, food safety, phytoextraction

In various agricultural fields metal concentrations have reached critical levels of heavy metals for food safety reason due to geogenic origin or anthropogenic activities such as e.g. mining or sludge applications. To minimize food chain contaminations by heavy metals or to improve phytoextraction as a possible clean-up technology a better understanding of the fundamental process of metal acquisition by plants have to be considered, which are: a) Transport to roots via mass flow and diffusion; b) mobilization and immobilization processes in the rhizosphere; c) uptake of heavy metals into roots; d) root-to-shoot translocation and e) accumulation and detoxification of heavy metals in leaf cells.

Due to a general low solubility of heavy metals in soils diffusion is of a higher relevance than mass flow for the transport of metals to the uptake sites at the root surface. Thus, soil water content as well as root growth characteristics (e.g. root hair density and length) are of significance for a high spatial accessibility of heavy metals. Via an efficient uptake system with a subsequent deep depletion at the root surface the concentration gradient as driving force for diffusion and therefore spatial accessibility can be increased. As a consequence of such an efficient uptake system for heavy metals in hyperaccumulating plant species a depletion up to 40% of total metal content (e.g. Cd) in the rhizosphere can easily be reached. However, the spatial extent of the rhizosphere is only fractions of a millimetre which will limit the efficacy of phytoextraction.

But also by direct changes in the rhizosphere chemistry the chemical availability of heavy metals can be managed for food safety (reduced solubility) or for enhanced phytoextraction (enhanced solubility). Chemical changes in the rhizosphere such as in pH, redox potential or root exudation can be reached by selection of distinct genotypes and/or by measures of a rhizosphere management such as the use of nitrogen fertilizer with different N-forms (nitrate versus ammonium), liming and supplementation with heavy metal mobilizing chemicals (chelators) or bioeffectors. The root-to-shoot translocation via xylem sap is hardly a liming step, in general. In hyperaccumulating plants with an excessive high accumulation of metals in shoots, an effective compartmentation and detoxification preferentially in vacuoles have to take place as a vital process of revival.

Beside various methods of phytoremediation, phytoextraction is often discussed as a possible technology for clean-up on the basis of model pot experiments. But field experiments on sites with realistic metal contaminations cannot support such an optimistic view, which is in agreement with the findings that till now no practical application does exist world-wide in spite of an intensive research on phytoextraction for more than two decades. In general, unrealistic long periods of years will be required to reach the requested decontamination, mainly because of a restricted spatial accessibility and limited kinetics for replenishment. To make phytoextraction more feasible, a GM approach with an insertion of a manipulated IRT1 transporter for specific toxic metals as indicated by M. Guerionot (2004) in crop plants with a high capacity for biomass formation will be needed, a trait which is not easy and not reached in short term.

Nevertheless, there are various measures which can improve in particular food safety such as rhizosphere management with consideration and evaluation of chemical and spatial accessibility of heavy metals at the same time.

Cd, Cu and Zn content in the above-ground parts of *Taraxacum officinale*

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Keywords: *Taraxacum officinale,* metal-uptake, soil-legislation, food-legislation

The soil we analysed was on an ancient landfill that was polluted by heavy metals – especially Cd, Cu and Zn. Previous analyses performed on trees (*Salix viminalis*, *Betula pendula*, *Alnus incana*, *Fraxinus excelsior* and *Sorbus mougeotii*) showed less or no uptake of metal in the vegetative tissues, except for *Salix* and, partially, for *Betula* (Rosselli *et al*. 2003[1]). Recently, we tested a common grassy species *(Taraxacum officinale)* in order to compare the concentration of Cd, Cu and Zn in its above-ground vegetative tissues with the metal concentration in the soil at different pollution levels (polluted, slightly polluted and non-polluted, according to the Swiss law) and to compare it with the Swiss legislation concerning food – *Taraxacum officinale* is traditionally employed for salad and in the natural pharmacopoeia. We wished to establish under which conditions the metal concentration in the plant tissues was higher than the values admitted by the Swiss legislation relating on nutrition. We observed that the metal concentration in the vegetative tissues was not correlated with the metal concentration in the soil analysed. The metal concentration in the soil samples was statistically very variable in each sample group, but the groups were clearly distinguishable from each other.

	Cd	Cu	Zn	pH
control	0.42 ± 0.05	18.90 ± 5.27	102.14 ± 55.53	7.72 ± 0.08
slightly polluted	0.67 ± 0.21	132.93 ± 88.65	178.62 ± 100.45	7.70 ± 0.16
polluted	1.22 ± 0.52	284.39 ± 154.02	322.34 ± 155.94	7.77 ± 0.08
OIS	0.8	40	150	–

Concentrations of Cd, Cu and Zn [mg kg^{-1}] and pH measured in the three soil groups and concentrations admitted by the Swiss federal Ordinance relating to impacts on the soil (OIS 1998).

The metal concentration we found in the vegetative tissues of *Taraxacum* was higher than the limits admitted by the Swiss federal ordinance relating to the substances and components of food (OSEC 1995), even in the plants growing on a non-polluted soil, except for Cd. Nevertheless, the concentration of Cd we measured in the plants growing on the polluted and slightly polluted soil was very variable, with peaks above the guideline concentrations. Similar high Cu or Zn concentrations were found in the samples growing on the polluted soil as well as in the control samples. Thus, the concentration of these metals in the tissues of *Taraxacum* does not seem to depend on the metal concentration in the soil.

[1] Rosselli W., Keller C., Boschi K. 2003. Phytoextraction capacity of tree growing on a metal contaminated soil. Plant Soil 256: 265-272.

