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Microorganism's application strategy for bio-phytoremediation of heavy metal: A review

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Abstract

Phytoremediation is a group of technologies that use plants to reduce, remove, de- grade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications. Phytoremediation efforts have largely focused on the use of plants to accelerate degradation of organic contaminants, usually in concert with root rhizosphere microorganisms, or remove hazardous heavy metals from soils or water. Phytoremediation of contaminated sites is a relatively inexpensive and aesthetically pleasing to the public compared to alternate remediation strategies involving excavation/removal or chemical in situ stabilization/conversion. Their potential role in phytoremediation of heavy metal (HM) contaminated soils and water is becoming evident although there is need to completely understand the ecological complexities of the plant-microbe-soil interactions and their better exploitation as consortia in remediation strategies employed for contaminated soils. The use of metal-accumulating plants to clean soil and water contaminated with toxic metals is the most rapidly developing component of this environmentally friendly and cost-effective technology. The recent discovery that certain chelating agents greatly facilitate metal uptake by soil-grown plants can make this technology a commercial reality in the near future.

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Introduction

Biosphere pollution by heavy metals and nucleotides was accelerated dramatically during the last few decades due to mining, smelting, manufacturing, treatment of agricultural soils with agro-chemicals and soil sludge, etc. Problems associated with the contamination of soil and water such as animal welfare, health, fatalities and disruptions of natural ecosystems are well documented (He *et al*., 2005). Heavy metals such as Pb, Cr, As, Cu, Cd, and Hg, being added to our soils through industrial, agricultural and domestic effluents, persist in soils and can either be adsorbed in soil particles or leached into ground water.

Human exposure to these metals through ingestion of contaminated food or uptake of drinking water can lead to their accumulation in humans, plants and animals. Lead, Copper, Zinc and Cadmium are also found naturally in soils and they can cause significant damage to environment and human health as a result of their mobility and solubilities. They can occur in soil and water in several forms and their speciation in soils is determined by sequential extraction using specific extract ants, which solubilize different phases of metals (Shuman, 1985). The physical and chemical characteristics of soil determine the speciation and mobility of heavy metals (Kabata-Pendias and Pendias, 1992).

Closely associated with the soil environment and have substantial impact on mankind (Doyle and Lee, 1986). We know very little about the enormous diversity of soil microbes, their properties, and behaviour in the soil environment. Soil microorganisms inhabiting the rhizosphere environment interact with plant roots and mediate nutrient availability, e.g. those forming useful symbiotic associations with the roots and contribute to plant nutrition. Implications of plants and their symbionts like mycorrhizal fungi, N-fixing rhizobia, and free living rhizosphere population of bacteria which promote plant growth need to be fully exploited and encouraged by inoculating nutrient poor agricultural soils with appropriate microbes (Khan, 2002a). The aim of this study review was to evaluate the effects of bio-fertilizers on the uptake of heavy metals by plants.

Biological mechanisms of phytoextraction

The best long-term strategy for improving phytoextraction is to understand and exploit the biological processes involved in metal acquisition, transport and shoot accumulation. In combination with the continuous search for novel phytoextracting plants, this understanding will enable improvements in phytoextraction efficiency. Recent advances in plant biotechnology should provide the means to rapidly capitalize on the mechanistic understanding of phytoextraction. Unfortunately, we know very little about the biological mechanisms involved in phytoremediation. Roots, which account for 20–50% of plant biomass, extract from the soil and deliver to the shoots most of the elements composing plant tissues, with the exception of carbon. Most of the work on the mechanisms of root and plant cell uptake has focused on the study of N, P, S, Fe, Ca, K and possibly Cl (Marschner, 1995). These studies produced some understanding of the processes involved in the acquisition of these essential elements. However, little is known about the mechanisms of mobilization, uptake and transport of most environmentally hazardous heavy metals, such as Pb, Cd, Cu, Zn, U, Sr, and Cs. It is clear that a large proportion of these metals remains sorbed to solid soil constituents. To acquire these 'soil-bound' metals, phytoextracting plants have to mobilize them into the soil solution. This so-called mobilization of 'soil-bound' metal can be accomplished in a number of ways:

1. Metal-chelating molecules can be secreted into the rhizosphere to chelate and solubilize 'soil-bound' metal. Until now, the major successes in phytoextraction were achieved by applying synthetic chelates to the soil (see above); however, there is a distinct advantage in using natural rootexuded compounds for this purpose. Only iron-chelating

compounds, termed phytosiderophores, have been studied well in plants (see below). These phytosiderophores are released in response to iron deficiency and can, in principle, mobilize Cu, Zn and Mn from soil. Mugineic and deoxymugeneic acids from barley and corn and avenic acid from oats are probably the best studied plant phytosiderophores (Kinnersely, 1993). Metal-chelating proteins, perhaps related to metallothioneins (Robinson Kramer *et al*.,1994) or phytochelatins (Rauser, 1995), may also function as siderophores in plants, although this has never been demonstrated; however, the contribution of phytosiderophores in toxic metal acquisition by the roots of phytoextracting plants remains largely unexplored. It has been recently reported that an Ni hyperaccumulator, *Alyssum lesbiacum,* may use histidine, an excellent Ni chelator, to acquire and transport Ni (Kramer *et al*.,1996).

