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Phytoremediaton: An Alternative Approach To Detoxify Heavy Metals With Arbuscular Mycorrhizal Fungi

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Abstarct
Arbuscular mycorrhizal fungi (AMF) are known to improve plant growth on nutrient-poor soils and enhance their uptake of P, Cu, Ni, Pb, and Zn. Many plants growing on metal-contaminated soils possess mycorrhizae, representing that these fungi have evolved a tolerance to heavy metals and that they play an important role in the phytoremediation of contaminated soils. These rhizomicroflora secrete plant growth-promoting substances, siderophores, phytochelators to alleviate metal toxicity, enhance the bioavailability of metals (phytoremediation) and complexation of metals (phytostabilisation). Grasses, due to their fibrous rooting systems, can stabilize soil and provide a large surface area for root-soil contact. With this extraordinary ability, these plants can be used in future environmental remediation activities, so present review highlights the role of AMF-plant interaction in heavy metals uptake, as this model can be a very potent biotechnological tool for the successful reclamation of soil.

Keywords: Arbuscular mycorrhizal fungi, Heavy metals, Medicinal plants, Glomalin

Introduction

Due to continued industrialization and urbanization preservation of environmental quality is the main concern in this century. The increasing industrialization has led to serious environmental problems. A broad variety of chemicals have been detected in soil, water and air (Cheng, S., 2003). Heavy metals cause a critical concern to human health and the environment due to their common occurrence as an contaminant, low solubility in biota and classification of several heavy metals as carcinogenic and mutagenic (Diels *et al*., 2002).

Heavy metal contamination occurs from variety of sources mine tailing, industrial practices, pesticides, car exhaust and sewage sludge treatment used as a fertilizer are major contributors to soil contamination (do Nacimiento et al., 2006; Jacob and Otte, 2004; Liphadzi et al., 2003; Madrid et al., 2003; Yang et al., 2005). It is also likely for metals to enter the environment from natural processes such as weathering of rocks, volcanic activity and continental dusts (Schutzendubel and Poole, 2001).Remediation of soils contaminated with toxic metals is particularly challenging. Unlike organic compounds, metals cannot be degraded, and the cleanup usually requires physical or chemical removal (Lasat, M.M., 2002). Although some of these metals are essential plant micronutrients and are required or are beneficial for plant growth and development (Zn, Cu, Fe, Mn, Ni, Mo, Co), high contents and long-term presence of heavy metals in soils, are generally considered a matter of concern to society as they may adversely affect the quality of soil and water, and compromise sustainable food production (Pandolfini et al.,1997; Keller et al., 2002; Voegelin et al., 2003; Kabata-Pendias and Mukherjee, 2007).

So it had become great demand to remove these heavy metal contaminations from soil as well as water in the era of continued industrialization and urbanization. Conventional soil crop management methods such as increasing the soil pH, draining wet soils and applying phosphate can help prevent the uptake of heavy metals by plants, leaving them in soil and the soil becomes sink of these toxic metals in due course of time (Ammaiyappan Selvam, Jonathan Woon- Chung Wong, 2009).

Phytoremediation

In recent years, phytoaccumulation/phytoextraction, i.e., the use of plants to clean up soils contaminated with non-volatile hydrocarbons and immobile inorganic 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 (Kling, 1997; Kumar et al., 1995). In the case of non-degradable pollutants such as heavy metals and metalloids, the precise terms covering the involved aspects of phytoremediation are rhizofiltration (metals in water), phytoextraction (metals in soil), phytovolatilization (metals that may be volatilized: e.g. Se and Hg) and phytostabilization (control of spread by erosion or leaching). When organic, biodegradable pollutants are the target, phytoremediation may comprise rhizodegradation (microbial degradation in the rhizosphere), phytodegradation (degradation of compounds absorbed by the plant), and hydraulic control (limiting the spread of a plume in soil by plant evapotranspiration) (EPA, 2000; Flathman et al., 1998).

A promising, relatively new technology for heavy metal contaminated sites is phytoremediation. Phytoremediation is the use of plants to remove organic and/ or inorganic contaminants from soil (phytoextraction), uptake and conversion into non-toxic forms (phytovolatilization), or stabilization of an inorganic into a less soluble form phytostabilization) (Reda AbouShanab *et al*., 2007).

