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Review Article

ARBUSCULAR MYCORRHIZAL FUNGI: A POTENTIAL BIOTECHNOLOGICAL TOOL FOR PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOILS

Satish A. Bhalerao
Environmental Sciences Research Laboratory, Department of Botany, Wilson College, Mumbai-400 007

ABSTRACT

High concentrations of heavy metals 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 is the direct use of living plants for in situ remediation of metal contaminated soil, sludges, sediments and ground water through contaminant removal, degradation or containment. It has gained popularity as an emerging clean up technology for environmental restoration during the last decade due to its convenience and low costs of installation and maintenance. However, as phytoremediation is a slow process, improvement of efficiency and thus increased stabilization or removal of heavy metals from soils is an important goal. Arbuscular mycorrhizal (AM) Fungi provide an attractive system to advance plant based environmental clean-up. AM associations are integral functioning parts of plant roots and are widely recognized as enhancing plant growth on severely disturbed sites, including those contaminated with heavy metals. They are reported to play an important role in metal tolerance and accumulation. Isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems. This review highlights the potential of AM fungi for enhancing phytoremediation of heavy metal contaminated soils.

KEYWORDS: Heavy metals, Phytoremediation, Arbuscular mycorrhizal fungi, Metal tolerance, Restoration *etc.*

INTRODUCTION

Soil contains numerous components and metals are one of them. Heavy Metals (HM) are grouped into one category of 53 elements with specific weight higher than 5g/cm^3 [1]. 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 [2]. Conversely, metals like Cd, Pb, Hg, and as are not essential [3]. Human activities such as mining and smelting of metals, electroplating, gas exhaust, energy and fuel production, fertilizer, sewage and pesticide application, municipal waste generation, etc. [4] have led to metal pollution become one of the most severe environmental problems today. Excessive accumulation of heavy metals is toxic to most plants. Heavy metals ions, when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduced growth [5,6]. At high concentrations, HM also interfere with essential enzymatic activities by modifying protein structure or by replacing a vital element resulting in deficiency symptoms [7]. Contamination of heavy metals in water and soil poses a major environmental and human health hazard on the other excessive metal concentrations in contaminated soil results in decreased, soil microbial activity and soil fertility leading to yield losses [8]. In soils they occurs as free metal ions, exchangeable metal ions, soluble metal complexes (sequestered to legends), organically bound metals, precipitated or insoluble compounds such as oxides, carbonate and hydroxides or they may form part of the silicate structure (indigenous soil content) [9]. Since HM are not biodegradable and may enter the food chain, they are a long-term threat to both the environment and human health [10]. Heavy metals are only transformed from one oxidation state or organic complex to another [11], therefore remediation of heavy metal contamination in soils is more difficult. Conventional methods used for their remediation such as excavation and land fill, thermal treatment, acid leaching and electro reclamation are not suitable for practical applications, because of their high cost, low efficiency, large destruction of soil structure and fertility and high dependence on the contaminants of concern, soil properties, site conditions and so on [12]. Furthermore translocation of HM polluted soil instead of solving the problem rather shifts it to upcoming generations. In contrast plants offer an inexpensive and sustainable on-site approach [13, 14]. Plants have a natural propensity to take up metals. 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) [7].

Phytoremediation, the use of plants for environmental restoration, is an emerging cleanup technology. To exploit plant potential to remediate soil and water contaminated with a variety of compounds, several technological subsets have been proposed. Phytoextraction is the use of higher plants to remove inorganic contaminants, primarily metals, from polluted soil [15]. In the approach, plants capable of accumulating high levels of metals are grown in contaminated soil. At maturity, metal-enriched aboveground biomass is harvested and a fraction of soil metal contamination removed.

Arbuscular Mycorrhizal (AM) fungi are soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing a direct physical link between soil and plant roots [16]. About 95% of the world's plant species belong to characteristically mycorrhizal families [17] and potentially benefit from AM fungus-mediated mineral nutrition due to the fundamental role played by these glomalean fungi in biogeochemical element cycling [18]. AM symbiosis occurs in almost all habitats and climates [19], including disturbed soils [20] and those derived from mine activities [21, 22, 23]. By acquiring and delivering phosphate, micronutrients and water to their hosts they enhance the nutritional state of their hosts. AM fungi can improve soil texture by binding soil particles into stable aggregates that resist wind and water erosion [24, 25]. 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) [7].