	Cd	Cu	Zn
control	0.00 ± 0.00	11.84 ± 2.48	59.94 ± 20.46
slightly polluted	0.06 ± 0.21	12.14 ± 2.16	44.06 ± 10.14
polluted	0.08 ± 0.20	11.82 ± 2.19	44.70 ± 9.60
OSEC	0.2	5	5

Concentrations of Cd, Cu and Zn [mg kg^{-1}] in the vegetative tissues of Taraxacum growing on the three soil groups and concentration admitted by the Swiss federal Ordinance relating to the substances and components of food (OSEC).

Trace metal measurements in atmospheric depositions in three sites in the north of Italy: methodology and preliminary results

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Keywords: atmospheric deposition, trace metals, ionic concentrations, ICP-MS, ICP-OES, metals fluxes

In the period January – December 2004 ARPA Piemonte and CNR ISE of Verbania Pallanza started a collaboration to analyse trace metals in atmospheric depositions. Since 1995 CNR Institute for Ecosystem Studies (CNR ISE) has acted as National Centre in Italy for the Project "ICP Waters", coordinated and directed by the International Environmental Protection Department of the Ministry of the Environment. In addition to the continuous monitoring of water chemistry at the ICP Waters sites, in 2004 a study on metal concentrations has been launched. It has been designed to cover lake and river waters, but extended to atmospheric depositions in order to distinguish between the fraction of metals deriving from transport and the fraction originating from weathering in the watershed. Atmospheric deposition analyses were performed in collaboration with researchers of ARPA Piemonte (Regional Environmental Protection Agency), which meant that analyses could be conducted using two different instrumental methods: plasma emission spectrophotometry (ICP-OES), by CNR ISE, and mass spectrometry (ICP-MS), by ARPA. The second method has the advantage of being more sensitive, so that some metals could be quantified to a lower level than the ICP-OES detection limit; concentrations of other metals which had not initially been included in the project, such as mercury, were also quantified. Besides metals also macroconstituents were analyzed (ionic concentrations) by CNR ISE. Analyses were performed on samples of atmospheric depositions collected in three stations, each site representing a different environmental condition: Bellinzago, at the edge of the Po Plain in an industrialized area; Pallanza, a town in the subalpine area; Alpe Devero, a high mountain valley far from anthropogenic sources in a remote area of the Veglia-Devero Regional Natural Park. The sampling station with the highest concentrations was Bellinzago, located close to industrial sites and urban centres. The subalpine area of Pallanza presented metals concentrations similar to Bellinzago. The Alpine station, Alpe Devero, located at 1,600 m, showed the lowest levels for metals and for the ionic concentrations. Measured fluxes of mercury, cadmium and lead were in good agreement with the fluxes estimated by the EMEP on the basis of the emissions of these metals in the atmosphere and according to dispersion models. In general concentrations showed high variability from one event to another. Cluster analysis applied to the atmospheric deposition data revealed the common origin of a number of metals, for example those of terrigenous origin (aluminium, boron, barium, iron, manganese and strontium) and those of clearly anthropogenic origin (copper, nickel and vanadium), due to metallurgical industries, road traffic and waste incineration. The same analysis also revealed expected differences between the sampling sites. The station of Pallanza, which is affected by road traffic to a greater extent than the other two sites, showed up the link between palladium and rhodium, used as catalysers in the new generation of car catalytic converters.

The effect of pH levels of 3.6, 4.6 and 6.0 on the growth and mineral composition of apple rootstocks: P 22, M 9 and M 26.

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Keywords: aluminium toxicity, low pH, apple rootstocks, aluminium, manganese, tolerance

Under low pH conditions the Al, Mn and H content in the soil solution significantly increases, as does their toxic effect on plants. Plants growing on acidic soils take up excessive amounts of ions characteristic of that environment, often those that are toxic and competitive (aluminium, manganese, some heavy metals), while such elements as magnesium, calcium, phosphorus and molybdenum are in general taken up in insufficient amounts. The difficulties in taking up basic cations from acidic soils are a result of the reduction in the transfer rate at which exchangeable H^+ ions pass through the plasma membrane to the rhizosphere.

The field experiment was carried out on vegetatively propagated apple rootstocks P 22, M 9 and M 26 grown on microplots (a clay pot buried in the ground, 1.2 m high and 40 cm in diameter) having 3 different pH values: 3.6, 4.6 and 6.0. The differences in the development and morphology of the shoots, roots and mineral composition of the rootstocks and soil solution were determined.

The results revealed the highest tolerance of the P 22 rootstock to soil acidification and Al ions, lower tolerance of M9 and the lowest of M 26. The roots and leaves of the plants growing on the most acidic soil (pH 3.6) contained the largest amounts of Al. The M 26 rootstock was the most sensitive to strong soil acidification, which was associated with the highest Al content in its roots and shoots, and the highest manganese content in its leaves, shoots and roots. In comparison with the M 26 rootstock, the Al and Mn content was lower in M 9, and the lowest in the more tolerant P 22. The largest amounts of Al were found in the roots (1038 mg/kg d.m.), less in the shoots (153), and the least in the leaves (105). Strong soil acidification (pH 3.6) significantly increased the total manganese content in the roots, shoots and leaves of the rootstocks. Irrespective of the soil pH and the type of rootstock, aluminium accumulated in the largest quantities in the roots while manganese in the leaves of the rootstocks. This indicates that manganese, unlike aluminium, was easily transported from the roots to shoots and that is why in the acidified soil environment symptoms of Mn toxicity were first observed on the leaves. The highest aluminium content of the roots and leaves of the rootstocks growing on the most acidic soil (pH 3.6) was associated with the highest level of this element in the soil solution and its high availability to plants.

Accumulation and solubility of heavy metals during leaf litter decomposition in non-polluted and polluted soil

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Keywords: leaf litter, heavy metals, degradation, solubility

The litter decomposition is a key process in the material cycle of a forest ecosystem and depends on the interaction between the soil, biota and the environment. During the decomposition process heavy metals in the litter may be released through leaching and mineralization or may be enriched if they are immobile. Most studies about leaf litter degradation investigated this process in forests containing a continuous litter layer. No information is available to date on the degradation of leaves when they fall onto bare soil. Such a situation can occur in forests without continuous litter layer, or when leaves are deposited outside the forest or in the first years of planting trees. Trees have been proposed for the phytostabilization of metal polluted soils and in such a situation leaves from trees grown on polluted soils can be deposited either on the bare polluted soil but also be carried to adjacent non-polluted soil.