With these extraordinary abilities, plants have been proved to be a very useful tool in the reclamation of the environment. However these are not only plants alone which have role in phytoremediation as there is always a close interaction between the microorganisms in the rhizosphere and the plant which leads to an increased activity related to soil remediation (Compant et al., 2010). Hence application of hyper accumulating plants in combination with a beneficial rhizo- and/or endo-spheric microbial community holds great promise for low cost & time effective tool for the reclamation of contaminated sites.

AMF are ubiquitous soil microflora and constitute an important functional component of the rhizosphere. This symbiotic interaction between plants and AMF is directly beneficial to the host plant's growth and development due to the acquisition of phosphorous and other mineral nutrients from the soil by the fungus. Arbuscular mycorrhizal fungi (AMF) are known to occur in a wide variety of ecosystems, including many stressfulenvironments (Cook *et al.*, 1993; Bhardwaj *et al.*, 1997; Barrow *et al.*, 1997; Bhaskaran and Selvaraj, 1997). So this stress tolerance capability of AMF also enhances the plant's resistance to biotic and abiotic stresses, when grown with AMF (Harrier and Sawczak, 2000). AMF protect the roots of plants from heavy metal toxicity by mediating interactions between metals and plant roots (Marschner H., 1995; Leyval C et al., 1997).

Recently, plants capable of forming association with AM fungi have been shown to accumulate a considerable amount of trace metals (Burke *et al*., 2000; Karagiannidis and Nikolaou, 2000). Nowadays, it has been proved that improvement of the interactions between beneficial rhizosphere microorganisms and plants can significantly lower the heavy metals stress on plants, increase the availability of metal for plant uptake and therefore are considered to be an important tool for phytoremediation technology (Glick, 2003, 2010). For example, AMF, by forming extra-radical mycelial networks, could enhance uptake of nutrient elements as well as water by host plants and protect the host plants against heavy metals toxicity (Leyval et al., 1997).

The perennial grasses are metal-tolerant plants (Rosselli W. et al., 2003).They are characterized like high dry matter yields producer, as well as high accumulators for heavy metals (Pichtel J., Salt C.A. 1998) and well reducer of metals toxicity in the soil (Schnoor J.L. *et al*., 2007). With this extraordinary ability, these plants can be used in future environmental remediation activities, however full scale applications have yet to achieve. One important reason for this lies with lack of knowledge on the biological processes involved in metal acquisition, transport and shoot accumulation. The uptake of heavy metals by mycorrhizal plants depends on several factors such as physico-chemical properties of the soil, particularly its fertility level and pH; the host plants and the fungi involved; and, above all, the concentration of the metals in the soil (Smith & Read 1997).

Role of arbuscular mycorrhizae in phytoremediation

AMF are among the most common soil microorganisms and constitute an important functional component of the soil-plant system occurring in almost all habitats and climates. More specifically, it has been shown that AMF can be affected by heavy metal toxicity, but in many cases mycotrophic plants growing in soils contaminated with heavy metals are colonized by AMF (Leyval et al., 1997). The influence of AMF on metal plant uptake depends on many factors such: “fungal genotype, uptake of metal by plant *via* AM symbiosis, root length density, competition between AMF communities, the rhizosphere (pH, CEC, etc.), the metal itself, concentrations of available metals, soil contamination conditions contaminated or artificially contaminated vs noncontaminated soil, interactions between P and metals (addition of P fertilizers), experimental conditions (light intensity, plant growth stage, available N and P), litter inputs, plant species and plant size” (Giasson et al., 2008). According to Gadd (1993), both live and dead components of the fungal cell wall can be involved in HM binding with help of free amino, hydroxyl, carboxyl and other groups. AMF form extraradical mycelium and intraradical hyphae that penetrate the intercellular spaces and enter cortical root cells. In the case of reduction of HM uptake, an important role in retention, binding and immobilization seems to be associated to *fungal vacuoles*. They are involved in the regulation of cytosolic metal ion concentrations and the detoxification of potentially toxic metal ions. The fungal cell wall, respectively chitin and glomalin from the fungal wall (Christie et al. 2004), are also important due to the presence of free amino, hydroxyl, carboxyl and other functional groups (Gadd, 1993).

