

Phytoremediation: principles and perspectives

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Abstract

Acute and diffuse contamination of soil and water by heavy metals and metalloids cause wide, environmental and social concern. Among the techniques used to cleanup affected sites, phytoremediation has recently emerged as a new, cost-effective, environment-friendly alternative. After a short introduction to the types of plant-based cleanup techniques, this review focuses on metal hyperaccumulator plants and their potential use in phytoextraction technology.

Keywords: Contamination, heavy metal, hyperaccumulator plant, phytoremediation.

Resum

Hi ha una preocupació social i científica creixent per la contaminació ambiental, aguda i difusa, dels sòls i de l'aigua per metalls pesants. Entre la diversitat de tècniques disponibles per a la neteja dels llocs afectats, la fitoremediació ha emergit recentment com una nova alternativa, efectiva de costos i sostenible ambientalment. Després d'una breu introducció als diferents tipus de tècniques basades en les plantes, aquesta revisió se centra principalment en les plantes hiperacumuladores de metalls i considera el seu potencial per a les tecnologies de fitoextracció.

Human evolution has led to immense scientific and technological progress. Global development, however, raises new challenges, especially in the field of environmental protection and conservation. Technological ingenuity has enhanced the potential for improving industrial development and rapid progress is being made not only in the field of electronics but also in biological, medical and pharmaceutical applications. In recent decades, increasingly precise knowledge of basic biological functions has brought about biotechnological advances. The possibility to produce transgenic organisms has opened up new fields of experimentation and perspectives for scientific and technological development which go beyond the limits of natural evolution.

In summary, the beginning of the XXI century is witness to an irreversible dimension of power, with global concern, in all fields: politics, economics, social and cultural affairs, science and technology. Technological potential and development, however, have not always had beneficial effects [15]. Social and cultural progress fall far behind technological evolution and the over-exploitation of natural resources

with short-term, fast profit-oriented management systems has severely damaged the environment.

Acute water and soil pollution are evident consequences that call for rapid and efficient solutions. However, in addition, diffuse contamination of large expanses of land [56] is an ever-growing problem that requires sustainable correction measures.

Remediation of contamination

The cleanup of soils contaminated by hazardous chemical substances is a cost-intensive, technically complex procedure. Conventional methods of *in situ* or *ex situ* remediation are based on a number of techniques such as [122]:

- Leaching of pollutant by flushing with water or a chelate. The leachate is recovered and treated on- or off-site
- Solidification/stabilization by either physical inclusion or chemical interactions between the stabilizing agent and the pollutant.
- Vitrification using thermal energy for soil fusion, allowing physical or chemical stabilization
- Electrokinetical treatment: ionic species of the pollutant migrate to electrodes inserted into the soil.
- Chemical oxidation or reduction of the pollutant to at-

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tain chemical species with lower toxicity that are more stable and less mobile.

- Excavation and off-site treatment or storage at a more appropriate site ("dig and dump")

In most cases, these techniques are expensive and technically limited to relatively small areas. These technical difficulties, together with improved knowledge of the mechanisms of uptake, transport, tolerance and exclusion of heavy metals and other potentially hazardous, contaminants in microorganisms and plants, have recently promoted the development of a new technology, named bioremediation. Bioremediation is based on the potential of living organisms, mainly microorganisms and plants, to detoxify the environment [2]. The capacity of plants to clean the environment has been known since the XVIII century when experiments by Joseph Priestley, Antoine Lavoissier, Karl Scheele and Jan Ingenhousz demonstrated that, in light, plants purify the atmosphere. The importance of green zones for the maintenance of air quality is universally accepted, albeit not always respected.

The use of plants for purifying contaminated soils and water has been developed much more recently. In the 1970s, reclamation initiatives of mining sites developed technologies for covering soil with vegetation for stabilization purposes and reduction of visual impact [142]. It was not until the 1990s that the concept of phytoremediation emerged as a new technology that uses plants for cleaning or decreasing the toxicity of soils and surface and waste waters contaminated by metals, organic xenobiotics, explosives or radionuclides [4, 34, 36, 40, 47, 80, 89, 98, 115, 121]. In this comprehensive review, we mainly refer to phytoremediation of soils contaminated by heavy metals.

Phytoremediation of soils contaminated by heavy metals

Plants show several response patterns to the presence of potentially toxic concentrations of heavy metal ions. Most are sensitive even to very low concentrations, others have developed resistance and a reduced number behave as hyperaccumulators of toxic metals [5, 16, 21, 27, 120, 123, 128]. This particular capacity to accumulate and tolerate large metal concentrations has opened up the possibility to use phytoextraction for remediation of polluted soils and waters [58, 131].

Plants with metal resistance mechanisms based on exclusion can be efficient for phytostabilization technologies. Hyperaccumulator plants, in contrast, may become useful for extracting toxic elements from the soil and thus decontaminate and restore fertility in polluted areas. In 1885, the German botanist A. Baumann had already found that the leaves of certain plant species grown on soils with high Zn levels concentrated high amounts of this metal. However, it was not until the end of the last century that metal hyperaccumulation was studied in detail. In recent years, improved knowledge of the mechanisms of uptake, transport and tolerance

of high metal concentrations in these plants [3, 62, 74, 75, 81, 106, 107, 127, 135, 136] has opened up new avenues for remediation by phytoextraction.

In practice, to be operative, phytoextraction requires the fulfillment of several basic conditions. An ideal plant species for remediation purposes should grow easily on soils contaminated by metals, have high soil-to-shoot transfer factors, tolerate high shoot metal concentrations, and produce high biomass quickly [1, 12, 13, 17, 23, 24, 55, 59, 60, 111].

Unfortunately, most metal hyperaccumulator plants grow quite slowly and have a low biomass, while plants that produce a high biomass quickly are usually sensitive to high metal concentrations. The energy costs of metal tolerance mechanisms are responsible for this phenomenon (trade-off hypothesis). There are, however, exceptions (e.g. the Ni hyperaccumulator *Berkheya coddii*) that indicate that the capacity to accumulate and tolerate high metal concentrations in shoots and to produce high amounts of dry matter are not always mutually exclusive [117]. The cost of Cu tolerance in the non-hyperaccumulator *Mimulus guttatus* is very small and no consistent effects on growth or competitiveness are observed [64]. These data indicate that there is no intrinsic reason why metal-tolerant plants produced for phytoremediation should be competitively inferior or slow-growing [90]. As this is a crucial point for developing efficient plants for metal extraction from polluted soils, the mechanisms of metal tolerance in hyperaccumulators are addressed later in more detail.

Phytoremediation of soils contaminated by heavy metals can be achieved by a number of techniques [122]:

1. Phytoextraction: This technique reduces soil metal concentrations by cultivating plants with a high capacity for metal accumulation in shoots. Plants used for

Table 1. Some examples of metal hyperaccumulators. Detailed information can be found in refs. [2, 8, 29, 46]

Species	Shoot metal concentration $\mu\text{g g}^{-1}$	Reference
<i>Arabidopsis halleri</i> (<i>Cardaminopsis halleri</i>)	13,600 Zn	Ernst, 1968 ^[44]
<i>Thlaspi caerulescens</i>	10,300 Zn	Ernst, 1982 ^[46]
<i>Thlaspi caerulescens</i>	12,000 Cd	Mádico <i>et al.</i> , 1992 ^[91]
<i>Thlaspi rotundifolium</i>	8,200 Pb	Reeves & Brooks, 1983 ^[116]
<i>Minuartia verna</i>	11,000 Pb	Ernst 1974 ^[1974]
<i>Thlaspi goesingense</i>	12,000 Ni	Reeves & Brooks, 1983 ^[116]
<i>Alyssum bertholonii</i>	13,400 Ni	Brooks & Radford, 1978 ^[31]
<i>Alyssum pintodasilvae</i>	9,000 Ni	Brooks & Radford, 1978 ^[31]
<i>Berkheya coddii</i>	11,600 Ni	Brooks, 1998 ^[27]
<i>Psychotria douarrei</i>	47,500 Ni	Baker <i>et al.</i> , 1985 ^[6]
<i>Miconia lutescens</i>	6,800 Al	Bech <i>et al.</i> , 1997 ^[24]
<i>Melastoma malabathricum</i>	10,000 Al	Watanabe <i>et al.</i> , 1998 ^[7]

