Mycorrhizal fungi in extreme environments and their impact on plant growth

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(Submitted in December, 2013 ; Accepted on 30 June, 2014)

ABSTRACT

This review focusses on mycorrhizal fungi-host plant adaptation to metal-contaminated and extreme environments in order to draw conclusions on the co-evolutionary strategies for the establishment of mycorrhizal associations on such sites. Mechanisms involved in amelioration of metal toxicity by mycorrhizal fungi are discussed. Mycorrhizas present in extreme environments like fly ash, bauxite residue and mining soils and their role in promoting the vegetation are illustrated. The introduction of plants in stressed or extreme environmental sites inoculated with mycorrhizal fungi is useful in reclaiming degraded ecosystems. The mycorrhizal fungi have the potential to increase the efficiency of the plant system by providing the seedlings with essential levels of P and other nutrients for growth.

Keywords: Mycorrhizal fungi, fly ash, metal toxicity, bauxite residue, revegetation, nutrient uptake

INTRODUCTION

Soil is the basic environmental element constituting ecosystem and the important material basis of human being for survival and development. The hallmarks of civilization like industrialization, urbanization and technological advancements have been increasing heavy metal release into the environment, which pose a significant threat to the environment and public health because of their toxicity, accumulation in the food chain and persistence in nature. The term 'heavy metals' in general includes elements (both metals and metalloids) with an atomic density greater than $6 \, gcm^{-3}$ with the exception of arsenic, boron and selenium. This group includes both biologically essential (cobalt, copper, chromium, manganese and zinc) and non-essential (cadmium, lead and mercury) elements. Both groups are toxic to plants, animals and/or humans at high concentrations. Heavy metals such as copper and zinc are essential for normal plant growth and development since they are constituents of many enzymes and other proteins. The elevated concentrations of both essential and non essential heavy metals in the soil can, therefore, lead to toxicity symptoms and inhibition of growth of most plants. The toxicity symptoms seen in the presence of excessive amounts of heavy metals may be due to a range of interactions at the cellular/molecular level. Toxicity may result from the binding of metals to sulphydryl groups in proteins, leading to inhibition of the activity or disruption of structure, or from the displacing of an essential element resulting in deficiency effects (Van Assche and Clijsters, 1990). In addition, heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress (Dietz *et al.*, 1999).

Pollution of the biosphere with toxic metals due to man-made activities poses a major environmental and human health problem. Metals in soil are present as free metal ions, soluble metal complexes, exchangeable metal ions, organically bound metals, precipitated or insoluble compounds such as oxides, carbonates and hydroxides or they may form part of the structure of silicate minerals. The toxicity of metals in soil depends on their bioavailability. According to Hartley *et al.* (1997), metal bioavailability is a function not only of their total concentration but also of physico-chemical (pH, organic matter, clay content) and biological (biosorption, bioaccumulation and solubilization) factors. The problem of toxic metals becomes more severe in acidic soils as the metals are present in the free ionic form in acidic pH range. Unlike organic pollutants, which are broken down to a greater or lesser extent dependent upon their reactivity, heavy metals cannot be degraded and will remain in the soil permanently unless they are leached out. However, when they are leached out, heavy metals move into surface and ground waters and may eventually end up in contaminating the water resources.

Plants and microorganisms are successfully used in the removal of some heavy metals. Plants which exhibit hyper accumulation can be used to remove heavy metals from soils by concentrating them in their bio matter. However, heavy metals at elevated levels are generally toxic to most plants impairing their metabolism and reducing plant growth. The interface between microbes and plant roots (rhizosphere) may affect nutrient uptake and metal toxicity (Smith, 1994). An alternate way of using rhizosphere microbes to reduce the toxicity of heavy metals to plants has been investigated. The amelioration of heavy metal toxicity in plants by microorganisms may be through reduction in the metal uptake by plants (Vivas *et al.*, 2006) or through reduction in the amounts of detrimental stress induced by heavy metals with no effect on their uptake (Rajkumar *et al.*, 2006). There is considerable interest in the interaction between mycorrhizas and pollution.

