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Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation

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Abstract

Phytoremediation is a site remediation strategy, which employs plants to remove non-volatile and immisible soil contents. This sustainable and inexpensive process is emerging as a viable alternative to traditional contaminated land remediation methods. To enhance phytoremediation as a viable strategy, fast growing plants with high metal uptake ability and rapid biomass gain are needed. This paper provides a brief review of studies in the area of phytoaccumulation, most of which have been carried out in Europe and the USA. Particular attention is given to the role of phytochelators in making the heavy metals bio-available to the plant and thier symbionts in enhancing the uptake of bio-available heavy metals. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

With increasing heavy metal contamination due to various human and natural activities, ecosystems have and are being contaminated with heavy metals (HMs). Migration of contaminants into non-contaminated sites as dust or leachate through the soil, and the spreading of sewage sludge are examples of events that contribute towards contamination of our ecosystems.

Contaminated soil can be remediated by chemical, physical or biological techniques (McEldowney et al., 1993). The available techniques may be grouped into two categories: (a) ex situ techniques which require removal of the contaminated soil for treatment on- or off-site and (b) in situ methods, which remediate without excavation of contaminated soil. In situ techniques are favored over the ex situ techniques due to their lower cost and reduced impact on the ecosystem. For a detailed overview and analysis of these technologies, the reader is referred to the excellent reviews of Rao et al. (1996) and Burns et al. (1996).

This paper focuses on the bioremediation of heavy metal contaminated soils using in situ techniques. Heavy metals form the main group of inorganic contaminants (Adriano, 1986, 1992; Alloway, 1990; Meeuseen et al., 1994). Remediation of metal compounds presents a different set of problems when compared to organics. Organic compounds can be degraded while metals normally need to be physically removed or be immobilised (Kroopnick, 1994). Whilst a number of on-site treatment techniques are available to decontaminate soils containing organics, there are comparatively few in situ methods for the removal of heavy metals and inorganic contaminants (Peters and Shem, 1992; Burns et al., 1996). Traditionally, remediation of heavy metal contaminated soils involves either on-site management or excavation and subsequent disposal to a landfill site (Parker, 1994).

In Australia, the most common remediation technique is off-site management. The metal contaminated soil is taken for burial at landfill sites (Elliott et al., 1989; McNeil and Waring, 1992; Smith, 1993; Shoebridge, 1993). This method of remediation merely shifts the contamination problem elsewhere (Smith, 1993). Additionally there are hazards associated with the transport of contaminated soil and migration of contaminant from landfill into adjacent environment (Williams, 1988).

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On-site management of heavy metal contaminated soils can be achieved either by diluting the contaminant to safe levels by using clean soil as dilutent, (Musgrove, 1991) or stripping and stockpiling clean top soils and redistributing it over the landfill. Deep ploughing to vertically mix heavily contaminated soil with less contaminated sub-soils can also be employed to dilute the heavy metal contents (Thompson-Eagle and Frankenburger, 1992).

Immobilization of inorganic contaminants is also a possible strategy (Mench et al., 1994). Immobilization can be achieved by complexing the contaminants (Wills, 1988), or by increasing the soil pH by liming. The solubility of metals such as Cd, Cu, Zn and Ni are reduced due to the formation of insoluble hydroxides (Adriano, 1986).

Soil washing or extraction for removing inorganic compounds from contaminated soils is the only alternative to off-site burial method (Elliott et al., 1989; Tuin and Tels, 1991). As with organic compounds, this technique produces a residue with high heavy metal contents which require further treatment or burial (Dennis et al., 1994). This method, though effective, is costly. Use of microbial bioremediation technology, well-known for decontamination of organic compounds (Flathman et al., 1994), is not available for large-scale transformation of inorganic contaminants.