The result of mycorrhizal colonization on clean-up of contaminated soils depends on the plant–fungus–HM combination and is influenced by soil conditions. Mycorrhizal fungi are of great importance in phytoremediation-potentially enhancing heavy metals availability and plant tolerance [26]. The fungi can accelerate the revegetation of severely degraded lands such as coal mines or waste sites containing high levels of heavy metals [27, 28]. Even if AM fungi are ubiquitous in terrestrial ecosystems, mechanical or chemical disturbance of the soil can substantially reduce AM fungal population vigour and functioning [29]. The number of spores and root colonization of plants occurring at sites are often reduced by soil disturbance [30]. However, AM fungal isolates adapted to local soil conditions can stimulate plant growth better than non-indigenous isolates. Indigenous AM fungal ecotypes result from long-term adaptation to soils with extreme properties [29].Therefore, isolation of indigenous stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants in disturbed ecosystems [31]. This review highlights the potential of AM fungi for enhancing phytoremediation of heavy metal contaminated soils.

Significance of AM Fungi

AM associations are important in natural and managed ecosystems due to their nutritional and non-nutritional benefits to their symbiotic partners. They can alter plant productivity, because AMF can act as biofertilizers, bioprotectants, or biodegraders [32]. AMF are known to improve plant growth and health by improving mineral nutrition, or increasing resistance or tolerance to biotic and abiotic stresses [33,34]. Their potential role in phytoremediation of heavy metal contaminated soils and water is also becoming evident [35, 36, 38]. AMF modify the quality and abundance of rhizosphere microflora and alter overall rhizosphere microbial activity. Following host root colonization, the AMF induces changes in the host root exudation pattern, which alters the microbial equilibrium in the mycorrhizosphere [38]. These interactions can be beneficial or harmful to the partner microbes involved and to the plant, and sometimes may enhance plant growth, health, and productivity [39]. Giovannetti M. and Avio L., 2002 [40] reviewed and analysed important data on the main parameters affecting AM fungal infectivity, efficiency, and ability to survive, multiply and spread, which may help in utilizing obligate biotrophic AMF in biotechnological exploitation and sustainable agriculture. There is a need to understand and better exploit AM symbionts in the different world ecosystems.

Although AMF are ubiquitous, it is probable that natural AM associations are not efficient in increasing plant growth [41]. Cropping sequences as well as fertilization and plant pathogen management practices also dramatically affect the AMF propagules in the soil and their effects on plants [42]. The propagation system used for horticultural fruit and micro-propagated plants, can benefit most from AM biotechnology. Micropropagated plants can withstand transplant stress from in vitro to in vivo systems, if they are inoculated with appropriate AMF [43]. In order to use AMF in sustainable agriculture, knowledge of the factors such as fertilizer inputs, pesticide use, soil management practices, etc. influencing AMF communities is essential [42,44]. This area deserves further research and efforts because sound scientific knowledge is necessary for the improvement of AM biotechnology aimed at selecting infective and efficient inoculants to be used as biofertilizers, bioprotectants, and biostimulants in sustainable agriculture, horticulture, and forestry.

The potential of arbuscular mycorrhizal fungi (AMF) to enhance plant growth is well recognized but not exploited to the fullest extent. They are rarely found in nurseries due to the use of composted soil-less media, high levels of fertilizer and regular application of fungicide drenches. The potential advantages of the inoculation of plants with AM fungi in horticulture, agriculture, and forestry are not perceived by these industries as significant. This is partially due to inadequate methods for large-scale inoculums production. Monoxenic root-organ in vitro culture methods for AMF inocula production have also been attempted by various workers [45, 46] but these techniques, although useful in studying various physiological, biochemical, and genetic relationships, have limitations in producing inocula of AM fungi for commercial purposes. Pot cultures in pasteurized soils, have been the most widely used method for producing AMF inocula but are time consuming, bulky, and often not pathogen free. To overcome these problems, soil-free methods such as soil-less growth media, aeroponics, hydroponics and axenic cultures of AM fungi have been used successfully to produce AMF-colonized root inocula [47, 48, 45]. Substrate-free colonized roots produced by these methods can be sheared and used for large-scale inoculation purposes. Mohammad A. *et al*., 2004 [49] compared the growth responses of wheat to sheared root and pot-culture inocula of AMF at different P levels under field conditions, and concluded that P fertilization can be substituted by AMF inoculum produced aeroponically to an extent of 5 kg/ha under field conditions.