In our study we studied the decomposition of alder *(Alnus glutinosa)* and poplar *(Populus tremula)* leaves from trees grown on polluted and non-polluted soils. The leaf litter was exposed to control and metal-polluted polluted soil.

The leaves differed in the initial Cd and Zn content depending on the growth place of trees whereas the lead and copper contents were similar. Between 40% and 60% of the leave mass was lost in the first year. Only slight differences were observed between the different treatments and neither initial leaf metal content nor metal pollution of the soil has a large influence.

The metal concentrations in litter placed in non-polluted soil showed little or no variation for Cu, Zn, and Cd over time and a slight increase for Pb. The heavy metal concentrations in leaf litter exposed to polluted soil increased considerably (factor up to 57) during decomposition.

Much higher dissolved cadmium and zinc were measured in extracts of leaves from trees grown on polluted soil compared to leaves from trees grown on non-polluted soil at the beginning of the experiment. The soluble cadmium and zinc contents decreased down to the level in the extract from control leaves during the first two months. The dissolved copper and lead amounts didn't vary between both extracts. The solubilized Cu, Zn and Cd concentrations were higher in leaves from polluted soil. The solubility of the heavy metals was between 1.5% and 5% when the experiment was started. During the decomposition the solubility of metals in the decomposed leaves in non-polluted soil remained about the same compared to fresh leaves. In contrast the solubility of the metals in leaves placed on polluted soil was markedly reduced.

The results show that on bare polluted soil a net uptake of metals by the decaying leaf litter is observed but that the relative solubility of the metals is reduced during that time.

Influence of heavy metals on the detoxification of organic xenobiotics in plants

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Keywords: multiple pollution, organic xenobiotics, detoxification, inducible metabolism

Environmental pollution by heavy metals is in many cases accompanied by pollution with organic foreign compounds and vice versa. Phytoremediation but also phytostabilization in such areas of multiple pollution is complicated, and only few plant species have been shown to survive under such adverse conditions. Heavy metals are well known for their ability to interfere with the plant´s metabolism and to induce the formation of reactive oxygen species (ROS), albeit through different reactions. Especially arsenic, cadmium, lead and copper have been investigated with respect to these effects. On the other hand, it has been shown that ROS may serve as signalling molecules for a number of defense reactions in plants, including alterations in the sulphur metabolism. Tightly connected to the sulphur metabolism in plants is the predominant detoxification pathway of halogenated organic pollutants and herbicides, i.e. the glutathione S-transferase dependent detoxication. Glutathione S-transferases (GST) catalyze the conjugation of reduced glutathione to the electrophilic centers of such xenobiotics. They comprise a very heterogeneous family of enzymes that can be subdivided into distinct classes with tasks in plant metabolism and defence. More than 50 gst genes have been identified in model plants. To date their functions are not known in too much detail, but it is clear, that their expression and activity is strongly modulated by oxidative stress and ROS. In order to obtain information on the physiological background of the ROS formation and the influence on the detoxification of organic pollutants, we have investigated the reaction of plants used in phytoremediation, e.g. *Typha* and *Phragmites*, but also a plant cell culture that has been used as a biomonitor of stress.

The general answer of these plants toward heavy metals in concentrations from 10 to 250 μM is an increase in GST activity and several other enzymes like peroxidase, ascorbate peroxidase and glutathione reductase, connected to strong alterations in the glutathione pool.

Interestingly, and different from the general opinion, we found that even mild heavy metal stress might lead to the inhibition of detoxification reactions in plants. Glutathione S-transferase actitvity for specific organic substrates was lacking after 6 to 24 hrs of incubation with heavy metals, whereas other substrates were conjugated at even higher rates. This development depends heavily on the heavy metal and its concentration. Mixtures of heavy metals show a tendency towards synergistic effects.

Under real life conditions and multiple pollution scenarios this might mean that the combination of heavy metals and organic pollutants (a) can be tackled by the plant, if the correct enzyme activity is induced, or (b) leads to rapid development of stronger stress due to the additional action of the undetoxified organic xenobiotic. Whether this effect is connected to a depletion of GSH, or an overload of storage pools of the plants, is critically discussed.

Phytoremediation of organic pollutants with *Typha latifolia* under the influence of heavy metals

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Keywords: *phytoremediation, organic polluants, heavy metals, wastewater*

Typha latifolia is very effective for phytoremediation of wastewater contaminated with low concentrations of antropogenic substances such as pharmaceuticals, pesticides and other xenobiotics.

The influence of heavy metals that may be present in sediments or in polluted wastewater on the removal of xenobiotics has so far not been addressed. Series of pilot experiments with xenobiotic model substrates showed that *Typha latifolia* has the potential to remove and detoxify xenobiotics from wastewater and bed-load sediments contaminated with both, heavy metals and organic pollutants.

Incubating *Typha* plants with Cu, Pb, Cd and As in concentrations between 20 to 250 μM for 24 hrs resulted in changes in the activity of the detoxificaton enzymes.

When calculated on a fresh weight basis, all metals apart from Cu caused decreases in GST activity. Under real life conditions, this would mean a loss of detoxification capacity. However, with regards to the specifical activity (calculated on soluble protein basis), arsenic increased GST activity dramatically. Here, GST proteins are induced at the cost of other proteins.

The efficacy of *Typha* to facilitate phytoremediation under multiple pollution stress is critically evaluated.

