Phytoremediation of Heavy Metal Contaminated Soil and its Mechanism*

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Abstract: Phytoremediation is being developed in recent years as a cost-effective and environmental friendly solution for heavy metal contaminated soils. After an initial focus on plants with an immediate promise of application, research is now being directed at understanding the underlying mechanisms involved in heavy metal hyperaccumulation in plants. In recent years, major scientific progress has been made in understanding the mechanisms of soil heavy metal bioavailability and heavy metal uptake and transport by hyperaccumulators even biochemical basis of the capacity for metal accumulation has not been clearly elucidated. So further studies on both the role of organic acids and amino acids in the heavy metal tolerance mechanism and ion transport systems are clearly required for understanding the phenomenon of metal hyperaccumulation and tolerance. Once the rate-limiting steps for uptake, translocation, sequestration and detoxification of metals in hyperaccumulator plants are identified, an improved understanding of the mechanisms underlying hyperaccumulation of heavy metals should open up new possibilities for the application of metal accumulating plants in the phytoremediation of contaminated soil.

Key words: heavy metals; soil contamination; phytoremediation; hyperaccumulator; mechanism
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1　Concept of Phytoremediation

Phytoremediation, which intended to address a wide variety of surfical contaminants, including heavy metals, metalloids, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents and industrial by-products[1], was put forward to use green plant-based systems for environmental restoration. A series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of phytoremediation into a promising, cost-effective, and environmentally friendly technique especially for several trace elements[2,3].

Although the consequences of heavy metal contamination and hyperaccumulators of trace elements have been becoming better identified and characterized in this decade, the mechanisms of heavy metal uptake by plants remain elusive. For the effective and successful phytoremediation, the better understanding of fundamental mechanisms of trace metal accumulation and heavy metal tolerance may be of great importance. So several important characteristics and research progresses on how hyperaccumulator can achieve this remarkable bioaccumulation of metals were discussed in this paper.

2　Effect of Heavy Metal Availability on Phytoremediation

If regardless of the plants used, availability of heavy metals to plant roots is considered a key factor limiting the efficiency of phytoextraction. This availability usually includes two aspects: one is the enhanced soil heavy metal solubility and/or translocation ability; the other is the increased possibility to be absorbed by the plant roots.

2.1　Role of Organic Chelators

Both soil heavy metal solubility and availability to plants were thought to be affected obviously by the organic chelators. The use of synthetic metal chelates to artificially hyperaccumulate (induced hyperaccumulation) toxic metals from soil into plant shoots has been shown to be important to the emerging field of phytoremediation by the increasing of trace element bioavailability. For this

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purpose, the use of complexing agents, such as DTPA or EDTA for Ni, citrate for U etc., has been proposed. These synthetic chelators can desorb heavy metals from the soil matrix to form water soluble metal complexes.

So, one interesting question is that which kinds of heavy metal ion species are the ones absorbed by the plant roots. Some people suggested that the heavy metal ion was absorbed as a ion type, while other people thought that, just as the work done on the inorganic chelators[4-6], only the organic-chelated ion was suitable for the absorption by plants. Li et al. (1994) found that the chelators decrease the concentration of Cd ion and increase the Cd complex, so one of the possible hypotheses to explain the enhancement of Cd uptake at elevated Cl concentration in soil is that chloro-complexes of Cd are available for root uptake[5,6].

Metal uptake may also have been limited by physiological barriers, inhibiting the uptake of synthetic chelates by plants, but considerable debate exists on that issue. Some authors reported that the use of chelating substance such as DTPA or did not enhance, or even decrease[7] heavy metal uptake, even others observed impressive increases after EDTA and DTPA application and suggest that synthetic chelates enhance translocation of certain metals from roots to shoots.