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 L. *et al*., 2002 (50] 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) [50]. SODs are metalloproteins that convert superoxide to hydrogen peroxide and molecular oxygen [51]. They act as a primary defense during oxidative stress by protecting cell membranes from damage caused by reactive oxygen species [52]. 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 [53]. 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 [54].

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 [55, 56, 57]. Mycorrhiza-induced high-affinity plant Pi transporter genes have also been reported [58, 59, 60, 61, 62]. Since plants take up as arsenate (AsO^{3-}) via their Pi transporter systems [63], 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 [64, 13].

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. 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 [65]. The expression of HM transporter genes under the control of a constitutive or even a mycorrhizainducible promoter would be attractive for phytoextraction: HM from the soil would be mobilized and transported to the plant via continuous fungal extraand 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 [62], leguminous [59] and poaceous [61] 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 [66]. 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 [67, 68] 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 [13, 14]. 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.

Role of AM Fungi in Phytoremediation of Heavy Metal Contaminated Soils

In nature, some plants hyperaccumulate HMs. Although hyperaccumulator plants are widely used in phytoextraction, they are generally of low biomass, inconvenient for phytoremediation of HM-contaminated soils. In other words, phytoremediation usually is time consuming, mostly as a result of low bioavailability of HMs in the soil environment and/or low biomass of hyper accumulators (69]. Mobility and bioavailability of HMs and therefore their possible phytotoxicity is strongly affected by affinity of the soil for sorption of a given metal, which in turn depends on soil properties [70].

Soil microorganisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants [70]. 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 [19], including disturbed soils [72, 73]. Degraded soils do, however, suffer from changes in diversity and abundance of AM fungal populations [74, 75, 76].

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 [77]. 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 [78, 79, 80, 81].

In the last few years research interest has focused on the diversity and tolerance of AMF in heavy metal contaminated soils trying to understand the basis underlying adaptation and tolerance of AMF to heavy metals in soils, since this could facilitate the management of these soil microorganisms, for restoration/ bioremediation programs. Vandenkoornhuyse P., 1998 [82] showed that AMF species diversity associated with maize plants in a long-term field experiment did not differ between three plots that had received different levels of heavy metals-containing sewage sludge. However, the number of spores of each species was lower in the soil with the highest concentration of heavy metals. Using the same long-term field experiment and the same plant variety, but more acidic soils, Del Val *et al.*, 1999b [83] found a reduction of number, but also of diversity of AMF spores in the soil receiving the highest rate of sludge.

On a highly polluted soil in Indian Thar Desert where only adapted plants can grow, *Anogeissus latifolia* roots were collected along a gradient of heavy metal concentration. Up to three different *Glomus* species were identified inside *Anogeissus* roots, which differed along the gradient of metals. The contribution of these AMF to plant tolerance to heavy metals or heavy metal accumulation by plants has not been established [9]. Four *Glomus* species were also found in the rhizosphere of another metal tolerant plant, *Viola calaminaria,* growing on a soil highly contaminated with heavy metals (20, 961 and 41 mg kg^{-1} Zn and Cd respectively) [84]. Only one of these fungi colonized clove roots growing in pots supplemented with Cd and Zn salts. This *Glomus* sp. Increased Cd and Zn concentrations in clover roots, but not in shoots and did not affect plant growth. On the contrary, *Glomus isolate* from the rhizosphere of *Viola calaminaria* increased the growth of maize and lucerne in, heavy metal polluted soils and reduced Zn concentration in roots and shoots [81, 85].

Although AMF have been recovered from numerous metal enriched habitats, their role in plant interaction with toxic metals is not well understood. At high metal concentrations reports show variations in metal accumulation and inter-plant translocation depending on the fungi, host-plant, root density, soil characteristics, metals and their availability [86,77]. Large variations have also been found between AM fungal species due to differences in hyphal growth outside the rhizosphere [87]. Metal tolerant AMF isolates can decrease metal concentration in shoots or in roots, or decrease translocation from root to shoots [88, 87]. The latter could be due to the high metal sorption capacity of these fungi, which could 'filter' metal ions during uptake [89]. The high concentrations of heavy metals in the intracellular hyphae of a heavy metal tolerant AMF colonizing maize roots [85] and in phosphate rich material in hyphal vacuoles of mycorrhizal roots of *Pteridium aquilinum* [90] strengthen the hypothesis of a sequestration of metals by AMF structures.

