Article

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High concentrations of heavy metals (HM) in the soil have detrimental effects on ecosystems and are a risk to human health as they can enter the food chain via agricultural products or contaminated drinking water. Phytoremediation, a sustainable and inexpensive technology based on the removal of pollutants from the environment by plants, is becoming an increasingly important objective in plant research. However, as phytoremediation is a slow process, improvement of efficiency and thus increased stabilization or removal of HMs from soils is an important goal. Arbuscular mycorrhizal (AM) fungi provide an attractive system to advance plant-based environmental clean-up. During symbiotic interaction the hyphal network functionally extends the root system of their hosts. Thus, plants in symbiosis with AM fungi have the potential to take up HM from an enlarged soil volume. In this review, we summarize current knowledge about the contribution of the AM symbiosis to phytoremediation of heavy metals.

Reference

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REVIEW

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Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation

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Abstract High concentrations of heavy metals (HM) in the soil have detrimental effects on ecosystems and are a risk to human health as they can enter the food chain via agricultural products or contaminated drinking water. Phytoremediation, a sustainable and inexpensive technology based on the removal of pollutants from the environment by plants, is becoming an increasingly important objective in plant research. However, as phytoremediation is a slow process, improvement of efficiency and thus increased stabilization or removal of HMs from soils is an important goal. Arbuscular mycorrhizal (AM) fungi provide an attractive system to advance plant-based environmental clean-up. During symbiotic interaction the hyphal network functionally extends the root system of their hosts. Thus, plants in symbiosis with AM fungi have the potential to take up HM from an enlarged soil volume. In this review, we summarize current knowledge about the contribution of the AM symbiosis to phytoremediation of heavy metals.

Keywords Arbuscular mycorrhizal symbiosis · Glomus · Heavy metal · Phytoremediation

Abbreviations AM: Arbuscular mycorrhizal · HM: Heavy metals · EC_{50}: Effective concentration reducing germination or hyphal growth to 50%

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Introduction

Heavy metals are grouped into one category of 53 elements with specific weight higher than 5 g/cm^3 (Holleman and Wiberg 1985; Weast 1984). Trace elements such as Cu, Fe, Mn, Ni and Zn are essential for normal growth and development of plants. They are required in numerous enzyme catalyzed or redox reactions, in electron transfer and have structural function in nucleic acid metabolism (Zenk 1996). Conversely, metals like Cd, Pb, Hg, and As are not essential (Mertz 1981). HM occur mainly in terrestrial or aquatic ecosystems although they can be also emitted into the atmosphere. For land plants the root is typically the organ, which is in immediate contact with metal ions. Acquisition at the root–soil interface, partitioning throughout the plant organism and cellular homeostasis of essential HM must be well controlled by the plant to avoid deficiency as well as excess. Essential HM are taken up by specific uptake systems, but at high concentrations they also can enter the cell via non-specific transporters. Non-essential HM can enter the root via passive diffusion, but also using low-affinity metal transporter with broad specificity (Hall and Williams 2003).

At high concentrations, HM interfere with essential enzymatic activities by modifying protein structure or by replacing a vital element resulting in deficiency symptoms. The plasma membrane is particularly vulnerable to HM toxicity since membrane permeability and thus functionality can be affected by alterations of important membrane intrinsic proteins such as H^+-ATPases (Hall 2002 and citations therein). Also the production of reactive oxygen species leading to oxidative damage of plant tissue occurs in response to elevated HM levels (Schützendübel and Polle 2002). As a consequence toxicity symptoms such as chlorosis, growth retardation, browning of roots, effects on both photosystems, cell cycle arrest and others can be observed. Plants have evolved several mechanisms to maintain ion homeostasis under elevated HM concentrations (Clemens 2001; Hall

