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## REVIEW

# Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation

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## Abstract

This article reviews recent developments in in situ bioremediation of trace metal contaminated soils, with particular reference to the microbial dynamics in the rhizospheres of plants growing on such soils and their significance in phytoremediation. In non-agricultural conditions, the natural role of plant growth promoting rhizobacteria (PGPR), P-solubilizing bacteria, mycorrhizal-helping bacteria (MHB) and arbuscular mycorrhizal fungi (AMF) in maintaining soil fertility is more important than in conventional agriculture, horticulture, and forestry where higher use of agrochemicals minimize their significance. These microbes initiate a concerted action when a particular population density is achieved, i.e. quorum sensing. AMF also recognize their host by signals released by host roots, allowing a functional symbiosis. AM fungi produce an insoluble glycoprotein, glomalin, which sequester trace elements and it should be considered for biostabilization leading to remediation of contaminated soils. Conclusions drawn from studies of metal uptake kinetics in solution cultures may not be valid for more complex field conditions and use of some combination of glasshouse and field experiments with organisms that occur within the same plant community is suggested. Phytoextraction strategies, such as inoculation of plants to be used for phytoremediation with appropriate heavy metal adapted rhizobial microflora, co-cropping system involving a non-mycorrhizal hyperaccumulator plant and a non-accumulator but mycorrhizal with appropriate AMF, or pre-cropping with mycotrophic crop systems to optimize phytoremediation processes, merit further field level investigations. There is also a need to improve our understanding of the mechanisms involved in transfer and mobilization of trace elements by rhizosphere microbiota and to conduct research on selection of microbial isolates from rhizosphere of plants growing on heavy metal contaminated soils for specific restoration programmes. This is necessary if we are to improve the chances of successful phytoremediation.

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**Keywords:** Arbuscular mycorrhiza; Heavy metals; Soil microbiota; Plant growth promoting rhizobacteria; Mycorrhiza helper bacteria; In situ mycorrhizoremediation; Mycorrhizosphere; Phytoremediation strategies

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## Introduction: soil pollution with trace elements

### Soil contamination sources

Metals such as lead, arsenic, cadmium, copper, zinc, nickel, and mercury are continuously being added to our soils through various agricultural activities such as agrochemicals usage and long-term application of urban sewage sludge in agricultural soils, industrial activities such as waste disposal, waste incineration and vehicle exhausts, as well as from anthropogenic sources. All these sources cause accumulation of metals and metal-oids in our agricultural soils and pose threat to food safety issues and potential health risks due to soil-to-plant transfer of metals. Co-existence and persistence of heavy metals in soils as multiple contaminants and human exposure to them through ingestion of heavy metal contaminated food or uptake of contaminated drinking water can lead to their accumulation in humans, plants and animals. They can also cause a considerable detrimental effect on soil ecosystems, environment and human health due to their mobilities and solubilities which determine their speciation [1]. In some cases, the soil may be contaminated to such an extent that it may be classified as a hazardous waste [2]. Soil contamination with heavy metal mixtures is receiving increasing attention from the public as well as governmental bodies, particularly in developing countries [3]. The remediation of such soils is important because these usually cover large areas that are rendered unsuitable for agricultural and other human use.

### Remediation technologies

Various physico-chemical and biological remedial technologies have been developed over the last three decades and selection of each technology is site specific [4–6]. Biochemical processes such as bioleaching involving *Thiobacillus* spp. bacteria and *Aspergillus niger* fungus, biosorption of low concentrations of metals in water by algal or bacterial cells, bio-oxidation or bio-

reduction of metal contaminants by *Bacillus subtilis* and sulphate reducing bacteria, and biomethylation of metals such as arsenic, cadmium, mercury or lead, have shown some promises and could be used for soil sediment treatments [4]. Several technologies exist for in situ chemically enhanced soil flushing by extracting solutions such as organic and inorganic acids, and complexation agents have also been proposed for remediation [7]. All these methods in many cases are expensive, labour intensive, and result in extensive changes to the physical, chemical, and biological characteristics of the treated soil. Physical and chemical methods of remediation of contaminated soils are mainly applicable to relatively small areas and are unsuitable for very large areas such as a typical mining site or industrially/agrochemically contaminated soils.