Many reports concerning this have quantified spores and estimated root colonization *in situ*. Others have gone further and described metal tolerant AMF in heavy metal polluted soils (Del Val et al., 1999; Weissenhorn and Leyval, 1995). Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition (Figure 3). 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 acquisition 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 (Chaudhry et al., 1998; Shetty et al., 1995) 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. Also, Gali 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.

Particular effects of AM fungi on different Heavy Metals

The alleviation of Zn toxicity towards plants by using AMF was reported in Christie et al. (2004) and Chen et al. (2004), and this phenomenon was shown to be dependent on direct and indirect mechanisms. As an example for a direct mechanism, Zn was bound in mycorrhizal structures and immobilized in mycorrhizosphere, while for an indirect effect, an influence of mycorrhiza on the plant's mineral nutrition, especially for P, lead to increased plant growth and enhanced metal tolerance. The mobility of Zn is greatly affected by the changes in soil pH. The Zn immobilization through the fungal activity might be an effect of these changes, contributing to the inhibition of Zn uptake into the mycorrhizal plant by storage in the arbuscles, but also in hyphae (Christie et al., 2004). In highly contaminated soil, Zn was found in higher concentration in roots while a decrease in the shoots was seen as effect of AMF. When Zn amounts in soil increased, a critical threshold exists, below which Zn uptake is enhanced, while above this level Zn translocation to the aboveground parts of host plants is inhibited. In some plant species, higher translocation rates may occur, but at the cost of poor plant biomass development and probable early death of the individuals (Chen et al., 2005). 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 related by Rufyikiri at al. (2004) demonstrated that the mobility of **U** in soil depends on the organic compound content, the bioavailability being highly dependent on soil pH. The same author found that the most mobile U forms are U(VI) salts, predominantly as UO22+ and carbonate complexes, while other forms are less bioavailable and remain bound to soil particles. The role of AM fungi in translocating U as uranyl cations to roots through fungal tissues is related to fungal mycelium HM binding capacity (Chen et al., 2005). Chen *et al.* (2005 cited by Babula at al., 2008) performed and confirmed such studies using *Medicago trunculata* as a model plant, inoculated with *Glomus intraradices.* They found higher concentrations of U in roots than in shoots of mycorrhizal plant, suggesting that the AM fungus has a potential to reduce the translocation of U from roots to shoots. Yu et al. (2010) reported that in the case of Hg**,** the uptake is lower by mycorrhyzal than by nonmycorrhyzal roots of maize, and AMF inoculation significantly decreased the total and extractable Hg concentrations in soil as well as the ratio of extractable to total Hg. Calculating mass balances for Hg in soil indicated a loss of Hg which can be attributed to Hg volatilization as a result of AMF influence. No significant difference of Hg concentrations was found between mycorrhizal and nonmycorrhizal shoots of maize which suggest that contribution of root uptake to shoot accumulation of Hg is very limited. The release of Hg into soil gases or into the atmosphere is a result of methylation (CH3Hg+), which leads to phytovolatilization, seen also with As and other metalloids. Some research has been carried out on Cs, with, e.g., Leyval et al. (2002) reporting that 134Cs radioactivity increased twofold in leaf tissue of *Paspalum notatum* in symbiosis with AMF while, in the case of mycorrhizal *Mellilotus officinalis* 1.7 to 2 times increased 137Cs was found. *Sorgum Sudanese* revealed only insignificantly increase. A significant decrease of 137Cs in mycorrhyzal *Festuca ovina* and *Agrostis tenuis* was found; this finding underlining that soil fungi represent a potential for Cs immobilization. On the other hand, Rosén et al. (2005) working with mycorrhizal ryegrass and leek found an enhanced 137Cs uptake by leek, but no effect on the uptake by ryegrass Similar studies were performed on mycorrhizal *Festuca ovina* in which shoots showed higher 137Cs concentration than roots, as well as on *Trifolium repens,* and AM plants took up less Cs with no increase in translocation of 137Cs to the shoots being found. In conclusion, AMF seems to play a role, with regard of both immobilization and phytoextraction being represented depending on plant species. Specifically grasses seem to respond with decreased uptake into shoot biomass.