Most of the above mentioned techniques have been shown to be efficient in lab-scale and pilot-scale studies. However, only a few field studies have been conducted to test their efficiency and efficacy (Burns et al., 1996). Furthermore, the physio-chemical technologies used for soil remediation render the land useless as a medium for plant growth as they also remove all biological activities, including useful microbes, such as nitrogen fixing bacteria and mycorrhizal fungi, as well as fauna. There is a need to develop suitable on-site techniques for the removal of non-volatile and non-mobile soil contaminants (Wheeler, 1994). Plants that uptake heavy metals from soil offer an alternative and less expensive method to strip heavy metals directly from the soil. Plants have constitutive (present in most phenotypes) and adaptive (present only in tolerant phenotypes) mechanisms for accumulation or tolerating high contaminant concentrations in their rhizospheres. The use of such plants to cleanup soils and water contaminated with organic and inorganic pollutants, a technique termed as phytoremediation, is emerging as a new tool for in situ remediation.

This paper provides a brief review of studies in the area of phytoremediation, most of which have been carried out in Europe and the USA. Particular attention is given to the role of phytochelators in making the heavy metals bio-available to the plant and their symbionts in enhancing the uptake of bio-available heavy metals.

2. Phytoremediation

In recent years, phytoaccumulation/phytoextraction, i.e., the use of plants to cleanup soils contaminated with non-volatile hydrocarbons and immobile inorganics is showing promises as a new method for in situ cleanup of large volumes of low to moderately contaminated soils. Plants can be used to remove, transfer, stabilize and/or degrade heavy metal soil contaminants (Anderson and Coats, 1994; Baker et al., 1994; Markert, 1994; Raskin et al., 1994; Salt et al., 1995; Kumar et al., 1995; Negri and Hinchman, 1996; Kling, 1997). The technique was first adapted to constructed wetlands, reed beds and floating plant systems for the treatment of contaminated ground and waste waters for years (Cunningham et al., 1995). Current efforts now focus on expanding the phytoremediation strategy to address contaminated soils and air pollutants in an attempt to preserve the biodiversity of soil and its biota (Markert, 1994). Phytoremediation has been tested by various green house and pilot-scale field experiments in the USA and Europe (Baker et al., 1994; Kumar et al., 1995). From this remediation method, the biological properties and physical structure of the soil is maintained, and the technique is environmentally friendly, potentially cheap, visually unobstructive and offer the possibility of bio-recovery of the heavy metals.

Contaminated sites often support characteristic plant species, some of which are able to accumulate high concentrations of heavy metals in their tissue (Baker and Brooks, 1989; Hegde and Fletcher, 1996; Chaudhry et al., 1998; Khan et al., 1998). Most plants that survive in toxic soils do so by either, avoiding heavy metals, or, hyper-accumulating them in their tissues. Such plants are uncommon (Cunningham and Ow, 1996), and, to date, approximately 400 hyper-accumulator species have been identified, according to the analysis of field collected specimens (Kramer et al., 1997). Most have been found in contaminated areas of temperate Europe and the USA, New Zealand and Australia. Examples of reported hyper-accumulators have been tabulated by Chaudhry et al. (1998) and Bonaventure and Johnson (1997). Besides the limited distribution of hyper-accumulators in the wild, such plants also tend to be contaminant specific. No plant species has yet been found that will demonstrate a wide spectrum of hyper-accumulation (Watanabe, 1997). Cultivating such plants on low to moderately contaminated industrial waste sites can provide a clean, cheap alternative to the suck, muck and truck cleaning approach to contaminated soil cleanup. In addition to the removal of contaminants, the technique also offers containment of leachates and maintenance/improvement of soil structure, fertility and bio-diversity (Cunningham et al., 1995; Watanabe, 1997). Phytoremediation covers a range of methods such as phytodegradation, phytostabilization, rhizofiltration, enhanced biodegradation and phytoaccumulation (for references and description of each, refer to Chaudhry et al., 1998).

