# Session 3

| Progress in phytoextraction of inorganic contaminants: critical factors for success in the field |

**Chair:** Maria Greger (SE) and Bernd Markert (DE)

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Metal hyperaccumulator plants: biological resources for exploitation in the phytoextraction of metal-contaminated soils

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Metal hyperaccumulation is a phenomenon generally associated with species endemic to metalliferous soils and it is found in only a very small proportion of such metallophytes. Most, but not all, hyperaccumulators are strictly endemic to metalliferous soils. The 430+ taxa described to date include representatives of many families, ranging in growth form from small annual herbs to perennial shrubs and trees. They have been discovered in all continents in temperate and tropical environments. Notable centres of distribution are for Ni: New Caledonia, Cuba, SE Asia, Brazil, Southern Europe and Asia Minor; Zn and Pb: NW Europe; Co and Co: Southcentral Africa. Some families and genera are particularly well represented; e.g., for Ni: Brassicaceae (Alyssum and Thlaspi), Euphorbiaceae (Phyllanthus, Leucocroton and Asteraceae (Senecio, Pentacalia); Zn: Brassicaceae (Thlaspi); Cu and Co: Lamiaceae, Scrophulariaceae.

It has been recognized for more than seventeen years that plant uptake could be exploited as a biological clean-up technique for various polluted rooting media including soils, composted materials, effluents and drainage waters. Before phytoextraction of soils is possible on a large scale, a number of important issues must be addressed. Firstly, metal hyperaccumulator plants are relatively rare, often occurring in remote areas geographically and being of very restricted distribution in areas often threatened by devastation from mining activities. Population sizes can be extremely small. There is thus an urgent need to collect these materials, bring them into cultivation and establish a germplasm facility for large-scale production for future research and development and trials work. Secondly, the potential exploitation of metal uptake into plant biomass as a means of soil decontamination is clearly limited by plant productivity. Many of the temperate hyperaccumulator plants are of small biomass, although considerable natural variation exists within populations. Selection trials are needed to identify the fastest growing (largest potential biomass and greatest nutrient responses) and most strongly metal-accumulating genotypes. However, such a combination may not be possible and a trade-off between extreme hyperaccumulation and lower biomass (or vice versa) may be acceptable. Selection could also identify the individuals with the deepest and most extensive and efficient root systems, and those of greatest resistance to disease. Breeding experiments are required to incorporate all these desirable properties into one plant.

Future work will involve genetic engineering to further improve metal-uptake characteristics, if the genes for metal accumulation can be identified and manipulated.
The possibility then exists to transfer genes for metal hyperaccumulation into a very productive (but inedible), sterile host plant. Excellent opportunities also exist through protoplast fusion techniques. There are very few hyperaccumulator plants discovered to date that have a capacity for multiple metal accumulation. Some, whilst primarily accumulating a single metal, do also show enhanced uptake of others. However, there is some experimental evidence to indicate metal antagonisms may limit uptake from multiply metal-contaminated soils. Increasing systematic effort in screening plant materials for these characteristics will most certainly reveal new hyperaccumulator plants - and new potentials for phytoextraction, phytomining and biorecovery. This paper will review the current status of phytoextraction technology and will consider the various 'bottle-necks' in its future development and commercial application.
Efficient heavy-metal-accumulating tobacco variants obtained from *in vitro* breeding and selection: results, conclusions and future prospects

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A strongly limited bioavailability of heavy metals in many top soils coupled with low uptake and translocation to the harvestable parts of high biomass producing plants are the actual 'bottle-necks' to overcome for improve of the phytoremediation technique and to bring it to a practical use.

The emphasis of this study, conducted in the frame of the Swiss Priority Programme 'Environment' was to significantly enhance the shoot heavy metal accumulation properties in two tobacco (*Nicotiana tabacum* L.) cultivars by means of *in vitro*-breeding and selection techniques (1-6). In two partner projects the development of suitable and controlled heavy metal mobilization agents, such as citric acid, NTA (7) and sulphur was investigated under lab and field conditions (8). The goal of this integrated optimization was the improvement of the biological extraction of heavy metals, which will reduce the time needed for gentle decontamination to a reasonable period.

In preliminary screening experiments on metal-spiked hydroponics with a good number of potentially accumulating crop species, such as tobacco, kenaf, hemp, sunflower, indian mustard and willows, two appropriate tobacco cultivars were selected for enhancing of their accumulation properties. Levels of Cd, Cu and Zn tolerance were established using root growth as a test parameter. Both *N. tabacum* cultivars reacted in a similar way in metal uptake, shoot to root metal distribution and toxicity symptoms. Metal treatments reduced dry matter in plants as concentration in solution increased. However, Zn affected yield less than Cd and Cu, the latter being the most toxic metal to tobacco.

Our in vitro-breeding program with tobacco was based on somaclonal variation that was used as the source of variability. A selection procedure was established that isolated four metal-tolerant tobacco callus cultures e.g. for Cd, Zn, Zn/Cd, and Cu. Selection pressure was applied at callus level for 18 months on culture media containing sublethal concentrations of metals. Shoot regeneration was induced in media without selection pressure at regular intervals during the
selection period. In a separate series of experiments growth and metal accumulation levels of selected cultures were compared with those of unselected cultures. Selected cultures grew more rapidly and showed higher metal contents than unselected ones, indicating that a more metal tolerant phenotype was selected. In total 812 variants were regenerated from the four metal specific callus sources. A subsequent screening on metal-spiked hydroponics lead to the identification of 120 variants (14.8% of all variants) showing increased shoot metal uptake, compared to controls. Variants that accumulated Cu 5-7 times, Cd 2-5 times and Zn 1.5 times greater than the mother plants were found. More than 30% of those metal-efficient variants derived from the Cu-selection line. In contrast, in the other three selection lines (Cd, Zn, and Zn/Cd) only a small percentage (3 -14 %) of enhanced variants were identified. The extent of somaclonal variation was then assessed by scoring phenotypic changes in regenerated populations and by comparing mean values and relative coefficient of variation (C.V.) of the characters: biomass and metal concentrations of shoots between micropropagated mother plants and somaclonal populations. Higher variability in regenerated plants demonstrated evidence for somaclonal effects of these in vitro cultures. Variability in regenerates was not affected by the time span of in vitro-cultivation, indicating that the optimized strategy for the exploitation of somaclonal variation may be through a short-term callus culture and selection procedure (< 12 months).