Table 2. Some examples of high biomass-producing species with potential use in phytoextraction or rhizofiltration

Species	Extracted contaminant/substrate	References
<i>Salix</i>	Heavy metals/soil, water	Greger and Landberg, 1999 ^[60]
<i>Populus</i>	Ni/ soil, water, groundwater	Punshon and Adriano, 2003 ^[112]
<i>Brassica napus</i> , <i>B. juncea</i> , <i>B. nigra</i>	Radionuclides, heavy metals, Se/soil	Brown, 1996 ^[32] , Bañuelos <i>et al.</i> , 1997 ^[12]
<i>Cannabis sativa</i>	Radionuclides, Cd/soil	Ostwald 2000 ^[105]
<i>Helianthus</i>	Pb,Cd /soil	EPA, 2000 ^[130] Elkatib <i>et al.</i> , 2001 ^[43]
<i>Typha sp.</i>	Mn, Cu, Se/mine wastewater	Horne, 2000 ^[65]
<i>Brassica juncea</i>	Se/saline drainage effluent	Bañuelos <i>et al.</i> 1997 ^[14]
<i>Phragmites australis</i>	Heavy metals/mine tailings-wetland	Massacci <i>et al.</i> , 2001 ^[95]
<i>Glyceria fluitans</i>	Heavy metals/mine tailings-wetland	MacCabe and Otte, 2000 ^[97]
<i>Lemna minor</i>	Heavy metals/water	Zayed <i>et al.</i> , 1998 ^[143]

this purpose should ideally combine high metal accumulation in shoots and high biomass production. Many hyperaccumulator species fulfill the first (see Table 1 for examples), but not the second condition.

Therefore, species that accumulate lower metal concentrations but are high biomass producers may also be useful (for examples see Table 2). When plants are harvested, the contaminants are removed from the soil. Recovery of high-price metals from the harvested plant material may be cost effective (e.g. phytomining [30] of Ni, Tl or Au.). If not, the dry matter can be burnt and the ash disposed of under controlled conditions.

- Rhizofiltration: This technique is used for cleaning contaminated surface waters or wastewaters by adsorption or precipitation of metals onto roots or absorption by roots or other submerged organs of metal-tolerant aquatic plants. For this purpose, plants must not only be metal-resistant but also have a high adsorption surface and must tolerate hypoxia [42, 65]. Some examples are listed in Table 2.
- Phytostabilization: Plants are used for immobilizing contaminant metals in soils or sediments by root uptake, adsorption onto roots or precipitation in the rhizosphere. By decreasing metal mobility, these processes prevent leaching and groundwater pollution. Bioavailability is reduced and fewer metals enter the trophic web.
- Phytodegradation: Elimination of organic pollutants by decomposition through plant enzymes or products.
- Rhizodegradation: Decomposition of organic pollutants by means of rhizosphere microorganisms [138].
- Phytovolatilization: Organic pollutants absorbed by plants are released into the atmosphere by transpiration, either in their original form or after metabolic modification. In addition, certain metals can be absorbed and volatilized by certain organisms. Several species of the genus *Astragalus* accumulate and volatilize Se. Uptake and evaporation of Hg is achieved by some bacteria. The bacterial genes responsible have al-

ready been transferred to *Nicotiana* or *Brassica* species, and these transgenic plants may become useful in cleaning Hg-contaminated soils [13, 101].

- Hydraulic control: This technique uses plants that absorb large amounts of water and thus prevent the spread of contaminated wastewater into adjacent uncontaminated areas. Phreatophytes can be used for cleaning saturated soils and contaminated aquifers [113]
- Phytorestitution: Revegetation of barren areas by fast-growing resistant species that efficiently cover the soil, thus preventing the migration of contaminated soil particles and soil erosion by wind and surface water run-off. This technique reduces the spread of contaminants and also visual impact. However, previous soil conditioning is required (e.g. liming or berengerite-amendments) to enable plants to colonize the polluted substrate [102, 133, 134.]

In recent years, the scientific and social interest in phytoremediation techniques has increased substantially for several reasons: extensive soil contamination, advanced scientific knowledge of the mechanisms and functions of living organisms and ecosystems, the pressure of public opinion, and political and economical concerns. Twenty years ago, studies on this subject were scarce, while today many scientists, especially in the USA and Europe are involved in basic and applied research projects aimed to make phytoremediation a commercially viable technique. Given the inherent limitations of biological systems and the diversity of problems present at polluted sites, it is unrealistic to conceive phytoremediation as an instant, high-profit, universal solution for contaminated soil. However, site-specific adaptation of general strategies developed in basic scientific research programs can provide sustainable, environment-friendly solutions for the cleanup of contaminated soils and sediments. The challenge of contamination cleanup and the crucial contribution of research in this field can be put into perspective by considering some statistic and economic data. In 1998, the European Environmental Agency estimat-

ed a total of 1,400,000 contaminated sites in Western Europe. A comparison of the economic costs between conventional, physical-chemical, decontamination procedures and new, plant-based, phytoremediation technology clearly favors the latter. According to several authors [39, 100], conventional procedures raise the average cost per contaminated hectare of soil from 0.27 to 1.6 million \$, while phytoremediation costs from about 10 to 1000 times less. From the period 1998-2000 to 2005, the market for phytoremediation in the USA is estimated to increase from \$16-29 million to \$214-370 million. The time factor is by far the most critical point in plant-based cleanup techniques. However, the long persistence of heavy metal contamination in soils (residence times of thousands of years) makes even long-term cleaning strategies attractive.

The complex scenario of acute or diffuse soil contamination by heavy metals therefore deserves attention by the scientific, economic, social, and political authorities in order to provide the means to study successful mechanisms for soil remediation. Only with a sustainable focus of this kind will harmony between nature and human evolution be restored.

Metal hyperaccumulator plants

At least 400 species distributed in 45 botanical families are considered metal hyperaccumulators [27]. By definition, hyperaccumulators are herbaceous or woody plants that accumulate and tolerate without visible symptoms a hundred times or greater metal concentrations in shoots than those usually found in non-accumulators. Baker and Brooks established 0.1% as the minimum threshold tissue concentrations for plants considered Co, Cu, Cr, Pb or Ni hyperaccumulators, while for Zn or Mn the threshold is 1% [5, 7].

Hyperaccumulators are metallophytes and belong to the natural vegetation of metal-enriched soils [48, 49, 108]. These species have evolved internal mechanisms that allow them to take up and tolerate large metal concentrations that would be extremely toxic to other organisms [37, 79]. These plants are perfectly adapted to the particular environmental conditions of their habitat and high metal accumulation may contribute to their defense against herbivores and fungal infections [26, 94, 127]. However, usually, the metabolic and energetic costs of their adaptation mechanisms do not allow them to compete efficiently on uncontaminated soil with non-metallophytes.

Metal hyperaccumulation has evolved in plants all over the world and important sites for collecting germplasm are, among others, New Caledonia, Australia, Central and South Europe, the Mediterranean Area, South-East Asia, Cuba, Dominican Republic, California, Zimbabwe, Transvaal in South Africa, Goiás in Brazil, Hokkaido in Japan, and Newfoundland in Canada [5, 45].