2002, Fig. 1). These rely on circumventing the generation of physiologically intolerable concentrations of HM at susceptible locations within the cell by regulating acquisition, enrichment, trafficking and detoxification of HM (Clemens 2001). Over the past years progress has been made in identifying components of such mechanisms. Several genes encoding metal uptake transporter proteins such as Nramp3 (Thomine et al. 2000), proteins involved in metal trafficking like RAN1 (Hirayama et al. 1999) or in transport to the vacuole e.g. AtHMA3 (Gravot et al. 2004) have been described from plants (reviewed in Clemens 2001; Guerinot 2000; Hall and Williams 2003; Himelblau and Amasino 2000). The basic principles of detoxification mechanisms include the extracellular HM-chelation by root exudates and/or binding of HM to the rhizodermal cell walls uptake of HM avoiding. Active plant efflux systems control cytosolic concentrations of HM. Intracellularly the plant cell produces chelating agents such as phytochelatins and metallothioneins, which have high-affinity HM binding properties. The resulting complex can finally be exported from the cytoplasm across the tonoplast and become sequestered inside the vacuole (Hall 2002, Fig. 1). Also other organelles are involved in storage. In plants Fe is stored bound to ferritin inside chloroplasts (Briat and Lobreaux 1997). In *Schizosaccharomyces pombe* a member of the cation diffusion facilator (CDF) family has been identified that has a role in accumulation of HM in the ER (Clemens et al. 2002). Proteins with similar function can be expected to exist in the ER of plants.

Man-made soil contamination resulting from mining, industry, agriculture and military activities resulted in high local concentrations of HM. In uncontaminated soil concentrations vary in orders of magnitudes, but on average the concentrations are, e.g., Zn: 80 ppm, Cd: 0.1–0.5 ppm and Pb: 15 ppm. However, in polluted soil dramatically higher concentrations were found, e.g., Zn: $>20{,}000$ ppm, Cd: $>14{,}000$ ppm and Pb: $>7{,}000$ ppm (http://www.speclab.com/elements/). Since HM are not biodegradable and may enter the food chain, they are a long-term threat to both the environment and human