Mycorrhiza, a specialized root organ is the result of symbiotic interactions between a plant and a compatible fungus. As a consequence of this symbiotic relationship, the fungus supplies the basic enzymatic machinery for absorbing, translocating and assimilating major mineral

ions. In turn the plant provides the peculiar ecological niche necessary for fungal growth and development including the completion of sexual cycle. The mycorrhizal condition is the rule among plants, not an exception as 95% of the plant species characteristically form mycorrhizae (Smith and Read, 2008). About two-thirds of the plants are symbiotic with AM glomalean fungi, while a relatively small number of plants develop ectomycorrhiza and they dominate forest ecosystems and are common in both Gymnosperms (*Pinaceae*, *Cupressaceae*) and Angiosperms (*Juglandaceae*, *Fagaceae*, *Betulaceae*, *Tiliaceae*, *Salicaceae*, *Ulmaceae*, *Rosaceae*, *Leguminosae*, *Sapindaceae*, *Aceraceae*, *Myrtaceae*, *Ericaceae* and *Dipterocarpaceae*) (Smith and Read, 1997). The association of mycorrhizal fungi with tree roots greatly alters both root morphology and physiology, enhance seedling growth and alter patterns of ion uptake and plant nutrition. The host derives many benefits from symbiotic association with mycorrhizal fungi; some of these include: (i) longevity of its feeder roots(ii) increased rate of nutrient uptake from soil, (iii) selective absorption of certain ions from the soil, (iv) resistance to feeder root pathogens, (v)) increased tolerance to soil toxins, cold temperatures and high temperatures and (vi) increased tolerance to adverse soil conditions (Smith and Read, 1997).

Mycorrhizal associations (including ericoid, arbuscular and ecto-mycorrhizas) are found colonising highly metal contaminated soils. The response of mycorrhizal fungi to toxic metals is important in view of their use in the reclamation of polluted sites and influence on plant growth and productivity. Trees are frequently used for the reclamation of contaminated and strip mined lands, in case the community structure of soil microorganisms is significantly affected, this could compromise the success of the site remediation. The rhizosphere hosts a large and diverse community of microorganisms that compete and interact with each other and with plant roots. Fungal survival in the presence of toxic metals mainly depends on intrinsic biochemical and structural properties, physiological and/or genetic adaptations, including morphological changes and environmental modification of metal speciation, availability and toxicity, the relative importance of each often being difficult to determine (Gadd, 1992). The mycorrhizal fungi improve the metal tolerance of their host plant by primarily accumulating metals in the walls of extramatrical hyphae and extrahyphal slime, and thus, the transport of metals to shoot is restricted (Galli *et al.*, 1994).

METAL TOLERANCE MECHANISMS

Mycorrhizas are useful in ameliorating the effects of metal toxicity on the host plant (Marschner *et al.*, 1999; Jentschke and Godbold, 2000). However, the mechanisms involved in conferring this increase in tolerance have been proved difficult to resolve; they may be quite diverse and show considerable species and metal specificity, since large differences in response to metals have been observed

between fungal species and to different metals within a species (Hartley *et al.*, 1997). To survive under metal-stressed conditions, the mechanisms employed by the fungi at the cellular level are probably similar to some of the strategies employed by higher plants, namely binding to extracellular materials or sequestration in the vacuolar compartment and various exclusion processes that restrict metal movement to the host roots (**Fig. 1**).
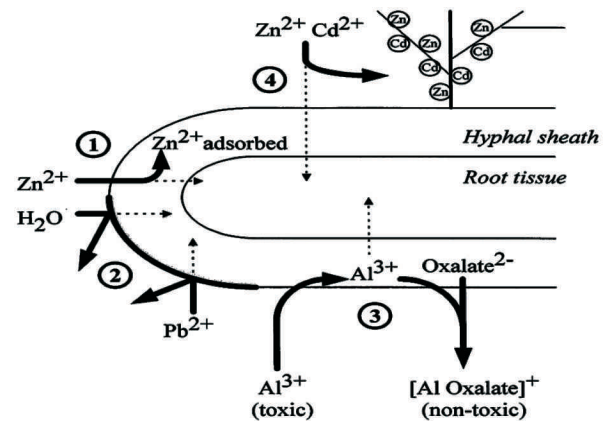