From the set of 120 metal-accumulating tobacco variants initially screened and selected on spiked hydroponics, 17 of them (15 %) also showed an increased shoot metal accumulation in pot experiments done on two metal-contaminated soils. Levels of improvement on these non-mobilized soils ranged between 20 and 70% compared to mother plants. This relatively poor differences in shoot metal accumulation between selected tobacco variants and controls (motherplants) was mainly be attributed to the limited bioavailiblity of metal in these soils.

In order to investigate the entire metal accumulation potential of two in vitro-grown tobacco variants a soil mobilizing experiment with an application of EDTA was performed. Under such optimized conditions the shoot uptake of Cu of both selected tobacco variants was enhanced to be 2.7- and 3.6-fold respectively that of controls after application of EDTA to soil, indicating that selected variants had a much higher metal-accumulation potential. The 'overall gain' of these improved tobacco clones, include the soil mobilization treatment was estimated to 10-16 -fold in comparison with controls without soil mobilization treatment. Improvements of the entire phytoextraction technique for soil decontamination of heavy metals needs to be at least of this order of magnitude to reach the goal for a future practical use of this promising 'green technique'. In a follow-up study within the Swiss COST 837 Programme, a 'fine tuning' of the in vitro-optimized tobacco variants with the greatest accumulating capacities and an assessment of their genetic stability has just started in order to clarify the potential of these promising variants for phytoextraction of heavy metals from soil.
References:


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Plant genes for hyperaccumulation and hypertolerance: prospects for genetic engineering in phytoremediation

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Heavy-metal-hyperaccumulating plant species are usually slow-growing and, therefore, unsuitable for phytoextraction purposes. Genetic modification of fast-growing crops might be a viable alternative. Hyperaccumulation evidently presupposes hypertolerance. Recent evidence suggests that these properties are under independent genetic control. The underlying molecular mechanisms are poorly understood.

Heavy metal tolerance in plants may be mediated by chelation, subcellular compartmentation, or, more likely, a combination of both. Phytochelatins (PCs) and metallothioneins (MTs) are the principal cytoplasmic heavy metal chelators. PC synthesis is essential for normal constitutive levels of tolerance to Cd, Hg, and As, but not essential for Cu and Zn tolerance. PC-based chelation as such may be essential but insufficient for normal Cd tolerance. Vacuolar transport of Cd-PC complexes and subsequent incorporation of acid-labile sulphide may be equally essential, such as shown in fission yeast. Vacuolar metal-PC complex transporters in plants have not been identified yet. Nevertheless, extreme Cd tolerance, such as found in mine plants, is entirely insensitive to inhibition of PC synthesis, suggesting the existence of PC-independent Cd sequestration and/or transport machineries. MT2 expression seems to be essential for normal constitutive tolerance to Cu, but not essential for Cd or Zn tolerance. Extreme Cu tolerance in mine plants is associated with overexpression of MT2. However, this gene seems to behave as a hypostatic enhancer, rather than a primary Cu tolerance gene. Extreme Cu tolerance seems to be mainly due to constitutively increased MgATP-driven efflux across the plasmamembrane. Heavy metal efflux transporters have not been identified in plants thus far, but they must be present. Extreme Zn tolerance is genetically correlated with enhanced MgATP-dependent vacuolar transport. Vacuolar Zn transporters have neither been identified in plants yet.

Overexpression of genes involved in PC synthesis, such as GSH1, GSH2, and PCS, encoding gamma-glutamylcysteine synthetase, glutathione synthetase, and PC synthase respectively, have been shown to increase Cd tolerance in various heterologous expression systems. Likewise, heterologous MT overexpression often leads to increased tolerance to Cu and, occasionally, Cd and Zn. In general, overexpression of metal sequestration traits is associated with marginally to moderately increased accumulation of the metals concerned, presumably due to a delayed down-regulation of the transporters involved in their uptake.
Hyperaccumulation, on the other hand, seems to depend on constitutive overexpression of root cell plasmamembrane transporter genes, such as ZTP2 and ZTP3 that were recently found in *Thlaspi caerulescens*. In normal plants these genes are exclusively expressed under conditions of Zn deficiency. The molecular basis of the strongly increased root to shoot metal transport and extreme metal tolerance levels in hyperaccumulators remains elusive. ZTP1 from *T. caerulescens* is particularly overexpressed in leaves and seems to be mainly involved in the foliar sequestration of Zn.
Genetic engineering of microbial amendments for increased immobilization of metals in soil: monitoring effects on plant growth

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Environmental disasters like that caused by the spill of the Aznalcsllar pyrite mine in Spain in 1998 are examples of the hazards arising from contaminating the environment with heavy metals. Due to the chemical nature of this type of pollutants, extensive remediation of metal ions in the soil and water around industrial plants is still a considerable challenge. Although most of the choices available so far rely on physicochemical procedures, the use of microorganisms and plants as biosorption agents is receiving an increasing attention because of the lower costs and higher efficiency at lower metal concentrations. Immobilization of toxic metals and radionuclides from waste streams employs different types of biomass as biosorbents, and a wide variety of fungi, algae and bacteria show a potential for this purpose. Immobilization is, in fact, the only mechanism available to higher organisms, including animals and humans, for counteracting heavy metal toxicity and may also become a useful biotechnological asset in environmental in situ applications. In this context, one interesting possibility is the use of soil bacteria as vectors for the introduction of metallothioneins in polluted sites, so that the immobilization of otherwise soluble heavy metals brought about by such proteins resembles the detoxification that occurs in higher organisms. Metallothioneins (MTs) are a group of small (around 60 amino acids) cysteine-rich eukaryotic proteins which are specialized in the binding of heavy-metals (e.g. Zn²⁺, Cd²⁺, Cu²⁺ and Hg²⁺). Previous studies have shown that human metallothioneins can be displayed functionally onto the surface of E. coli cells when expressed as fusions to outer membrane (OM) proteins like LamB, OmpA and PAL, thus leading to an increased heavy metal-adsorption by E. coli cells expressing the chimaeric protein. However, E. coli is not a satisfactory biological agent for soil bio-remediation, since it is not a microorganism adapted to sites polluted with heavy metals.