Effect of heavy metals contamination on AMF

Microorganisms in the soil are responsible for nitrogen fixation, assimilation, and degradation of organic residues to release nutrients (Baath, 1989; Brookes, 1995). When heavy metals are retained in the soil by repeated and uncontrolled additions, they interfere with these key biochemical processes which alter ecological balance.AMF occur in almost all habitats and climates (Barea J. M. et al.,1997), including in disturbed soils such as those derived from mine activities (Bundrett M. C et al.,1996), but soil degradation usually produces changes in the diversity and abundance of AMF populations (Jasper D. A. et al., 1991; Koomen I. et al., 1990; Loth C.1996). Gildon and Tinker (1981) reported that high amounts of heavy metals can delay, reduce or even completely eliminate AMF spore germination and AM colonization at concentrations at which phyto-toxic effects were not observed. High concentrations of heavy metals in soil have an adverse effect on micro-organisms and microbial processes due to their toxicity for living organisms. Toxic effects of Heavy metals on microorganisms manifests in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling (Baath, 1989), impaired enzyme synthesis and activity in soil, sediments and water. Among soil microorganisms, mycorrhizal fungi are the only ones providing a direct link between soil and roots, and can therefore be of great importance in heavy metal availability and toxicity to plants (Leyval C et al., 1997).

Heavy metals can delay, reduce, and even completely eliminate AM colonization and AMF spore germination in the field and a negative correlation between Zn concentrations and AM colonization has been reported in soil treated with urban-industrial sludge. There is evidence that heavy metals affect mycorrhizae (Chen X et al., 2005). Mycorrhizal infection rate of maize (Zea mays L) was reduced by the addition of heavy metals including Zn, Cu, Ni, Cr, Pb, and Cd (Chao CC et al., 1990).

In other studies, however, the addition of metal-containing sludge did not significantly affect AM development under field conditions, probably because different AMF ecotypes can exhibit different degrees of metal tolerance. Thus, a relatively high rate of mycorrhizal colonization can be found in plants growing in much polluted soils. A higher tolerance to Cu, Zn, Cd, and Pb of indigenous fungi from sludge-polluted sites in comparison to those of reference isolates from unpolluted soils has been described previously.

Chao and Wang (1990)found that mycorrhizal infection rate of maize (Zea mays) was reduced by the addition of heavy metal (Zn, Cu, Ni, Cr, Pb and Cd).Del Val et al. (1999)reported that mycorrhizal colonization and growth of external hyphae were inhibited by sewage sludge-contaminated soil containing Zn, Cd and Pb. Chao and Wang (1990)found that mycorrhizal infection rate of maize (Zea mays) was reduced by the addition of heavy metal (Zn, Cu, Ni, Cr, Pb and Cd).Del Val et al. (1999) reported that mycorrhizal colonization and growth of external hyphae were inhibited by sewage sludge-contaminated soil containing Zn, Cd and Pb. However, Tonin et al. (2001) found that a polluted soil with Cd and Zn enhanced mycorrhizal diversity index of roots of clover (Trifoliumrepens).Whitfield et al. (2004) showed that the metal contaminated soil with Cd, Pb and Zn enhanced mycorrhizal vesicular numbers of Thymus polytrichus.

Glomalin and heavy metal sequestration

Heavy metals in soil are associated with a number of soil components which determine their behavior in the soil and influence their bioavailability (Boruvka and Drabek, 2004). The cell wall components such as free amino, hydroxyl, carboxyl and other groups of soil fungi can bind to potentially toxic elements such as Cu, Pb, Cd, etc. (Kapoor and Viraraghavan, 1995). Many filamentous fungi can absorb these trace elements and are used in their commercial biosorbants (Morley and Gadd, 1995). The proteins in the cell walls of AM fungi appear to have similar ability to absorb potentially toxic elements by sequestering them. There is evidence that AMF can withstand potentially toxic elements. Gonzalez-Chavez et al.(2004) showed that glomalin produced on hyphae of AMF can sequester them. AMF play a significant ecological role in the phytostabilization of potentially toxic trace element polluted soils by sequestration and, in turn, help mycorrhizal plants survive in polluted soils.