Fig. 1. Metal exclusion mechanisms possibly operating in ectomycorrhizas. (1) Sorption of metals in the hyphal sheath, (2) Reduced apoplastic mobility as a result of hydrophobicity of the fungal sheath, (3) Exudation of chelating substances, (4) Sorption on the external mycelium. (Jentschke and Godbold, 2000)

1) Metal mobility in the fungal apoplast

Many studies into the role of mycorrhizal fungi in achieving metal resistance of their hosts have concentrated on the extracellular binding of metals to hyphae. Various studies have concentrated on the hypothesis that fungal hyphae act as a filter, removing toxic ions before they reach the plant. This hypothesis requires that the metals themselves do not damage the fungal cells, should stay immobilised on the fungal cells and are not in equilibrium with the growth medium and that the fungal biomass is high enough to immobilise the metals.

Gadd (1993) reported that fungi can effectively bind metals to the cell walls or extracellular polysaccharides. In addition, intracellular uptake and accumulation of certain metals in vacuoles may be significant. Binding of toxic metals to cell walls has been suggested as a tolerance mechanism both in higher plants (Ernst *et al.*, 1992) and fungi (Ross, 1993). It was suggested that sorption of metals to fungal tissues or intracellular uptake and detoxification in fungal vacuoles subsequently reduce metal uptake into the host plant (Jones and Hutchinson, 1986). Metals have been found in high concentrations in fungal hyphae in contaminated media. Frey *et al.* (2000) investigated zinc and cadmium localisation in *Hebeloma crustuliniforme* – *Picea abies* root tips. Cadmium was predominantly bound extracellularly in the Hartig net, while zinc accumulated mainly in cell walls of mantle hyphae, Hartig net hyphae

and in cortical cells. It was proposed that zinc accumulates in high quantities in fungal tissues, protecting the host plant. A range of elements were shown to accumulate in the fungal mantle and rhizomorphs in *Suillus luteus–Pinus sylvestris* associations on polluted soils (Turnau *et al.*, 1996).

2)   Fungal hydrophobicity

Besides metal sorption to fungal cells, metal uptake into host tissues may be affected by the degree of hydrophilicity of the fungal apoplast. The fungal sheath formed by a fungus (Unestam, 1991) could provide a barrier to apoplastic radial transport of water and ions. In ecto- and endo-mycorrhizal fungi, heavy metals were bound to cell wall components such as chitin, cellulose derivatives and melanin (Galli *et al.*, 1994). Extrahyphal slime and polyphosphate linkage of copper and zinc was observed to be the amelioration mechanism in *Pisolithus tinctorius* tolerance to Cu and Zn (Tam, 1995).

3)   Chelation by organic acids and other substances

In addition to the large surface area produced by mycorrhizas, there are reports that mycorrhizal fungi, like certain plant hosts, can excrete organic acids into the rhizosphere (Landeweert *et al.*, 2001). The benefits of organic acid excretion might be to liberate base cations from soil minerals to mobilise phosphate from insoluble iron and aluminium phosphates, to counteract aluminium and iron toxicity by complexing their ions in soil. Increasing evidence suggests that exudation of organic acids plays a major role in aluminium tolerance of higher plants (Jones, 1998). In fungi, metal tolerance in some cases has been linked to extracellular chelation by organic compounds (Gadd, 1993). Since mycorrhizal fungi exude a range of organic acids (Lapeyrie *et al.*, 1987) or produce slime capable of binding metals, it is possible that organic compounds released by mycorrhizal fungi are responsible for the amelioration of metal toxicity in mycorrhizal plants.

Ahonen-Jonnarth *et al.* (2000) showed that in contrast to non-mycorrhizal pine seedlings, seedlings colonised with *Suillus variegatus* or *Rhizopogon roseolus* responded to aluminium exposure with a strongly increased exudation of oxalic acid, which is an efficient aluminium chelator. Although the data does not prove that organic acids released by ectomycorrhizal fungi affect the metal sensitivity of the host plant, they highlight the potential role organic acids may have in the amelioration of metal toxicity by mycorrhizas. Oxalate synthesis by fungi could be an efficient way of improving host mineral nutrition. As compared to other organic acids, oxalic acid would be most efficient in mineral weathering because of its complexant as well as its acid properties. By forming complexes with metal ions such as calcium, aluminium and iron, oxalate would release phosphate from insoluble phosphates (Cromack *et al.*, 1979). Malajczuk and Cromack (1982) have reported the accumulation of calcium oxalate in the mantle of mycorrhizal