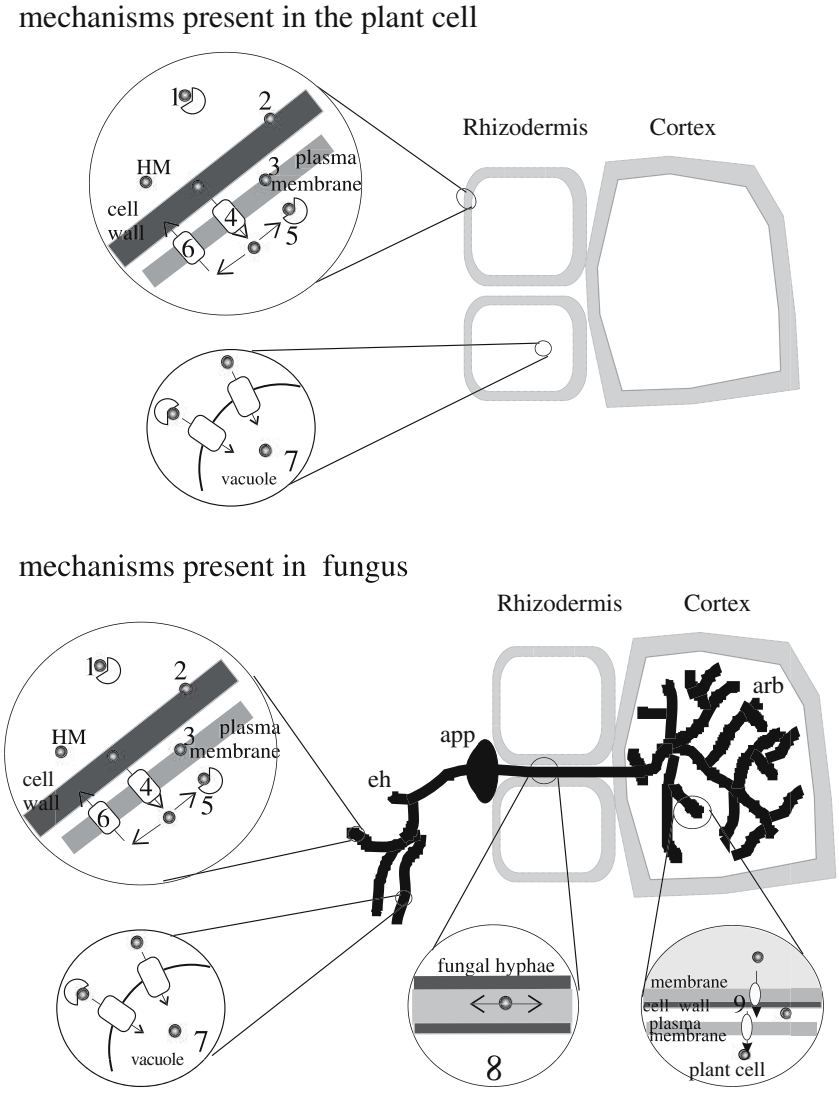

Fig. 1 HM detoxification mechanisms of plants and fungi in arbuscular mycorrhiza symbioses. *1* Chelating agents are secreted that bind metals in the soil, e.g. histidine and organic acids from the plant, glomalin from the fungus. *2* Binding of HM to cell wall components in plants and fungi. *3* The plasma membrane as a living, selective barrier in plants and fungi. *4* Specific and non-specific metal transporters and pores in the plasma membrane of plants and fungi (active and passive import). *5* Chelates in the cytosol, e.g., metallothioneins (plants and fungi), organic acids, amino acids, and metal-specific chaperons (shown for plants, assumed for AM fungi). *6* Export via specific or non-specific active or passive transport from plant or fungal cells. *7* Sequestration of HM in the vacuole of plant and fungal cells. *8* Transport of HM in the hyphae of the fungus. *9* In arbuscules, metal export from the fungus and import into plant cells via active or passive transport

health (Jarup 2003). Conventional soil remediation practices in the past have relied mainly on the excavation of the contaminated soil. However, physical displacement, transport and storage or alternatively soil washing are expensive procedures and leave a site behind devoid of any soil microflora. Furthermore translocation of HM polluted soil instead of solving the problem rather shifts it to upcoming generations (Vidali 2001). In contrast plants offer an inexpensive and sustainable on-site approach (Krämer 2005; Peuke and Rennenberg 2005; Salt et al. 1998), which relates to the above-described mechanisms for HM detoxification (Hall 2002 Fig. 1). There are two main strategies that use plants either to bind HM in the soil (phytostabilization) or to import and store HM in the plant's above-ground tissues (phytoextraction).

Arbuscular mycorrhizal (AM) fungi occur in the soil of most ecosystems, including polluted soils. By acquiring phosphate, micronutrients and water and delivering a proportion to their hosts they enhance the nutritional state of their hosts. Similarly, HM are taken up via the fungal hyphae and can be transported to the plant. Thus, in some cases mycorrhizal plants can show enhanced HM uptake and root-to-shoot transport (phytoextraction) while in other cases AM fungi contribute to HM immobilization within the soil (phytostabilization). The result of mycorrhizal colonization on clean-up of contaminated soils depends on the plant–fungus–HM combination and is influenced by soil conditions. The significance of AM fungi in soil remediation has lately been recognized (reviewed in Gaur and Adholeya 2004; Khan 2005). In this present review, we summarize current knowledge of the suitability of AM fungi for soil remediation, focusing on recent developments and novel molecular aspects. Other important factors affecting the rhizosphere and thus phytoremediation e.g. pH and soil microbes such as bacteria and ectomycorrhiza are not considered here.

AM fungi in heavy metal contamination

Arbuscular mycorrhizal fungi of the phylum of the *Glomeromycota* (Schüssler et al. 2001) are a natural constituent of the soil of most ecosystems. They interact with the roots of more than 80% of terrestrial plants and can be considered functional extensions of plant roots considerably enlarging the soil volume for nutrient uptake (Harrison 1999). To use the AM symbiosis for phytoremediation, it is important to understand how the fungus itself and establishment of the symbiosis are affected by contaminated soils. Spores and pre-symbiotic hyphae are generally sensitive to HM in the absence of plants. EC_{50} values (effective concentration reducing germination or hyphal growth to 50%) vary with the strain, but overall negative effects at high HM concentrations are observed (Shalaby 2003): spores from HM-polluted and unpolluted soils were isolated and their germination and subsequent hyphal growth were assessed in vitro (monoxenical cultures) in the presence of Zn, Pb and Cd. Germination and hyphal growth were inhibited by HM in all cases. However, spores from polluted soils were more tolerant to elevated concentrations of each of the three HM than spores from uncontaminated soils. This naturally occurring resistance is likely due to phenotypic plasticity rather than genetic changes in the spores, because tolerance is lost after one generation in the absence of HM (Shalaby 2003). Enhanced tolerance to specific HM of fungi isolated from soils contaminated with Zn, Pb, Cd or Cu has been observed frequently (reviewed in Gaur and Adholeya 2004). Furthermore, independent studies examined spore count and colonization efficiency of sewage sludge-treated sites and revealed that spores tolerant to increased HM application readily colonize host roots despite low spore counts (Del Val et al. 1999; Jacquot-Plumey et al. 2001). It remains to be studied, if the spore count decreases due to spore formation efficiency or due to spore death in the soil. Examination of the effect of Zn, Pb and Cd on pre-symbiotic (e.g., spore germination and hyphal extension), and symbiotic (e.g., extraradical mycelial growth and sporulation) fungal life stages of two *Glomus* species showed *Glomus intraradices* to be more tolerant to each of the metals than *Glomus etunicatum* (Pawlowska and Charvat 2004). Thus, tolerance varies depending on the fungal genotype. Mixtures of HM lead to synergistic or antagonistic interactions, increasing or decreasing toxicity of one metal alone. For example, addition of Zn was antagonistic to the toxicity of Pb and/or Cd on pre-symbiotic hyphal growth, while Pb and Cd acted synergistically (Shalaby 2003). In current phytoremediation practices, plants are generally introduced into soil without established symbioses. The fact that AM fungi occur in contaminated soil makes "adapted", indigenous inocula a promising tool for soil remediation efforts. Remediation could be carried out on-site without moving large amounts of soil to decontamination facilities. If the soil has already been treated and thus the mycorrhizal population is decreased or destroyed, inoculation with HM-resistant fungi from similar soils could speed up establishment of the symbiosis and improve soil remediation.