Phytoextraction of heavy metals by herbal plant (Basil)

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Keywords: phytoextraction, herbal plant, basil, heavy metals

Phytoextraction has become attractive due to its relatively low cost coupled with its effectiveness and aesthetic nature of using plants to clean up sites. Hyper-tolerance of metals is the key plant characteristic required for hyperaccumulation and biomass yield; vacuolar compartmentalization appears to be the source of hyper-tolerance of natural hyperaccumulator plants. The use of plants such as mustard and potato were for phytoextraction of the heavy metals reported in other studies (Bennet *et al*. 2003; Baghour *et al*. 2001). Basil is a hairy and labiate plant, growing about 3 feet high. It is a highly aromatic plant of the Ayurvedic tradition, and have adaptogenic, antifungal, antibacterial and immune enhancing properties. Basil has been occasionally used for mild nervous disorders and for the alleviation of wandering rheumatic pains- the dried leaves, in the form of snuff, are said to be a cure for nervous headaches. An infusion of the green herb in boiling water is good for all obstructions of the internal organs, arrests vomiting and allays nausea. The seeds have been reckoned efficacious against the poison of serpents, both taken internally and laid upon the wound (www.botanical.com). In the present work, the Sweet Basil (*Ocimum basilicum*, Family: Labiatae), commonly used in medicine and for culinary purposes in India was selected for the heavy metal phytoextraction studies. The different parts of basil (i.e. leave, stem, root and seed) and rhizospheric soil were collected from contaminated sites i.e., steel plant, thermal power plant, etc. in Chhattisgarh, India in November, 2004 as prescribed in the literature (Lawrence 1991). They were dried, crushed and sieved out the particles of mesh size, < 1 mm. The powdered samples were acid digested, and the concentration of the heavy metals i.e. As, Se, Cr, Mn, Fe, Ni, Cu, Zn and Pb are determined using techniques: flame- atomic absorption spectrometry (F-AAS) and hydride generation- atomic absorption spectrometry (HG-AAS). The accumulation of heavy metals (i.e. i.e. As, Se, Cr, Mn, Fe, Ni, Cu, Zn and Pb) by the most common herbal plant i.e. basil was investigated for its phytoextraction ability. The heavy metal contents in various parts of the basil, in addition to morphological and spatial variations in its accumulation are discussed. The available amount of the heavy metals in the soils and accumulation factor in the basil are also described.

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Phytoextraction of heavy metals by giant trees

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Keywords: heavy metals, phytoextraction, giant trees, central India

Bad air is filtered through photosynthesis and evapo-transpiration by the tree, cleaned, cooled, and released back into the atmosphere. An 80-foot beech tree has been shown to remove daily carbon dioxide amounts equivalent to that produced by two single-family dwellings [1]. The species i.e. neem (*azadirachta indica* A. Juss), banyan (*ficus bengalensis* linn), peepal (*ficus religiosa* linn), etc. are Indian evergreen holy tree. They grow in a desirable climate and tolerate drought and poor soils. Banyans are a type of tropical fig tree and **spread over a large area**. They are of great medicinal importance, and their leaves, flower, bark and seed have wide pharmaceutical applications. They are sources for the organic compounds i.e. tannin, lasone, etc. A tree i.e. neem, peepal, etc. is planted in the gardens purifies the surrounding air and helps in maintaining hygienic conditions due to the largest emitters of oxygen. These trees are very tall with long life, > 100 years. They absorb sunlight, dust, gases and cool the environment especially in summer. The aim of this work is to investigate the heavy metal (i.e. As, Se, Cr, Mn, Fe, Ni, Cu, Zn, Pb, etc.) contents in three trees (i.e. neem, peepal and banyan) to know their role in phytoremidiation of the heavy metals.

The samples are collected from the contaminated sites of the Chhattisgarh sate (central India) in the year 2005 using the established methodology. Their leave, bark, stem and fruit or seed, and soil samples are dried, powdered and sieved out the particles of mesh size, < 1 mm. The samples are digested with acids and the metal content is analyzed using technique i.e. inductively coupled plasma-optical emission spectrometer (ICP-OES).

The contents of heavy metals in various parts of trees (i.e. root, leave, stem, bark and soil are investigated. The translocation and accumulation factor of the heavy metals are reported. The phytoextraction potentiality of these trees for the heavy metals (i.e. Cr, Mn, Fe, Ni, Cu, Zn, Pb, As, Se, etc.) is critically discussed.

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Phytoextraction of heavy metals by green vegetables

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Keywords: heavy metals, vegetables, phytoextraction, Mandala city

Fluoride in excess is a health hazard, responsible for the prevalence of fluorosis. Pollution of the environment by the heavy metals (HMs) such as Cr, Mn, Co, Ni, Cu, Zn, Sn, Sb, Cd, Hg, Tl, Pb, etc. has been recognized as one of the most urgent environmental problems. Additionally, the heavy metal contamination of the environment enhances the toxicity factors of water significantly.

The fluorosis endemic area of Mandla city (lies at 22°60' N latitude and 80°38'E longitude) has been selected for the investigation of HMs (i.e. Ni, Cu, Pb, etc.) in the proposed work. At least 1 kg of soil samples (0–10 cm depth) derived from 4 different sites of the Mandla city in summer (March, 2002) were collected. They were stored in a polyethylene bottle and air dried for 1 week. The samples were ground to a fine powder with mortar and passed through a sieve of 0.2 mm mesh size. X-Ray fluorescence spectrometry: Energy dispersive XRS with polarized radiation (: Spectro X-Lab 2000) was used for the measurement of the trace metals in the soil samples at the Technical University, Darmstadt, Germany. The standard soil sample was used for the calibration. The total contents of elements i.e. K, Mg, Ca, Al, Si, Ti, Co, Ni, Cu and Pb were determined. The soil is black in color with high contents of elements i.e. Mg (0.28%), Ca (2.34%), Al (1.90%), Si (7.07) and Ti (1.44%). The total contents of Co, Ni, Cu and Pb in the surface soil of Mandla city were found in the range of 41–104, 41–66, 291–393 and 7.4–17.5 mg kg^{-1} with mean values of 70.5, 56, 312 and 11.1 mg kg^{-1}, respectively. The analytical techniques i.e. i.e. inductively coupled plasma-optical emission spectrometer (ICP-OES) is used for monitoring of the HMs in the environmental samples. The distribution of the total concentration of the heavy metals in the soil and common vegetables are described. Their mesh size and depth profile distribution, available amount of the HMs present in soil and their correlation with other ions are discussed.