roots of *Pinus radiata* and *Eucalyptus marginata* and increase in the phosphatase activity at the surface of mycorrhiza. The excretion of oxalic acid appears to increase in response to Al addition to *Populus deltoides* plants colonized by *Paxillus involutus*. The maximum amount of oxalate was recorded at 100 mg/l of Al concentration in mycorrhizal plants (Khosla *et al.*, 2009)

4)   Metal sorption on the external mycelium

Much attention has been focussed on the extent to which the external mycelium reduces net metal exposure. The hypothesis is based on the observation that the efficiency of fungal strains to reduce Zn and Cd translocation to the shoot was correlated with the amount of external mycelium produced by these strains (Colpaert and van Assche, 1993), whereas detoxification of Cd in *Paxillus involutus* involved binding of Cd to the cell walls and accumulation of Cd in the vacuole (Blaudez *et al.*, 2000). The significance of these processes may vary as a function of the metal involved, its concentration, and the location of the primary lesion caused by the metal. Mycorrhizal fungi may alter metal sensitivity of their hosts by any of the mechanisms outlined above, by either directly affecting metal availability and speciation or indirectly modifying plant physiological processes.

5)   Production of Peptides

In addition to the tolerance mechanisms discussed above, some fungi have preferentially developed the production of peptides capable of binding heavy metals. These molecules, such as cysteine-rich and metal-binding peptides, the metallothioneins (MTs) and the phytochelatins are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions, thus preventing or neutralizing their potential toxicity (Cobbett and Goldsbrough, 2002).

Metallothioneins (MTs) are ubiquitously present in eukaryotic and prokaryotic organisms (Cobbett and Goldsbrough, 2002). They are characterized by their small size (< 7 KDa), a high content of amino acid Cys (up to 33%) and a high degeneracy in the remaining residues; they are encoded by a multigene family and contain metal binding Cys-rich domains (Cobbett and Goldsbrough, 2002). MTs are involved in metal homeostasis and detoxification (Cobbett and Goldsbrough, 2002; Gadd, 1993). Transcription of MTs is typically induced by the same metal ion(s) that bind to the protein, thus providing a direct activation of their protective function (Waalkes and Goering, 1990). Among fungi, *Candida glabrata*, like higher eukaryotes, contains a large family of MT genes. However, the transcription of *C. glabrata* MT genes is activated in response to Cu and Ag but not by other heavy metals such as Cd, a potent activator of higher eukaryote MTs (Mehra *et al.*, 1989). By contrast, only two MTs have been identified in *Saccharomyces cerevisiae*. The

first one is encoded by the *CUP1* locus and is induced by Cu and Ag, but not by other heavy metals (Butt *et al.* 1984). The second one, *Crs5*, is regulated by Cu, Zn and oxidative stress (Pagani *et al.*, 2007). One MT has been identified in *Agaricus bisporus* (Münger *et al.*, 1985), *Gigaspora margarita* (Lanfranco *et al.*, 2002), *Neurospora crassa* (Münger *et al.*, 1985), *Pyrenopeziza brassicae* (Singh and Ashby. 1998), *Podospora anserina* (Averbeck *et al.*, 2001) and *Paxillus involutus* (Bellion *et al.*, 2007). In *Hebeloma cylindrosporum*, two metallothioneins *HcMT1* and *HcMT2* were reported in metal detoxification, and the yeast complementation assay revealed that *HcMT1* and *HcMT2* encode functional polypeptides capable of conferring increased tolerance against Cu and Cd. Growth studies of yeast strains complemented with *HcMT1* and *HcMT2* suggested that both these genes provide tolerance to Cu and Cd (**Fig. 2**).
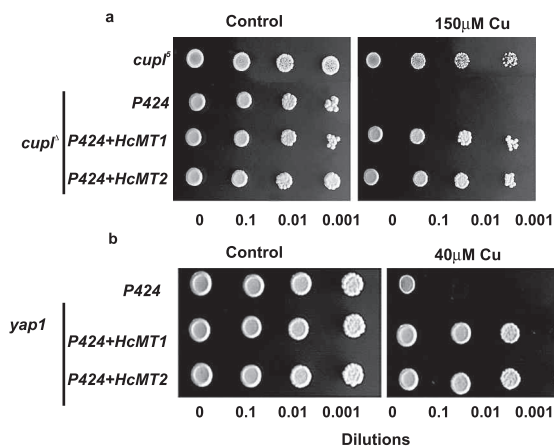
Fig. 2 Functional complementation and growth of the yeast mutants on selective media: a) Cu tolerance of *cup1"* cells; b) *yap1* mutant cells transformed with empty vector p424 or with p424-*HcMT1* and p424-*HcMT2* and a parental cells with a single CUP1 gene copy (*cup1ˢ*) (Ramesh *et al.*, 2009).