Phytostabilization

Phytostabilization prevents spreading of HM into the soil environment as well as their leakage from the soil due to erosion. Metal-tolerant plant species with extensive root systems and good soil cover prevent wind and/or water erosion of HM and therefore serve well for phytostabilization strategies. Immobilization of HM within the rhizosphere is accomplished by precipitation of HM within the soil, adsorption onto the root surface or uptake and accumulation within roots. Commercially available plant species include those with tolerance to single or multiple HM (reviewed in Wong 2003). AM fungi contribute to the immobilization of HM in the soil

beyond the plant rhizosphere and thereby improve phytostabilization. The fungus employs strategies similar to those of its host. Among these are immobilization of metals by compounds secreted by the fungus, precipitation in polyphosphate granules in the soil, adsorption to fungal cell walls, and chelation of metals inside the fungus (Fig. 1, Gaur and Adholeya 2004).

Glomalin is an example of an insoluble glycoprotein that is produced and released by AM fungi and binds HM in the soil (Gonzalez-Chavez et al. 2004; Wright and Upadhyaya 1996, 1998). Glomalin can be extracted from the soil together with significant amounts of bound HM. Gonzalez-Chavez et al. (2004) found that up to 4.3 mg Cu, 0.08 mg Cd and 1.12 mg Pb per gram glomalin could be extracted from polluted soils that had been inoculated with laboratory cultures of AM fungi. In an in vitro experiment, *Gigaspora rosea* sequestered 28 mg Cu per gram glomalin. Since there is a correlation between the amount of glomalin in the soil and the amount of HM bound, fungal strains with significant secretion of glomalin should be more suitable for biostabilization efforts.

Binding of HM to chitin in the fungal cell wall reduces its local concentration in the soil. Passive adsorption to the hyphae leads to binding of up to 0.5 mg Cd per mg dry biomass (Joner et al. 2000). Due to the large surface area presented by fungi in the soil hyphal binding is an important sink for HM. Furthermore, HM-tolerant fungi have a higher affinity for HM (2–4 times more than roots) and are therefore particularly suitable for fixing HM in the soil (Joner et al. 2000).