Several hypotheses have been proposed to explain the mechanisms of metal hyperaccumulation and the evolutionary advantage of this strategy.

1. *Complex formation and compartmentation*: Hyperaccumulators synthesize chelators that detoxify metal ions by complex formation. The soluble, less-toxic, organic-metal complex is transported to cell compartments with low metabolic activity (cell wall, vacuole) where it is stored in the form of a stable organic or inorganic compound. [18, 19, 20, 54, 62, 129, 135, 136]
2. *Deposition hypothesis*: Hyperaccumulators separate metals from the root, accumulating them in plant parts that are abscised (old leaves), leached by rain (epidermis, hairs) or burnt.
3. *Inadverted uptake*: Hyperaccumulation of the metal is thought to be the by-product of an adaptation mechanism to other adverse soil characteristics (e.g. Ni hyperaccumulation in serpentinophytes)
4. *Hyperaccumulation as a defense mechanism against abiotic or biotic stress conditions*. Metal effectiveness against certain pathogenic fungi and bacteria and on leaf-consuming herbivores has been reported [25]. Phloem parasites [53], however, are unaffected, probably because of low phloem mobility of the metals. High metal concentrations in leaves can act as feeding deterrents or, after ingestion, may reduce the reproduction rate of herbivores or poison them. Trade-off of organic defenses by metal hyperaccumulation may also confer advantage [127]. It has been suggested that high leaf metal concentrations may be used in osmotic adjustment under drought stress [8, 109]. Extensive studies in *Alyssum murale* (Ni hyperaccumulator) and *Thlaspi caerulescens* (Zn hyperaccumulator) have not confirmed this hypothesis [141].

At present, the adaptive advantages of the hyperaccumulation strategy are unclear. The strategy probably involves complex interactions of diverse factors and mechanisms that cannot be ascertained by a single, reductionistic interpretation. From both the scientific and practical viewpoint, however, the way in which plants achieve metal hyperaccumulation is of much greater interest than the *raison d'être* of the mechanism. Only by studying the basic mechanisms of hyperaccumulation will phytoextraction technologies be successfully developed.

Mechanisms of metal tolerance and hyperaccumulation in plants

Metal hyperaccumulators are highly specialized models of plant mineral nutrition. Seventeen elements are considered essential for all higher plants (C, H, O, N, S, P, K, Ca, Mg, Fe, Mn, Cu, Zn, B, Mo, Cl, and Ni). Macronutrients are those necessary in high concentrations (mM level) while micronutrients are required only in μM tissue concentrations. Hyperaccumulators concentrate, in a specific way, certain trace metals or metalloids that may be essential (Cu, Mn, Zn, or Ni) or not (e.g. Cd, Pb, Hg, Se, Al, As) at amounts that would be

extremely toxic to other plants [3, 5, 27, 62, 67, 93, 110, 128]. Some examples are given in Table 1.

Most metallophytes that can colonize metal-polluted soils base their metal resistance on efficient exclusion of metal ions from root tip meristems and shoots [16, 18, 20, 50, 54, 82]. In contrast, hyperaccumulators, preferentially accumulate the metal in shoots and, in the case of hyperaccumulation of essential trace elements, this capacity is frequently accompanied by plant requirement for unusually high substrate availability of the metal in order to avoid deficiency [128, 129]. This may be the result of a constitutively active mechanism that inactivates incoming metal ions in hyperaccumulators. Rapid complexation and compartmentation not only detoxify metal ions but also make them less available for essential metabolic processes. To understand the physiological, biochemical and molecular mechanisms that underlie metal hyperaccumulation in plants, it is necessary to consider metal uptake, transport and metabolic processes involved in the entire organism, from the rhizosphere to the leaf cell compartment.

Rhizosphere interactions

The availability of metals in the soil around roots is strongly affected by root exudates and root depositions (mucilage and border cells) but also by microbial activities such as siderophore release and redox reactions [41, 70, 84, 93]. The mutual influence of plants and soil microorganisms and the selective force of soil metal concentrations on microbe populations make research on this topic very difficult. Given the possible environmental problems associated with metal mobilization by synthetic chelators [22, 61, 85, 110, 118], enhancement of metal availability in the rhizosphere by hyperaccumulators would be a useful mechanism for improving phytoextraction technologies. At present, however, data on the role of root exudates from hyperaccumulators in metal mobilization are contradictory. While in acidic and calcareous soils some authors found that *Thlaspi caerulescens* tends to decrease rhizosphere pH [63, 71], others observed a decrease [99]. However, pH changes do not appear to be a relevant mechanism for metal mobilization by this kind of plant. Release of histidine and citrate into the rhizosphere may play a crucial role in the reduction of Ni uptake and toxicity in the Ni sensitive *Thlaspi arvense*, while in the Ni hyperaccumulator *Thlaspi goesingense* no Ni-enhanced release of these chelators into the rhizosphere was found [119]. The involvement of bacteria in Zn mobilization in the rhizosphere of *Thlaspi caerulescens* has been reported [140]; bacterial production of siderophores may be responsible for enhanced bioavailability [83].

Metal uptake and root-to-shoot transport

Metal resistance in species with exclusion strategy is frequently based on reduced metal uptake into roots, preferen-

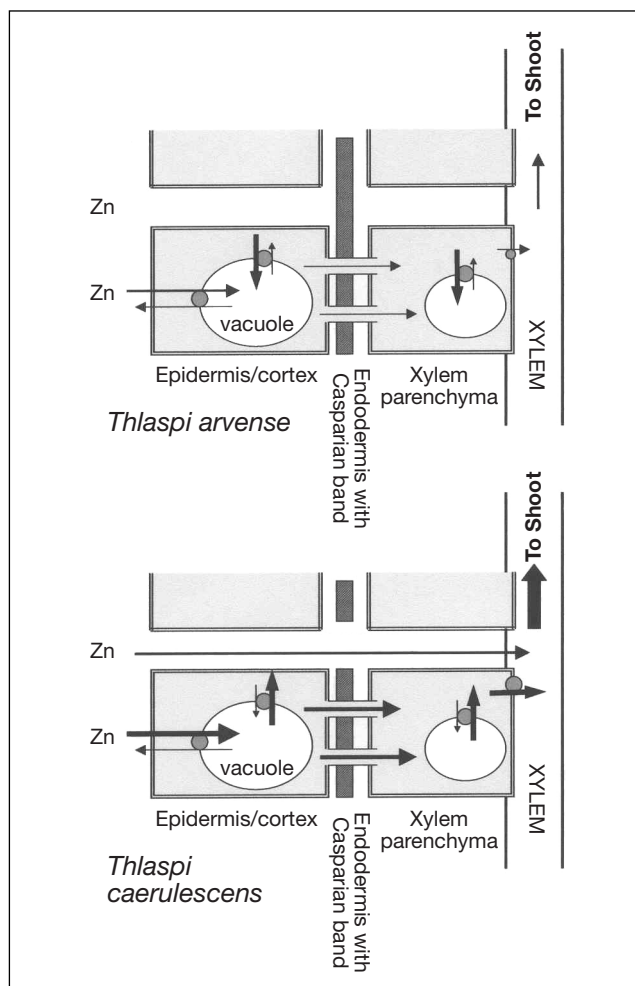


Figure 1. Differences in Zn transport in roots of the Zn hyperaccumulator *Thlaspi caerulescens* and the non-accumulator *Thlaspi arvense*. Thin arrows, low transport; thick arrows, high transport. (modified after Lasat and Kochian, 2000 [76].)

tial storage of metals in root vacuoles and restricted translocation into shoots. Hyperaccumulators, in contrast, take up more metals, store a lower proportion of them in root vacuoles, and export higher amounts to shoots (Figure 1).