How to improve the phytoremediation effects

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Keywords: phytoremediation, wheat, fertilizers

Major advantages reported for phytoremediation as compared to traditional remediation technologies include the possibility of generating less secondary wastes, minimal associated environmental disturbance and the ability to leave soils in place and in reasonable conditions. Main disadvantage is a long time required for the clean up metal contaminated soils. Therefore, the primary aim of phytoremediation research is to find suitable ways to enhance the rate of metal uptake. The choice of an appropriate plant species is an important stage for the successful application of phytoremediation techniques. Among others, wheat is a promising species for the phytoremediation of metal contaminated soils. Certain sorts of wheat can uptake large amounts of metals and produce rather high root and shoot biomass in various environmental conditions (different types of soil, different levels of soil contamination). The appropriate manipulation of contaminated soil can improve yields of crops. Besides, the amendment of soil with specific fertilizers can stimulate transfer of certain elements, including heavy metals, to more available to plants forms and enhance accumulation of the metals in the plants. The main purpose of the research was to find specific fertilizers that can affect biomass of wheat and simultaneously influence the uptake of several metals (cadmium, copper, lead, zinc). Soils with different type and level of metal contamination were chosen for the experiment. The effects of urea, manure and ispolin (mixture of organic acids and industrial populations of worms) on the yield of wheat *Triticum vulgare* sort Umanka, metal uptake and variations in metal concentrations in the rhizosphere were tested in greenhouse conditions. Urea was more effective for biomass growth when plants were grown in loam soil. Manure and ispolin provided the best effect for the plants grown in loam soil. Although treatments of the experimental soils with fertilizers did not automatically result in an increase of metal uptake by plants, an application of manure and ispolin led to increased uptake of Cd, Cu, Pb, and Zn by the wheat. Growth of wheat in non-fertilized contaminated soil resulted in a decrease (1.4 times) of Cd content in the rhizosphere. Amendment of the contaminated soil with urea enhanced the effect: the decrease of Cd concentration in the soil was more significant. The best effect was observed after application of ispolin: over a short period (six weeks) concentrations of Cd, Cu, Pb, and Zn in the rhizosphere decreased 1.2–1.4 times as compared with those in the initial contaminated soil (the differences were statistically significant at $P<0.05$). Thus, wheat can serve as a perspective plant species for the aims of phytoremediation of metal contaminated soils. The soil fertilization allowed to improve the soil quality, increase biomass of plants and stimulate metal mobility. Growth of wheat in contaminated soil and amendment of the soil with certain fertilizers affected significantly growth of root and shoot biomass and mobility of heavy metals in the rhizosphere. As a result, concentration of several metals in wheat increased while concentration of the metals in the contaminated soil decreased within rather short period.

Identifying genes in order to quantify the biological effects of trace metal uptake fluxes by *Chlamydomonas reinhardtii*

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Keywords: genes, cadmium, stress, biouptake

C. reinhardtii is a freshwater unicellular algae used in eco-toxicological and trace metal bioavailability studies. Its genome has been sequenced and techniques for its genetic manipulation are now well known. Therefore, it is highly suitable organism for the study of the defence mechanisms to toxic metals. In order to detect genes expressed specifically in the presence of cadmium and nickel, a differential method based on PCR amplification of cDNA restriction fragments from control and metal exposed cultures was employed. Algae were exposed to increasing free Cd^{2+} concentrations (10^{-8}–10^{-6} M) or to 10^{-7} M Ni. Free metal ion concentrations were determined by thermodynamic calculations. Biouptake fluxes appeared to be directly related to the solution physicochemistry and the measured biological response of the organisms. Using the differential display technique (ADDER[1]), twenty-eight genes were differentially expressed (induced or repressed) of which seven induced genes were cloned. Preliminary analysis revealed that one gene was involved in the cell wall composition (protein gp1 precursor) and another was a disulfide isomerase involved in the rearrangement of disulfide bonds. The other sequenced genes are ESTs that are currently being characterised.

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Comparison on mechanism of arsenic accumulation between hyperaccumulating and non hyperaccumulating fern

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Keywords: arsenic, hyperaccumulator, micro-PIXE, Pteris cretica, *Microlepia strigosa*