3. Limitation of phytoremediation

Phytoremediation is not a cure-all for contaminated soils. As with many new technologies, various mechanisms are either still unknown or poorly understood. Before this technology can become a technically efficient and cost-effective on a commercial scale, there are some limitations that need to be overcome. For example, very little is known about the molecular, biochemical and physiological processes that characterize hyper-accumulation. Many hyper-accumulator plants remain yet to be discovered and identified, as pointed out by Raskin et al. (1994). Furthermore, a long duration is needed before remediation to an acceptable level is achieved. Most heavy metal accumulating plants have root penetration to only shallow depths and a small biomass and are slow growing. To allow remediation within a reasonable period (e.g. <5 yr), the plant yield and metal uptake have to be enhanced dramatically. This may be achieved by cultivating rapid growing plants, or by engineering common plants with as yet unidentified hyper-accumulating genes. Another limitations is the potential contamination of the food chain if animals graze on the heavy metal contaminated vegetation. Also, the disposal of the harvested biomass is still to be resolved. Various techniques including air drying, ashing or incineration, composting, pressing and compacting for landfill and leaching are some of the options (Salt et al., 1995). Recovery of rare and expensive trace metal contaminants from the post harvest biomass (phytomining) is currently an option of great interest. The phytoremediation technique has long term applicability and is not a quick fix strategy. The costs, however, are lower than those of conventional methods and can have large-scale applications. Its source of energy is mostly solar and it allows the maintenance of soil ecosystems.

4. Role of mycorrhizae in phytoremediation

4.1. Arbuscular mycorrhizae

Since heavy metal uptake and tolerance depend on both plant and soil factors including soil microbes, we require information on interactions between plant root and their symbionts such as arbuscular mycorrhizal (AM) fungi and nitrogen-fixing microbes. It is the generally held view that the majority of plants growing under natural conditions have mycorrhizae (Smith and Reed, 1997). Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The extramatrical fungal hyphae can extend several cm into the soil and uptake large amounts of nutrients, including heavy metals, to the host root. The effectiveness of AM root colonization in terms of nutrient acquisiton differs markedly between AM fungi and host plant genotype (Ahiabor and Hirata, 1995; Marschner, 1995).

Mycorrhizae have also been reported in plants growing on heavy metal contaminated sites (Shetty et al., 1995; Weissenhorn and Leyval, 1995; Pawlowska et al., 1996; Chaudhry et al., 1998; Chaudhry et al., 1999) indicating that these fungi have evolved a HM-tolerance and that they may play a role in the phytoremediation of the site. Noyd et al. (1996) reported that AM fungal infectivity of native prairie grasses increased over three seasons on a coarse taconite iron ore tailing plots which helped to establish a sustainable native grass community that will meet reclamation goals. The reported symbiotic associations in the plants colonizing heavy metal contaminated soils further suggests a selective advantage for these plants as pioneering species on such sites and that they may be largely responsible for the successful colonization of such habitats.

Various authors have reported isolating spores of arbuscular mycorrhizal fungal texa such as *Glomus* and *Gigaspora* associated with most of the plants growing in heavy metal polluted habitats (Raman et al., 1993; Raman and Sambandan, 1998; Chaudhry et al., 1999). Raman et al. (1993) identified *Glomus* and *Gigaspora* spp. in the mycorrhizospheres of fourteen plant species colonising a magnesite mine spoil in India. Whereas Weissenhorn and Leyval (1995) isolated only *Glomus mosseae* and Duek et al. (1986) isolated *Glomus fasciculatum* alone from the heavy metal polluted soils. Pawlowska et al. (1996) surveyed a calamine spoil mound rich in Cd, Pb and Zn in Poland and recovered spores of *Glomus aggregatum*, *G. fasciculatum* and *Entrophospora* spp. from the mycorrhizospheres of the plants growing on spoil. Galli et al. (1994) suggested that mycorrhizae can play a crucial role in protecting plant roots from heavy metals. The efficiency of protection, however, differs between distinct isolates of mycorrhizal fungi and different heavy metals. Joner and Leyval (1997) reported that extra-radical hyphae of AM fungus *G. mosseae* can transport Cd from soil to subterranean clover plants growing in compartmented pots, but that transfer from fungus to plant is restricted due to fungal immobilization. The authors also reported no restriction of fungal hyphal growth into soil with high extractable Cd levels. Our preliminary (Chaudhry personal communications) studies have also showed very little, if any, translocation of Zn absorbed by mycorrhizal maize seedlings grown in contaminated soil, to the shoots. Turnau (1998) studied the localization of heavy metals within the fungal mycelium and mycorrhizal roots of *Euphorbia cyparissias* from Zn contaminated wastes and found higher concentrations of Zn as crystaloids deposited within the fungal mycelium and cortical cells of mycorrhizal roots. Studies by various researchers (Galli et al., 1994; Hetrick et al., 1994; Leyval et al., 1995) have shown that mycorrhizal fungal ecotypes from heavy metal contaminated sites seem to be more tolerant to heavy metals (and have developed resistance) than reference strains from uncontaminated soils.