One of these components is Glomalin, a glycoprotrien produced by the hyphae of AMF fungi (Wright and Upadhyaya, 1998), which is released into soil from AMF hyphae (Driver et al., 2005). These authors, using an in vitro system of Ri T-DNA transformed roots infected with Glomus intraradices, an AMF, showed that glomalin is tightly bound in AMF hyphal and spore walls. Small amounts (<20%) of glomalin were found to be adhered to soil via release into liquid medium from hyphae and not through passive secretion and their function is physiological in the course of the life of the organism. It has been hypothesized that glomalin has a role in the immobilization ('filtering') of heavy metals at the soil-hypha interface, i.e. before entry into fungal-plant system.

The extra-radical mycelium of AMF, in addition to its crucial role in enhancing nutrition of host plant, also plays a role in soil particle aggregation and soil stability (Dodd et al., 2000; Wright and Upadhyaya, 1998). There have been few analytical studies of AM in polluted soils. While some workers observed that the external mycelium of AMF was the main site for trace element localization (Kaldorf et al., 1999; Turnau, 1998), others reported selective exclusion of toxic and non-toxic elements by adsorption onto chitinous cell walls (Zhou, 1999), or onto extra-cellular glycoprotein, glomalin (Wright and Upadhyaya, 1998), or intra-cellular precipitation. All these mechanisms have implications in reducing a plant's exposure to potentially toxic elements, i.e. mycorrhizoremediation technology. Gonzalez-Chavez et al.(2002) studied the form and localization of Cu accumulation in the extra-radical mycelium of three AM fungi isolated from the same polluted soil contaminated with Cu and As. The authors reported differential capacity of AMF to absorb and accumulate Cu as determined by TEM and SEM. However, the nature of accumulation and mechanisms involved require further studies in order to better understand the participation of AMF in plant tolerance and its ecological significance in polluted soils.

Extracellular Heavy Metal Inactivation Mechanisms

Different mechanisms of heavy metal exclusion by mycorrhizal fungi have been suggested, among them extracellular chelation, cell wall binding and heavy metal accumulation in extraradical mycelium (Colpaert et al. 2011). Mycorrhizas can inactivate heavy metals through the exudation of complexing agents into the soil solution. According to Meharg (2003), organic acid exudation has a clear role in mycorrhizal adaptation to metal-contaminated sites. Citric, malic and oxalic acids are known to be produced by mycorrhizal fungi (Ahonen-Jonnarth et al. 2000; Meharg 2003); they can mobilize or immobilize metals by complexation, depending on various factors, especially rhizosphere pH (Gimmler et al. 2001; Hinsinger et al. 2009). Phenolic compounds produced by ECM are also involved in metal immobilization in soil (Schu¨tzendu¨bel and Polle 2002). Machuka et al. (2007) highlighted different metal-chelating compounds in in vitro culture of ECM fungi collected from pine plantations (species of Scleroderma, Suillus and Rhizopogon). Oxalic, citric and succinic acids but also hydroxamate- and catecholate-type compounds were found in the liquid medium. Cabala et al. (2009) reported the presence in the rhizosphere of different AM and ECM mycorrhizal plants of metal-bearing aggregates formed during symbiotic action between mycorrhizas and bacteria. These structures enhanced the binding of Zn, Pb and Mn in the rhizosphere.