Twelve species (13 varieties) of ferns were screened for arsenic (As) tolerance and their ability to accumulate As in the fronds by exposing the ferns, at 4–5 frond stage, to 50 and 100 mg As/L for 7 and 14 days. It was found that *Pteris cretica* var. *albolineata* was an As hyperaccumulator and was highly tolerant to As, while *Microlepia strigosa* was a non As hyperaccumulator and less tolerant to As. These 2 ferns were used in further studies. Using growth (fresh weight) as an endpoint, the 56-day EC50s of the 2 ferns were 7.8 and 0.61 mg As/L, respectively. *P. cretica* var. *albolineata* was 12-times more tolerant to As than *M. strigosa*. Thus, in this study, *M. strigosa* was classified as an As sensitive and non-hyperaccumulator while *P. cretica* var. *albolineata* was an As tolerant and hyperaccumulator. The mechanism of As accumulation by these 2 ferns was further studied using Micro-Particle Induced X-ray Emission (μ-PIXE) and Inductively Couple Plasma Atomic Emission Spectroscopy (ICPAES). The As accumulated in the frond of *P. cretica* var. *albolineata* was correlated with the initial As concentration in the test solutions (R^2 = 0.932) and the exposure time (R^2 = 0.998). The As accumulation in *P. cretica* var. *albolineata* was higher in the frond than in the root when As in the test solution was higher than 1.4 mg/L and the exposure time was more than 11 days. *M. strigosa* accumulated more As in the root than in the frond at every As concentration tested. Concentration and localization of some elements, including potassium (K), calcium (Ca), phosphorus (P), aluminium (Al), Iron (Fe), copper (Cu), sulphur (S), zinc (Zn) and chloride (Cl^-), as well as As, were determined by μ-PIXE analysis. In the root of *P. cretica* var. *albolineata*, at the location where As and Fe was found, the ratio of As to Fe was 1:1.5. In the frond, As and Fe were not found together. These results agree with a previous study that found As-Fe plaque only at the root of a moss plant growing in the As contaminated soils. In *P. cretica* var. *albolineata*, the As concentration was highest in the vein, up to 7700 μg As/g dw, and it was significantly increased from the base of the frond to the tip. For *M. strigosa*, As was not significantly different in the various parts of the frond. In the fronds of both ferns, Ca and P showed similar distribution patterns to As, while K and Cl^- was the opposite and the other minerals analysed were found non significantly different from the control. Phosphorus was found to be 2 fold higher, while As was 10 fold higher in *P. cretica* var. *albolineata* (hyperaccumulator) than *M. strigosa* (non-hyperacumulator).

Recent organic matter accumulation in ombrotrophic peat bogs exposed to heavy metal pollution

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Keywords: age dating, copper, nickel, emissions

Although metal contents of age-dated peat cores from ombrotrophic peat bogs have successfully been used to establish records of atmospheric heavy metal pollution in the past, the impact of metal pollution itself on the organic matter (OM) accumulation in peatlands has barely been studied. We studied accumulation of OM in four ombrotrophic peat bogs in Finland: Harjavalta (vicinity of a Cu-Ni smelter), Outokumpu (near a closed Cu-Ni mine), Alkkia (Ni treated site) and Hietajärvi (a background). At each sampling site two peat cores (15x15x100cm) were taken. Age-dating of peat was determined using 210Pb method (CRS model). Our aim was to compare recent (last 125 years) OM accumulation rates of heavy metal polluted ombrotrophic peat bogs with those of a pristine bog.

Based on 210Pb age-dating, the upper 16cm peat layer at Harjavalta, 35cm at Outokumpu and 25cm at Hietajärvi represents 125 years of peat formation, yielding the following average OM accumulation rates at Harjavalta 1.3 mm a^{-1}, 2.8 mm a^{-1} at Outokumpu and 2.0 mm a^{-1} at Hietajärvi. At the Alkkia site, the Ni treatment in 1962 had completely stopped the peat accumulation. The lower OM accumulation rate at Harjavalta compared to the other study sites gives some support to the hypothesis of retarded NPP due to the moss growth interference by metal toxicity. Hence smelter derived pollutants might have caused reduced peat growth. In contrast at the Outokumpu site, even though it is in the vicinity of an old Cu-Ni mine, such a effect could not observed. On the contrary OM accumulation rates were greatest at the Outokumpu site, even exceeding those at the background site Hietajärvi.

Bioaccumulation of Cd an Zn in *Salix cinerea* rooting in seasonally flooded contaminated sediments

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Keywords: hydromorphic conditions; oxidation–reduction; seasonal inundations

A hydrological regime aiming at wetland creation is a potential management option for contaminated land that favours reducing bioavailability. The hydrological conditions on a site constitute one of the many factors that may affect the availability of potentially toxic trace metals for uptake by plants. Metal concentrations in the wetland plant species *Salix cinerea* growing on a contaminated dredged sediment landfill in field conditions were measured to evaluate bioavailability of Cd, Mn and Zn as affected by differences in length of submersion periods at the different locations on the site. Foliar metal uptake on contaminated plots was compared with concentrations on reference plots.

Longer submersion periods in the field coincided with lower Cd and Zn concentrations in the leaves during the first weeks of the growing season. At the end of the growing season, foliar Cd and Zn concentrations were highest on the plot that initially was flooded but emerged early in the growing season. Foliar Zn concentrations were also high at a sandy-textured oxic plot with low soil total metal concentrations. Zn uptake in the leaves throughout the growing season was markedly slower than Cd uptake for trees growing on soils with prolonged waterlogging during the growing season, pointing at a different availability between these metals. Zn transfer to leaves was lowest when soil was submerged, but metal transfer from stems and twigs to leaves may mask a lower availability of Cd in submerged soils. Especially for Cd, a transfer effect from one growing season to the next season was observed: oxic conditions at the end of the previous growing season seem to determine at least partly the foliar concentrations for *S. cinerea* through this metal transfer mechanism. Duration of the submersion period is a key factor for bioavailability inasmuch as initially submerged soils emerging only in the second half of the growing season resulted in elevated Cd and Zn foliar concentrations at that time.

The advantage of biomonitoring willow leaf concentrations for research on metal bioavailability is that they reflect time-integrated accumulated concentrations for the studied soil profiles. For example, the studied landfill might gradually evolve to more oxidising conditions. This long-term process will be reflected in foliar concentrations. Monitoring of foliar quality thus is a valuable tool in ecological risk management. Imposing a hydrological regime aiming at wetland creation is a possible management option for wetlands polluted with metals. It causes a reduced bioavailability provided submersion can be maintained until the end of the growing season.