In this work, we have engineered an autotransporter (AT) protein secretion system to anchor functional mouse MT-I molecules onto the cell surface of Alcaligenes eutrophus CH34, a Gram-negative strain that possesses multiple resistances to heavy metals and thrives in soils heavily polluted with metal ions. Autotransporters are a widespread family of secreted proteins found in Gram-negative bacteria which are able to translocate through the OM without the concourse of an auxiliary machinery. Their mechanism of export, first exemplified by the IgA protease of Neisseria gonorrhoeae, involves the production of a large protein precursor containing a canonical N-terminal signal peptide that directs the transport
through the inner membrane (IM) into the periplasm. This is then followed by the insertion into the OM of the C-terminal domain of the protein (the helper or -domain) which, by forming an amphipathic -barrel, allows the accompanying passenger N-domain to cross the OM. By using this secretion strategy, and a mini-transposon vector for stable chromosomal integration of DNA, we have constructed a recombinant *A. eutrophus* strain endowed with an enhanced ability to adsorb Cd2+ from the media. The resulting bacterial strain, named *A. eutrophus* MTB, was found to have an enhanced ability for immobilising Cd2+ ions from the external media. Furthermore, the inoculation of Cd2+-polluted soil with *A. eutrophus* MTB decreased dramatically the toxic effects of the heavy metal on the growth of tobacco plants (*Nicotiana bentamiana*). These results can be traced to the increase of the number of metal-binding sites on the bacterial surface brought about by the production of the metallothionein in combination with chemical precipitation processes stimulated by the displayed MT_ hybrid.
Phytostabilization as a working technology

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Current remediation methods for soils contaminated with heavy metals are primarily based upon civil-engineering techniques (e.g. excavation of the contaminated soil). They are expensive and environmentally invasive. To redress these disadvantages an alternative remediation technique that is low cost and environmentally sound, but equally protective of human health and the environment, should be developed. The focus of much recent experimental work has been directed towards these ends, developing techniques that exploit biological (plant and micro-organisms) and chemical (use of metal-binding agents) processes to reduce the inherent risk associated with metal-contaminated soils. Strategies of this nature are classified under the generic heading of phytoremediation.

Experiments carried out over the last decade, based upon the above principles, have shown promise as viable soil treatment techniques. Such 'soft' technology can be subdivided into two possible alternative approaches, both reliant upon a reduction in the biologically-active soil metal pool. These are a) phytostabilization or phytorestoration with metal inactivation arising as a result of revegetation either with or without the use of metal immobilizing agents and b) phytoextraction, based upon accelerated metal removal from soil by cultivation of metal-hyperaccumulating plants.

This presentation will consider the current state-of-the-art of phytostabilization and in situ inactivation together with a detailed consideration of their advantages and limitations in the marketplace. Results of existing large scale field experiments will be discussed. In situ application of soil amendments modifying the physicochemical properties of the contaminating heavy metals combined with the development of biological communities for further metal immobilization indeed was shown to be a very promising alternative. Plant-based phytostabilization reduces the risk presented by un- or sparsely-vegetated contaminated soil by the use of a combination of plants and soil amendments to establish a stable vegetation cover which may progressively reduce the soil labile metal pool. This technology does not achieve a clean up of the soil, but changes the mobility of potentially toxic elements by either reducing concentrations in the soil water and other freely exchangeable sites within the soil matrix or by reducing re-entrainment of toxic particulates following the development of a stable and permanent vegetation cover. Both processes alter the speciation of soil metals, reducing potential environmental impact. These technologies draw upon fundamental plant and soil chemical processes as well as established agricultural practices. The development of a stable and self-perpetuating ecosystem as a result of this type of treatment may be a further beneficial process, as in some circumstances,
plant root activity may change metal speciation (changes in redox potential, secretion of protons, chelating agents); the microflora associated with their root systems may produce similar effects. The technique also has significant implications for 'polishing' less contaminated soils.

Soil amendments have a long history of use in the field. Lime, phosphates (calcium phosphate, hydroxyapatite), metal (Mn/Fe) oxyhydroxides and organic materials (sludges, composts) have been commonly used for metal immobilization. More recent research has targeted the use of more effective agents, such as modified aluminosilicates (Beringite), synthetic aluminosilicates (synthetic zeolites) and steel shots/grits.
Metal immobilization by constructed wetlands: regulating factors, limitations and future prospects

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The majority of constructed wetlands have been designed to treat wastewater and there are published European Guidelines for their design, operation and maintenance. Relatively few constructed wetlands are in use for the treatment of metal contaminated mine drainage and urban and road runoff. Horizontal surface flow (SF) and sub-surface flow (SSF) systems are normally used with the former requiring a larger area.

The metal removal performance of constructed wetlands has been shown to be more efficient than in natural wetlands, although the results of studies have shown high variability. The performance depends on a number of factors including wetland and detention pond area for which 3% of the catchment area has been recommended for urban runoff systems, although this may not be feasible. An aspect or length to width ratio of 2-4:1 is recommended for SSF systems and up to 10:1 for SF systems. Pre-treatment structures including oil and grit interceptors, a settlement trench or pond and rip-rap zone to attenuate flow and a post-treatment pond are also recommended. An overflow bypassing the wetland should allow excess flows to pass to the watercourse. A detention time of a minimum 4 hours is desirable. Flow rates should not exceed 0.3-0.5 m/s at the inlet zone if sufficient sedimentation is to be achieved. Gravel provides the most suitable substrate for constructed wetlands for supporting emergent plants with a high hydraulic conductivity 10-3 m/s to 10-2 m/s. A mixture of gravel and soil is recommended to improve metal removal performance by surface adsorption but an acid pH should be avoided to prevent the release of adsorbed metals. Phragmites australis and Typha latifolia have commonly been used for their metal tolerance, uptake and filtration ability.

The limitations of using constructed wetlands for metal immobilization include their relatively slow rate of operation and the land area required. There are few studies of performance and plant and associated microbial growth rates in different climates. Metal saturation of the substrate and plant senescence will eventually occur and require a monitoring programme. Storm events as well as background flow should be monitored at the inlet and outlet of the systems and the detention time should be allowed for in the collection of samples. Pollutant loadings should be determined as concentrations can provide misleading data due to the loss of water from evaporation and evapo-transpiration. The flow characteristics of the system should be monitored annually using tracer dyes to check that short-circuiting is not taking place. A satisfactory maintenance and management programme is essential if metal removal performance is to be sustained. The removal of metal contaminated
sediment in the trenches and ponds is required and the replacement of diseased and dead plants.