The uptake of Pb and its immobilization were found to be higher in roots of mycorrhizal than non-mycorrhizal plants (Chen et al. 2005). Enhanced influx of Pb into plant roots is generally observed upon mycorrhizal colonization. Furthermore, sequestration of Pb in the roots was found to be correlated with an increase in the number of fungal vesicles in highly colonized species. Similar to plant and fungal vacuoles, fungal vesicles may be involved in storing toxic compounds and, thereby, could provide an additional detoxification mechanism. Enhanced metal tolerance of mycorrhizal plants has been frequently observed (Gaur and Adholeya 2004). For example, the *Glomus* isolate Br1 obtained from roots of *Viola calaminaria* grown on HM contaminated soil colonized maize, alfalfa, barley and *V. calaminaria* and allowed each plant species to complete their life cycle on highly HM polluted soil. A common, not HM-adapted, isolate of *G. intraradices* also permitted growth, but to a lower extent, whereas non-colonized plants died on the same soil (Hildebrandt et al. 1999). An explanation for this observation is offered by another study in maize where it has been found that HM are selectively retained in the inner parenchyma cells coinciding with fungal structures (Kaldorf et al. 1999). Since hyphae of HM tolerant AM fungi display a higher affinity to HM than plant cells (Joner et al. 2000) HM possibly become immobilized at or within the fungus. Specific accumulation of HM in colonized tissue may represent the predominant mechanism of AM fungi-mediated intraradical detoxification. This fungus-associated "loss" of HM from plant tissue could also account for the reduced gene expression of HM-inducible plant genes upon mycorrhizal colonization ("dilution effect" Burleigh et al. 2003; Ouziad et al. 2005). Similarly, in pea (Rivera-Becerril et al. 2002), clover (Medina et al. 2005) and ribwort (Hutchinson et al. 2004) Cd is stabilized in the root system of AM plants. Rivera-Becerril et al. (2002) suggested a "mycorrhiza-buffering" of Cd-stress, which the authors attributed to detoxification mechanisms described earlier in this review. Parádi et al. (2003) later assigned this effect to changes in polyamine metabolism. These authors hypothesized that alterations in polyamine content and ratio in AM plants lead to Cd tolerance. Thus, AM symbioses seem to create a more balanced environment that ultimately allows roots to cope with higher HM concentrations possibly by enriching HM at or in fungal structures. On the fungal side induction of oxidative stress-related genes is observed in extraradical mycelium upon HM stress (Ouziad et al. 2005). It remains to be determined whether or not the fungus mounts a protection to oxidative stress also within the intraradical structures.

In clover and maize, increased retention of Zn in the roots of AM plants has been reported in several studies (Chen et al. 2001, 2003; Zhu et al. 2001). However, under Zn limitation, mobilization of Zn and transfer to the shoot is improved by the AM symbiosis (Chen et al. 2003), reflecting the role of Zn as a micronutrient and the beneficial role of the symbiosis on nutrient supply.

These examples illustrate mechanisms by which AM fungi immobilize HM within the soil or within roots and reflect the suitability of AM fungi for phytostabilization applications (Fig. 2). However, it must be emphasized that the system is complex and general predictions are difficult to be made. For example, each contaminated site has a specific profile of pollutants for which an appropriate combination of fungal and plant genotypes must be established. Second, other interactions take place in the soil that could positively or negatively affect the expected efficiency of HM stabilization. However, in summary it can be concluded that indigenous fungi from contaminated soils are most suitable for phytostabilization.

Phytoextraction

Phytoextraction is a rather recent technology and represents the most effective and hence attractive strategy to clean up contaminated soils (Krämer, 2005). It relies on plants with high root-to-shoot transfer accumulating high amounts of HM in their above-ground parts. Alternatively it employs plants producing high biomass with "normal" concentrations of HM (Fig. 2, Salt et al. 1998). Consequently, HM become removed together with the plant upon harvest and can be either recaptured (phytomining), used to produce energy by combustion