However, the competivity of such metal tolerant AMF in the field is often unknown and should be investigated. Further, the potential benefit of a consortium of AMF, which corresponds to the situation in the roots, to improve phytoremediation, should be considered. Phytoextraction studies often use hyper accumulators (plants accumulating high concentrations of heavy metals, e.g., 1% Zn in their dry matter), which are in most cases non-mycotrophic plants belonging to the *Brassicaceae.* One objective is to use plants with high concentrations of heavy metals in shoots, which may limit the potential use of AM plants. However, many of these hyperaccumulating plants, such *Thlaspi caerulescens,* are small and grow slowly, which may limits phytoextraction rates. Other accumulators producing a higher biomass, such as sunflower and willow, are now receiving attention and these are mycorrhizal plants. Highly productive crops associated with metal-tolerant AMF may therefore be considered for decontamination of slightly contaminated soils [92].

Lasat M.M., 2002 [93] observed that the effect of AMF associations on metal root uptake appears to be metal and plant specific. Greater root length densities and presumably more hyphae enable plants to explore a larger soil volume thus increasing access to cations (metals) not available to non-mycorrhizal plants [94].

Rahmanian M. *et al.*, 2011 [95] showed that the introduction of HM-resistant microbes caused a significant decline in plant biomass. They attributed his reduction to the increased access of plants to the relatively immobile Cd existed in the soil and also to more metal contaminants absorption caused by the microbes.

Gharemaleki T. *et al.*, 2010a, b [96,97] reported that co-inoculation of plant growth-promoting rhizobacteria (PGPR) and AMF resulted in increasing Cd and Zn uptake and their accumulation by corn (*Zea mays*) with comparison to sterile condition. However, in the given level of soil contamination, the plant accumulated higher Cd and Zn in PGPR inoculated soil rather than AMF inoculated soil. They concluded that in plant-microorganism system for co-remediation of Cd and Zn, PGPR was effective than AMF.

Role of AMF in PAH Polluted Soils

Polycyclic Aromatic Hydrocarbons (PAH) are hydrophobic organic molecules consisting of two or more fused benzene rings. A selection of 16 PAH is commonly quantified for characterization and monitoring of these pollutants. While in fact 200-300 PAH compounds and their derivatives are commonly extracted and may putatively be identified in polluted soil samples. The concern for PAH pollution derives from their ubiquitous distribution, their recalcitrance towards degradation and their proven or suspected mutagenic properties I [91]. The origin may partially be natural (organic residues after tire), or anthropogenic (mainly processing and incomplete combustion of fossil fuels). Thus, oil spills and industrial sites. e.g., for coke distillation etc. commonly give rise to extreme pollution event!; for which physical, chemical and biological remediation strategies are employed for clean-up. These include, among others, bio-venting, land-farming, bio-augmentation and phytoremediation. The latter is only applicable when pollution levels and physical conditions of the polluted matrix permit the establishment of plants, but offers a cost-effective and efficient treatment that simultaneously restores an ecosystem, limits erosion and improves the esthetical impression of a polluted site [98, 99].

Arbuscular mycorrhiza fungi may play a role in two aspects of bioremediation of PAH. The establishment of a plant covers on polluted soil and modification of PAH degradation rates or pathways.

Improved plant establishment on fallowed, degraded or polluted soils, waste materials or soil-waste mixtures in the presence of AMF is well known [100, 101, 102, 103]. The major mechanism behind the success of AM under such conditions is an improvement of plant nutrient acquisition, water relations, pollutant tolerance and sequestration. The impact of the latter factors are often difficult to distinguish due to the confounding effect of plant nutrition on plant size, but, in the case of enhanced survival in the absence of competitors/predators, the role of AM may be clear. Identification of the symbiotic features that have permitted continued subsistence is however difficult and such investigations are still in their infancy. One mechanism that may be involved is the oxidation of the contaminant by activated oxygen species and concomitant enhancement of oxidoreductases to protect the plant from oxidative stress. Indeed, enhanced levels of hydrogen peroxide in AM roots [104] as well as enhanced levels of peroxidase activity in mycorrhizal roots and the rhizosphere [105] has been demonstrated.