### Phytoremediation

The health hazards associated with soil contamination with trace elements having toxic effects together with high cost of removal and replacement of polluted soil have prompted to develop alternative and cheaper technologies to recover the degraded land. Current research in this area now includes plants to remediate polluted soils and to facilitate improvement of soil structure, the innovative technique being known as phytoremediation [8]. Plant-based technologies are applicable for removing metals from areas of low concentrations with shallow soils and water, although longer treatment times may be required [9]. Use of plants that have constitutive and adaptive mechanisms for tolerating or accumulating high metal contents in their rhizosphere and tissues, is the emerging in situ remediation technology employed in the clean up of soils, sediments and water that have been polluted by organics and heavy metals [10–13]. This technology is termed phytoremediation and it aims to use metal accumulating plants to remove, transfer or stabilize

these contaminants from soil, sediments and water. It is a natural, clean and economic alternative to physical and chemical methods of clean up.

Phytoremediation can be categorized under five major subgroups:

- (i) Phytoextraction – removal and concentration of metals into harvestable plant parts.
- (ii) Phytodegradation – degradation of contaminants by plants and their associated microbes.
- (iii) Rhizofiltration – absorption of metals by plant roots from contaminated waters.
- (iv) Phytostabilization – immobilization and reduction in the mobility and bioavailability of contaminants by plant roots and their associated microbes.
- (v) Phytovolatilization – volatilization of contaminants by plants from the soil into the atmosphere [11].

Phytoremediation, however, is a relatively slow process. It may take some years to reduce metal contents in soil to a safe and acceptable level due to small size and slow growth of most identified metal hyperaccumulator plants. Their employment in phytoremediation is restricted due to these reasons. To make phytoremediation a viable technology, we have to either find fast growing and metal tolerant and or hyperaccumulating plants with extensive root system, or, engineer common plants with as yet unidentified hyperaccumulation genes. Many fast growing and high biomass producing plants such as vetiver grass and hemp may not be defined as metal hyperaccumulators, but are metal tolerant allowing them to grow in soil with high metal concentrations. The possibilities of using such plant species which are easily growing in different climates, and using their biomass in non-food industries, can make them ideal plants for phytoremediation purposes [14,15].

Phytoremediation must be considered as a long-term strategy [16]. Various means have been tested in the last decade or so to find ways of enhancing the process. Plant uptake of metals is frequently restricted by limitations of contaminant bioavailability and in order to enhance metal uptake, soil amendments with metal chelating agents such as EDTA, HEDTA, DTPA, EGTA, NTA, citrate and hydroxylamine to make metals bioavailable and absorbed by plant roots have shown promises [17–19]. The type of chelate and its time of application are important considerations. It has also been suggested that if the plant biomass can be increased, then metal phytoextraction can be increased more than the plant can take up normally [20]. Use of plant growth regulators (PGR) such as auxins and cytokinins have shown to enhance phytoremediation abilities of non-hyperaccumulating plants by increasing their growth and biomass [21,22]. Patten and Glick [23] reported enhanced bioavailability of iron by applying plant hormone indol-acetic acid (IAA) via a mechanism

different from that involving siderophores. IAA is also produced by many plant growth promoting rhizobacteria (PGPR) such as *Pseudomonad* and *Acinetobacter* strains which result in enhanced uptake of iron, zinc, magnesium, calcium, potassium and phosphorus by crop plants [24]. Usefulness of PGPR is, however, limited under nutrient deficient conditions. Fertilizers have been used to help plants to increase their biomass and to extract more metals [25]. Further research needs to be carried out to find suitable combination of plant, PGPR, and soil type in order to investigate their potential(s) in increasing metal uptake by hyperaccumulator plants and improving the process of phytoextraction.

This review will now discuss the occurrence and potential role of both free-living and symbiotic soil microbes in the rhizosphere of plants growing on metal contaminated soils in increasing plant biomass production and enhancing phytoremediation process.

## Rhizospheres

Since 1904, when the term ‘rhizosphere’ was first coined by Hiltner [26], rhizosphere processes of plants have been widely investigated; however, little attention has been paid to the microbial community of rhizospheres of plants growing on metal contaminated sites. Soil microorganisms, including plant root associated free-living as well as symbiotic rhizobacteria and mycorrhizal fungi in particular, are integral part of the rhizosphere biota. The overall result of plant–rhizosphere microbe interactions is a higher microbial density and their metabolic activity in the rhizosphere, even in metal contaminated soils [27]. Plant root exudates provide nutrition to rhizosphere microbes, thus increasing microbiological activity in the rhizosphere, which in turn stimulate plant growth. Marschner and Baumann [28], studied the changes in bacterial community structure in the rhizosphere induced by mycorrhizal colonization in split-root infection by non-cultural techniques such as polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) and concluded that the effects may, at least in part, be due to changes in root-exudation with plant age.