MYCORRHIZAS IN EXTREME ENVIRONMENTS

It is well known that mycorrhizal associations are ubiquitous in natural ecosystems. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. The significance of mycorrhizas in plant uptake of metals from metal contaminated soils merits systematic studies. The wide distribution of mycorrhizal fungi on metal contaminated sites has shown general adaptation and tolerance of these symbionts to heavy metals and metal tolerant fungal strains have been isolated by several research groups. In order to utilize native plants for future revegetation schemes, more detailed investigations should be conducted to improve our understanding of plant adaptation to metal contaminated or extreme sites, to

identify the potential role of mycorrhizas in phytostabilization of metals in contaminated environments and stimulating plant growth so that both plant and fungal symbionts can be used for successful revegetation (Reddy *et al.*, 2002).

1) Mycorrhizal fungi and fly ash

Fly ash disposal is a major environmental concern throughout the coal based power generating countries. Disposal of fly ash has significant impact on terrestrial and aquatic ecosystems due to leaching of toxic substances from the ash into soil and ground water, as well as reduction in plant establishment and growth (Haynes, 2009; Wong and Wong, 1990; Wu *et al.*, 2009). In many cases, the reclamation practices have been ineffective in reducing mortality of tree seedlings due to deficiency of essential nutrients (usually N and P), low soil microbial activity, high soluble salt concentrations of trace elements, and the presence of compacted and cement layers on ash disposal sites (Selvam and Mahadevan, 2002). The lack of mycorrhizal symbionts, which enhance nutrient supply to the host plants and alleviate biotic stress, may be partly responsible for the problems in revegetation (Sylvia and Williams, 1992). Arbuscular mycorrhizal fungi (AMF) are considered to be ecologically important as they play important roles in the restoration of contaminated ecosystems by improving plant nutrition and fertility of the degraded lands (Chen *et al.*, 2001). Spontaneous selection of infective and effective AM fungi can be a long process in fly ash ponds. Application of selected metal tolerant AMF strains may increase the rate of restoration, efficiency of phytoremediation and speeds up these processes (Vivas *et al.*, 2005). Isolation and mass inoculum production of AM fungi that are adapted to fly ash may have beneficial effect compared to other AM fungi. It has also been demonstrated that the use of adapted AM fungal strains can be more effective in restoration and bioremediation studies than the non-adapted strains (Vivas *et al.*, 2005). Several researchers have reported the effects of selected isolates of AM fungi on the plant growth, nutrient uptake and aggregation of fly ash (Enkhtuya *et al.*, 2005). Babu and Reddy (2011) studied the root colonization and diversity of arbuscular mycorrhizal (AM) fungi in plants growing in fly ash pond. They have identified seven different species of AM fungi based on phylogenetic analysis. Inoculation of plants with the spores of these fungal consortia along with colonized root pieces increased the growth (84.9%), chlorophyll (54%) and total P content (44.3%) of *Eucalyptus tereticornis* seedlings grown on fly ash compared to non-inoculated seedlings. The growth improvement was the consequence of increased P nutrition and decreased Al, Fe, Zn and Cu accumulation. These observations suggested that the inoculation of tree seedlings with stress adapted AM fungi aid in the reclamation of fly ash ponds (**Table 1**).