Galli et al. (1995) reported that although there was an increase in the contents of cystein, gamma EC and GSH in the mycorrhizal maize roots grown in quart sand with added Cu, no differences in Cu uptake were detected between non-mycorrhizal and mycorrhizal plants. These results do not support the idea that AM fungi protects maize from Cu-toxicity. Mycorrhizae are also known to produce growth-stimulating substances for plants, thus encouraging mineral nutrition and increased growth and biomass necessary for phytoremediation to become commercially viable strategy for decontamination of polluted soils.

For arbuscular mycorrhizae the results are conflicting. Some reports indicate higher concentrations of heavy metals in plants due to AM, even resulting in toxic levels in plants (Killham and Firestone, 1986; Weissenhorn and Leyval, 1995; Joner and Leyval, 1997), whereas others have found a reduced plant concentrations of, e.g. Zn and Cu in mycorrhizal plants (Schuepp et al., 1987; El-Kherbawy et al., 1989; Heggo et al., 1990). Diaz et al. (1996) studied influence of Zn and Pb uptake by *Lygeum spartum* and *Anthyllis cytisoides* plants inoculated with *G. mosseae* and *G. macrocarpum* AM fungi in soils with different levels of these metals. The authors showed that, at low doses, mycorrhizal plants had equal or higher Zn or Pb concentrations than non-mycorrhizal controls; at higher doses, however, metal concentrations in the plants inoculated with *G. mosseae* were lower than those found in the corresponding controls, while the plants inoculated with *G. macrocarpum* showed similar or even higher levels than the controls.

In addition to the damaging effects on plants, the effect of heavy metals on the soil microorganisms and soil microbial activity also need to be considered. The impact of heavy metals in the field on *Rhizobium leguminosarum* bv. *Trifolii* and AM were estimated by various workers (Mench et al., 1994; Weissenhorn and Leyval, 1995). A negative effect of Zn on the indigenous rhizobial population was suggested by Mench et al. (1994). On the contrary, no adverse effect was found on spore number and mycorrhizal colonization of maize (Weissenhorn et al., 1992). Various soil factors such as the clay contents and mobility of heavy metals effect plants and soil biota. As metal uptake by plant roots depends on soil and their associated symbionts, it is important to monitor metal mobility and availability to plant and its symbionts when assessing the effect of soil contamination on plant uptake and related phytotoxic effects.

The prospect of symbionts existing in heavy metal contaminated soils has important implications for phytoremediation. Mycorrhizal associations increase the absorptive surface area of the plant due to extra-matrical fungal hyphae exploring rhizospheres beyond the root hair zone, which in turn enhance water and mineral uptake. The protection and enhanced capability of greater uptake of minerals result in greater biomass production, a pre-requisite for successful remediation. The potentials of phytoremediation of contaminated soil can be enhanced by inoculating hyper-accumulator plants with mycorrhizal fungi most appropriate for contaminated site.