## Rhizospheres and plant growth promoting rhizobacteria

The PGPR enhance plant growth by atmospheric nitrogen fixation, phytohormone production, specific enzymatic activity, plant protection from diseases by producing anti-biotic and other pathogen-depressing substances such as siderophores and chelating agents (for references see [29]).

Microbial cells can produce and sense signal molecules, allowing the whole population to spread as a biofilm over the root surface and initiating a concerted action when a particular population density is achieved. This phenomenon is known as quorum sensing, which, in combination with other regulatory systems, expands the range of environmental signals that target gene expression beyond population density [30]. The nitrogen-fixing rhizobial bacteria, chemotactically attracted towards legume roots by certain root exudates, adhere to and colonize the root surface, and activate rhizobial nodulation genes, Nod factors. Many quorum sensing signal molecules such as *N*-acyl-homoserine lactones (AHLs) are produced which regulate expression and repression of the symbiotic genes [30].

Free-living as well as symbiotic PGPR can enhance plant growth directly by providing bio-available phosphorus for plant uptake, fixing nitrogen for plant use, sequestering trace elements like iron for plants by siderophores, producing plant hormones like auxins, cytokinins and gibberellins, and lowering of plant ethylene levels [31]. The use of PGPR in phytoremediation technologies is now being considered to play an important role as adding PGPR can aid plant growth on contaminated sites [32] and enhance detoxification of soil [33]. PGPR are also beneficial to plants growing on derelict soils by conferring resistance to water stress in tomatoes and peppers [33]. The properties of plants used for phytoremediation, e.g. biomass production, low level contaminant uptake, plant nutrition and health, are improved by PGPR but it is important to choose PGPR which can survive and succeed when used in phytoremediation practices. Pairing PGPR with arbuscular mycorrhizal fungi (AMF) may be a good way of increasing the efficiency of phytoremediation. Although the role of PGPR is potentially important in the phytoremediation strategies, research in this area, as pointed out by Lucy et al. [34], is very limited and requires field studies to support greenhouse or growth chamber results.

### Rhizosphere and arbuscular mycorrhizal fungi

AMF are ubiquitous soil microflora and constitute an important functional component of the rhizosphere. These fungi form symbiotic relationships with roots of 80–90% land plants in natural, agricultural, and forest ecosystems [35]. Such associations are also common in aquatic plants under oligotrophic conditions [36].

AM symbiosis is >460 million years old and the most widespread type of mycorrhizal associations with plants possessing true roots, i.e. pteridophytes, gymnosperms and angiosperms [37]. Approximately 160 fungal taxa of Glomeromycota have been described on the basis of their spore morphology and root infection patterns [38].

The number of AM fungal taxa may be much higher based on recent molecular analyses [39].

AMF may also play a role in the protection of roots from heavy metal toxicity by mediating interactions between metals and plant roots [40,41]. The external fungal hyphae exploit a larger volume of nutrient deficient resources in the soil that are otherwise unavailable for uptake by roots alone [42]. Therefore, AMF may be important for the revegetation of metal polluted soils. Wang et al. [43] studied the effect of chitosan and AM fungus on copper, zinc, and lead accumulation by *Elsholtzia splendens* grown in soil contaminated by copper smelt factory fly ash and reported enhanced uptake of these metals by mycorrhizal plants without showing any symptoms of heavy metal toxicity as compared to the controls. The authors also found that AMF or chitosan alone did not significantly increase the metal concentrations in *E. splendens*.

AM fungi also recognize their host by signals released by host roots, allowing a functional symbiosis [44–46], and in the absence of the host root these fungi do not produce mycelia and consequently complete their life cycle. Root exudates from the AM host plants are known to stimulate spore germination and early growth of AMF hyphae. On the contrary, root exudates from non-mycorrhizal hosts such as mustard, spinach, sugar-beet and lupin inhibit asymbiotic [47] as well as symbiotic extra radical hyphal growth of AMF from spores and level of root colonization [48]. Reduced root colonization by AMF is regarded to be due to inhibitory compounds in the root exudates of crucifers, which play a role in the expression of non-host status of some plants [49]. Recently, a C-glycosylflavonoid was detected in the non-mycorrhizal roots but not in the mycorrhizal roots of melon [50], and application of this flavonoid to AMF-inoculated melon plants enhanced root colonization, suggesting that root colonization-stimulating compounds in root exudates are involved in mycorrhization. Recent studies by Piche and associates [48], however, showed that root exudates of non-mycorrhizal cucumber plants stimulate root colonization, whereas root exudates from the mycorrhizal and the non-mycorrhizal sides of a split-root system did not show this stimulatory effect and were slightly inhibitory. These studies indicate that root exudates in mycorrhizal plants may be partially involved in systemic resistance of mycorrhizal plants to soil-borne fungal pathogens.