More recently, different studies showed the role of glomalin, a very abundant AM fungal glycoprotein released into the soil where it participates in soil aggregation. Glomalin seems to be involved in heavy metal inactivation in soil (Ferrol et al. 2009; Gamalero et al. 2009). Glomalin extracted from polluted soil or from hyphae irreversibly sequesters metals such as Cu, Cd, Zn and As (Gonzalez-Chavez et al. 2002). Cornejo et al. (2008) showed that a glomalin-related soil protein was more abundant in polluted soils with high concentrations of Cu and Zn. Up to 27 % of the total Cu was bound by this protein, and in a highly polluted soil, with a low pH, up to 90 % of the soil organic carbon was represented by the glomalin-related protein. Similar results were obtained by Vodnik et al. (2008) for the sequestration of Pb and Zn Heavy Metal Binding in Fungal Wall Some of the metals inactivated in mycorrhizal plants are retained by fungal walls. Joner et al. (2000) exposed extraradical mycelium of different Glomus spp. isolates to high concentrations of Cd and Zn and measured their capacities to bind these metals. The most tolerant isolate adsorbed more metals than the others. The fungal wall was responsive for 50 % of the metal retained. Orlowska et al. (2008), analysing the elemental distribution in mycorrhizal plants of the Ni-hyperaccumulator Berkheya coddii, also reported a high binding capacity of the extraradical mycelium for Zn, Cu and Ni. Using EDXS analyses, with 248 H. Amir et al. monoxenic cultures of G. intraradices, Gonzalez-Guerrero et al. (2008) showed that Cu, Zn and Cd at toxic concentrations were partly localized in the fungal cell wall. Marques et al. (2007) and Zhang et al. (2009) reported that Zn and Cu were mainly deposited in the cell wall of the root cortex of the mycorrhizal plants, including the AM fungal wall. Several cell wall-binding molecules have been reported, such as glucan, chitin and galactosamine polymers, minor peptides and proteins, all presenting potential binding sites as free carboxyl, amino, hydroxyl, phosphate and mercapto groups (Bellion et al. 2006). Glomalin is also partly located at the AM fungal wall (Purin and Rillig 2008). In ECM, Cd and Zn are predominantly bound in cell wall of mantle hyphae, Hartig net hyphae and cortical cells (Meharg 2003).

Tolerance and adaptation of AMF to heavy metals

The number of spores and subsequently root colonization of host plants are often reduced by soil disturbance (Waal and Allen, 1987). However, AMF species adapted to local soil conditions could be able to stimulate plant growth better than non-indigenous species. Indigenous AMF ecotypes result from long-term adaptation to soils with extreme properties (Sylvia and Williams, 1992; Bae et al., 2003; Rahmanian et al., 2011).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 Adholeya2004).

The effect of heavy metal exposure on presymbiotic functioning of AM fungi can be quantified easily and can potentially be used to predict the functioning of AM symbiosis under metal stress, so considerable attention has been devoted to the impact of elevated metal concentrations on spore germination. Spore germination is highly sensitive to metal stress in AM fungi derived from low-metal habitats, while ecotypes from metal-enriched environments have higher levels of metal tolerance.This may be explained due to the fact that different AMF ecotypes can exhibit varying degrees of tolerance to metals (Hasel wandter et al., 1994). A higher tolerance to Cu, Zn, Cd and Pb of indigenous fungi from sludge-polluted sites, in comparison to reference isolates from unpolluted soils, has been reported (Gildon and Tinker, 1983; Weissenhorn et al., 1993; Diaz et al., 1996),Studies by various researchers (Galli et al., 1994; Hetricket 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.

Effect of AMF on phyto-remediating activity of grasses

The perennial grasses are metal-tolerant plants (Rosselli W., Keller C., Boschi K.,2003), and are studied frequently in the literature (MarseilleF. *et al.,* 2000;Palazzo A.J. *et al*., 2003; Caggiano R. *et al*., 2005; Bidar G., Garçon G. *et al.,* 2007).They are characterized like high dry matter yields producer, as well as high accumulators for heavy metals (Pichtel J., Salt C.A., 1998) and well reducer of metals toxicity in the soil (Schnoor J.L. *et al*., 2005).