4.2. Ectomycorrhizae

Evidence for the role of ectomycorrhizal fungi in ameliorating heavy metal toxicity in their hosts is still developing. It is possible that protection against heavy metals is by mycelia affording a physical barrier or mantle (Donnelly and Fletcher, 1994; Smith and Reed, 1997) and may include metabolic processes such as intracellular metal accumulation and the extracellular precipitation of metals by metabolites in exudates as is known in saprophytic fungi but this has been shown in only a few mycorrhizal fungi (Turnau, 1993). In most of the studies which report ectomycorrhizal fungi to be beneficial, the mechanism suggested for the protective effect of the fungus is the prevention of translocation of heavy metals into the host. For example, in *Picea abies* mycorrhizal with *Laccaria laccata*, most Cd was found to be associated with cell walls of the latter (Galli et al., 1993). The outer pigmented layer of the cell wall of *Pisolithus tinctorius* was where Cd, Cu and Fe were revealed to accumulate (Turnau et al., 1994). In ecto- and endomycorrhizal fungi heavy metals were demonstrated to be bound to cell wall components such as chitin, cellulose derivatives and melanin (Galli et al., 1994). Extrahyphal slime and polyphosphate linkage of Cu and Zn was observed to be the amelioration mechanism in *P. tinctorius* (Tam, 1995). All this means that protective effect is directly proportional to the amount of extramatrical mycelium, as has been found in a study of Cd and mycorrhizal *Pinus sylvestris* (Colpaert and Vannasche, 1993). Concentrations of heavy metals were usually found to be little altered in roots of mycorrhizal birch, pine and spruce but were high in extramatrical hyphae of the symbionts *Amanita*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Scleroderma and Suillus* spp. (Wilkins, 1991). In *Rhizopogon roseolus* and *P. sylvestris* associations, Cd and Al were found to accumulate in the fungal mantle and their concentrations were found to decrease along the Hartig net towards the root interior (Turnau et al., 1996). A few studies report that ectomycorrhizal fungi do not limit heavy metal concentration in their hosts. For example, *Thelephora terrestris* was claimed to increase Zn concentration in its host (Colpaert and Vanassche, 1992) and the tolerance of *Picea abies* to Cd conferred by *Paxillus involutus* could not be related to decreased Cd uptake (Godbold et al., 1998). However, whether Zn transport is allowed through pine ectomycorrhiza to the host has been shown to be dependent in part, on the concentration of the metal external to the ectomycorrhiza (Bucking and Heyser, 1994). At low external concentrations, Zn uptake is increased. At high concentrations, ectomycorrhiza can maintain shoot tissue concentration at a low level. It is possible that this effect also applies to other heavy metals and may explain some contradictory reports on uptake.

The tolerance of ectomycorrhizal fungi to heavy metals varies. In growth studies on agar and liquid culture, *L. laccata* proved sensitive at 10 ppm to Cu and Al but not Zn (Jones and Meuhlchen, 1994). The same study revealed high tolerance of *Thelephora terrestris* to Cu (500 ppm) and Zn (1000 ppm). A liquid culture study indicated that *Hymenogaster* spp., *Scleroderma* spp. and *P. tinctorius* were able to withstand high concentrations of Al, Fe, Cu and Zn (Tam, 1995). Naturally, all this has implication for the selection of appropriate ectomycorrhizal fungi for use in remedial plantings on contaminated sites. However, neither selection based on in vitro growth trials nor selection of presumably adapted fungi will guarantee success. For example, in vitro tolerance of an ectomycorrhizal fungus to Zn did not always predict its tolerance as a symbiont (Colpaert and Vanassche, 1992). Isolation of different *Paxillus involutus* Fr. strains from polluted and non-polluted sites did not influence their tolerance to aluminium (Rudawska and Leski, 1998). Similarly, Cd contaminated soil was not found to be a better source of Cd tolerant ectomycorrhizal fungi (Colpaert and Vanassche, 1992). Mycorrhizal fungi adapted to contaminated soil did not increase plant growth compared to fungi from uncontaminated sites (Shetty et al., 1994).