The obligatory biotrophic AMF, in the absence of a host, do not maintain their viability and host infection capability. Many cellular events are involved in the survival of individual AMF growing in the absence of the host plant root [51]. The fate and behaviour of individual AM fungal spores that germinate in the absence of appropriate hosts may affect their survival due to many active cellular events taking place in the



cytoskeleton of AMF hyphae which undergo prolonged growth arrest and resource reallocation [51]. Prolonged presence of AMF spores in the soil without host plant has been shown to induce a progressive increase in the proportion of empty AMF hyphae [52], suggesting the occurrence of a senescence phase in the mycelium of AM fungi. However, these resting AMF spores are still viable and capable of renewed growth in response to host roots [51,53,54].

### Arbuscular mycorrhizae in plants growing in metal contaminated soils

AM fungi are ubiquitous soil microbes occurring in almost all habitats and climates, including metal contaminated soils [55] and are considered essential for the survival and growth of plants growing in nutrient especially phosphorus deficient derelict soils. However, polluted wastelands contain reduced population diversity and number of autochthonous AMF strains which are heavy metal tolerant [56].

Studies with AM fungi have focused on their ability to enhance nutrient uptake in a nutrient deficient soil and have ignored the role they may play in phytoremediation. The prospect of fungal symbionts existing in metal contaminated soils has important implications for phytoremediation (mycorrhizoremediation) of metal contaminated soils as AM fungi help plant growth through enhanced nutrient uptake.

### Arbuscular mycorrhizae in metal hyperaccumulating plants

Plant species belonging to plant families Chenopodiaceae, Cruciferaeae, Plumbaginaceae, Juncaceae, Juncaginaceae, Amaranthaceae and few members of Fabaceae, are believed not to form a symbiosis with AMF [42]. Many hyperaccumulators belong to the family Brassicaceae but there are conflicting reports regarding their mycotrophic status. Hirrel et al. [57] reported them to be non-mycorrhizal but 1–5% AMF root colonization occurred in 7 species of crucifers when grown in the presence of a mycorrhizal companion plant. DeMars and Boerner [58] made an extensive literature survey of crucifers and revealed that roots of 18.9% of the 946 members investigated were found to be colonized with internal hyphae, occasional vesicles but no arbuscules. Since arbuscules are the major site of nutrient exchange, including metals, between the plant and AMF, these associations are non-functional [59]. Pawlowska et al. [60] found roots of *Biscutella laevigata*, a Brassicaceous plant colonizing the calamine mounds in Poland, to be mycorrhizal but without arbuscules, a criterion of a functional mycorrhiza. However, a latter study by Orlowska et al. [61], who re-examined the

mycorrhizal status of *B. laevigata* and the role of restoration of zinc-wastes on mycorrhization of this cruciferous plant species, observed AM hyphae, vesicles, as well as arbuscules in its roots collected prior to seed maturity. However, arbuscules were found by the authors in the roots collected during the flowering period, whereas only hyphal coils and vesicles were observed after seed formation. Similar observations were made by Saif and Khan [62] who reported the extent of arbuscule formation influenced by the stage of development of the wheat plants, i.e. arbuscular infection during the vegetative growth period followed by a sharp fall at the time of flowering and ripening when vesicles were abundant in old tissues before they sloughed off. DeMars and Boerner [58] also reported absence of arbuscules in Brassicaceous plants under green house condition.