In a study on Dactyloctenium aegyptium it was found that the elevated concentration of metals in the roots and low translocation to the above ground parts of the grass indicated their suitability for phytostabilization. Phytostabilization is a process which depends on roots ability to limit the contaminant mobility and bioavailability in the soils which occurs through the sorption, precipitation, complexation or metal valance reduction (Ghosh & Singh, 2005).Their thick growth habit makes it ideal for providing a dense mat on the soil surface which can prevent erosion and at the same time remove heavy metals from the soil. It spreads by both tillering and seedling which makes its establishment easy. Grasses are therefore more preferable in use for phytoaccumulation than shrubs or trees because of their high growth rate, more adaptability to stress environment and high biomass production.(Garba et. al., 2012).

Wild grasses can be suitable to reduce metal pollution in contaminated soils (McLaughlin et al., 2000). Cynodondactylon has been found to be hyper accumulator for many heavy metals (Leung et al., 2006).

Many researches showed that Bermuda grass (Cynodondactylon) as a widespread creeping grass could be useful for stabilizing spill-affected soils (Smith et al., 1998; Madejon et al., 2002). Begonia et al. (2005) reported that tall fescue was relatively tolerant to moderate levels of Pb in soil bynon-significant differences in root and shoot biomass. Tall fescue was identified as a potential phytoextraction species because of its high biomass yield under elevated Pb levels and its ability to translocate high amount of Pb from roots to shoots (Begoniaet al., 2001).

Effect of AMF on phyto-remediating activity of Medicinal plant

In order to study the effect of mycorrhizal fungi (inoculated and non-inoculated) and heavy metals stress [0, Pb (150 and 300 mg/kg) and Cd (40 and 80 mg/kg)] on pot marigold *(Calendula officinalis L.),* a factorial experiment was conducted based on a randomized complete block design with 4 replications in Research Greenhouse of Department of Horticultural Sciences, University of Tehran, Iran, during 2012–2013. Plant height, herbal and flower fresh and dry weight, root fresh and dry weight and root volume, colonization percentage, total petal extract, total petal flavonoids, root and shoot P and K uptakes, and Pb and Cd accumulations in root and shoot were measured. Results indicated that with increasing soil Pb and Cd concentration, growth and yield of pot marigold was reduced significantly; Cd had greater negative impacts than Pb. However, mycorrhizal fungi alleviated these impacts by improving plant growth and yield. Pot marigold concentrated high amounts of Pb and especially Cd in its roots and shoots; mycorrhizal plants had a greater accumulation of these metals, so that those under 80 mg/kg Cd soil^{-1} accumulated 833.3 and 1585.8 mg Cd in their shoots and roots, respectively. In conclusion, mycorrhizal fungi can improve not only growth and yield of pot marigold in heavy metal stressed condition, but also phytoremediation performance by increasing heavy metals accumulation in the plant organs.

Contribution of AM fungi to Uptake of Heavy Metals

AM fungi supply plants with essential nutrients from the soil through uptake by extraradical hyphae. Toxic elements like Cd may also be transported by hyphae, but the fungus may constitute a biological barrier against transfer of heavy metals to shoots. Thus, there are different effects of AM fungi on heavy-metal uptake. In some cases, AM fungi reduce excess plant uptake of trace elements like Zn, Cd and Mn , whereas in other cases they enhance or have no effect on the uptake. Kaldorf M. *et al*., 1999 [85] showed that maize grown in two different heavy-metal soils contained lower metal concentration(including Pb) in roots and shoots when colonized with heavy metal tolerant *Glomus* isolate, compared to plants grown with common *Glomus* isolate. Diaz G. *et al*., 1996.

Table 1. Summary of AM fungi-heavy metal interactions and application in phytoremediation