Good initiation of mycorrhiza is a necessary first step in exploiting the benefits of mycorrhizal fungi. There is evidence from work on *P. tinctorius* and *Eucalyptus urophylla*, that at high enough concentrations, Cr and Ni can reduce the percentage of root tips colonized by the fungus (Aggangan et al., 1989). While this would have a consequence for natural revegetation of contaminated sites by plants which would normally become ectomycorrhizal but for the contamination, this might be a lesser problem in remedial plantings because the required ectomycorrhizal seedlings can be produced in nurseries. Thus, the contaminated sites would be outplanted with plants with ectomycorrhiza already established. If the mechanism of protection of plants against heavy metal toxicity by ectomycorrhizal fungi is interception by the fungal sheath and extramatrical mycelium, then two issues can be attended to with benefit. Firstly, fungal types which can initiate a high percentage of mycorrhizal roots and which can also subsequently produce much extramatrical biomass under the prevailing environmental conditions should prove the most useful. The fungus chosen should also be appropriate for the stage of growth of the host (usually seedling). Secondly, the conditions which optimize the soil environment for mycorrhizal initiation and extramatrical growth should be identified and applied if possible. These conditions include the soil nutrient, water, pH and porosity regimes. While studies to date have understandably concentrated on challenges with concentrations of heavy metals, the confounding effect of choice of ectomycorrhizal fungus and soil conditions other than that of contaminant presence and concentration, will require investigation in future studies.

5. Role of plant chelating agents in phytoremediation

The ultimate sink for heavy metal pollutants is atmospheric deposition and burial in soils and sediments. They often accumulate in the top layer of soil, and are, therefore, accessible for uptake by plant roots which are the principal entry points of metals into the food chain. The success of phytoremediation depends upon the selection of plant species and soil amendments that maximize the removal of heavy metals from this top layer of contaminated soil. For phytoremediation to be possible, the contaminant(s) must be within the plant's root zone, be bioavailable and be biologically absorbed. Heavy metals are retained by soil in three ways: by adsorption onto the surfaces of mineral particles, by complexation by humic substances in organic particles, and by precipitation reactions (Walton et al., 1994). Recently, by-products of industrial processes such as bearings and steel shots and sewage sludge have been used to immobilize heavy metals (Mench et al., 1994). Amendment of contaminated soils with lime, phosphate and organic acids generally reduce the bioavailability of heavy metals (Marschner, 1995).

Plants, depending on their species and genotype, differ in their efficiency in acquisition and utilization of nutrients (Baird, 1997). Some plants release phytosidophores (PS) under Zn or Fe deficiencies (Walter et al., 1994; Marschner, 1995; Marschner and Romheld, 1995) which mobilizes Mn, Zn and Cu in the rhizosphere, uptake of which is also enhanced (Zhang et al., 1991; Graham et al., 1994).

Some plants are able to tolerate an excess of heavy metals by involving processes like sequestration in the cell vacuole with organic acids and complexation with metal detoxifying peptides induced on their exposure to heavy metals (Rauser, 1984; Rauser, 1990; Steffens, 1990).

The unique superfamily of thiol-containing metal binding proteins called metallothioneins (MT) are known to modulate internal levels of metal concentrations between deficient and toxic levels by binding toxic metals through closely spaced cystein thiol groups. These polypeptides have been given the name phytochelators. Various researchers in the past two decades have provided evidence to show that plants, algae and certain fungi also produce MT, which differs from the classical MT first discovered by Margoshes and Vallee (1957). Rauser (1990) tabulated the eukaryotic organisms in which MT have been found. Glutathione (GSH) is the most abundant cellular thiol-rich heavy metal-binding peptide (PC) in plants, animals and fungi (Singh et al., 1997). The role of PCs in metal detoxification has largely been studied using Cd and plant cell suspension cultures. Cd-tolerant cells bound most of the cellular Cd as Cd-binding complexes; little binding of Cd occurred in non-tolerant cells, which grew poorly and subsequently died (Klapheck et al., 1994). Formation of Cd-binding complexes allowed the Cd-tolerant cells to survive excess Cd due to lower contents of the free metal in the cells, allowing undisturbed metabolism. Sequestration of heavy metals by PCs confers protection for heavy metal sensitive enzymes. Keltjnes and Vanbeusichem (1998) tested the use of PCs as biomarkers and concluded that PCs seem to be a useful early warning system for heavy metals stress in plants.