Comparative studies on Zn uptake and transport in the non-hyperaccumulator *Thlaspi arvense* and the Zn–hyperaccumulator *Thlaspi caerulescens* indicate that these differences are caused by altered tonoplast Zn transport in roots and stimulated Zn uptake into leaves [77, 78]. Stimulation of ZNT1 expression, a genes that encodes a Zn transporter that belongs to the ZIP family of plant micronutrient transporters, was higher in *Thlaspi caerulescens* than in *Thlaspi arvense*. Other transporter systems of special interest in hyperaccumulators are the cation diffusion facilitators (CDF type), the ABC transporters for phytochelatin and the metal chaperones [38, 81, 103, 104, 106, 125, 132]. Detailed studies on the characterization and the differential distribution of these transporter systems in hyperaccumulators and non-hyperaccumulators will clarify the basic genetic and molecular mechanisms responsible for metal hyperaccumulation. The results from this research may lead to the development of specific strategies to produce efficient plants for metal ex-

traction technologies by molecular engineering and conventional breeding [10].

The pathways of radial root transport of metals and their entrance into the vascular cylinder are a further issue of debate in hyperaccumulation research. It has been proposed that apoplastic transport of Zn to the xylem is required for sustaining rapid Zn transport to the leaves [106], while factorial experiments with Zn and Cd [86, 92] support the hypothesis of a common, but Cd-preferent, symplastic transport system for Zn and Cd to the xylem [52].

Formation of less toxic metal complexes is essential in metal hyperaccumulation in plants. The toxicity of metal cations is mainly due to their tendency to form organic complexes with distinct ligands, which interfere with membrane functions, enzyme reactions, electron transport etc. Uptake and root-to-shoot transport of high metal concentrations is only possible when these toxic interactions are avoided by the synthesis of strong chelators that efficiently bind the metals in a non-toxic form, thereby allowing flux to and through the xylem up to the leaves. Organic acids, aminoacids, phytochelatins have been implied in metal detoxification. The Zn and Cd hyperaccumulator *Thlaspi caerulescens* contains constitutively high organic acid levels [129]. Many Al hyperaccumulators contain high concentrations of either or both organic acids and flavonoid-type phenolics, which form strong complexes with the metal [18, 19]. High concentrations of organic acid anions in leaf tissues are a crucial, widely distributed, mechanism that allows plants to maintain cation/anion homeostasis under conditions of excess ion stress. The ability to accumulate high organic acid levels in tissues may be considered a prerequisite, but is not sufficient, for metal tolerance [18, 129].

The sulfhydryl-rich phytochelatins (PC) have high affinity for binding Cd, Hg, Cu or even As. *Arabidopsis cad1* mutants, deficient in PC synthase, are very sensitive to Cd [66]. Nonetheless, treatment of Cd-exposed plants with BSO, an inhibitor of PC synthesis, increased Cd sensitivity only in plants that lacked Cd hypertolerance. These results indicate that Cd hypertolerance is not based on PC-mediated sequestration. In contrast, PC-based sequestration may be essential in both constitutive As tolerance and As hypertolerance [124].

A metal can be bound by a number of ligands within distinct plant organs and compartments. In this regard, in 1975, Mathys [96] and Ernst [51] proposed the malate shuttle hypothesis. According to this hypothesis, in Zn-resistant plants excess Zn is bound to malate in the cytoplasm and, after transport to the vacuole, a ligand exchange occurs. Zn forms more stable complexes with citrate, oxalate or other ligands, while malate returns to the cytoplasm. Recent research shows that ligand exchange can also play a critical role in hyperaccumulators. Studies with the Al hyperaccumulator *Fagopyrum esculentum* revealed the importance of ligand exchange (oxalate-citrate-oxalate) during Al transport from the rhizosphere through roots to the leaves [88]. Furthermore, in the Ni hyperaccumulator *Thlaspi goesingense*, Ni is bound by several ligands. Cytoplasmic Ni seems

to be detoxified by binding to histidine, while vacuolar storage of Ni is probably in the form of citrate [74].

Data indicate that metal hyperaccumulation is not based on the capacity of a plant to produce a high concentration of a particular chelator, but that it involves the coordinated action of a number of ligands and differential distribution of diverse metal transport systems. Current research is beginning to identify the components that interact in metal hyperaccumulation. However, the regulatory processes responsible for this particularly altered metal homeostasis in hyperaccumulators remains to be elucidated. Progress in this field of basic research is essential to identify or produce effective plants for phytoextraction technologies.

Conventional breeding and genetic engineering for efficient phytoextraction

As stated at the beginning of this review, the development of commercial phytoextraction technologies requires plants that produce high biomass and that accumulate high metal concentrations in organs that can be easily harvested, i.e. in shoots. There are two main approaches to this problem:

- (1) Domestication and breeding of improved hyperaccumulator species [33] and
- (2) Application of genetic engineering to develop fast-growing high biomass plants with improved metal uptake, translocation and tolerance [69, 73]. Some examples are shown in Table 2.

The first approach, mainly developed in the USA by the group headed by Chaney, involves several crucial steps: selection of hyperaccumulator plants that are likely to be domesticated, collection of seeds from wild plants and bioassay of their phytoextraction utility, breeding for improved cultivars and development of adequate soil and crop management practices [33]. The usefulness of this system has been shown for Co and Ni and has obtained a utility patent [35]. However, the authors recognize that in situations where available hyperaccumulator species are too small to afford economic cleanup procedures, biotechnology may be required to combine hyperaccumulation and high biomass production.

The V Framework Research Program of the European Community includes two projects [68] on the production of genetically modified plants for phytoremediation, PHYTAC and METALLOPHYTES. The former (<http://www.uku.fi/~atervaha/PHYTACHome.htm>) aims to transfer genes from the hyperaccumulator *Thlaspi caerulescens* to the high biomass-producing *Brassica* or tobacco. While the METALLOPHYTES project is devoted to engineering *Festuca* for improved metal tolerance and or accumulation (<http://biobase.dk/~palmgren/metallophytes.html>).

To date, the most successful approach has been the transformation of plants using modified bacterial *merA* gene (*merA9*) for detoxifying Hg (II). The *merA* gene codifies for a mercuric ion reductase that removes Hg from stable thiol salts by reducing it to volatile metallic Hg. When grown in a 5

μM Hg(II) solution, transformed *Arabidopsis* plants expressing the *merA9* gene volatilized 10 ng of metallic Hg per minute and mg plant tissue. Hg reductase has also been successfully transferred to Brassica, tobacco and yellow poplar trees [101].

There are, however, ecological, social, and legal objections to the practical application of genetically modified organisms in the field. The potential of transgenic plants to efficiently cleanup contaminated sites may help to change adverse public opinion. Nonetheless, future research should address not only the "know-how" of producing efficient plants for phytoremediation and their integration into sustainable cropping and management systems, but should also clarify the potential impact of transgenic plants on the target habitat and the fate of the introduced genes in the surrounding environment.

Concluding remarks

Phytoremediation is a new, attractive technique that has emerged over recent years. This technique offers excellent perspectives for the development of plants with the potential for cleaning metal-contaminated soils, at least under certain, favorable conditions and for using adequate crop management systems. Advances in molecular biology and genetic engineering of plants have been indispensable for this progress. However, this spectacular development would not have been possible without the invaluable contribution of a small group of researchers. More than thirty years ago they showed extraordinary scientific insight by recognizing the enormous potential of plants that can colonize metal-contaminated soils and they dedicated many years of conscientious research to the geobotany and ecophysiology of metallophytes. Exploratory studies of this kind are still necessary and should be supported in order to preserve the immense natural genetic resources of metalliferous habitats and to increase our basic knowledge about the natural adaptation mechanisms of hyperaccumulators.