But even such organic chelators can enhance the heavy metal bioavailability, restrictions still apply, however, to the use of complexing agents. Many synthetic chelators, e.g. EDTA, show a low degree of biodegradability. *In situ* application of such chelators bears the risk of uncontrolled metal mobilization and water pollution through leaching processes, also, most kinds of chelators are not only expensive, but may threaten leaching of target and non-target metals. Recent research found that some restrictions that apply to chelate-assisted phytoextraction (induced phytoextraction) may be overcome by usage of easily biodegradable chelating agents, such as Nitrilotriacetate (NTA), and injection to the root zone of metal-accumulating plants rather than surface application. It should be pointed out that the research on the natural and environmental friendly chelators is still needed for the induced phytoremediation.

2.2 Role of Root Exudate

Phytoextraction will benefit from rhizosphere processes primarily through root exudation or plant-microbe induced solubilization prior to uptake by the plant. Excretion of protons and exudation of organic compounds carrying acidic functional groups, such as organic acids, can decrease the pH and thus solubilize metal cations. This hypothesis can be supported by the finding that the rhizosphere soils tend to have higher concentrations of mobile heavy metals than the non—rhizosphere soils. Metal cations can also be solubilized through the formation of metal-organic chelates with phytosiderophores and organic acids released by plant roots or microbes.

McGrath et al. postulated that the ability of *T. carelescenes* to remove Zn from moderately to highly contaminated soils maybe related to root exudate, while not to the pH change in the rhizosphere[8], because no significant differences between the hyper and non-hyperaccumulator in the degree of rhizosphere acidification can be found. Naidu et al.[9] also thought that the soil solutions contain a variety of low molecular weight dissolved organic substances from breakdown of plant residues and exudation from plant roots, and complexation of metals by these organic ligands can play an important role in controlling metal solubility.

But it should be pointed out that, up to now, no direct evidence of higher secretion of organic ligands exudated by heavy metal hyperaccumulator and mobilize soil heavy metals has been presented. So the previous work highlights the need to investigate the role of root exudates on the mobilization of heavy metals by the hyperaccumulator[10]. And the work on the analysis of soil organic chelators gives a possibility of such kind of research work.

3 Plant Heavy Metal Tolerance and Sequestration

3.1 The Role of Heavy Metal Tolerance

In general, metal resistance is genetically determined and metal-specific. Resistance in higher plants is mainly based on tolerance, which implies uptake of heavy metals and the ability to tolerate excess internal metal concentrations. Hypertolerance is the key property which makes hyperaccumulation possible[11] and the hypothesis of an underlying, mechanistic association between metal hyperaccumulation and tolerance seems perfectly plausible[12]. Further, more and more works on the tolerance of hyperaccumulators have concluded that the tolerance should be caused by organic sequestration. The organic chelators would be able to bind heavy metals and transport them into the vacuole, where they could be bound to other organic chelators or exist as free ions. Chelation reaction and vaculor compartmentalization are believed to result in the hypertolerance. The chelators related to metal tolerance and metal transportation includes organic acids, amino acids or PCs which were investigated in past years.

But metal tolerance is a complex phenomenon which involves several different mechanisms, it is still not very clear about the relationship between hyperaccumulation and tolerance. It is found that zinc tolerance and hyperaccumulation segregate independently in the F2 produced by the crossing of Zn tolerant species and hyperaccumulator *Cardaminopsis halleri* and non-accumulating and non-tolerant species *C. petraea*, which suggest that the two characters are genetically independent. Furthermore, they also find that zinc tolerance and Cd tolerance are genetically independent.

3.2 Organic Acids and Heavy Metal Tolerance

In many tolerant ecotypes a high level of organic acids has been observed. High concentrations of citric acid and/or malic[13-15] and also of malic and malonic[16, 17] or malic and oxalic acids[18] have been found associated with the tissue fraction showing a high metal concentration, and therefore they have been considered involved in complexing the metal, favouring its accumulation. Ernst (1975) firstly suggested Zn-malate shuttle hypothesis, holding that zinc tolerance is based on the ability of a zinc-tolerant clone to accumulate organic acid. After that, many research results give support on this Zn-malate shuttle hypothesis, where the significant correlation between soluble heavy metals and a lot kinds of organic acids was found in shoots, is in line with this hypothesis.