One peculiarity of PAH polluted soil that may be overcome by AM plants is the hydrophobicity and resulting limitations in uptake of water and water-dissolved inorganic nutrients [103]. Again confounding effects of improved mineral nutrition prevents the distinction of mycorrhizal effects on uptake of water and mineral nutrients, but even if the effect is limited to mineral uptake it is of no less importance.

Effects of AMF on PAH degradation in the rhizosphere may be direct or indirect. As PAH are not absorbed by plants [106,98] and are metabolized intracellularly, all degrading activity would take place in soil or inside soil organisms other than AM. Furthermore, AMF have poor saprophytic capacities, so the only probable direct effect of AMF on PAH degradation would be through enhanced production of extracellular peroxidases. Indirect effects would be due to changes in the microbial community e.g. due to stronger competition for mineral nutrients, direct antagonistic or synergistic effects of AMF or changes in root exudation patterns, phenomena that are well documented.

Binet P. *et al*., 2000b [106] found no effect of AM on the dissipation on light compounds like anthracene (3 rings) in spiked soil on the short term (<40 days). Continued phytoremediation treatments of spiked soil resulted in an almost complete (98%) dissipation of anthracene after only 56 days in the rhizosphere of clover/ryegrass [9]. Heavier compounds (4-6 rings) are more persistent and often rely on degradation by co-metabolism rather than direct metabolism [107, 108]. The rhizospheric effect on dissipation of these compounds is thus more pronounced than for the lighter PAH [109] as the root exudates may drive co-metabolism. Mycorrhizal effects on PAH dissipation was in this case accompanied by a modification in biomass partitioning (but not total plant biomass) between two co-occurring plant species (clover and ryegrass) [110], as well as changes in soil microbial community structure. The effect of colonization by mycorrhizal fungi on the competitive ability of clover on PAH-polluted soil is perhaps similar to that observed in non- polluted soil [111], *i.e*., a phenomenon linked to the improved nutrient acquisition of the mycotrophic legume.

The modification of the soil microbial community may on the other hand either be due to inherent qualitative [112] or quantitative [113] differences in root exudation between the two plant species coupled with a proportional change in plant biomass (indirectly mediated by the mycorrhizal effect on competitive ability/plant size of the two plants], or more direct effects of AMF, i.e., alterations in root exudation as a consequence of AMF colonization [114,115] and direct antagonistic/synergistic effects between the AMF hyphae and the soil microtlora [116, 117]. The exploitation of root-free soil by AMF hyphae has a potential for modifying microbial composition [118, 119] and activity. Ephemeral hyphae serving as a source of C outside the rhizosphere may ultimately result in a microbial community with an improved capacity of PAH degradation.

Potential Use of AMF in Bioassays for Soil Pollution

Arbuscular mycorrhizas are not only an aid to ecosystem remediation. They should also be considered as a key indicator for soil pollution or soil quality because:

- mycorrhizal fungi are ubiquitous microorganisms
- many plants are highly dependent on mycorrhizas for their growth,
- they provide a direct link between soil and roots and
- are involved in the transfer of elements including pollutants from soil to plants

Furthermore, AMF can be affected by pollutants in soil and can be more sensitive to pollutants than plants [120].

The toxicity of compounds such as xenobiotics, PAH and heavy metals on AMF has been studied using techniques based on estimation of spore germination [120], mycorrhizal colonisation of roots in pot cultures using nested PCR [121], mycorrhizal infectivity [122] and mycorrhizal colonization of Ri T-DNA transformed roots [123]. AMF spore germination in soil can be used as an early indication of the toxicity towards the ecosystem, while mycorrhizal colonisation of roots indicates toxicity at a later stage of the association between symbiotic fungi and roots. AMF can be affected by pollutants but also by soil properties such as pH and phosphorus content [122]. The lack of specificity towards heavy metals or other pollutants is often the case for other indicator organisms such as earthworms, algae, fish and plants, which are used for the assessment of ecological risk due to toxic substances. AMF could be included as additional indicator organisms in the existing battery of bioassays. There is a need for a standardized technique using arbuscular mycorrhizas as a bioassay, which should be made easier since commercial AMF inoculum is now available. The technology of phytoextraction utilizes hiperaccumulating plants in order to extract heavy metals from the environment. Most hyper accumulating plants belong to the families Brassicaceae (pl. *Thlaspi*, *Alyssum*), *Compositae*, *Euphorbiaceae*, *Fabaceae*, *Liliaceae*, *Scrophulariaceae*, *Poaceae*, *Violaceae*, which occur also in the native Hungarian flora. Nearly all of them are not agricultural plants, thus their economic utilization is problematic (also often because of the low biomass, the complicated harvesting and propagation). Another problem is the mentioned selectivity of accumulation. Until now there are some new innovations in order to solve these problems, e.g., the heavy metal uptake can be enhanced among larger biomass-producing, but not hiperaccumulating plants with chelating soil treatment like with EDTA [124].