In order to enhance the performance of constructed wetland systems, there is a need for further research into metal immobilization mechanisms, not only by the plants and substrate but in particular by microorganisms. The collation of performance data from existing metal immobilization systems is also required to establish guidelines for their design.
Risk assessment for metals with the help of 'mobile' and total metal concentrations in soils as regulated by the Swiss Ordinance relating to impacts on the soil

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In July 1997, the revised Environmental Protection Law was enforced in Switzerland. Central to this revision, was the introduction of a the risk assessment of contaminated soils as well as physical soil protection from compaction and erosion. The revised and new law entitled Ordinance on Soil Impacts was enforced in July 1998. The federal law, the ordinance and several manuals specifying the legal regulations are the basis of soil protection. The Cantons (Swiss states) are still responsible for the implementation of these regulations. Soil contamination is assessed by means of Guide, Trigger and Clean-up values. The Guide values at the lowest pollutant level are a measure to assess soil fertility. If a guide value is exceeded, long-term soil fertility is no longer assured. The Cantons have to investigate the source of contamination and stipulate measures in the fields of air quality control (e.g. emission abatement), hazardous substances or waste control to avoid a further increase of pollutant levels in the affected area. Pathway-specific Trigger values are thresholds for possible but not yet confirmed hazards. They trigger case-specific investigations along the respective pathway to the risk receptors to assess the presumed hazard. If the Trigger values for food or fodder plant are exceeded, the pollutant concentration in the plants must be analyzed and assessed with the help of quality objectives for food and fodder, respectively. A hazard is given, if a quality objective is exceeded. In that case the land use must be restricted. Land-use-specific clean-up values indicate a level of contamination at which the current land use is not possible any longer without causing a hazard to man, animals or plants. Curative measures must be taken immediately (land use ban or change, clean-up measures). Guide values are set for cadmium, chromium, copper, lead, nickel, molybdenum, zinc, fluorine, PAH, PCB and PCDD/F. Trigger and clean-up values are set for cadmium, copper, lead and zinc, PAH, PCB and PCDD/F. Trigger values for food plants are determined on the basis of the Reasonable Worst Case scenario (RWC): It is assumed that (1) the transfer into the plant is maximum, (2) the food plant is highly accumulating and (3) the degree of self-sufficiency of consumer is high. To meet the last requirement, the quality objectives used are the tolerance concentrations of metals in foods as laid down in the Swiss legislation, supplemented by German guideline values for foods. The Trigger values for fodder plants contain the sub-pathways soil-plant-transfer, direct soil ingestion and contamination of the fodder through adhering soil particles. These pathways are...
aggregated mathematically. The RWC scenario is interpreted as follows: (1) For each pollutant the most sensitive animal, (2) the feeding method with maximum exposition is chosen and (3) the transfer into plant is assumed to be maximum. Fodder plants are assessed in accordance with the maximum levels set in Swiss legislation or with handicap threshold values.

For the clean-up values, several pathways depending on land use must be considered. For agriculture and horticulture the pathways food plant, fodder plant and plant growth are relevant, for domestic gardens and allotments the pathways food plant, plant growth and direct uptake by children. The assessment of direct uptake by children is not subject of this paper. The pathways food plant and fodder plant are assessed similarly to the Trigger values. Instead of the RWC the Best Case (BC) scenario is applied: Although all land use restrictions have been stipulated to avoid a hazard (the "best" case), the hazard is still existing. For the pathway food plants it is assumed that (1) the transfer soil-plant is minimal and (2) the quality objectives used are threefold the tolerance values. For fodder plants (1) the most usual feeding method is used, (2) the animal chosen is little sensitive and (3) the ratio of direct soil ingestion is minimum. For clean-up values the pathway plant-growth is additionally included, because a minimum yield must be guaranteed for agricultural and horticultural land use. It is assessed with yield reduction data (decrease of 25%) or qualitative description of plant damage due to a quantified soil contamination. For each pathway the maximum tolerable soil concentration is established on the basis of the BC-scenario. To calculate the clean-up value for a specific land use, the relevant pathways have to be aggregated.

The availability of a pollutant in soil is important in assessing the soil-plant-transfer. Since 1986, two extraction methods have been favoured: the potential hazard is assessed with the pseudo-total content (1M HNO$_3$ extraction). This is a first order approximation to quantify the mobilizable fraction in soil. The actual hazard is assessed with the soluble content (0.1M NaNO$_3$ extraction), which is a measure for the plant-available fraction. The Trigger and clean-up values are set for both contents. The transfer is assessed with qualitative data analysis. Other methods are not suitable because several requirements for their application are not met.

References:


Cadmium uptake by *Salix viminalis* as affected by root CEC and dependence of root CEC on soil characteristics

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Cadmium uptake by plants varies with soil characteristics. The CEC (cation exchange capacity) of plant roots also seems to be affected by soil factors. If root CEC is involved in Cd uptake this may have implications in understanding phytoextraction.

In this work we tested the hypothesis that the root CEC is dependent upon some soil characteristics (including Cd concentrations in soil) and that Cd uptake increases with increasing root CEC. This was done in two steps:

1) Cuttings of one clone of *Salix viminalis* were grown in a) 6 soils with various physico-chemical characteristics and b) 4 soils with the same soil characteristics but with 4 different Cd concentrations. After 7 weeks growth under controlled conditions, the soil was removed from the plant roots. Half of the plant material was dried and analyzed for the root CEC as well as Cd concentration in roots;

2) The second half of the plants was used to study the $^{109}$Cd uptake during 8 hours in a nutrient solution containing 2.43 µCi $^{109}$Cd/l. The different soils were analyzed for pH, particle size distribution, total, mineral and organic carbon, nitrogen, soil CEC, manganese, iron and aluminium forms, Cd, Cu, Pb and Zn concentrations in EDTA, DTPA, NaNO$_3$ and HNO$_3$ extracts.

The results show that:

1) An increased total Cd in roots (after the soil step) is related to an increase in the root CEC but a decrease in the amount of $^{109}$Cd taken up by the plant (in solution). This means that the amount of $^{109}$Cd taken up by the plant decreased with increasing root CEC probably due to adsorption site saturation by Cd from the soil.

2) The root CEC is affected by soil characteristics such as pH, sand, clay, Cd(NaNO$_3$) and Mn (Mehra-Jackson).