In this regard, we dedicate this review to Prof. W.H.O Ernst, an eminent pioneer in research into metallophytes.

Acknowledgements

Part of the authors work cited in this paper was supported by the *Generalitat de Catalunya* (Autonomous Government of Catalonia) (2001 SGR-00200), the European Union (ICA4-CT-2000-30017), and the Spanish Government (DGICYT, BFI2001-2475-CO2-01).

References

- [1] Alcántara E, Barra R, Benloch M, Ginha A, Jorrián JV, Lopez JA, Lora A, Ojeda MA, Puig M, Pujadas A, Requejo R, Romera J, Riso J, Sancho ED, Shibo SI, Tena
- [2] Anderson TA, Coats JR 1994 Bioremediation through rhizosphere technology. ACS Symposium Series, vol 563. American Chemical Society, Washington, DC, 249 pp.
- [3] Assunção AGL, Da Costa Martins P, De Folter S, Vooijs R, Schat H, Aarts MGM 2001 Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 24, 217-226.
- [4] Baker AJM, McGrath SP, Sidoli CMD, Reeves RD 1994 The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Res. Conserv. Recycl. 11, 41-49.
- [5] Baker AJM, Brooks RR 1989. Terrestrial higher plants which hyperaccumulate metallic elements— a review of their distribution, ecology and phytochemistry. Biorecovery 1, 81-126.
- [6] Baker AJM, Brooks RR, Kersten WJ 1985. Accumulation of nickel by *Psychotria* species from the Pacific Basin. Taxon, 34, 89-95.
- [7] Baker AJM, McGrath SP, Reeves RD, Smith JAC 2000. Metal Hyperaccumulator Plants: A Review of the Ecology and Physiology of a Biological Resource for Phytoremediation of Metal Polluted Soils. In: N Terry, G Bañuelos (eds.) Phytoremediation of Contaminated Soils and Waters. CRC Press LLC, Boca Raton, FL, USA, pp 85-107.
- [8] Baker AJM, Walker PL 1989. Ecophysiology of metal uptake by tolerant plants. In: AJ Shaw (ed.) Heavy Metal Tolerance in Plants. Evolutionary Aspects. CRC Press, Boca Raton FL, USA, pp 155-177.
- [9] Baker AJM, Proctor J, Van Balgooy MMJ, Reeves RD 1992. In AJM Baker, J Proctor, RD Reeves (eds.) The Vegetation of Ultramafic (Serpentin) Soils, Intercept, Andover, UK, pp 291-304.
- [10] Baker AJM, Whiting SN 2002. In search of the Holy Grail – a further step in understanding metal hyperaccumulation? New Phytol 155, 1-7.
- [11] Bañuelos GS 2000. Factors influencing field Phytoremediation of selenium-laden soils. In: N Terry, G Bañuelos (eds.) Phytoremediation of Contaminated Soils and Waters. CRC Press, Boca Raton, FL, USA, pp 41-59.
- [12] Bañuelos GS, Ajwa HA, Mackey B, Wu LL, Cook C, Akohoue S, Zambrzuski S 1997. Evaluation of different plant species used for phytoremediation of high soil selenium. J Environ. Qual. 26, 639-646.
- [13] Bañuelos GS, Ajwa HA, Wu LL, Zambrzuski S 1998. Selenium accumulation by *Brassica napus* grown in Se-laden soil from different depths of Kesterson Reservoir. J. Contam. Soil 7, 481-496.
- [14] Bañuelos GS, Ajwa HA, Terry N, Zayed A 1997. Phytoremediation of selenium laden soils: A new technology. Journal of Soil and Water Conservation 52, 426-430

- [15] Barceló J, 1989 Home-planta-medi ambient: una harmonia trencada (Model d'estudi planta-metalls pesants). Discurs d'Ingrés Reial Acadèmia de Farmàcia de Barcelona. Servei Publicacions Universitat Autònoma de Barcelona, Bellaterra, 69 pp.
- [16] Barceló J, Poschenrieder C 1989. Estrés vegetal inducido por metales pesados. *Investigación y Ciencia* 154, 54.
- [17] Barceló J, Poschenrieder C, Lombini A, Llugany M, Bech J, Dinelli E 2001. Mediterranean plant species for phytoremediation. In: Abstracts COST Action 837 WG2 workshop on Phytoremediation of Trace Elements in Contaminated Soils and Waters with Special Emphasis on Zn, Cd, Pb and As. Ed. Universidad Complutense Madrid, Faculty of Chemistry, Madrid, page 23. (<http://lbewwww.epfl.ch/COST837>)
- [18] Barceló J, Poschenrieder C 2002 Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. *Environ. Exp. Bot.* 48, 75-92.
- [19] Barceló J, Poschenrieder C, Tolrà RP 2003. Importance of phenolics in rhizosphere and roots for plant-metal relationships. In: G. Gobran (ed.) Extended Abstracts 7th ICOBTE Upsala 15-19 June, pp 162-163.
- [20] Barceló J, Poschenrieder C. 1999 Structural and ultrastructural changes in heavy metal exposed plants. In: MNV Prasad, J Hagemeyer (eds.) *Heavy Metal Stress in Plants. From Molecules to Ecosystems*. Springer Verlag, Berlin, pp 183-205
- [21] Barceló J., Vázquez M.D., Mádico J., Poschenrieder C. 1994. Hyperaccumulation of zinc and cadmium in *Thlaspi caerulescens*. In: S.P. Varnavas (ed.) *Environmental Contamination CEP Consultants Ltd.*, Edinburgh, pp 132-134.
- [22] Barona A, Aranguiz I, Elias A 2001. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. *Environ. Pollut.* 113, 79-85
- [23] Bech J, Poschenrieder C, Barceló J, Lansac A 2002. Plants from mine spoils in the South American area as potential source of germplasm for phytoremediation technologies. *Acta Biotechnol.* 22, 5-11.
- [24] Bech J, Poschenrieder C, Llugany M, Barceló J, Tume P, Tobias FJ, Barranzuela JL, Vázquez ER 1997 Arsenic and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru. *Sci. Total Environ.* 203, 83-91.
- [25] Boyd RS 1998. Hyperaccumulation as a plant defensive strategy. In: RR Brooks (ed.) *Plants that Hyperaccumulate Heavy Metals. Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining*. CAB International, Wallingford, UK; pp 181-201
- [26] Boyd RS, Martens SN 1998. The significance of metal hyperaccumulation for biotic interactions. *Chemoecology* 8, 1-7.
- [27] Brooks RR (ed.) 1998. *Plants that Hyperaccumulate Heavy Metals*. CAB International, Wallingford, UK, 380 pp.
- [28] Brooks RR, Morrison RS, Reeves RD, Dudley TR, Akma Y 1979. Hyperaccumulation of nickel in *Alyssum Limnaeus*. *Proc. Roy. Soc. London, sct. B* 203, 387-403.
- [29] Brooks RR 1987 *Serpentine and its Vegetation. A Multidisciplinary Approach*. Dioscorides Press, Portland, Oregon.
- [30] Brooks RR, Chambers MF, Nicks LJ 1998. Phytomining. *Trends in Plant Science* 3, 359-362.
- [31] Brooks RR, Radford CC 1978. Nickel accumulation by European species of the genus *Alyssum*. *Proc. Royal Soc. (London) sect. B* 203, 387-403.
- [32] Brown KS 1996. The green clean: The emerging field of phytoremediation takes root. *BioScience. American Inst. Biol. Sci.*, Washington DC, 45, 579
- [33] Chaney RL, Li Y-M, Brown SL, Homer FA, Malik M, Angle JS, Baker AJM, Reeves RD, Chin M 2000 Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 129-158.
- [34] Chaney RL 1983. Plant uptake of inorganic waste constituents. In: JF Parr, PB Marsh, JM Kla (eds.) *Land Treatment of Hazardous Wastes*. Noyes Data Corp., Park Ridge, USA, pp 50-76.
- [35] Chaney RL, Angle JS, Baker AJM, Li Y-M, 1998. Method for phytomining of nickel, cobalt and other metals from soil. U.S. Patent 5,711,784.
- [36] Chaney RL, Malik M, LI YM, Brown SL, Brewer EP, Angle JS, Baker AJM 1997. Phytoremediation of soil metals. *Curr. Opin. Biotechnol* 8, 279-284.
- [37] Clemens S 2001. Molecular mechanisms of plant metal homeostasis and tolerance. *Planta* 212, 475-486.
- [38] Clemens S, Bloss T, Vess C, Neumann D, Nies DH, zur Nieden U 2002. A transporter in the endoplasmic reticulum of *Schizosaccharomyces pombe* cells mediates zinc storage and differently affects transition metal tolerance. *J Biol Chem.* 277, 18215-18221.
- [39] Cunningham SD, Berti WR 2000. Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-lead issue. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 359-375
- [40] Cunningham SD, Berti WR, Huang JW 1995 Remediation of contaminated soils and sludges by green plants. In: RE Hinchee, JL Means, DR Burris (eds.) *Bioremediation of Inorganics*. Battelle Press, Columbus, OH, USA, pp33-54.
- [41] Deiana S, Manunza B, Palma A, Premoli A, Gessa C 2000. Interactions and mobilization of metal ions at the soil-root interface. In: GR Gobran, WW Wenzel, E Lombi (eds.) *Trace Elements in the Rhizosphere*. CRC Press, Boca Raton, FI, USA, pp 127-148.
- [42] Dushenkov V, Nanda Kumar PBA, Motto H, Rakin I