Leopold and Gunther (1997) reported the induction of PCs and the binding of heavy metals to these complexes by exposure of *Silene vulgaris* cell cultures to different concentrations of Cd, Cu, Pb and Zn. Choi et al. (1996) found that different ratios of PC:Cd complexes were stimulated in Cd-treated seedlings of *Canavalia lineata*. Salt et al. (1998) reported Cd-binding PCs in Indian mustard seedlings exposed to Cd. Zenk (1996) isolated PC from plants and plant suspension cultures and suggested that PC synthase will be an interesting target for biotechnological modifications of heavy metal tolerance/accumulation in higher plants. Gwozdz et al. (1997) showed that there is a complex defense system, comprising of specific proteins, antioxidant enzymes and PCs, against metal phytotoxicity in the roots of *Lupinus luteusi* L. exposed to Pb, Ca and Cu. Recently Schaefer et al. (1998) found massive formation of PCs in the roots of *Brassica juncea* L. exposed to Cd, indicating Cd-induced PC systhesis. PCs occurred in roots of *Acer pseudoplatanus* and *S. cucubalus* growing on a Zn mine waste site (Grill et al., 1989). Harmens et al. (1994) studied SH–GSH concentrations in Zn-sensitive and Zn-tolerant *S. vulgaris* exposed to Zn and found higher concentrations of SH–GSH in the roots of Zn-sensitive plant compared to that of the tolerant plant due to the production of PCs as well as cystein and non-identified thiols. Tukendorf (1993) reported stimulation of PC contents in spinach plants exposed to higher levels of Cd and Cu. Klapheck et al. (1994) reported the formation of metal-induced HM-PCs in several species of *Poaceae* exposed to Cd. Guo and Marschner (1995) also suggested that PCs induced in Cd exposed plants may be involved in the translocation of Cd from root to shoot. Inouhe et al. (1994) reported that the synthesis of a Cd-binding complex containing PCs in cereal roots exposed to Cd and that this has an important role in its tolerance of Cd. Grill et al. (1989) showed that roots of plants growing in a HM-contaminated mine dump contained 10–100 times greater concentrations of PCs than in the leaves. Exposure of intact plants or their cell cultures to relatively high concentrations of metals such as Cd, Cu, Zn causes the appearance of Cd-binding complexes (Rauser, 1990). As suggested by Rauser (1990) integrated biochemical and physiological studies in roots are most likely to clarify the phenomena of phytoaccumulation.

Macnair (1993) reviewed the genetics of the phenomenon of metal tolerance in vascular plants and discussed the role of phytochelatins and metallothionein-like proteins in metal tolerance. Much has been learned in recent years on how plants and certain fungi chelate and transport heavy metals. Fission yeast is shown to produce PCs in response to excess Cd (Wu et al., 1997; Mehra et al., 1998) and target genes for heavy metal tolerance have been identified in it (Ow, 1993, 1996). The sequence of these target genes can be modified for expression in a host plant cultivar for commercial use in phytoremediation. Hunter and Mehra, 1998 transformed a Cd-sensitive mutant *Candida glabrata* with a gene from the wild type to restore Cd tolerance and formation of Cd-glutathione and Cd-phytochelatin complexes. As plant nutrient uptake is intrinsically linked to associations with mycorrhizal fungi, elucidating metal sequestration in these fungi offers additional opportunities for engineering mycorrhizal plants to assist phytoextraction. A better knowledge of the biological processes governing heavy metal uptake and accumulation should allow the application of modern genetic engineering techniques to improve the application of phytoremediation. A study of the genetics of tolerance and hyper-accumulation is of importance in unraveling tolerance mechanisms and in breeding plants for heavy metal tolerance.

