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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.

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.

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

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

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

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

2. 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.
3. 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.
4. Phytodegradation: Elimination of organic pollutants by decomposition through plant enzymes or products.
5. Rhizodegradation: Decomposition of organic pollutants by means of rhizosphere microorganisms [138].
6. 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 already been transferred to *Nicotiana* or *Brassica* species, and these transgenic plants may become useful in cleaning Hg-contaminated soils [13, 101].
7. 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]
8. Phytorestauration: 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 estimated 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 politic 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, Goiàs 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, preferential 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).

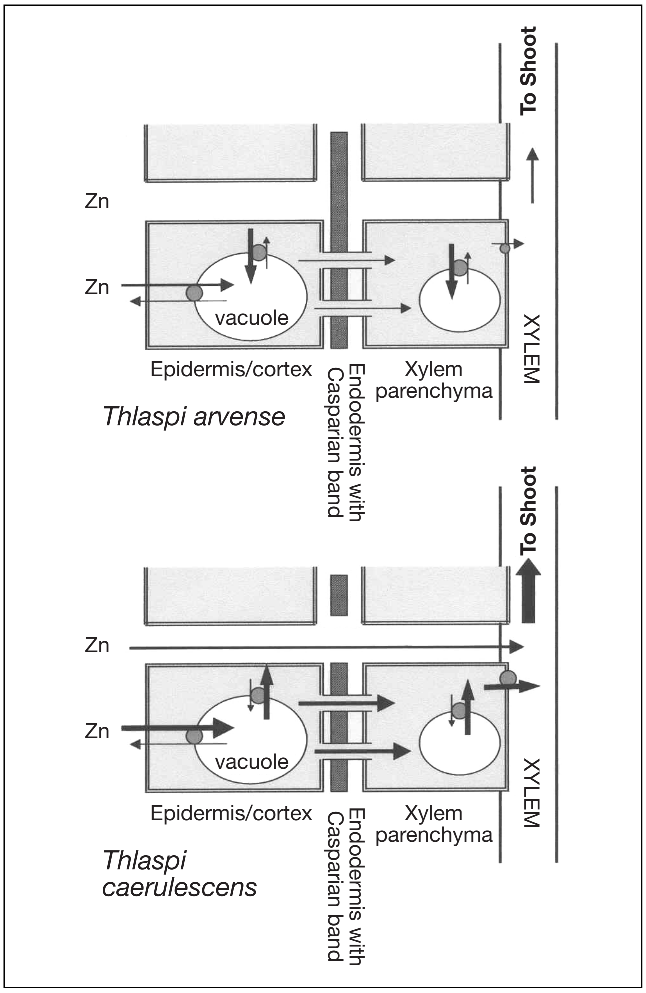

Figure 1. Differences in Zn transport in roots of the Zn hyperaccumulator *Thlaspi caerulescens* and the non-accumulator *Thlaspi arvense*. Thin arrows, low transport; thick arrows, high transport. *(modified after Lasat and Kochian, 2000 [76].)*

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 phytochelatins 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 extraction 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. Cytoplastic 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 (*mer A9*) 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 metallipherous 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.

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About the authors

Joan Barceló was born in Palma de Mallorca in 1938. He graduated in Pharmacy from the University of Barcelona in 1964 and obtained Ph.D. in 1970 with his work on the effect of UV radiation on plants. In 1973 he became an associate lecturer at the Faculty of Pharmacy of the same university and in 1976 he was appointed Full Professor of Plant Physiology at the Complutense University of Madrid. From 1980 to 1982 he held the post of Professor of Plant Physiology at the University of the Balearic Islands, where he was also the Dean of the Science Faculty. Since 1982 Joan Barceló has been a Professor and Head of the Plant Physiology Laboratory at the Science Faculty of the Autonomous University of Barcelona. He is full member of the Royal Academy of Pharmacy of Catalonia.

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