1995. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environ. Sci. Technol.* 29, 1239-1245.
- [43] Elkatib EA, Thabet AG, May AM 2001. Phytoremediation of cadmium contaminated soils: role of organic complexing agents in cadmium phytoextraction. *Land Contamination & Reclamation* 9, 301-306.
- [44] Ernst WHO 1968 Zur Kenntnis der Soziologie und Ökologie der Schwermetallvegetation Grossbritanniens. *Ber. Deutsch. Bot. Ges.* 81, 116-124
- [45] Ernst WHO 1974 Schwermetallvegetation der Erde. Gustav Fischer Verlag, Stuttgart.
- [46] Ernst WHO 1982 Schwermetallpflanzen. In: H. Kinzel (ed.) *Pflanzenökologie und Mineralstoffwechsel*. Verlag Eugen Ulmer, Stuttgart, pp 472-506
- [47] Ernst WHO 1995. Decontamination or consolidation of metal contaminated soils by biological means. In: U Förstner, W Salomons, P Meder (eds.) *Heavy Metals: Problems and Solutions*. Springer Verlag, Berlin, pp 141-149.
- [48] Ernst WHO 2000 Evolution and ecophysiology of metallophytes in Africa and Europe. In: SW Breckle, B Schweizen, U Arndt (eds.) *Results of Worldwide Ecological Studies. 1st Symposium AFW Schimper Foundation (H & E Walter) Stuttgart 1998*, Heimbach Verlag, Stuttgart, pp 23-35
- [49] Ernst WHO 2000. Evolution of metal hyperaccumulation and phytoremediation hype. *New Phytol.* 146, 357-358.
- [50] Ernst WHO 2002 *Leven aan de grenzen van leven*. Vrije Universiteit, Amsterdam.
- [51] Ernst WHO, 1975. Physiology of heavy metal resistance in plants. In: *Int Conf. Heavy Metals in the Environment*, Toronto, Can Vol. 2. CEP Consultants Ltd., Edinburgh, pp121-136.
- [52] Ernst WHO, Assunção AGL, Verkleij JAC, Schat H 2002. How important is apoplastic zinc xylem loading in *Thlaspi caerulescens*? *New Phytol* 155, 1-7.
- [53] Ernst WHO, Schat H, Verkleij JAC 1990 Evolutionary biology of metal resistance in *Silene vulgaris*. *Evolutionary Trends in Plants* 4, 45-51.
- [54] Ernst WHO, Verkleij JAC, Schat H 1992. Metal tolerance in plants. *Acta Bot. Neerl.* 41, 229-248.
- [55] Escarre J, Lefebvre C, Gruber W, LeBlanc M, Lepart J, Riviere Y, Delay B 2000. Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites in Mediterranean area: implications for phytoremediation. *New Phytol.* 145, 429-437.
- [56] ETCS (European Topic Centre Soil), 1998. Topic report– Contaminated Sites. European Environmental Agency, Copenhagen 142 pp.
- [57] Frérot H, Petit C., Lefèbvre C., Gruber W, Collin C, Escarré J. 2003. Zinc and Cadmium accumulation in experimental hybrids between metalicolous and non-metallicolous *Thlaspi caerulescens* (Brassicaceae). *New Phytologist* 157, 643-648.
- [58] Garbisu C, Alkorta I 2003 Basic concepts on heavy metal soil bioremediation. *Min. Proc. Environ. Protect.* 3, 229-236.
- [59] Gonelli C, Marsili-Libelli S, Baker AJM, Gabbriellini R 2000 Assessing plant phytoextraction potential through mathematical modelling. *Int. J. Phytoremediation* 2, 343-351.
- [60] Greger M and Landberg T 1999. Use of willow in phytoextraction. *Int. J. Phytorem.* 1, 115-123
- [61] Gupta SK, Herren T, Wenger K, Krebs R, Hari T 2000. In situ gentle remediation measures for heavy metal polluted soils. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 303-322.
- [62] Hall JL 2002 Cellular mechanisms for heavy metal detoxification and tolerance *J Exp. Bot.* 53, 1-11.
- [63] Hammer D, Keller C 2002. Changes in the rhizosphere of metal accumulating plants evidenced by chemical extractants. *J Environ Qual* 31, 1561-1569.
- [64] Harper FA, Smith SE, MacNair MR 1997. Where is the cost in copper tolerance in *Mimulus guttatus*? Testing the trade-off hypothesis. *Functional Ecology* 11, 764-774.
- [65] Horne AJ 2000. Phytoremediation by constructed wetlands. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 13-39
- [66] Howden R, Goldsbrough PB, Anderson CR, Cobbet CS 1995. Cadmium-sensitive cad1 mutants of *Arabidopsis thaliana* are phytochelatin deficient. *Plant Physiol.* 107, 1059-1066.
- [67] Jansen S, Broadley MR, Robbrecht E, Smets E 2002. Aluminum hyperaccumulation in Angiosperms: a review of its phylogenetic significance. *The Bot. Rev.* 68, 235-269.
- [68] Kärenlampi S 2002 Risk of GMO's: General introduction, political issues, social and legal aspects. In: Abstracts of COST Action 837. 4. WG2 Workshop Risk assessment and sustainable land-management using plants in trace element contaminated soils. 25-27 April, Bordeaux, pp 99-100. (<http://lbewwww.epfl.ch/COST837>)
- [69] Kärenlampi S, Schat H, Vangronsveld J, Verkleij JAC, vander Lelie D, Mergeay M, Tervahauta A.I. 2000. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environ. Pollut* 107, 225-231
- [70] Kidd PS, Llugany M, Poschenrieder C, Gunsé B, Barceló J 2001 The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). *Journal of Experimental Botany* 52, 1339-1352.
- [71] Knight B, Zhao FJ, McGrath SP, Shen ZG 1997. Zinc and cadmium uptake by the hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metal in soil solution. *Plant Soil* 197, 71-78.