In re-cultivation processes the adaptation mechanism of hyper-accumulating plants is a competitive advantage to be a pioneer and on a longer scale it alters the environment so that weaker or non-tolerant species can distribute. Hence, hyper-accumulating plants offer an environment-friendly way of land decontamination and controlled agro-ecosystem formation. Such succession can be accelerated through the co-application of hyper-accumulating plants and selected metal-tolerant microbes.

Several publications report on the role of ecto and endo-mycorrhizal fungi in the re-colonization of heavy metal contaminated soils by pioneer plants. Mycorrhizal fungi offer a better nutrient supply, higher metal tolerance for the macrophyte symbiotic partner [125, 126]. The more abundant and more ancient mycorrhizal type is the arbuscular one (AM), which offers a better soil exploitation, higher nutrient and water uptake, thus results in a higher biomass production [127]. AM fungi are among the most abundant soil fungi.

The effect of AM fungi on the heavy metal uptake of the host plant depends on the physical and chemical properties of the contaminated soil [128], on the heaviness and duration of the contamination load, on the plant [129, 130] and fungal species [131], thus, the efficiency of the symbiosis. The published data demonstrate that by selected coupling of compatible symbiotic partner's plant metal uptake can be altered parallel to higher plant vitality. The scientific and economic establishment and the necessity of the planned research, research-innovation is proved by our previous experimental results and the country's environmental problems. Inorganic contaminants like heavy metals are not biodegradable thus possible management like Phytoremediation aims in the decreasing of soil heavy metal content (by extraction), parallel to the prevention of food chain contamination. Based on toxicity, level of contamination and ecological conditions *heavily contaminated* (lethal for most organisms) and contaminated (tolerable) sites are differentiated. Level of contamination changes in time and this dynamics is affected by the successive order of the applied phytoremediation methods.

According to these one can plan the development of phytoremediation methods (and method-combinations) that could be applied together in space and successively in time:

Cultivation of Heavy Metal Accumulating Plants (On Tolerable Contaminated and Heavily Contaminated Sites)

An important aim in the application of hyper accumulating plants to maximize the level and speed of metal uptake. Important factor is the allocation of uptake metals into aboveground organs that can be easier harvested and further incinerated. The rate of the heavy metal extraction can be increased by the choice of the plant species, through intra specific selection, by the application of uptake-enhancing additives [132], by increasing plant biomass (through agricultural techniques), by increasing plant life cycles that are active in heavy metal uptake (e.g., rotation of species with different temperature optima).

Cultivation of Heavy Metal Accumulating Plants That Are Able to Form Mycorrhizal Partnership (On Heavily Contaminated Sites)

The choice of plants based on their ability to form symbiosis with AM fungi. Coupling with proper fungal partner has positive effect on water and nutrient uptake and thus on biomass production. Coupling of such plant and fungal species in which symbiotic fungal partner enhance metal uptake in order to extract higher amount of contaminant.

Cultivation ofNon-Hiperaccumulating but Mycorrhizal Plants That Produce Large Amount of Biomass (On Tolerable Contaminated Sites)

In the choice of the proper plant and fungal partners it is important to have a positive mycorrhizal effect on metal uptake (controlled mycorrhization with heavy metal uptake enhancing AM fungi). The last step of a phytoremediation technology is the one when metal contamination has decreased or on a weekly contaminated site the heavy metal entrance to food chains should be prevented (e.g., grazing wild animals). At this step a negative AM-fungal effect on metal uptake is beneficial, in order to decrease the amount of metals entering the food chain.