3) In uncontaminated soils total Cd in roots is influenced by the clay content of the soil and in the Cd-contaminated soils it is correlated with pH, soil CEC, Cd(HNO$_3$) and Zn(HNO$_3$).
Lead uptake by native Mediterranean plants grown on the metalliferous soils of Lavrio-Attica, Greece

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Lavrio is a unique area in the Mediterranean region. It is perhaps the largest and oldest industrial area in Europe and includes tens of square kilometers of metalliferous mines, ore-washeries and furnace complexes dating from the very earliest years of the Bronze Age up to the beginning of the Byzantine period. The intensive metallurgical and mining activities in Lavrion over a period of over 2,700 years have resulted in the formation of vast spoil heaps of mining, milling and metallurgical wastes and tailings, most of them characterized as toxic and hazardous. Previous studies have shown that total Pb in Lavrion soils varies from 24-70,032 ppm, with mean and median values 2,883 and 692, respectively. Lavrio is a developing region with a great future potential of cultural, economical and tourist development. The environmental restoration of the Lavreotiki peninsula is therefore essential and imperative.

Results from a field survey of Mediterranean vascular plants growing on metal-contaminated soil in the Lavreotiki peninsula are presented. The study was conducted in order to identify species which accumulate large amounts of lead in their roots and shoots, and included the following plant species: Glauceum flavum, Chrysanthemum coronarium, Ballota acetabulosa, Matthiola fruticulosa, Silene colorata, Carthamus sp, Lithospermum sp. Results for metal uptake and tolerance of Chrysanthemum coronarium growing under glasshouse hydroponic culture with controlled microclimatic conditions are presented and discussed to evaluate its Pb phytoextraction efficiency. It is concluded that Lavrio is of great interest as a resource of Mediterranean plants for use in phytoremediation studies.

The study is part of a vegetation-based bioremediation technology which is being developed in our Laboratory to provide a sound scientific tool for clean up the heavily contaminated site at Lavrio and with potential usefulness in phytoremediation projects under Mediterranean climate conditions elsewhere.
Important physiological factors for optimizing phytoremediation

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Many plant physiological properties are of importance for phytoremediation such as low or high metal accumulation, low or high root-to-shoot translocation of metal, high biomass production and, often, high tolerance to the specific metal. A physiological understanding of these properties is critical for optimizing phytoremediation processes. The aim of the present study was to investigate the most important physiological factors in tolerance to, and accumulation of heavy metals in a woody plant species frequently used in phytoremediation projects. More than 140 clones of Salix (from 10 different species) were investigated for their tolerance to and accumulation of Cd, Cu and Zn. Additionally, several detailed physiological screening studies were performed, such as uptake and leakage of metal ions by roots, root-CEC, phytochelatin production, antioxidative stress, carbohydrate production and utilization in relation to tolerance and metal accumulation.

None of the results obtained indicated any particular mechanisms that may have a major role in regulating uptake, translocation, accumulation or tolerance to the metals in the Salix clones studied. Furthermore, the absence of any correlation between root and shoot for the measured parameters and between the three metals leads to the conclusion that the most important factors are clonal differences in quality and quantity of internal sites where the metal ions interact. This concerns both the toxic actions of the metal ions as well as the pathways of uptake, translocation and accumulation. The degree of interaction with these sites may differ between the clones because of genetic differences. This may have major significance when optimizing plants for high phytoremediation capacity in breeding programmes.
Toxic metals uptake and physiological responses of trees and some medicinal plants

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The poster summarizes experiments on the uptake and some physiological responses of trees (Populus, Quercus, Salix, Betula, and Fagus) and medicinal plants (Karwinskia and Hypericum) to five toxic metals (Cd, Cu, Al, Hg and Ni), which are important environmental pollutants.

Cuttings of Populus clone P-090 grown hydroponically responded to Cd with significant inhibition of number of roots (77% inhibition at 10^-5 mol Cd dm^{-3} and 53% at 10^-7 mol Cd dm^{-3}), the number of shoots was not affected. The most conspicuous inhibition was observed for total length of roots (87% inhibition at 10^-5 mol Cd dm^{-3} and 50% at 10^-7 mol Cd dm^{-3}).

RuBPC activity and protein content in the leaves of seedlings of three oak species (Quercus cerris, Q. robur and Q. dalechampii) were significantly lowered in forest stands with different degrees of damage by Cu and Al. In injured leaves a higher concentration of both metals was found. On the basis of these findings the RuBPC activity and protein content could be used as a diagnostic marker for determination of the degree of forest stand damage.

Oxygen evolution rate (OER) in chloroplasts of Salix sp. Populus nigra, Betula pendua and Fagus silvatica was inhibited by toxic metals in the following order: Cu>Hg>Ni>Cd. A study on metal accumulation in Populus nigra chloroplasts and leaves showed a rise in Cu accumulation with increase in metal concentration. The accumulation of copper was dependent on the pH value. At higher concentrations of Cu (10^{-3} and 10^{-4} mol dm^{-3}) the accumulation increased at pH 5.5 compared to pH 7.5. However, at lower concentrations (10^{-5} - 10^{-7} mol dm^{-3}), Cu accumulation decreased at lower pH.

The effects of Cd on growth, plant biomass (root, shoot) and root dark respiration rate were studied in medicinal plant Hypericum perforatum. Plants treated with the metal respired faster than control plants. The species accumulated large amounts of Cd without reduction of growth and biomass. Higher concentrations of Cd were found in roots compared to stems and leaves. The OER in H. perforatum and Karwinskia humboldtiana chloroplasts was inhibited by Cu, Hg, and Ni. The toxicity
of the metals in these plants decreased in following order Cu>Hg>Ni>Cd. The degree of tolerance of poplar callus cells (*Populus alba* L.) showed surprising results. In a first subculture with 10^{-3} and 10^{-4} mol dm^{-3} Cd cultures grew very well compared with the control (evaluated by fresh mass, dry mass and growth index -GI). This green callus showed, however, in the next subcultures changes in morphology, loss of pigmentation and a reduced growth. Tolerance indices for different Cd concentrations ranged between 80 and 95%.
Plant biotechnology applied to environmental monitoring: the case of toxic metals

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Environmental monitoring of pollutants with higher plants presents several advantages as compared to other systems based upon animals or microorganisms, mainly because of the wider range of situations, which can be monitored with these organisms. Monitoring of toxic metals in the environment can effectively address their effects at DNA level, which could be classified as related to the function or with the structure of nucleic acids: functional effects concern regulation of specific-gene expression, whereas structural effects deal with mutagenesis and genotoxicity.