- [72] Köhl KI, Harper FA, Baker AJM, Smith JAC 1997. Defining a heavy metal hyperaccumulator plant. The relationship between metal uptake, allocation and tolerance. *Plant Physiol* 114, 124.
- [73] Krämer U, Chardormens AN 2001. Use of transgenic plants in the bioremediation of soils contaminated with trace elements. *Appl. Microbiol Biotechnol* 55, 661-672.
- [74] Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE 2000. Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. *Plant Physiol.* 122, 1343-1353.
- [75] Küpper H, Lombi E, Zhao FJ, Wieshammer, G., McGrath SP 2001. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. *J. Exp Bot.* 52, 2291-2300
- [76] Lasat MM, Kochian LV 2000. Physiology of Zn hyperaccumulation in *Thlaspi caerulescens*. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press, Boca Raton, FL, USA, pp. 159-169.
- [77] Lasat MM, Baker AJM, Kochian LV 1996. Physiological characterization of root Zn²⁺ absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiol* 112, 1715-1722.
- [78] Lasat M M, Baker AJM, Kochian LV 1998. Altered Zinc compartmentation in the root and stimulated Zn absorption both play a role in Zn hyperaccumulation in *Thlaspi caerulescens*. *Plant Physiology* 118, 875-883.
- [79] Lasat MM 2002 Phytoextraction of toxic metals: a review of biological mechanisms. *J. Environ. Qual.* 31, 109-120.
- [80] Lasat MM, Fuhrman M, Ebbs SD, Cornish JE, Kochian LV 1998. Phytoextraction of radiocesium-contaminated soil: evaluation of cesium-137 bioaccumulation in the shoots of three plant species. *J. Environ. Qual.* 27, 165-169.
- [81] Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV 2000 Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. *J. Exp Bot* 51, 71-79.
- [82] Llugany M, Lombini A, Poschenrieder C., Dinelli E, Barceló J 2003. Different mechanisms account for enhanced copper resistance in *Silene armeria* ecotypes from mine spoil and serpentine sites. *Plant and Soil* 251, 55-63.
- [83] Lodewyckx C, Mergeay M, Vangronsveld J, Clijsters H, Van Der Lelie D 2002. Isolation, characterization, and identification of bacteria associated with the zinc hyperaccumulator *Thlaspi caerulescens* subsp. *calamianaria*. *Int J Phytoremediation* 4, 101-115.
- [84] Lombi E, Wenzel WW, Gobran GR, Adriano DC 2000. Dependency of phytoavailability of metals on indigenous and induced rhizosphere processes: a review. In: GR Gobran, WW Wenzel, E Lombi (eds.) *Trace Elements in the Rhizosphere*. CRC Press, Boca Raton, FL, USA, pp 3-24.
- [85] Lombi E, Zhao FJ, Dunham SJ, and McGrath SP 2001. Phytoremediation of heavy metal contaminated soils: natural hyperaccumulation versus chemically-enhanced phytoextraction. *Journal of Environmental Quality* 30, 1919-1926
- [86] Lombi E, Zhao FJ, McGrath SP, Young SD, Sacchi GA 2001. Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. *New Phytol* 149, 53-60.
- [87] Lombini A, Llugany M., Poschenrieder C, Dinelli E, Barceló J (2003) Influence of the Ca/Mg ratio on Cu resistance in three *Silene armeria* ecotypes adapted to calcareous soil or to different, Ni- or Cu-rich serpentine sites. *J. Plant Physiol* 160, 1451-1456.
- [88] Ma JF, Hiradate S 2000 Form of aluminium for uptake and translocation in buckwheat (*Fagopyrum esculentum* Moench). *Planta* 211, 355-366.
- [89] Macek, T, Mackova M, Kas J 2000. Exploitation of plants for the removal of organics in environmental remediation. *Biotechnology Advances* 18, 23-34.
- [90] MacNair MR, Tilstone GH, Smith SE 2000. The genetics of metal tolerance and accumulation in higher plants. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 235-250.
- [91] Mádico J, Poschenrieder C, Vázquez MD, Barceló J (1992) Effects of high zinc and cadmium concentrations on the metallophyte *Thlaspi caerulescens* J. et C. Presl. (Brassicaceae). *Suelo y Planta* 2, 495-504
- [92] Mádico J, Poschenrieder C, Vázquez MD, Barceló J 1992.. Effects of high zinc and cadmium concentrations on the metallophyte *Thlaspi caerulescens* J. et C. Presl. (Brassicaceae). In: *Contaminación: Efectos fisiológicos y mecanismos de actuación de contaminantes*. Ed. Publ. Univ. Alicante, Alicante, pp 89-97.
- [93] Marschner H 1995 Mineral Nutrition of Higher Plants. 2nd ed. Academic Press, London
- [94] Martens SN, Boyd RS 2002 The defensive role of Ni hyperaccumulation by plants: a field experiment *Am. J. Bot.* 89, 998-1003
- [95] Massacci A, Pietrini F and Iannelli MA (2001) Remediation of wetlands by *Phragmites australis*. The biological basis. *Minerva Biotecnologica* 13, 135-140
- [96] Mathys W 1975. Enzymes of heavy-metal-resistant and non-resistant populations of *Silene cucubalus* and their interaction with some heavy metals *in vitro* and *in vivo*. *Physiol Plant* 33, 161-165.
- [97] McCabe OM and Otte ML (2000) The wetland grass *Glyceria fluitans* for revegetation of mine tailings. *Wetlands* 20, 548-559.
- [98] McGrath SP 1990. Phytoextraction for soil remediation. In: RR Brooks (ed.) *Plants that Hyperaccumulate Heavy Metals*. CAB International, Wallingford, UK, pp261-287.
- [99] McGrath SP, Shen ZG, Zhao FJ 1997. Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. *Plant Soil* 188, 153-159