Phytoremediation of metal contaminated soils

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Keywords: Phytoremediation

The use of green plants to remove, contain, inactivate or degrade harmful environmental contaminants (generally termed phytoremediation) is an emerging technology. An overview will be given of existing information concerning the use of plants for the remediation of metal contaminated soils. Both, site decontamination (phytoextraction) and stabilization techniques (phytostabilization) will be discussed, but focus will be on phytoextraction. There exists some evidence that metal phytoextraction is a promising approach but it is still at its infancy stage and needs further research and development. Recent studies investigated the feasibility of phytoextraction and confirmed that both biomass production and metal concentration factor (metal (hyper)accumulation) determine the efficiency of the remediation process. Several studies claiming to have demonstrated a high potential of different plant species for remediation of contaminated sites in fact were using plants showing a bioconcentration factor of lower than 0.5. A simple calculation using realistic biomass production levels leads to the conclusion that remediation of a moderately contaminated soil should take at least more than 100 years. In (hyper)accumulator plants, the bioconcentration factor is usually higher than 1 and in some cases even up to 100. For easy harvesting, the root to shoot transport should be efficient, resulting in a *of metal concentration higher that 1. Few studies report on the use of natural metal hyperaccumulators under field conditions. The metal concentration factor is not only depending on plant but also soil factors (soil type, pH, organic matter content, ...). Metal availability in soils plays an important role in the efficiency of the remediation process. The use of amendments for mobilization (f.i. chelating or acidifying agents) of metals in the soil can sometimes improve the metal accumulation by plants. Chemically assisted phytoextraction is based on the use of non-accumulator plants with metal accumulation levels far below those of hyperaccumulators, but with high biomass potential. Restrictions apply, however, to both the use of complexing agents and artificial soil acidification. It was found that EDTA and EDTA-heavy metal complexes are toxic for some plants and that high dose of EDTA inhibited f.i. the development of arbuscular mycorrhiza. Furthermore, EDTA is poorly photo-, chemo- and biodegradable. *In situ* application of both poorly degradable but also easily degradable chelating agents can cause groundwater pollution by uncontrolled metal dissolution and leaching. Since in many cases metal uptake by plants is limited by low metal solubility, it is necessary that the efforts for selection of appropriate rhizosphere manipulation be continued. There is a need to find cheaper, environmentally benign chemical compounds with chelating properties as well as to better understand the role of rhizospheric bacteria in metal solubility, plant uptake and tolerance. Another possibility that should be considered is the use of Plant Growth Promoting Bacteria that stimulate root formation by plants and also produce siderophores. These siderophores can interact with heavy metals, in certain cases reducing their toxicity and increasing their bioavailability and uptake by plants. Endophytic bacteria can be engineered for increased heavy metal sequestration. The (combined) activities of these bacterial strains could enhance heavy metal uptake and translocation by the host plants. Bacterial siderophores can be considered as natural chelators and the bacterial production of which is in tight equilibrium with plant activity, thus improving heavy metal uptake and translocation as part of the phytoextraction process. Besides of the more biological and technical aspects, the economical impacts of changed land-use, eventual valorisation of biomass and cost-benefit aspects of phytoremediation will be briefly discussed. An opinion exists that phytoextraction will get only economically feasible if, in addition to metal removal, plants produce biomass with an added economical value. An increasing number of reports are confirming the rationale of this option.

It is clear that, in spite of the a growing public and commercial interest and success, more fundamental research still is needed to better exploit the metabolic diversity of the plants themselves (knowledge of the molecular mechanisms of (hyper)accumulation of metals), but also to better understand the complex interactions between metals, soil, plant roots and micro-organisms (bacteria and mycorrhiza) in the rhizosphere. Improvement also could be achieved by optimization of agronomic practices Further, more demonstration experiments are needed to measure the underlying economics, for public acceptance and last but not least to convince policy makers.

Microlocalisation and mapping of zink in poplar with cytochemical methods

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Keywords: cytochemistry, microlocalisation, zink, light microscopy, element mapping

There are astonishingly few publications dealing with the microlocalisation and mapping of heavy metals (HM) which used methods from the cytochemistry. During the project “From Cell to Tree”, we developed several cytochemical methods to understand how HM contributed to the detected stress reactions. Two of these methods specifically revealing Zn and based on different reaction principles are presented in this paper. In comparison to the more frequent methods from the analytical electron microscopy, their advantages include 1) a higher sensitivity, 2) the microlocalisation of Zn at the organelle level in the whole cell and 3) the mapping of Zn in several mm of sample sections allowing the observer to analyze Zn allocation and relative frequency in different tissues along Zn transport pathway. The results shown here focus on roots and leaves of poplar young trees (*Populus tremula* L.) exposed to moderate HM soil pollution (Cd 10 mg/Kg, Cu 385 mg/kg, Pb 63 mg/kg and Zn 2700 mg/kg) in forest soil and which showed significant HM importation in their above-ground parts, reduced biomass, altered leaf physiology and specific HM leaf symptoms. Both staining methods gave comparable results with one method better showing microlocalisation details and the other one better suited for tissue mapping. Zn apparent concentrations increased from the absorption to the conducting zone in roots with a primary structure, then further in roots with a secondary structure and finally in leaves where the strongest signals were found. Each tissue accumulated Zn at specific levels and in specific compartments either in the symplasm or the apoplasm. Except in primary xylem, cells in the root absorption zones showed principally a symplasmic allocation. In symplasm of root cells, Zn was never detected in nucleus but in cytoplasmic compartments; it remained however rare in the vacuole. In assimilation tissues of the leaf blade, the most frequent microlocalisation site for Zn was in the chloroplasts followed by cytoplasmic strands and vacuoles. At tissue level, tissues showing the strongest signals included phloem and xylem in the leaf vein and lower epidermis, and mesophyll in the leaf blade. Phloem microlocalisation indicated Zn recycling processes. Zn characteristically accumulated in an irregular and patchy way especially next to leaf veins with decreasing gradients extending towards the leaf blades. Zn maps from the whole microscopical samples showed that Zn principally accumulated in the lower leaf blade tissues along the water exchange pathway. They were most efficient for targeting Zn zones during X-ray microanalyses. In conclusion, the two cytochemical methods were efficient in detecting and mapping Zn and their comparable results were confirmed by X-ray microanalyses. They allowed an overview of Zn allocation and frequency showing characteristic and specific cellular and histological patterns. In poplar, Zn was found in many different places, including some highly sensitive localisations to oxidative stress like the chloroplasts. Together with a very patchy distribution at the tissue level, it suggests that Zn allocation, especially in leaves, was little managed by the plant. Zn could thus contribute to the observed sensitive stress reactions and specific visible leaf symptoms.

Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.). Microlocalization and cellular effects of cadmium

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Keywords: autometallography, heavy metals, histochemistry, oxidative stress, plant defense, senescence

Leaves largely control the phytoextraction of cadmium in terrestrial ecosystems but can be injured by cadmium toxicity. Cadmium sinks at the cell and tissue level vary between different species of plants. Cadmium has several still debated direct and indirect toxic effects on leaf physiology. In this study, the cadmium microlocalization and the associated structural changes were investigated in leaves of a tolerant clone of Salix viminalis to assess cadmium distribution, stress and tolerance. Rooted stem cuttings were exposed during 13 weeks in hydroponics to increasing concentrations of CdCl2 (0–200 μM). Cadmium was cytochemically revealed with the method of physical development in leaves from the 0, 10 and 50 μM treatment. The resulting cellular injuries and defence reactions were analysed with several histochemical techniques using light and fluorescence microscopy. The main cadmium sink was in the pectin-rich layers of the collenchyma cell walls of the veins. Active storage was indicated by homogeneous cell wall thickenings with cellulose and proanthocyanidins. Cadmium microlocalization and cell injury in the conducting phloem indicated metal cycling. In the leaf blade, oxidative stress and accelerated cell senescence increased in those areas of the mesophyll with a low cadmium content. Local cadmium accumulation in veinlets near the leaf edges caused tannin plugging in xylem and necroses in the surrounding mesophyll and upper epidermis. When sinks approached saturation, random accumulation of cadmium appeared at sites in the leaf blade. Higher exposure to cadmium also enhanced the intensity of stress reactions. The role of different markers in metal binding and stress mitigation is discussed.

Environmental factors affecting metal mobility

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Keywords: metal mobility, nitrate, chloride, sodium, acidification, flooding

Mobility of most heavy metals is generally considered rather low compared to other soil compounds such as nitrate, chloride or sodium.

However, mobility varies with a range of environmental conditions. The effect of soil acidification and flooding has been extensively studied. It is well known that metal concentrations in soil solution and consequently leaching can be much enhanced in soils with low pH and/or low redox potential. Changes of land use such as deforestation or afforestation typically result in changes of pH, redox potential and content of organic matter and subsequent mobilisation or immobilisation of metals. Droughts and changes in temperature can modify metal mobility as a consequence of changes of organic matter content, quality and solubility. These processes can even occur as seasonal phenomena, for instance resulting in increased metal solubility during dry and warm summers.

Plants and micro-organisms have developed strategies to control metal mobility at their interface with the soil. Plant metabolisms and associated processes in the rhizosphere can change the solubility, speciation and mobility of metallic micro-nutrients as well as of pollutant metals. One example is the well-known strategy of plants and bacteria to increase the availability of Fe and some micro-nutrients by exudation of siderophores. Plants and associated micro-organism can also change other soil factors that control metal mobility, including pH, redox potential and dissolved organic compounds.

This contribution will provide an overview on environmental factors that control metal mobility in soil and in the rhizosphere of plants. An attempt will be made to quantify the magnitude of changes in the mobility of metals induced by different environmental factors and changes in environmental conditions and to evaluate their environmental relevance.

The mobilization and transport of heavy metals in the presence of plants: a direct observation and modelling in column experiment

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Keywords: mobilization, heavy metal, column experiment, exudation, copper, zinc

Heavy metals are normally rather immobile in soils but can be mobilized when the physical, chemical, or biological conditions change, for example the water flow, pH, or the vegetation. Plant exudation may play a role in the mobilization and transport of heavy metals in soils. The low-molecular-weight organic acids as an important part of the exudates are potential ligands for heavy metals.

To study these possible processes in soils we have set up unsaturated column experiments with and without the presence of *Lupinus albus* and *Salix viminalis*. Suction cups were installed along the column to directly obtain soil solution. A mesh between the polluted topsoil and the unpolluted subsoil restricted the root growth to the topsoil. The column was irrigated with synthetic rainwater plus different amounts of nitrogen fertilizers. Different flow rates were applied. Labile and dissolved heavy metal concentrations were measured together with general chemical parameters. At the end soil samples were taken at each 2 cm along the column and water content, total organic matter and total and extractable heavy metals were measured.

The experimental set-up gives a direct view of the processes in the soil with a spatial resolution of a few centimeters without disturbance of the soil. The mobilization of the metals was influenced by soil chemistry (pH, DOC mobilization), which in turn was influenced by the amount and composition of infiltrating water. The mobilization of Cu was strongly coupled to the slow mobilization of DOC. Dissolved Zn increased with depth, again indicating the importance of slow mobilization reactions. Rapid immobilization of Cu and Zn occurred within few centimeters of the polluted topsoil- clean subsoil boundary. There was no significant difference in heavy metal concentrations with or without Lupine. The strong transpiration of willow reduced the water content of the planted soil and this in turn influenced the dissolved heavy metal concentrations.

Our results show that small-scale and temporal changes in pH and DOC mobilization were the main factors that determined Cu and Zn mobilization and transport in the polluted soil that we have used. Lupine had no effect on the mobilization of heavy metals because its exudation only appears temporarily; the effect of willow was due mainly to the strong transpiration.

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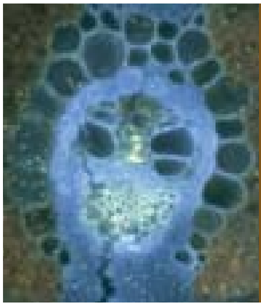

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