If organic acid do have an effect on the plant tolerance by chelation reaction, such chelation processes might be plant-specific and metal-specific[14, 16]. But some researchers suggested that although organic acids such as citrate, malate and malonate participate in heavy metal chelations in the shoots of hyperaccumulations, they are present constitutively at high concentration in most terrestrial plants and account for neither the metal specificity nor the species specificity of hyper-accumulation[19]. Considering that no specific organic acid could be observed, maybe there is another possibility that organic acids is just absorbed as a balance of metal absorption, after that, it can play a chelation role and may be used as a metal transporator.

So, it should be acknowledged that the complexing of heavy metals by such universal plant metabolites as citrate and malate is insufficient to explain the special behavior of hyperaccumulators of this element. It is desirable for further investigation to focus on the root systems and xylem fluids of the hyperaccumulators to discover the origin of the species- and element-specificity of this unusual form of plant behavior. To give a more complete picture of the role of chelating substance in translocation of Zn, Cd, and Ni etc. in hyperaccumulators, further systematic investigation on the concentration of organic acids in roots, leaves and xylem exudates and speciation by computer programs are necessary. Unfortunately, such studies are often restricted by the low biomass production and the low xylem exudation rates in many hyperaccumulators.

3.3 Amino Acids and Phytochelations (PCs)

Previous research studied the relationship between Ni concentration and Histidine and other free amino acids. After that, over a wide range of nickel treatments, a linear relationship between xylem nickel and histidine concentrations in Alyssum lesbiacum was found[20], indicating that enhanced production of histidine is responsible for the nickel hyperaccumulation.

Specific functions might also have some proteins able to complex metals as metallothioneins, particularly studied in copper and cadmium toxicity. In recent years, the research on Cd induced metallothiones-link and Cd phytochelations which were extracted from many kinds of plants including hyperaccumulator and non-hyperaccumulator gave some new idea about the heavy metal tolerance mechanism[21], even different opinion also exists on the study of heavy metal induced PCs[22].

3.4 Vacuole Compartments and Heavy Metal Sequestration

The plant vacuole has long been suspected of being a site for accumulation of Cd[23], Ni[16, 24] and Zn[25] in plant roots and vacuole compartment has been strongly regarded as the possible mechanism of heavy metal tolerance in many hyperaccumulators[26].

Salt[24] studied the role of vacuolar compartment-alization by a direct comparison of the Ni contents of intact vacuoles from leaves of both hyper and non-hyperaccumulators. The results revealed that 53% of the intracellular Ni was found within the vacuole of the hyperaccumulator, approximately 2 fold more than in the non-hyperaccumulator, which supports the hypothesis that the vacuole plays an important role in the cellular Ni resistence machanism in the hyperaccumulator. It is suggested that the presence of nickel in the mitochondria can presumably block the citric acid cycle by deactivating malic dehydrogense leading to build-up of malic acid in the vacuoles which could then absorb excess nickel by a complexing reaction and lead to its diffusion back into vacuoles from the mitochondria, hence unblocking the citric acid cycle[16].

However, the biological basis of this cellular heavy metal tolerance mechanism is still not clear. The occurrence of soluble complexes in the vacuoles can eliminate from metabolism another fraction of the element, which however must pass through the cytoplasm to reach the vacuoles. This higher plasmatic resistance possibly depends on the presence of organic complexes that can reduce the number of free ions in the cytoplasm and then make the hyperaccumulators tolerant unfavorable edaphic factors such as heavy metal rich soils.

3.5 Other Tolerance Mechanisms

Some researchers also suggested several other tolerant mechanisms. Metal binding to cell walls has been considered an important detoxification mechanism, but it can represent only one aspect of the detoxification as only a fraction of the element can be blocked in this way, as it has been shown by zinc desorption experiments on *Descbampsia caespitosa* and Ni-tolerant experiment[11] with the leaf proplasts of hyperaccumulator *T. goesingense* and non-hyperaccumulator *Thlaspi* species.