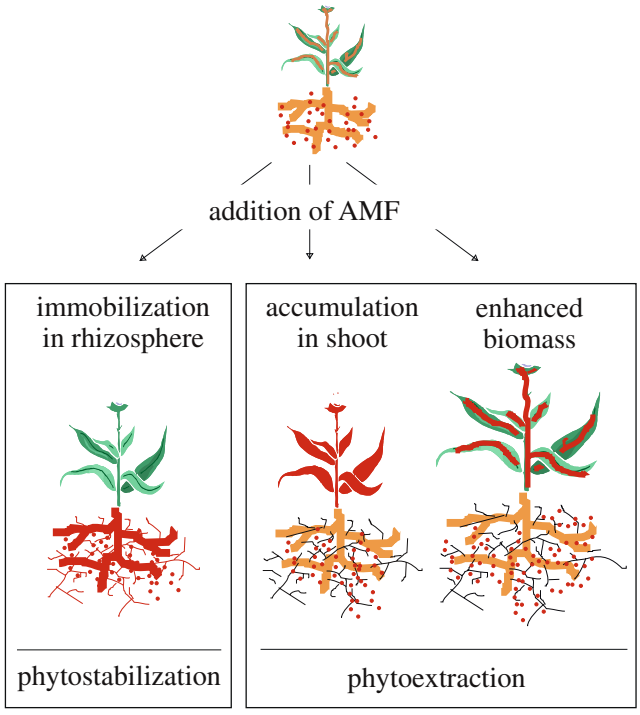

Fig. 2 Contribution of AM fungi to phytoremediation of HM. Top: Non-mycorrhizal plant in HM-polluted soil. *Left panel* Improved stabilization of HM in soil upon mycorrhizal colonization; favored for phytostabilization. *Right panel* Enhanced uptake and transfer of HM to the shoot (*left plant*) and increased biomass of plants resulting from AM-fungi enhanced nutrition leading to increased removal of HM from soil (*right plant*); beneficial for phytoextraction. (*red dots* HM in the soil; orange or red plant or fungal parts: low and high concentrations of HM, respectively)

or stored as low-volume dried material (Krämer 2005; Peuke and Rennenberg 2005). However, the soil clean-up is slow requiring many years to decrease soil contamination by half (McGrath and Zhao 2003). Bioavailability of HM in the soil represents one of the major constraints for rapid phytoremediation. The efficiency of phytoextraction depends on biomass production by the plants and their metal tolerance. Naturally occurring hyperaccumulators can enrich HM by 100- to 1,000-fold relative to non-accumulators without displaying toxicity symptoms (Peuke and Rennenberg 2005 and citations therein). However, the majority of these species produce modest amounts of biomass. Brake fern (*Pteris vittata*) is one plant species suitable for phytoextraction and one variety has been used commercially for As removal from contaminated sites ("edenfern"; http://www.edenspace.com/index.html). Possible ways to accelerate the clean-up process even in non-hyperaccumulators include the addition of chelates resulting in "induced" HM accumulation by plants (Salt et al. 1998). Different synthetic chelates show selective high affinity to certain HM, e.g. EDTA (ethylenediamine tetraacetate) to Pb, enabling chelate-assisted phytoremediation to soils contaminated by different HM. Improved soil remediation can also be achieved by increasing HM acquisition by root cells coupled to boosting transport to the shoot and detoxification within plant cells (Krämer 2005 and citations therein).

It was discovered recently that addition of AM fungi further enhances the uptake and accumulation of As in *P. vittata* (Leung et al. 2006). At the highest As concentration tested (100 mg per kg soil), non-colonized plants accumulated 60.4 mg As per kg while plants colonized by AM fungi isolated from an As mine accumulated 88.1 g As per kg. This was accompanied by enhanced growth, possibly due to improved phosphate (Pi) nutrition reaching 36.3 mg per pot in non-colonized and 257 mg per pot in colonized plants. Both effects combined allow for higher recovery of HM.

Berkheya coddii belongs to the Asteracea family and like *P. vittata* is also recognized as a phytoextraction crop. It is used for phytomining, i.e. the recovery of metals from plant tissues (Salt et al. 1998). The biomass of this Ni-hyperaccumulator was found to be at least twice as high in plants colonized by "adapted" AM fungi than in non-mycorrhizal controls. Furthermore, mycorrhizal plants accumulated 30% more Ni (Turnau and Mesjasz- Przybylowicz 2003). Commercial efforts have already been made towards phytomining of Ni, since this technology is less expensive than classical extraction methods. It would be worthwhile to combine clean-up and recovery assays to minimize costs and maximize recyling of HM. Non-hyperaccumulators can also be used for phytoextraction if they are sufficiently tolerant to HM and produce high biomass. Mycorrhizal tomato plants, for example, had at least 30% higher root and shoot biomass than non-colonized controls at As concentrations up to 75 mg As per kg soil, which coincided with higher Pi uptake (Liu et al., 2005). No effect was observed at higher concentrations. Elevated shoot As concentrations paralleled the increase in biomass. A maximum of 39% (As in shoot/total As) was reached at 75 mg As per kg soil in colonized plants. However, it should be noted that, in general, As was accumulated preferentially in the roots.

In thyme (*Thymus polytrichus*), enhanced biomass was linked to elevated Pi acquisition and Zn concentration within the shoots. This effect was more pronounced in plant cuttings exhibiting low Zn tolerance: 15 mg as against 210.6 mg dry weight (dw) of control and colonized plants, respectively, which corresponded to 140 and 210 mg Zn per kg dw (Whitfield et al. 2004).