Regvar et al. [63] examined roots of pennycresses, i.e. *Thlaspi* spp. of the Brassicaceae, collected from diverse locations in Europe for AM fungal infections by applying PCR approaches such as 18S-rDNA PCR products and found that the meadow species were sparsely but distinctly colonized by *Glomus intraradices*, as indicated by the occurrence of hyphae, vesicles and arbuscules. Species from other locations, including those collected from metal contaminated soils, were found to be poorly colonized but with non-discernible arbuscules. Sequencing of the rDNA PCR-products revealed that, although colonization was by a common AM fungus *G. intraradices*, none of the sequences obtained from the *Thlaspi* roots was identical to any other *G. intraradices* sequences, indicating slightly different sequences from habitat to habitat [64]. This might indicate, as stated by the authors, that a species continuum exists in *G. intraradices* clade. An AM fungal ecotype specifically adapted to heavy metals may exist at such locations. Regvar et al. [63] failed to establish an effective colonization of *Thlaspi* roots by AMF under greenhouse conditions and concluded that it is doubtful whether an affective symbiosis with the mutual exchange of metabolites is formed by both partners. The authors also concluded that the use of AMF-*Thlaspi* combination for phytoremediation is not possible. Maybe a complex control mechanism and signals are involved in this plant-AMF interactions [65].

### Phytoremediation strategies involving rhizosphere microbes

#### Inoculating plants used for phytoremediation with rhizobial microflora

Contaminated soils, which are often nutrient poor and with low water holding capacities, have been related

to the incidence of AM fungi, suggesting a selective advantage of plants colonized by these fungi, acting as pioneering species on such sites [17]. Recently, Andrade et al. [66] studied the influence of lead additions on AM and *Rhizobium* symbiosis under soybean plants and found that in the presence of both microsymbionts, AMF and *Bradyrhizobium*, mycorrhizae benefited soybean plants due to their better nutritional state under conditions of high lead availability in the soil. Plant growth promoting bacterium *Kluyvera ascorbata* SUD165 isolated from metal contaminated wetland near Sudbury, Ontario, Canada, protected canola plants against nickel toxicity when grown in soils supplemented with nickel, lead, zinc, and chromate [67]. It may be possible to inoculate plants with such rhizobial microbes in order to increase plant biomass and thereby stabilizing, revegetating, and remediating heavy metal polluted soils.

Managing the microbial population in the rhizosphere by using an inoculum consisting of a consortium of PGPR, mycorrhiza-helping bacteria (MHB), nitrogen-fixing rhizobacteria, and AMF as allied colonizers and biofertilizers, could provide plants with benefits crucial for ecosystem restoration on derelict lands [68–70].

It is important to use indigenous AMF strains which are best adapted to actual soil and climatic conditions to produce site-specific AMF inocula. The role of AMF in reducing cadmium stress was investigated by Rivera-Becerril et al. [71], who found that, even though the AMF *G. intraradices* BEG141 used was not previously been exposed to cadmium, it attenuated the toxic effect of cadmium in pea. The authors also found that genetic variability can exist between plant species, depending on the level of metals present in soils. Many soil-less techniques have now been developed to produce efficient AMF inocula [72]. If indigenous AMF exist in the contaminated soil to be phytoremediated, management of the indigenous AMF and their associate rhizobial microflora would be an important strategy. During soil restoration, the evaluation of mycorrhiza development and other soil microflora could be used as an important indicator of ecosystem efficiency, in order to biomonitor the success of mycorrhizoremediation [73]. Phytoremediation using plants associated with efficient and metal tolerant/adaptive AMF isolates, will be enhanced in soils devoid of AMF [74]. The structure of the plant community appears to be driven by the biodiversity of AMF [75,76]. Further research is needed on AMF ecotypes isolated and selected from heavy metal contaminated soils and being used for specific restoration programs. Molecular tools such as taxon specific primers could be successfully used to assess the success of AMF in colonizing plants used for restoration.

Different mechanisms have been proposed to explain the protective effect of AM and metal stress [77–79]. As stated by Burd et al. [67], in heavily contaminated soils

where the metal content exceeds the limit of plant tolerance, it may be possible to treat plants with rhizosphere microbes, increasing plant biomass and thereby stabilizing, revegetating, and remediating metal-polluted soils.

### Intercropping system

Wu et al. [80] used an intercropping system to examine the interactions of mycorrhizosphere and rhizosphere on metal uptake by growing mycorrhizal non-hyperaccumulator *Zea mays* and non-mycorrhizal hyperaccumulator *Brassica juncea* in a split-pot experiment. Their results showed that the intercropping system achieved higher phytoremediation efficiency in metal contaminated soil, especially with dual inoculation of beneficial rhizobacteria and AM fungi.