Monitoring functional effects: Transgenic tobacco plants have been produced by inserting as stable integration a construct containing a gene fusion between the reporter gene \textit{uidA} encoding for beta-glucuronidase (GUS) and the promoter of the heat shock inducible gene \textit{Hvhsp17} isolated from barley and encoding for a low-molecular weight heat shock protein. This promoter also contains regulatory elements conferring inducibility upon exposure to heavy metals, in particular to Cd, Zn and Al. The transgenic plants express the reporter gene after elicitation with heat shock, especially in stem tissues. Four independent transgenic lines of ascertained efficacy have been back-crossed with the parental line or self-fertilized for the production of genetically uniform populations. Two lines have been chosen after three self-fertilization cycles: LO3, from NT3-6-3, and LO8, from NT2-6-3. The choice of the lines was made on the basis of response to treatment and of low basal level of expression of the reporter gene. Genetic analyses showed that the lines contained two copies of the construct in homozygote state. Response to treatment is evaluated through a histochemical reaction (based upon transformation of p-nitrophenyl-D-glucouronide) directly on plant sections or through spectrophotometric determination after protein extraction. Exposure of plants to heat shock treatments at 40°C for 2h or to 20 mM solutions of cadmium nitrate or zinc nitrate for 24h leads to an induction of GUS activity ranging from 2- to 6-fold over control. Low concentrations of metals can also induce GUS activity if exposure is prolonged. The response of the biosensor is therefore regulated by severity of the stress, depending on duration and concentration.

Monitoring structural effects: A method for scoring mutagenic effects in model plants has been developed with \textit{Arabidopsis thaliana}, a small plant widely used in biology for its several advantageous features. Arabidopsis seeds contain a small embryo in which a limited number of cells is present, being the progenitors of the cells which will constitute the adult plant. Mutagenic changes exerted at this early
stage will be manifest in the adult plant as visible sectors with phenotypic differences, if the mutation is dominant, or will be detectable with sophisticated molecular analyses. Starting from *Arabidopsis* seeds exposed to non-lethal concentrations of heavy metals (Pb, Cd, Mn) it was shown that detection of polymorphic alleles originated by mutation could be obtained by amplifying different portions of the genome by means of randomly chosen oligonucleotides in the technique called RAPD (Random Amplified Polymorphic DNA). *Arabidopsis* plants were exposed for different time periods, starting from sowing, to a situation of environmental pollution represented by the emissions of a municipal waste incinerator plant containing Cu, Cd, Cr and Pb at significant levels. By increasing the time of exposure it corresponded to an increase in the number of polymorphism scored after PCR amplification with 14 different oligonucleotides, in comparison with control plants which had been kept in the same environment in a box with filters to avoid exposure to the contaminants.
An inventory of trace elements in agricultural ecosystems: the INCO European project FERTILIA

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Several trace elements are necessary for animal and plant nutrition; however, anthropic activities can lead to excess of some metals in natural substrates: soil, water, air. Detrimental effects upon the health of living organisms at different levels can arise from this contamination, depending on the chemical speciation, mobility and availability of the elements. In particular, agricultural ecosystems receive metal inputs from several sources including amendants and fertilisers, such as sewage sludge, manure and slurries: this practice is however considered to be an appropriate way for disposal of organic wastes, encouraged by EU directives. In the 4th Framework the European Commission financed the INCO project designated 'FERTILIA' with the aim of tracing the cycling of elements into inorganic, organic and biological components of agroecosystems of different European countries: Italy, Spain, Poland and Czech Republic. The project comprises six phases.

The first Action is the evaluation of parameters of amendants and fertilisers applied to agricultural soils, together with the existing legislation in the different countries. Special attention has been paid to heavy metal content: Cd, Cr, Cu, Ni, Pb and Zn. Experiments with lysimeters have provided data about mobility and leaching of the different metals in typical soils.

The second Action concerns the description of trace metals contents in agricultural soils differing for geopedological features, geographic location, and agronomic practices. Efforts towards standardisation of sampling and analysis techniques have been undertaken to achieve comparable results in the different laboratories involved. Differences in the level of some heavy metals have been described according to fertilisation practices.

The third Action will describe metal content in different living components of the agroecosystems, particularly crop plants and invertebrates. Semiquantitative methods for mapping the metal distribution in plant tissues, based on X-ray emission, have been applied. Interesting data about the different transfer coefficients of metals from soils to edible plant parts have been gathered.
Actions 4 and 5 deal with biomonitoring of metal effects on ecosystems. Different biomarkers of effect have been developed: microbial activities, determined as respiration quotients or enzymatic activities; growth of soil fungi under the influence of heavy metals; reporter gene induction in transgenic plants, under the control of a metal responsive promoter; genetic polymorphisms in model plants deriving from mutagenesis by heavy metals.

Finally, Action 6 involves the construction and management of a database which will store all the data produced, with the final aim of building a Geographic Information System (GIS) for revealing the European diffusion and effects of metals in agroecosystems. To facilitate collection of data and exchange among participants, a project WWW site has been included within the PHYTONET network: http://www.dsa.unipr.it/phytonet/fertilia.
Accumulation of zinc and lead in *Phragmites australis* and *Typha domingensis*

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The accumulation of the heavy metals Zn and Pb were studies in two macrophyte species: *Phragmites australis* and *Typha domingensis*. The plants were grown in solution culture containing increasing concentrations of Zn (2, 8 and 16 ppm), and Pb (1, 2 and 10 ppm). Seeds were germinated in Petri dishes, using CaCl2 solution and then transferred to vermiculite, moistened with CaCl2 solution. When seedlings had the first two leaves (4-5 cm) they were transferred to 1:10 Hoagland solution (pH 6.0). After three weeks the metal treatments were applied, those of Zn in 50% Hoagland solution, and those of Pb in solution containing NH4NO3, KNO3, Ca(NO3)2 and Pb(NO3)2 (pH 5.0), equivalent to 50% Hoagland solution for N, K and Ca; to avoid Pb precipitation in the solution. The Zn concentration in shoots of *P. australis* was between 1100 and 1300 ppm (on a dry matter basis) for the Zn treatments, while root levels increased from 11460 ppm (in 2 ppm Zn) to 1790 ppm (in the 16 ppm treatment). Propagation by rhizome did not show significant differences in Zn concentration of shoots, but it was lower in roots (7700 ppm). Treatment with 16 ppm Zn in *T. domingensis* reduced the weight of the plant but to a lesser extent than for *P. australis*, although having a similar Zn concentration in shoots (9700 ppm). The accumulation of Pb in *P. australis* reached 243 ppm in shoots and 4900 ppm in roots with 10 ppm Pb treatment, and 122 ppm and 1900 ppm in shoots and roots respectively in the 2 ppm treatment. *T. domingensis* tolerated lower Pb concentrations in the growing media, having similar Pb concentrations in shoots (200 and 230 ppm) in the 1 and 2 ppm Pb treatments.
Polyamine treatment and lead accumulation and translocation in maize seedlings: interesting perspectives for phytoremediation strategies