As mentioned previously, addition of chelating agents enhances the bioavailability of HM and thus the efficiency of phytoextraction in the absence of the AM symbiosis (Salt et al. 1998). Application of EDTA (or EDDS, ethylene-diaminedisuccinate) had no negative effect on the infectivity of AM fungi (Grcman et al. 2003). Using maize as a model plant Chen et al. (2004) found that addition of EDTA led to phytotoxic concentrations of Zn in the plant reflected by growth retardation. Colonization by AM fungi diminished the phytotoxic effect of higher Zn levels and thereby contributed overall to increased mobilization of Zn from the

soil. In addition, other chelating agents should be tested since each chelate exhibits a different HM-specific efficiency in inducing HM accumulation in the plant (Salt et al. 1998). However, it should be noted that the use of chelates holds the risk of leachates: HM might be mobilized efficiently, but cannot be taken up to the same extent and thus may be washed into the ground water. It is therefore important to adjust the degree of mobilized HM to the uptake capacity of the cultured plants.

These examples illustrate that colonization by AM fungi can lead to increased uptake and subsequent accumulation of HM in above-ground tissues of plants (Fig. 2). It has to be emphasized, however, that in several cases mycorrhizal colonization leads to accumulation of HM in the root as described earlier within this review. Although this scenario could be desirable for enhanced plant HM tolerance, it may interfere with efficient phytoextraction. It is therefore crucial to determine the appropriate conditions (such as plant–fungus combination) for a given contaminated site to maximize the utility of the AM symbiosis.

Concepts for improving phytoremediation by plant engineering

AM fungi are asexual organisms and refractory to transformation. Therefore, genetic or transgenic approaches cannot be undertaken to improve fungal phytoremediation properties. Instead, the focus here must be on the plant. It is important, however, to enhance our knowledge of molecular mechanisms in AM fungi either to effectively employ them for soil remediation or to define fungal genes attractive for introduction into plant backgrounds. For example, during analysis of differentially expressed genes in the presymbiotic versus the symbiotic stage of *Gigaspora margarita*, Lanfranco et al. (2002) identified a fungal metallothionin *GmarMT1*. Metallothionins are ubiquitous proteins that are involved in HM sequestration in plants. In heterologous complementation assays, the *G. margarita* protein conferred resistance to Cd and Cu, suggesting a similar function in AM fungi. *GmarMT1* expression occurs throughout the whole life cycle of the fungus but is higher in presymbiotic than in symbiotic fungal structures. While Cu induced expression of *GmarMT1* in symbiotic mycelia, there was no effect of Cd treatment. The authors suggested that the protein was involved in the HM resistance similar to plant metallothionins. The same screen yielded a gene encoding a functional Cu/Zn superoxide dismutase (SOD) (*GmarCuZnSOD*) (Lanfranco et al. 2005). SODs are metalloproteins that convert superoxide to hydrogen peroxide and molecular oxygen (Fridovich 1995). They act as a primary defense during oxidative stress by protecting cell membranes from damage caused by reactive oxygen species (Natvig et al. 1996). Thus, they might protect AM fungi against oxidative stress resulting from HM exposure. A fungal Zn-transporter has been identified in *G. intraradices* (*GintZnT1*) that belongs to the CDF family (Gonzalez-Guerrero et al. 2005). The gene is upregulated in the extraradical hyphae upon Zn exposure, suggesting a role in Zn homeostasis. The authors suggested that GintZnT1 is involved in Zn efflux and, thus, in protection of *G. intraradices* against Zn stress. The identification of fungal genes with beneficial properties for soil remediation routines when expressed in phytoremediation crops represents an important goal. The choice of the appropriate transgene candidate, host plant and fungal isolate will condition the efficiency of such approaches and some experimental designs might not be applicable as illustrated by a recent publication (Janouskova et al. 2005).