Heavy Metal Tolerant AM Fungi

The literature presents a range of "classic" ecological principles explaining the processes that increase the tolerance or resistance of a community [133]. Resistance refers to the ability of microorganisms to withstand the effects of a pollutant usually effective against them, while tolerance refers to the ability of microorganisms to adapt to the persistent presence of the pollutant. As stated by Leyval C. and Joner E.J., 2001 [134], tolerance and resistance to the toxic effect of HMs depends upon the mechanism involved. Briefly, as mentioned in epidemiological studies [135, 136], metal tolerance could be defined as a phenomenon by which microorganisms increase resistance towards stress resulting from exposure to HM toxicity.

Metal tolerance of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi have been assessed using several observation methods including: AM spore numbers, root colonization and the abundance of ECM fruiting bodies [83]. Unfortunately, such methods did not give information concerning conditions, limitations and threshold values ensuring the survival and growth of AMF, or about the genetic basis for multi-metal resistance and tolerance. Moreover, AMF coexist with other microbial communities and plant roots that can tolerate and accumulate metals, and this could confound the real interactions between AMF and metals in the medium. More recently, to evaluate the tolerance of microorganisms in soils polluted with metals, specialists have adopted the concept of pollution-induced community tolerance (PICT) [137]. This perspective stipulates that with time, in an ecosystem, contamination exposure increases tolerance in microbial communities. Davis M.R.*et al*., 2004 [138] used the PICT method to assess the effects of long-term exposure to Zn on the metabolic diversity and tolerance to Zn of soil microbial community. They showed that long-term exposure to Zn imposes stress on soil microbes, resulting in an increased tolerance. They concluded that the long-term accumulation of Zn in soils provides the microbial community with time to adapt to this metal. This adaptation has been attributed to two factors [139]. The first one is a gradual decrease in metal availability due to immobilization reactions occurring in the rhizosphere. The other factor is a gradual change in microbial community structure, based on changes in phospholipid fatty acid profiles [140] which results in more tolerant organisms.

Although metals may induce changes in the microbial community, resulting in microorganisms more resistant to metals [139], most essential and non essential metals exhibit toxicity above a certain concentration. This toxicity stress, appreciated by a threshold value [134], will vary depending on many factors including the type of microorganism, the physico-chemical properties and concentration of the metal, and the edaphic and environmental conditions [141].

Even though metals can exhibit a range of toxicities toward soil microorganisms [142, 8, 143, 144, 137], AMF isolates, particularly the ecotypes living in metal-enriched soils, metalliferrous sites and mine spoils heavily polluted with metals, can, depending on intrinsic and extrinsic factors, tolerate and accumulate HMs [145, 146, 147, 17]. Field investigations have indicated that AMF can colonize plant in metal contaminated sites [148, 149] and in agricultural soils contaminated with metals of different origins, including atmospheric deposition from smelter and sludge amendments. Mycorrhizal fungi have also been shown to be associated with metallophyte plants on highly polluted soils. Nevertheless, it should be kept in mind that in some extreme metal conditions, AMF inoculation can be entirely inhibited [147]. Del Val C. *et al*., 1999a [80] reported that spore numbers decreased with the increasing amounts of HMs, whereas specie richness and diversity increased in soils receiving an intermediate rate of sludge contamination but decreased in soils receiving the highest rate of HM contaminated sludge.

Several reports and reviews suggested that AMF from metal-contaminated sites have developed tolerance against metal toxicity and are well adapted [146, 147, 134, 150, 151]. The evolution of metal tolerance is showed to be rapid in MF. As stated by Sudová R. *et al*., 2007 [151], tolerant strains of some MF may develop within one or two years [147, 152]. Gonzalez-Chavez C. *et al*. 2002a, b [153, 154] reported that AMF have evolved arsenate resistance and conferred enhanced resistance on Holocus lanatus. Heavy metal concentration may decrease the numbers and vitality of AMF as a result of HM toxicity [155] or may have no effect on mycorrhizal colonization [77]. Biró B. *et al*., 2005 [156] studied the stress buffer effect of the AMF and their colonization behavior in metal spiked soil on a long-term level in controlled conditions. The soils used were collected after a 12 year metal-adaptation process, where 13 trace element salts, such as Al, As, Ba, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sr and Zn were applied in four gradients (0, 30, 90 and 270 mg/kg dry soil). Barley (*Hordeum vulgare L.*) was used as a test plant. They found a strong dose dependency at the arbuscular richness in general. The sporulation of the AMF was found as the most sensitive parameter to longterm metal(loid) stress. They reported that Al, As, Ba, Cd, Cr, Cu, Pb, Se, Sr and Zn reduced significantly the spore numbers of the AMF, while the Ni loadings (at 36 g/soil) increased mycorrhizal sporulation.