Research work in recent years has already focused on the mechanism of heavy metal tolerance and accumulation by plants. However, the biochemical basis of the capacity for metal accumulation has not been elucidated. So further studies on both the role of organic acids and amino acids in the heavy metal tolerance mechanism and ion transport systems are clearly required for understanding the phenomenon of metal hyperaccumulation and tolerance. An improved understanding of the mechanisms underlying hyperaccumulation of heavy metals should open up new possibilities for the application of metal-accumulating plants in the phytoremediation of contaminated soil.

4 Heavy Metal Translocation from Roots to Shoots

Usually, contrary to non-hyperaccumulator, the heavy metal concentrations are much higher in plant shoots than in roots in hyperaccumulators[19,27]. So, it is supported that the hyperaccumulator is more effective at translocating heavy metals from solution to shoots. Just for the strong translocation ability, hyperaccumulators can accumulate much higher heavy metals in plant shoots than non-hyperaccumulators.

4.1 Heavy Metal Translocation

First, before finally appearing in the xylem, the heavy metal solutes have to cross two cell membranes —— one at the point of entry into the symplast (rhizodermal cells, or conceivably cortical cells), and the other at the point of exit (xylem parenchyma cells) when being loaded into the xylem elements for translocation.

Usually the higher metal influx across the root cell plasma membrance and enhanced absorption into the root is one of the mechanisms involved in heavy metal hyperaccumulation.

Then, the transport sites other than entry into the root symplasm are also stimulated in hyperaccumulators. Often a smaller fraction of the absorbed heavy metal was stored in the root vacuole and higher levels of metals are shown in xylem and are translocated into the shoot of hyperaccumulators compared with non-hyperaccumulators. However, a significant fraction of symplasmic heavy metal was stored in the root vacuole of non-hyperaccumulator, and presumably became unavailable for loading into the xylem and subsequent translocation to the shoot[28,29].

Finally, transport across the plasma membrane and tonoplast and uptake by leaf cells must also be critical sites for heavy metal accumulation in hyperaccumulators.

So, marked differences should be shown in all the metal translocation procedures including heavy metal compartmentation in the root, heavy metal transport into the xylem, and heavy metal absorption into leaf cells between hyperaccumulators and non-hyperaccumulators.

4.2 The Role of Organic Chelators on Translocation

During all procedures of heavy metal translocation in hyperaccumulators, organic chelators may play a great role. All metals move to some degree in plants, but their distribution and diffusion rates may depend on their stability constants with various natural complexing or binding organic ligands. To determine whether selective metal chelation is involved in metal translocation from root to shoot, xylem sap was studied[20] and the results showed that histidine is involved both in the mechanism of nickel tolerance and in the high rate of nickel transport.

The histidine response observed in nickel-hyper-accumulators appears to have evolved more than once within the plant kingdom. The response, however, has not been observed in hyperaccumulators of Mn or Zn, and so seems to be intrinsically specific for Ni. The ways in which Ni is transported and sequestered within plant, and the effect on metal accumulation of contamination of the root medium with organic pollutants, are currently under investigation.

Because metals move acropetally via the xylem fluid, it is reasonable to study chemical species and metal complexation with that system. However, most information on xylem solutes is fragmentary and not easy to compare, so that it is still difficult to establish both a comprehensive view of the range of ligands and other solutes under known growth conditions and the effects of plant species. To understanding metal-complex formation in xylem fluid, most major organic and inorganic solutes and their concentrations must be systematically studied. Critical compilation of this information from published values is still impossible because of confounding factors and metal and plant specific organic chelators.

5 Discussion

5.1 Rare Hyperaccumulators

Metal hyperaccumulator plants are relatively rare taxa, often occurring in remote areas geographically and being of very restricted distribution in areas often threatened by devastation from mining activities. Also population sizes are can be extremely small. There is thus an urgent need to collect these materials, bring them into cultivation and establish a germplasm facility for large scale production.