In most of the examples above, enhanced HM uptake by mycorrhizal plants was associated with improved Pi nutrition, a well-known beneficial effect of this symbiosis. Pi transporter genes have been identified in AM fungi and plants. High-affinity Pi transporters that are expressed at the hypha–soil interface during the symbiotic interaction have been cloned from AM fungi (Benedetto et al. 2005; Harrison and van Buuren 1995; Maldonado-Mendoza et al. 2001). Mycorrhiza-induced high-affinity plant Pi transporter genes have also been reported (Glassop et al. 2005; Harrison et al. 2002; Nagy et al. 2005; Paszkowski et al. 2002; Rausch et al. 2001). Since plants take up As as arsenate (AsO_3-) via their Pi transporter systems (Meharg and Macnair 1994), it is likely that such Pi transporters could contribute to As removal from the soil. Exploiting transgenic approaches towards enhanced arsenate shoot concentrations should lead to the production of plant lines with improved As phytoextraction properties. This may simultaneously improve As mobilization, acquisition and "deposition" in above-ground organs. A similar strategy has been suggested previously for the model plant *Arabidopsis* in the absence of an AM symbiosis (Dhankher et al. 2002; Krämer 2005).

As described above, AM fungi can induce the accumulation of HM other than As in host roots. In analogy to symbiotic Pi transport, it may be assumed that also other HM are released to the periarbuscular interface and then taken up by plant-encoded transporters. For example, Turnau and Mesjasz-Przybylowicz (2003) observed dense depositions close to the end of the fine arbuscular branches of mycorrhizal Ni-hyperaccumulating *B. coddii*. Although the nature of these depositions was not clear, they may indicate agglomerations of HM-related substances delivered by the fungus. HM enter plant cells via specific and unspecific transporter systems operating at the plant plasma membrane. Many transporters involved in HM uptake in plants have been identified as members of multigene families (Hall and Williams 2003). The expression of HM transporter genes under the control of a constitutive or even a mycorrhiza-inducible promoter would be attractive for phytoextraction: HM from the soil would be mobilized and transported to the plant via continuous fungal extra- and intracellular structures. Constitutive expression or

induction of HM transporters during the symbiosis could improve translocation to the plant. Powerful candidates are promoters of the symbiotic Pi transporters. These are available from solanaceous (Rausch et al. 2001), leguminous (Harrison et al. 2002) and poaceous (Paszkowski et al. 2002) plant species. The combination of enhanced uptake with enhanced root-to-shoot transport is particularly promising for phytoextraction strategies (as previously already mentioned for As) and could involve homologs of genes such as *AtHMA4* (Verret et al. 2004). Also an appropriate choice of the plant system is critical. Despite important information gained from the model plant *Arabidopsis thaliana*, it is not a host of AM fungi and hence is not suitable in this context. Tomato, bean and maize, for example, are promising non-hyperaccumulators previously used in HM stress experiments (Guo et al. 1996; Liu et al. 2005) that can be transformed and are hosts of AM fungi. The genomes of rice and poplar (both hosts of AM fungi) have been completely sequenced and both offer microarray platforms to identify candidate genes, for example, for HM uptake in the presence or the absence of AM fungi. Furthermore, poplar is already being used in phytoremediation practices because it (a) produces extensive root systems and above-ground biomass, (b) grows rapidly and has an efficient root–shoot transport due to intense water uptake and transpiration rate, and (c) is transformable (Krämer 2005; Peuke and Rennenberg 2005). Since AM fungi can compensate for inefficient plant nutrient and HM uptake, they should be integrated into the design of future soil clean-up strategies with, for example, poplar. Studies of contaminated sites provide fungal isolates highly suitable for phytoremediation. Also, mechanistic data are being obtained in artificial laboratory experiments that might help the design of new strategies. However, such data must be validated in field experiments.

Conclusions

A vast amount of literature is available on the effects of mycorrhizal colonization on plants under HM stress. Until recently, contradictory observations and wide variation in results were reported and are reflected in two recent reviews (Gaur and Adholeya 2004; Khan 2005). It was, therefore, challenging to try to draw general conclusions about the usefulness of AM fungi for soil remediation. However, enhanced understanding of mycorrhizal biology and of the HM tolerance of plants and fungi has defined valuable parameters for improving phytoremediation. One example is the use of adapted indigenous fungal strains that are more suitable for phytostabilization and extraction purposes than laboratory strains. As a result, the number of mycorrhizal publications reporting the successful employment of AM fungi for these two phytoremediation strategies has multiplied over the last 5 years and can be expected to increase further. It was our intention with this review to draw attention to research illustrating the utility of AM fungi in soil remediation (schematically summarized in Fig. 2). It will be important in future to include the AM symbiosis in the design of both research plans and applications, with the ultimate goal of increasing the efficiency of phytoremediation.

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