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Polyamines are small aliphatic amines that are essential for life and participate in a bewildering number of seemingly unrelated processes. The most common polyamines are spermidine (SPD) and spermine (SPM), together with their diamine precursor putrescine (PUT). Their intracellular concentrations vary greatly with the differentiation status of the plant cell and also in response to a wide variety of external conditions, such as light, temperature, and various chemical and physical stress factors. The purpose of our research is to investigate the possible role of polyamines in the phytoremediation of heavy metal ions from soils. This idea arises from the knowledge that in animal cells polyamines have an antioxidative effect due to a combination of their anion- and cation- binding properties. The binding of polyamines to anions (nucleic acids, phospholipid membranes) concentrates these molecules at cellular sites particularly prone to oxidations, whereas the binding to cations prevents oxygen radical generations in specific cell sites, including polyamines in the group of metal-chelating molecules. Polyamines form complexes with several metal ions with the stability constants increasing with the number and the position of N-groups within the molecule. In this context, we hypothesise that formation of a metal-polyamine complex also occurs in plants and it could play a role in the absorption and translocation to shoots of heavy metal ions. An increase of polyamine content by an exogenous treatment or by biotechnological techniques could strengthen the capability of plants to hyperaccumulate heavy metals. Experiments were carried out using maize seedlings treated with SPM and lead chloride in hydroponic solution in short- and long-term treatments. Short-term treatments were performed in a growth chamber (dark, 28°C) using three-day old seedlings pre-treated with or without SPM 2mM for 18 hours and then transferred in growth solution (CaSO₄ 0.5 mM) added with or without 30 _M lead chloride for 24 hours. SPM treatment induced about 12-fold and 3-fold enhancement of endogenous content of SPM and PUT respectively. ICP spectroscopy analysis showed a 2.5-fold lead accumulation in roots of SPM-pretreated seedlings respect to untreated ones. Analogous results were obtained using a maize cell-culture system. In a long-term experiment, three-day old maize seedlings were transferred in a glasshouse and fed with half-strength Hoagland nutrient solution with or without SPM 2mM and/or lead chloride 20 _M for 15 days. At the end of experiment analyses of polyamine and lead content were performed in plants separated into roots, stems and leaves. SPM treated plants showed an increase of SPM and PUT level, progressively lowering along the
plant axis, compared to control plants. Lead analysis by ICP spectroscopy showed an increase in the Pb content of the aerial parts of plants treated with SPM with respect to control ones. These preliminary results indicate that an enhancement of endogenous SPM content ameliorates accumulation and translocation of lead in maize plant confirming the hypothesis about the formation of polyamine-metal complexes and opening up interesting perspectives for phytoremediation purposes.
Phytoremediation of toxic metals and radionuclides at the laboratory scale

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The amount of land contaminated with heavy metals has increased during the last century due to mining, smelting, manufacturing, urban and other industrial activities. At present, remediation technologies consist primarily of removal and replacement of contaminated soil. An alternative soil remediation technology has been proposed that would use rare, heavy-metal-tolerant plant species that are able to hyperaccumulate metals in plant shoots. It is possible that the tolerance mechanisms can be exploited to remove heavy metal pollutants from soil. This proposed technology, called phytoremediation, involves successive cropping of hyperaccumulator plants to translocate polluting metals from soil to plant shoots. Harvested plant shoot biomass can then be smelted to recycle the accumulated metals.

Before phytoremediation can be developed for commercial use, the behaviour of hyperaccumulator species must be more clearly understood. An understanding of the patterns of heavy metal uptake and the specificity of tolerance will enable growers to maximize metal pollutant concentrations in plant shoots.

Bioaccumulation of heavy metals by hairy-root culture of Armoracia rusticana L. was studied. The adsorption of heavy metals on to glass and the surfaces of dead roots was also studied. The highest adsorption on to the surface of dead roots was found with the Hg²⁺ ion. Tolerance of hairy-root cultures to different concentrations (0.1 - 10 mM) of heavy metals was studied. The toxic metal ions used were: Be²⁺, Cd²⁺, Cr³⁺, Cr⁶⁺, Cu²⁺, Hg²⁺, Pb²⁺ and Zn²⁺.

The ¹³⁷Cs uptake was analyzed from a hydroponic medium (14 MBq L⁻¹; 0.5 mM CsCl) during cultivation of three plant species (Phragmites australis L., Helianthus annuus L., Populus simonii L.). The best results on ¹³⁷Cs uptake were obtained using in vitro culture of poplar. In this case up to 41% initial activity was accumulated after 16 days. From the results achieved it can be concluded that the radionuclide ¹³⁷Cs is accumulated to a certain extent by all plants tested. Localization of the radionuclide was found mostly in areas of cell expansion and active metabolism and thus in parts of increased requirement for water and nutrients i.e. in the node, leaf tips and young leaves and in the young shoot meristems. The results allow both a comparison of the capability and rate of ¹³⁷Cs remediation and an assessment of the application of the biotechnology for biosensing.
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A study of accumulation of heavy metals by \textit{in vitro} culture of plants

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Due to intensive anthropogenic activity, high concentrations of hazardous chemicals not occurring naturally in the environment continuously enter aquatic and terrestrial ecosystems. Contaminated soil, water and the atmosphere together with harmful impact on living organisms including humankind take place as a result. In attempting to preserve our environment, new methods of remediation using physical, chemical and biological principles are being studied \cite{1}. In some cases, it has become clear that plants with specific properties can be useful for remediation of soils contaminated with both organic \cite{2} and inorganic \cite{3} xenobiotics.