S.No.	Heavy metal	Tolerant fungi	Host used	Reference
1	Cd	*Glomus mosseae*	*Trifolium repens*	Vivas et al 2003
2	Cd	*Glomus* sp. And *Gigaspora* sp.	*Hordeum vulgare*	Tullio et al 2003
3	Cd	*G. mosseae*	*Trifolium subterraneum*	Joner et al 1997
4	Cd	*G. mosseae*	*Allium porrum*	Weissenhorn et al 1993
5	Ni	*Gigaspora* sp. And *Glomus tenue*	*Berkheya coddii*	Turnau et al 2003
6	Zn	*Glomus* sp.	*Viola calaminaria*	Kaldorf et al 1999, Tonin et al 2001
7	Zn	*Glomus fasiculatum*	*Festuca rubra*	Dueck, et al 1986
8	Pb	*Glomus intraradices*	*A. capillaris, Zea mays*	Malcova et al 2003
9	Cd, Cu	*Glomus caledonium*	*Z. mays*	Liao et al 2003
10	Zn, Pb	*G. mosseae, Glomus macrocarpum*	*Lygeum spartum*	Diaz et al 1996
11	Cd, Zn	*G. mosseae*	*T. subterraneum*	Joner et al 2000
12	Cd, Zn, Pb	AM fungi	*Biscutella laevigata*	Orlowska et al 2002
13	Cd, Zn, Cu	*G. mosseae*	*T. subterraneum*	Joner et al 2001
14	Zn, Cd, Cu, Ni, Pb	*G. caledonium*	*Sorghum bicolor*	Del et al 1999

Mechanism of the mycorrhizal extraction of heavy metals from soil

Mycorrhiza is a versatile beneficial relationship. The most popular root-fungus association is Vesicular Arbuscular mycorrhizal association also known as glomeromycota association (Read, 1997, Burdett, 2002). Mycorrhiza term was first used by Frank (1885), who claimed that plant fungus relationship is essential for survival of both and absorption of substance from soil takes place, through this relationship (Harley and Smith, 1983). Because of this intimate relationship plant and fungal association is very different from other associations (Nehls *et al*., 2001, Pfeffer and Bago *et al*., 2001). The VAM fungi is benefited from this association as they get the nutrients and in turn they increase the phosphorus and trace metal uptake by plants (Burgus *et al*., 1983, Jasper *et al*., 1988) by Electrochemical potential gradient (–120 and –180 mV). The amount of spare metals in the soil is considered as bio-available fraction of metal. To understand the process of metal uptake through the plant the studies were carried out on the process of uptake of iron. In these experiments two processes were identified: in first process iron chelates Fez^3 were formed which were reduced to Fez^2 by the reductant (Chaney *et al*., 1972, Brown and Ambler, 1973, Olsen and Brown, 1980, Welch *et al*., 1983). In second process some acids such as mugeneic acids and avenic acids were used as reluctant which are known as phytometallophores (Kochian, 1993, Fan *et al*., 1997). \

The release of phytometallophore is generally related to deficiency as compared to sufficiency. Commonly VAM fungi occur in rhizosphere but some may also be present in stem and thallus (Smith and Read, 1997, Read *et al*., 2000). As Arbuscular mycorrhiza increases the nutritional status of the host, in the same way fungal hyphae absorbs heavy metals, and transfer it to the plant. Therefore on one hand mycorrhizal plant express increased heavy metal uptake and on other hand VAM fungi helps in immobilization of heavy metals in soil. Therefore the cleanup of the ecosystem depends on the Heavy Metals- Plant- Mycorrhizal relationship through the removal of heavy metal from the soil. During stress condition i.e. increased concentration of toxic heavy metals plants employs various mechanisms to maintain the metal-ion equilibrium internally as well as in their surrounding environment (Clemens, 2001, Hall, 2002). In this mechanism various types of genes are also involved which helps in accomplishing the mechanism of remediation of heavy toxic metals which in turn helps in detoxification of heavy metals from contaminated soil (Thomine *et al*., 2000, Hirayame *et al*., 1999 Gravot *et al*., 2004, Himelblau and Amasino, 2000). The main purpose of detoxification is: chelation of toxic.

PROCESS OF DETOXIFICATION IN VAM CELL

In the case of fungal cell wall a different protein called glomalin is secreted by the cells of fungi to form a complex with heavy toxic metals which helps in its binding to plant cell wall. In fungal cells plasma membrane is the selective barrier as a result of which active as well as passive transport takes place by the specific and non specific metal transporters and also from the pores of the plasma membrane. After seizure of heavy metals in the fungal cell wall, the heavy metals are transported in the hyphae of the fungus. Intracellularly the cells of the plants secreting chelating agents like phytochelatins and metallothinein have high affinity for heavy metals. They also secrete organic acids, amino acids and specific metal chaperons.

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