At present, potential interaction mechanisms between AMF and metals, and the cellular and molecular mechanisms of HM tolerance in AMF, are poorly understood [134,157]. Metal transporters and plant-encoded transporters are involved in the tolerance and uptake of HMs [7, 158] from extracellular media, or in their mobilization from intracellular stores [159]. Göhre V. and Paszkowski U., 2006 [7] hypothesized that metals could be released at the pre-arbuscular interface and then taken up by plant encoded transporters.

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 [67], but the fungus may constitute a biological barrier against transfer of heavy metals to shoots [87]. 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 [160, 161], whereas in other cases they enhance or have no effect on the uptake [36, 87, 162]. 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 [88] observed lower Pb concentration in the shoots of plants inoculated with an AM isolate from contaminated soil (*G. mosseae*) than in the shoots of plants inoculated with an isolate from non-contaminated soil (*G. macrocarpum*).

The effect of AM fungal inoculation on metal accumulation has been shown to vary among plant species. The inoculation with two *G. intraradices* isolates significantly reduced Pb concentration in maize plants, while Pb concentrationin *Agrostis* plants was not changed or even increased by inoculation [163]. Dependence of heavy metal-AM fungal interaction on plant species was also shown in two studies on AM isolates from the rhizosphere of a metallophyte zinc violet, *V. calaminaria* [84]. Kaldorf M. *et al.*,1999 [85] found much lower concentrations of heavy metals in maize plants inoculated with *Glomus* Br1 isolate than in non-inoculated control plants. Tonin C. *et al.*, 2001 [84] reported that colonization of clover roots with mixed population of AM fungi from the zinc-violet rhizosphere significantly increased Cd and Zn concentration in clover roots, without significantly affecting heavy-metal concentration in theshoots. A range of factors like fungal properties, inherentheavy metal-uptake capacity of plants and soil absorption/desorption characteristics can influence heavy-metal uptake by mycorrhizal plants [77]. Mycorrhiza functioningdepends on exploitation of non-rhizosphere soil by extraradical hyphae [164]. Functional mycorrhizas can reduce excessivepassive uptake of potentially harmful elements through the roots while maintaining an adequate supply of the other elements like N and P through active hyphal uptake. The hypha are also less sensitive than roots with respect to heavy-metal toxicity [87]. Thus, hyphal growth andnutrient uptake may be maintained when roots are impaired.

Conclusion and Future Prospectus

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 [26]. It was, therefore, challenging to try to draw general conclusions about the usefulness of AM fungi for soil remediation. However, the prospect of AM fungi existing in heavy metal-contaminated soils has important implications for phytoremediation. Since heavy metal uptake and tolerance depend on both plant and soil factors, including soil microbes, interactions between plant root and their symbionts such as AM fungi can play an important role in successful survival and growth of plants in contaminated soils. Mycorrhizal associations increase the absorptive surface area of the plant due to extrametrical fungal hyphae exploring rhizospheres beyond the root-hair zone, which in turn enhances water and mineral uptake. AM fungi can further serve as a filtration barrier against transfer of heavy metals to plant shoots. The protection and enhanced capability of uptake of minerals result in greater biomass production, a prerequisite for successful remediation.

Indigenous AM isolates existing naturally in heavy metal-polluted soils are more tolerant than isolates from non-polluted soils, and are reported to efficiently colonize plant roots in heavy metal-stressed environments. Thus, it is important to screen indigenous and heavy metal-tolerant isolates in order to guarantee the effectiveness of AM symbiosis in restoration of contaminated soils. It is further suggested that the potential of phytoremediation of contaminated soil can be enhanced by inoculating hyper-accumulator plants with mycorrhizal fungi most appropriate for the contaminated site. It is therefore of great importance that we combine selected plants with specific AM fungal isolates adapted to high concentrations of heavy metal in future research for phytoremediation programmes. However, there is need to develop new methods and optimize the conditions to grow in large quantities and characterize, develop and screen large number of AM fungi for tolerance to metals. The lack of correlation between colonization rates and a beneficial or detrimental host response perhaps suggests the need to look more closely at the diversity and competition among AM fungi-colonizing roots. Identifying and culturing the most effective isolates could then be undertaken to select or develop genetically improved strains customized for a particular set of conditions or host plants.

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