The aim of this study is to observe the ability of selected plants to accumulate heavy metals in their tissues. The study is carried out on the aspen (\textit{Populus tremula} \textit{x} \textit{tremuloides}) plantlets grown \textit{in vitro} and on callus culture of rhubarb (\textit{Rheum palmatum}). Concentrations of 0.1 mM and 0.5 mM of Pb\textsuperscript{2+} and Ni\textsuperscript{2+} cations have been tested. The highest value for accumulation was found for aspen which was able to accumulate about 3500 µg of Pb\textsuperscript{2+} per g of biomass in the 0.5 mM Pb\textsuperscript{2+} treatment. In the case of 0.1 mM Pb\textsuperscript{2+}, 1400µg of Pb\textsuperscript{2+} per g d.w. was accumulated by this plant culture.

References:

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Searching for genes for the phytoremediation of metal-polluted soils

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The abstract is not available. Please contact the authors for further information.
The influence of wetland plants on the mobilization of metals in submerged lead-zinc tailings

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The use of wetlands in phytoremediation of toxic mine drainage has focused primarily on flow-through systems including constructed wetlands and marshes. Alternatively, phytoremediation of stagnant systems such as highly polluted storage ponds for fine-grained mine waste (tailings) has not been thoroughly examined. Due to constant submergence, these tailings ponds can maintain the anaerobic conditions necessary for toxic metal immobilization via precipitation as metal sulphides. These reducing conditions may be altered, however, by the introduction of wetland plants. Some species are known to increase the redox potential of the surrounding sediment through root-oxygen diffusion, thus potentially increasing metal solubility by oxidation and dissolution of metal sulphides. To test the effect of two wetland macrophyte species (Glyceria fluitans and Typha latifolia) on metal (Fe, Zn) solubility, pH and redox potential in submerged lead-zinc mine tailings, a specialized lysimeter chamber was designed allowing porewater sampling at specific distances from the roots. G. fluitans showed little effect on the sediment chemistry, but the presence of T. latifolia resulted in decreased pH, increased redox potential and increased soluble Zn near the roots (as far as 1 cm). Results of this study may have implications for the development of wetland cover on tailings ponds and long-term substrate metal retention.
Cadmium detoxification by aquatic plant *Hygrophila Corymbosa* ‘Stricta’, *in vitro* grown

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Heavy metal pollution in soil and water has becoming a serious problem for agriculture and health. Some heavy metals are natural micronutrient in the vegetable kingdom; they are rapidly absorbed by roots and translated into whole plant. The toxic effect is checked when the heavy metals concentration in soil is higher than the normal trace amounts. However, many species developed some physiological mechanisms that allow the survival also in that status. Physiological studies on plants able to live also in presence of high concentration of heavy metals have explained the metals attachment and binding as main mechanisms for detoxification. The main classes of intracellular ligands for heavy metals are organic acids (malic acid and citric acid are essential for Zn detoxification).

Aquatic plants appear a good cure for heavy metals accumulation and, therefore, for water detoxification. As reported in literature, some of them are well known to accumulate pollutants (Cd, Co, Pb, Hg, Cu, and Zn).

For present experiments the aquatic plant *Hygrophila corymbosa* ‘Stricta’ (*Acanthaceae*), grown in vitro on two-layer medium containing CdSO$_4$ were used. This plant was selected for its high attitude to in vitro regeneration from leaf disks.

For test cadmium tolerance of *H. corymbosa*, this plant was grown on liquid medium containing the heavy metal in a range of concentration between 0 and 1000 ppm.

Cd content in whole plant tissues was carried out by ICP (Inductively Coupled Argon Plasma Emission Spectroscopy).

The results of this assays showed that Cd have not toxic effects on plants up to 50 ppm. For plants grown in medium containing Cd concentration more than 50 ppm, the uptake of this xenobiotic achieves values very high, up to 750 ppm, but, in these cases, plant growth is very difficult, for Cd concentration higher than 100 ppm, plant fresh weight decreased, because the heavy metal begin to be toxic for plant metabolism.

From these experiments it is also obvious that the biggest uptake of pollutant is in the second week of culture on liquid medium containing cadmium; in fact we have not only a bigger uptake, but also an increment in fresh weight of plants, compared to the control.
Since the concentration of Cd in the natural environment exceeds 1 ppm very rarely, in successive experiments a range of concentration between 0 and 6 ppm was adopted.

In these conditions the plants did not decrease the growth, even in the medium containing the highest concentration of cadmium. After subculturing, the following parameters were analyzed: plant growth (dry weight/fresh weight), variation of medium pH, and Cd content in the tissues.

In all treated tissues Cd was detected, especially in leaves (100 ppm) and in roots (90 ppm), whereas in culture medium, Cd content is lower than instrumentation sensibility (< 1 ppb).

In control sample tissues Cd was never found.

The fact that cadmium did accumulate in Hygrophila tissues suggest that plant might play some role in heavy metals detoxification.
Cynara cardunculus L. cell suspensions, grown in a bioreactor as a biological model for phytoremediation

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Cynara cardunculus (wild cardoon) cell suspensions were grown in 2-L bioreactors, mechanically stirred, to study the kinetics of cell growth in a batch system (Lima-Costa, 1994). Carbon, nitrogen and phosphorus metabolism were all correlated with cell growth and a structured model was proposed (Cabral et al. 2000). This biological model is being used to study the abiotic stress caused in cell metabolism by copper and zinc in large amounts. The use of a bioreactor allows the study of cell metabolism in a controlled environment and has a high potential for an accurate and rapid study of xenobiotic metabolism. Copper and zinc are micronutrients that are required by plants in relatively small amounts but they do have some important biological functions. The physiological significance of copper and zinc lie mainly in their being constituents of a number of proteins and enzymes mostly involved in oxidation-reduction reactions.

Cell suspension cultures were prepared by adding 2g fresh weight of calli to 100ml of Gamborg B5 medium supplemented with 2% sucrose, 1 mg/ml 2,4 D and 0.1 mg/ml BAP at pH 5.5 and 25°C. The stocks were subcultured weekly by inoculating cell suspension into fresh medium (1:1). Biomass was measured by fresh and dry weight determinations, respiratory rate and protein contents. Copper and zinc quantifications were performed by atomic absorption spectrophotometry. In this work, the effect of copper and zinc concentrations on the growth and metabolism of suspension cultures of Cynara cardunculus were studied using 2-L bioreactors, aerated and mechanically-stirred to find at what level these ions become toxic and if they are accumulated intracellularly. Our goal is:

• To screen several plant cell cultures and several toxic metals to evaluate the ability of the plant species for phytoextraction.
• To study the effect of xenobiotics on the cell metabolism and eventual changes by using a controlled growth environment.

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