2) Mycorrhizal fungi and bauxite residue

Bauxite residue (red mud) is a waste material generated in large quantities during alumina extraction from bauxite ore by Bayer's process. World production of this waste has been estimated at 30 million Mg per year (Menzies *et al*., 2004) and the majority of this material is disposed on land. Generally, bauxite residue ponds are extreme ecosystems characterized by high pH (pH>10), high electrical conductivity (EC> 30 dSm^{-1}), high exchangeable sodium percentage (>70) (Snars *et al*., 2004). The major components in the red mud are iron oxide, silica, un-reacted alumina and residual NaOH as Na_2CO_3 as well as alkali bound in the form of sodalite, ferrite, etc. High pH, high concentration of soluble ions such as sodium and carbonate are toxic and competitively inhibit the uptake of nutrients in plants. Limited information is available on rehabilitation of these habitats especially changes in the key physico-chemical and microbiological characteristics and their impact on the vegetation cover (Babu and Reddy, 2011). The published work on the establishment of plant cover on RM has been demonstrated by amending with different organic and inorganic substrates (Benjamin *et al*., 2011; Courtney and Timpson, 2005). Babu and Reddy (2011) collected the root samples along with the red mud flooded rhizospheric soil samples from plants such as *Acacia pennata* and *Lantana camara* growing adjoining

to red mud pond due to absence of vegetation in red mud pond. It has been shown that inoculation of AM fungi significantly increased the plant growth (**Fig. 3**), nutrient uptake and reduced Fe, Al accumulation in plant tissue and also improved the soil physico-chemical and biochemical properties. Their results provided evidence for the potential use of Bermuda grass in combination with AM fungi for ecological restoration of bauxite residue sites.

3) Mycorrhizal fungi in bauxite mining area

Degradation of forest land by way of mining leads to very serious environmental hazards. Mining in general and open cast mining in particular may lead to severe environmental degradation. Mining activities generate a variety of wastes whose presence in soils had adverse effects on plant growth such as low water infiltration rates, rough surfaces, poor aeration, high levels of heavy metals, low fertility, salinity and extremes of pH. Mycorrhizal fungi associated with plants growing in such environments are more adapted to these extreme conditions and promote the plant growth much better than the mycorrhizal fungi associated with non polluted environments. Khosla and Reddy (2008) tested the efficacy of the ectomycorrhizal fungus *Pisolithus albus* isolated from bauxite mining area on the growth and establishment of *Eucalyptus tereticornis* in

Table 1: Influence of arbuscular mycorrhizal (AM) fungi on the growth of *Eucalyptus tereticornis* seedlings grown in fly ash (Babu and Reddy, 2011).

Parameters	Control	AM fungi
Plant height (cm)	20.38±1.3	37.6±1.0*
Shoot dry weight (g)	0.83±0.1	1.5±0.1*
Root dry weight (g)	0.10±0.0	0.2±0.0*
AM fungal colonization (%)	18.4±0.6	76.1±2.3*
Chlorophyll a+b (mg/g. fw)	0.91±0.0	1.4±0.1*
Plant total P (mg/kg)	243.4±3.9	351.2±5.8*

*significant at P < 0.05
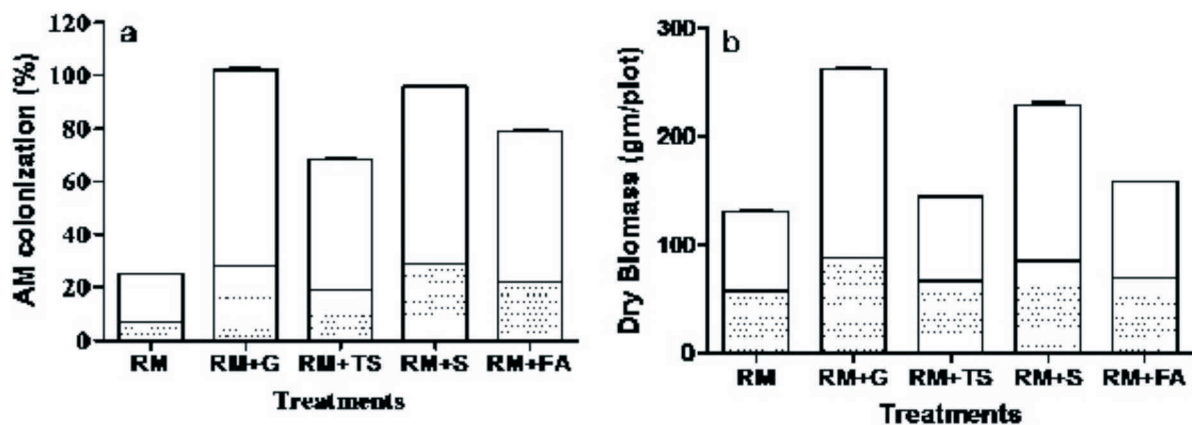
Fig. 3. a) Arbuscular mycorrhizal fungal colonization; b) Grass biomass of different treatments inoculated with AM fungi (empty bars) and respective controls (dotted bars). (RM, red mud; RM+G, red mud with gypsum; RM+TS, red mud with top soil; RM+S, red mud with sludge and RM+FA, red mud with fly ash) (Babu and Reddy, 2011)