Similar studies were conducted by Zhang et al. [81] who grew peanuts (leguminous crop) and maize (non-leguminous crop) and found that the iron-deficient maize released phytosiderophores which improved iron nutrition of peanuts through influencing its rhizosphere processes. Similarly, improved growth of maize and faba beans in an intercropping system was found by these authors to be due to improved nitrogen and phosphorus uptake compared with corresponding sole crop.

### Co-cropping system

Wu et al. [82] used a co-crop system with the metal hyperaccumulator plants *Thlaspi caerulescens* and *Sedum alfredii* and the low-accumulating but highly mycorrhiza dependent corn, *Zea mays* L. to increase phytoextraction efficiency from Zn-contaminated sewage sludge. The authors compared the co-crop systems with the mono-crop cultures and found that the hyperaccumulators, when grown with maize as a co-crop, extracted more Zn from the sewage sludge as compared with the mono-cultures of these plants. This is a step in the right direction to better understand the interactions between the non-mycorrhizal hyperaccumulators and the mycorrhizal plant species in order to increase the efficiency of the process and further experiments are needed using different combinations of co-crop systems.

### Pre-cropping with mycotrophic crops

Another possible strategy to exploit AM fungi for phytoremediation purposes is to use mycorrhiza-dependent plant species pre-inoculated with metal tolerant AMF on the site to be remediated followed by growing mycorrhiza-responsive plant species which can benefit from the AMF potential of soil left by the pre-crop.

Recently, Panja and Chaudhuri [83] applied this strategy and carried out a nursery experiment to study the effect of short season pre-cropping with different mycotrophic herbaceous crops followed by growing AM-dependent mandarin orange. The authors recorded greater growth benefit to mandarin oranges by mycorrhizal pre-crops such as soybean and onion than by the non-mycorrhizal crops such as mustard and ginger. Their results show that an AM-dependent crop grown even for a short season can substantially alter the inherent AMF potential of soils to significantly influence the performance of the succeeding mycorrhiza-dependent plants. This strategy allows managing the native AMF through choice of crops and cropping systems to ecological management of derelict and low input land.

## Conclusion

The above studies imply that it is possible to develop new phytoextraction strategies with an inoculation of plants used for phytoremediation with rhizobial microbes, co-cropping/intercropping systems, or pre-cropping with mycotrophic crops in order to enhance phytoextraction of metals from contaminated soils. A better understanding of the physical, chemical and biological rhizosphere processes and the interactions between hyperaccumulator and non-accumulator plant species in an intercropping or pre-cropping with mycotrophic crops system is needed to optimize phytoremediation and further investigations at field levels should be carried out.

Because of the ecological implications discussed above, AM associations along with the associated integral microbial components of a very diverse soil biota should be considered in plans for the ecological restoration of functional ecosystems on metal contaminated land and should be a significant component of studies assessing derelict land ecosystem dynamics. It is clear that AMF are an important component of natural ecosystems, and that they can influence the development of plant community composition and ecosystem function. The phytoremediation of disturbed lands, and the course of plant succession in such environments may be strongly influenced by inoculation with AM fungi and their associate rhizobacteria. Recently, Mohammad et al. [84] reported improved growth of wheat in a field containing low levels of phosphorus and a low population of indigenous AMF, when inoculated with commercially produced sheared-root inoculum of *Glomus intraradices*, indicating that the introduced AMF can compete with the indigenous AMF and benefit plant growth. Our group [85] may have been the first to demonstrate the potential value of pre-inoculating plants with AM fungi and transplanting them into

nutrient deficient field soil with its indigenous AMF population, but we must admit that we do not know how long such introduced strains persist. The composition of AMF community, including associated rhizobacteria, and their interactions clearly have relevance to mycorrhizoremediation of contaminated soils and water. However, the significance of apparently obligate symbiotic bacteria-like organisms within spores and hyphae of AM fungi, with ability to fix nitrogen, to the biology of the plant and the fungus has yet to be elucidated. Further research is also needed to investigate various chemical aspects of metal accumulation, e.g. diffusion of metals, release of specific chelators in the rhizosphere by accumulating and non-accumulating plants, the dynamics and persistence or decomposition of chelates and metal-chelate complexes in the rhizospheres/soil, and other constraints on the process of phytoextraction. This knowledge may enable us to understand other contributing soil and environmental processes. In addition, we need to understand the mechanisms involved in mobilization and transfer of metals in order to develop future strategies and optimize the phytoextraction process.

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