- [100] McNeill KR, Waring S 1992. Vitrification of contaminated soils. In: JF Rees (ed.) *Contaminated Land Treatment Technologies*. Elsevier Applied Science for SCI
- [101] Meager RB, Rugh CL, Kandasamy MK, Gragson G, Wang NJ 2000. Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 201-219.
- [102] Mench M, Vangronsveld J, Clijsters H, Lepp NW, Edwards R 2000. *In situ* immobilization and phytostabilization of contaminated soils. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 323-358.
- [103] O'Halloran TV, Culotta VC 2000. Metallochaperones. An intracellular shuttle service for metal ions. *J Biol Chem* 275, 25057-25060.
- [104] Ortiz DF, Ruscitti T, McCue KF, Ow DW 1995. transport of metal-binding peptides by HMT1, a fission yeast ABC-type B vacuolar membrane protein. *J Biol Chem* 270, 4721-4728.
- [105] Ostwald A. 2000. Physiologische Grundlagen der Akkumulation von Schwermetallionen beim Faserhanf (*Cannabis sativa* L.) und das Nutzungspotential bei der Phytoremediation. Ph.D. Thesis Universität Wuppertal.
- [106] Pence NS, Larsen PB, Ebbs SD, Letham DLD, Lasat MM, Garvin DF, Eide D, Kochian LV 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc. Natl Acad. Sci. USA* 97, 4956-4960.
- [107] Persans M, Salt DE 2000. Possible molecular mechanisms involved in nickel, zinc and selenium hyperaccumulator plants. *Biotech. Gen. Eng. Rev.* 17, 385-409.
- [108] Pollard AJ, Dandridge KL, Shee EM 2000. Ecological genetics and the evolution of trace element hyperaccumulation in plants. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 251-264.
- [109] Poschenrieder C, Barceló J 1999. Water relations in heavy metal stressed plants. In: MNV Prasad, J Hagemeyer (eds.) *Heavy Metal Stress in Plants. From Molecules to Ecosystems*. Springer, Berlin, pp 207-229.
- [110] Poschenrieder C, Barceló J. 2004. Estrés por metales pesados. pp 413-442 In: M. Reigosa, N. Pedrol, A. Sánchez (eds.) *La Ecofisiología Vegetal. Una Ciencia de Síntesis*. Ed. Paraninfo, Madrid, 413-442.
- [111] Poschenrieder C, Bech J, Llugany M, Pace A, Fenés E, Barceló J 2001. Copper in plant species in a copper gradient in Catalonia (North East Spain) and their potential for phytoremediation. *Plant Soil* 230, 247-256.
- [112] Punshon T, Adriano DC 2003. Assessing phytoextraction potential in a range of hybrid poplar. <http://www.uga.edu/srel/Nipoplar/introduction.htm>
- [113] Quinn JJ, Negri MC, Hinchman RR, Moos LP, Wozniak JB 2001. Predicting the effect of deep-rooted hybrid poplars on the groundwater flow system at a large-scale phytoremediation site. *Int. J. Phytorem.* 3, 41-60.
- [114] Raskin I, Kumar PBAN, Dushenkov S, Salt DE 1994. Bioconcentration of heavy metals by plants. *Current Opinion Biotech.* 5, 285-290.
- [115] Raskin I, Smith RD, Salt DE 1997. Phytoremediation of metals: using plants to remove pollutants from the environment. *Curr. Opin. Biotechnol.* 8, 221-226.
- [116] Reeves RD, Brooks RR 1983. Hyperaccumulation of lead and zinc by two metallophytes from a mining area in Central Europe. *Environ. Pollut.* 31, 277-287.
- [117] Robinson BH, Brooks RR, Howes AW, Kirkman JH, Gregg PEH 1997. The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *J Geochem Explor* 60, 115-126.
- [118] Römkens P, Bouwman L, Japenga J, Draaisma C 2002. Potentials and drawbacks of chelate-enhanced phytoremediation of soils. *Environ Poll.* 116, 109-121.
- [119] Salt DE, Kato N, Krämer U, Smith RD, Raskin I 2000. The role of root exudates in nickel hyperaccumulation and tolerance in accumulator and nonaccumulator species of *Thlaspi*. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 189-200.
- [120] Salt DE, Smith RD, Raskin I 1998. Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 643-668.
- [121] Salt, DE, Blaylock M, Nanda Kumar PBA, Dushenkov V, Ensley BD, Chet I, Raskin I 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol.* 13, 468-474.
- [122] Saxena PK, Raj SK, Dan T, Perras MR, Vettakkorumakankav NN, 1999. Phytoremediation of heavy metal contaminated and polluted soils. In: MNV Prasad & J Hagemeyer (eds) *Heavy Metal Stress in Plants. From Molecules to Ecosystems*. Springer Verlag, Berlin, pp 305-329.
- [123] Schat H, Llugany M, Bernhard R 1999. Metal specific patterns of tolerance, uptake, and transport of heavy metals in hyperaccumulating and non-hyperaccumulating metallophytes. In: N Terry, G Bañuelos (eds.) *Phytoremediation of Contaminated Soils and Waters*. CRC Press LLC, Boca Raton, FL, USA, pp 171-188.
- [124] Schat H, Llugany M, Vooijs R, Hartley-Whitaker J, Bleeker PM 2002. The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. *J. Exp. Bot.* 53, 2381-2392.
- [125] Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI 2000. cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to Nramp genes. *Proc. Natl. Acad. Sci. USA* 97, 4991-4996.
- [126] Tolrà RP 1997. Mecanismos de tolerancia a la toxicidad por Zn en *Thlaspi caerulescens*. Ph. D. Thesis, Universitat Autònoma de Barcelona
- [127] Tolrà RP, Poschenrieder C, Alonso, R, Barceló D,

- Barceló J 2001 Influence of zinc hyperaccumulation on glucosinolates in *Thlaspi caerulescens*. *New Phytol* 151, 621-626.
- [128] Tolrà RP, Poschenrieder C, Barceló J 1996. Zinc hyperaccumulation in *Thlaspi caerulescens*. I. Influence on growth and mineral nutrition. *J. Plant Nutr.* 1531-1540
- [129] Tolrà RP, Poschenrieder C, Barceló J 1996. Zinc hyperaccumulation in *Thlaspi caerulescens*. II Influence on organic acids. *J. Plant Nutr.* 19, 1541-1550.
- [130] United States Protection Agency (USEPA). 2000. Introduction to Phytoremediation. EPA 600/R-99/107. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.
- [131] Van der Lelie D, Schwitzgübel JP, Glass DJ, Vangronsveld J, Baker AJM 2001. Assessing Phytoremediation Progress in the United States and Europe. *Environ. Sci. Technol.* November, 446 A-452.
- [132] Van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonens AN, Schat H, Verkleij JAC, Hoykass PJI 1999, Overexpression of a novel Arabidopsis gene related to putative zinc transporter genes from animals can lead to enhanced zinc resistance and accumulation. *Plant Physiol.* 119, 1047-1056.
- [133] Vangronsveld J, Cunningham SD 1998. Metal-contaminated soils. In situ inactivation and phytoremediation. Springer Verlag, Berlin.
- [134] Vangronsveld J., Mench, M., Lepp NW, Boisson J, Ruttens A, Edwards R, Penny C, van der Lelie, D 2000. *In situ* inactivation and phytoremediation of metal and metalloid contaminated soils: field experiments. In: D Wise, E Toronto, Cichon H, Inyang H, Stotmeister U (eds.) *Bioremediation of Contaminated Soils*. Marcel Dekker Inc., New York, pp 859-884.
- [135] Vázquez MD Poschenrieder C, Barceló J, Baker AJM, Hatton P, Cope GH 1994. Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens* J & C Presl. *Bot. Acta* 107, 243-250.
- [136] Vázquez MD, Barceló J, Poschenrieder C, Mádico J, Hatton P, Baker AJM, Cope GH 1992 Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. *J Plant Physiol* 140, 350-355.
- [137] Watanabe, T., Osaki, M., Yoshihara, T., Tadano, T., 1998. Distribution and chemical speciation of aluminium in the Al accumulator plant, *Melastoma malabathricum* L. *Plant Soil* 201, 165-173.
- [138] Wenzel WW, Lombi E, Adriano DC 1999. Biogeochemical processes in the rhizosphere: role in phytoremediation of metal polluted soils. In: MNV Prasad, J Hagemeyer (eds.) *Heavy Metal Stress in Plants. From Molecules to Ecosystems*. Springer Verlag, Berlin, pp273-303.
- [139] White PJ, Whiting SN, Baker AJM, Broadley MR 2002. Does zinc move apoplastically to the xylem in roots of *Thlaspi caerulescens*? *New Phytol* 153, 199-211.
- [140] Whiting SN, Souza MP, Terry N 2001. Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. *Environ. Sci Technol* 35, 3144-3150
- [141] Whitting SN, Neumann PM, Baker AJM 2003. Nickel and zinc hyperaccumulation by *Alyssum murale* and *Thlaspi caerulescens* (Brassicaceae) do not enhance survival and whole -plant growth under drought stress. *Plant, Cell Environ* 26, 351-360.
- [142] Williamson A, Johnson MS, 1981. Reclamation of metalliferous mine wastes. In: NW Lepp (ed.) *Effect of Heavy Metal Pollution on Plants. Vol. 2, Metals in the Environment*. Applied Science Publishers, London, pp185-212.
- [143] Zayed A., Gowthaman S, Terry N 1998. Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *Journal of Environmental Quality* 27:715-721

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