Table 2: Effect of *Pisolithus albus* on the growth of *Eucalyptus tereticornis* plants grown in bauxite mined out soil (Khosla and Reddy, 2008)

	Non-mycorrhizal Plants	Mycorrhizal Plants
Shoot height (cms)	17.37	26.61 *
Shoot dry weight (gm)	0.56	1.10 *
Root dry weight (gm)	0.13	0.33 *
S/R ratio	4.38 *	3.31
Mycorrhizal colonization (%)	0.0	57.80 *

* significant at P <0.05 as determined by t- test.

bauxite mined soils. The ability of mycorrhizas to enhance plant growth and mineral nutrition on contaminated soils is illustrated in **Table 2**. The results clearly shows that growth and mineral nutrition of *Pisolithus* colonized *E. tereticornis* plants growing on bauxite mined out soil in the nursery conditions increases considerably as compared to plants not colonized with mycorrhizal fungi (Khosla and Reddy, 2008).

Jourand *et al*. (2010) reported *Pisolithus albus* isolated in nickel-rich serpentine soils from New Caledonia (a tropical hot spot of biodiversity) and showing *in vitro* adaptive nickel tolerance. Results showed that plants inoculated with *P. albus* were able to tolerate high and toxic concentrations of Ni (up to 60 µg g^{-1}) while uninoculated controls were not. The improved growth in ECM plants was associated with a 2.4-fold reduction in root Ni concentration but a massive 60-fold reduction in transfer of Ni from root to shoots. Similar observations had also been reported for the AM fungi that the inoculation of *E. tereticornis* seedlings with *Glomus aggregatum*, *G. fasciculatum* and *G. geosporum* and grown in bauxite mine spoils as potting medium under nursery conditions improved the biomass of the seedlings of *E. tereticornis*. The AM fungi inoculated seedlings of *E. tereticornis* showed 95% survival over the control seedlings and their growth was also significantly higher. Tissue nutrients (N, P, K) were also found higher in AM fungi inoculated seedlings than un inoculated control seedlings (Karthikeyan and Krishnakumar, 2012).

CONCLUSIONS

The introduction of plants in stressed or extreme environmental sites after inoculation with mycorrhizal fungi aids in the revegetation of degraded ecosystems. The mycorrhizal fungi have the potential to increase the efficiency of the plant system by providing the seedlings with essential levels of P and other nutrients for growth. There have been a large number of studies aimed at unravelling the mechanisms involved, and as to how mycorrhizal fungi affect the metal sensitivity of their hosts. Some ectomycorrhizal types may exclude metals from the host plant, although the mechanisms responsible for this (exudation of organic acids, adsorption, intracellular sequestration or hydrophobic restriction of apoplastic mobility) are not known, but binding to the external mycelium may be an important means by which metal exposure of the host plant is reduced. Other possible mechanisms including metal chelation by organic substances released from the mycorrhizal fungi have recently been demonstrated to be potentially important in ameliorating Al toxicity in ectomycorrhizal associations. There is a need not only to determine the function of potential mechanisms under laboratory conditions using few model organisms, but also to ascertain the ecological significance of these mechanisms working in real soil environment and understanding the broad range of mycorrhizal fungi present in forest ecosystems.

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