Plant roots exude organic acids, for example malic and citric acids, and/or acid phosphatases under P deficiency (Hoffland et al., 1989; Liu et al., 1990; Ohwaki and Hirata, 1992; Marschener, 1998). This localized enhanced excretion of organic acids increases the effectiveness of exudates for the mobilization of nutrients such as P, Zn, Fe and Mn (Marschener, 1998). The population density and composition of symbiotic and non-infecting microorganisms in the rhizosphere can enhance root exudation and the concentration of organic acids, chelators, and acid phosphatases released as ectoenzymes from roots, or from microorganisms including arbuscular mycorrhizal fungi as microbial metabolites (Meharg, 1994; Trafdar and Marschner, 1994).

6. Phytoremediation assisted by synthetic metal chelators

Metals can exist in various chemical forms (or species). These forms often exist in a complex equilibrium governed by many soil factors and properties. For any given heavy metal, only a fraction is bioavailable and thus potentially it is only this fraction that can be taken up by the plants. More of the metal could be converted to the bioavailable fraction as it is gradually removed by the plant but the extent to which this happens and the kinetics of such processes are not known and would invariably be soil specific.

Recently, low toxicity multidendate chelating agents such as EDTA, have been used to enhance the bioavailability of heavy metals for plant uptake (Leyval et al., 1995; Turnau, 1998). The resulting chelates are very stable and do not normally release their metal ions back into a free form, unless there is a significant drop in soil pH. Salt et al. (1995) have shown that the shoots of Indian mustard plant (*B. juncea*) grown for four weeks in soil containing 0.9 mmol/kg Cd and 1 mmol/kg EDTA yielded 875 μgCd/g dry weight of plant. This compared to only 164 μgCd/g dry weight of plant in the absence of the chelator. Glasshouse studies using heavy metal contaminated soil from an abandoned gold mine in Australia have shown that after a six week growth period there was enhanced uptake of Fe, Mn and Cu by *Zea mays* if the soil was dosed with EDTA or DTPA (1g chelator/kg soil) prior to planting (Chaudhry unpublished results). In a pot experiment, using Zn-contaminated soil amended with EDTA, Ebbs and Kochian (1998) compared the phytoextraction of Zn by oat, barley and Indian mustard and found that the addition of EDTA to soil significantly increased Zn accumulation by plants. Barley accumulated 2–4 times more Zn than oat in the presence of EDTA, suggesting it has a phytoremediation potential equal to, if not greater than, that for Indian mustard. Huang et al. (1997) investigated the effect of organic acids amendment of uranium contaminated soils and found that citric acid was the most effective in increasing metal availability and enhancing uranium accumulation many fold in the shoots of selected plants. These and other studies indicate that the accumulation of heavy metals in plant shoot can be enhanced through the application of synthetic chelates to the soil and that with proper management, chelate-assisted heavy metal phyotoextraction may provide a cost effective decontamination strategy. Care needs to be taken, however with the addition of metal chelators as the resultant increased mobility of the metals may lead to its increased leaching into surrounding water systems.

7. Conclusion

Phytoremediation is emerging as a biobased and low cost, alternative technology in the cleanup of contaminated soils. The future of the technique is still in the development and research phase and there are some technical barriers which need to be addressed such as optimization of the process, greater understanding of how plants absorb, translocate and metabolize heavy metals, the identification of genes responsible for uptake and/or degradation of the contaminant, decreasing the length of time needed for phytoremediation to work, disposing the biomass so produced and protecting wild life form feeding on plants used for remediation. In addition, since contaminant uptake and tolerance depend on both plant and soil factors including soil microbes, information on microbial interactions such as nitrogen fixing bacteria and the ubiquitous mycorrhizal fungi are also required. The contribution of mycorrhizal, actinorrhizal and rhizobial symbionts to soil productivity and enhanced heavy metal uptake have not yet been seriously considered and is hitherto neglected or overlooked. In addition to optimizing metal bioavailability, it is recommended to introduce actinorrhizal, mycorrhizal and rhizobial plants as soil improvers to rehabilitate polluted sites by optimizing the uptake of bioavailable metals due to modification of the root/rhizosphere systems.

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