5.2 Efficiency of Hyperaccumulators

Many plant species have been reported to have a great potential for the extraction of heavy metals from contaminated soils. The identification of these metal hyperaccumulator plant species demonstrates that the genetic potential does exist for successful phytore-mediation. Despite its ability to accumulate high levels of metals in the shoot, the use of most hyperaccumulators for commercial-scale soil remediation is severely limited by its small size and slow growth (plant productivity). Even some researchers thought that crop plants were more effective in extracting heavy metals from soil than the hyperaccumulator species because the differences in yield between plant species were higher than species differences in metal accumulation.

Further, recent research found that hyper-accumulators are less effective in extracting heavy metals from the relative "clean" soil. Hyperaccumulators may have a high physiological requirement for heavy metal and will not grow at low soil heavy metal levels.

So, except the collecting and evaluating wild germplasm, breeding improved genotypes, and identifying fertilization requirements and agronomic practices for optimum production, studies on the effective artificial and natural organic chelators that can solubilize heavy metals are really needed to incorporate all these desirable properties into one plant.

5.3 Root Exudates of Hyperaccumulators

The ability of hyperaccumulator plants to take up large amounts of metals can be also related to differences in the specificity and number of soil metal transporter per unit of membrane area in hyperaccumulator roots compared to nonhyperaccumulators.

It was found that acidification was not the mechanism used to mobilise heavy metal in the soil. Recent research works[30] highlight the need to investigate the role of root exudates on the mobilisation of heavy metals by the hyperaccumulator. But, unfortunately, no direct evidence of higher secretion of organic ligands from hyper-accumulators, which solubilize soil heavy metals, has been presented for the difficulties of research on soil organic chelators. Recent progresses on analytical techniques give a possibility for the research of soil root exudates especially on the change of organic acids and soil-root heavy metal influx.

5.4 Mechanisms of Hyperaccumulator Phenotypes

Metal hyperaccumulators are interesting model organisms to study for the development of a phyto-remediation technology, the use of plants to remove pollutant metals from soils. However, little is known about the molecular, biochemical, and physiological processes that result in the hyperaccumulator phenotype. Progress in the commercial technology is hindered by a lack of understanding of the fundamental mechanisms involved in metal transport and accumulation in plant roots and shoots. It is believed that improved understanding of the mechanisms of metal uptake, accumulation, translocation and tolerance in hyperaccumulator plants should aid in development of commercially useful phytoremediation plants.

But from above literature, we can see some problems existing in the mechanism research of hyperaccumulators. To understanding the metal-complex formation and translocation in hyperaccumulators, the overall research on chemical composition and concentrations of xylem fluid must be needed.

5.5 Gene-transfering and Some Other Technologie

The transfer of zinc-hyperaccumulating properties from *T. caerulescens* into high-biomass producing plants has been previously suggested by Brown[27] as a potential avenue for making phytoremediation a commercial technology. Further work could involve genetic engineering to further improve metal-uptake charac-teristics, if the genes for metal accumulation can be identified and manipulated. The possibility would then exist to transfer genes for metal hyperaccumulation into a very productive (but inedible) host plant.

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重金属污染土壤的植物修复及其机理研究

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摘　要：近年来，作为一种成本低廉、对环境友好的修复方法，重金属污染土壤的植物修复成为环境修复领域的研究热点。早期的研究侧重于超富集植物的发现与实际应用，近来超富集植物重金属富集机理的研究得到了重视。尽管重金属植物累积的生化机制尚未能被完整阐述，但在土壤重金属生物活性、超富集植物重金属的吸收、转运、储存等方面还是取得了大量进展。综述了近年来国内外相关研究工作成果，探讨了该研究领域存在的主要问题，认为在有机螯合剂如有机酸与氨基酸等对重金属活性、耐性和离子迁移转运系统中所起作用等方面尚需要深入研究。如果在植物重金属吸收、迁移、解毒机理及其影响因素等重金属富集的关键方面能取得进展，则将大大丰富污染土壤重金属修复的研究与应用领域。

关键词：重金属；土壤污染；植物修复；超富集植物；富集机理
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