2. Roots can reduce 'soil-bound' metal ions by specific plasma membrane bound metal reductases, which may increase metal availability. Pea plants deficient in Fe or Cu have an increased ability to reduce Fe3+ and Cu2+, which is coupled with an increased uptake of Cu, Mn, Fe and Mg (Welch *et al*., 1993).

3. Plant roots can solubilize soil-bound toxic metals by acidifying their soil environment with protons extruded from the roots. A similar mechanism has been observed for Fe mobilization in some Fe-deficient dicotyledonous plants (Crowley *et al*., 1974).

4. Roots can employ rhizospheric organisms (mycorrhizal fungi or root-colonizing bacteria) to increase the bioavailability of metals. However, the significance of microorganisms in the phytoremediation of metals remains largely unknown. It is believed that plant uptake of certain mineral nutrients such as Fe (Crowley *et al*., 1974), Mn (Barber and Lee, 1974), Cd (Salt *et al*., 1995) and possibly Zn (Y Kapulnik, personal communication) may be facilitated by rhizospheric microorganisms. Mobilized metals are taken up by plant roots from the soil solution and exported to the shoots. Very little is known about toxic metal transport into roots and their subsequent movement within the plant; however, some information is available on the transporter systems involved in the uptake of free and chelated Fe (for a review, see Marschner, 1995; Guerinot and Yi, 1996; Briat *et al*., 1995). A putative iron transporter has recently been cloned from *Arabidopsis* (Eide *et al*., 1996). Ca2+ and Mg2+ ions, which are present at high concentrations in soil solution and may not require mobilization, may enter the root via either extracellular (apoplastic) or intracellular (symplastic) pathways. These metal ions enter plant cells by an energy-dependent, saturable process via specific or generic metal ion carriers or channels (Clarkson and Luttge, 1989). Theoretically, toxic metals may compete for the same transmembrane carriers as those used by Ca and Mg; however, the high concentrations of these ions in soil solution makes this unlikely.

Most environmentally hazardous metals are too insoluble to move freely in the vascular system of the plant. Many form sulfate, carbonate or phosphate precipitates immobilizing these metals in apoplastic and symplastic compartments. Apoplastic transport of these metals is further limited by the high cation-exchange capacity of cell walls, unless the metal ion is transported as a noncationic metal chelate. Earlier studies showed that in hyperaccumulating and non hyperaccumulating plant species, some toxic metals may be transported to the shoot complexed to organic acids, mainly citrate *(Baker and Brooks, 1989)*. (*Senden et al*., 1995). Recent studies of Cd movement in *B. juncea*, a good Cd accumulator, showed that, in roots, Cd was present as a CdS4 complex, which may contain phytochelatins (Salt *et al*., 1995). In the xylem sap, Cd was coordinated predominantly with oxygen or nitrogen ligands, consistent with the involvement of organic acids (Salt *et al*., 1995). In the leaves, Cd preferentially accumulated in trichomes.

arbuscular mycorrhizae and phytoremediation

Despite the importance of the role that AM play in soil-microbe-plant interactions, relatively few studies

have focused on their potential in phytoremediation efforts. This is first due to the fact that earlier phytoremediation studies focused on the predominantly non-mycorrhizal plant families such as *Brassicaceae* and *Caryophyllaceae*, and second AM have not been considered by earlier workers as important component of phytoremediation practices. It is possible to improve the phytoremediation capabilities of plants by inoculating them with appropriate AM fungi.

Significance of arbuscular mycorrhizae

Arbuscular mycorrhizae 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 (Xavier and Boyetchko, 2002). AMF are known to improve plant growth and health by improving mineral nutrition, or increasing resistance or tolerance to biotic and abiotic stresses (Clark and Zeto, 2000; Turnau and Haselwandter, 2002).

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 (Pfleger and Linderman, 1994). 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 (Paulitz and Linderman, 1989; Lynch, 1990). Giovannetti and Avio (2002) 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 (Fitter, 1985). 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 (Bethlenfalvay and Linderman, 1992). 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 (Lovato *et al*., 1996; Azcon-Aguilar *et al.*, 2002). 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 (Bethlenfalvay and Linderman, 1992; Allen, 1991; 1992). 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 inoculum production. Monoxenic root-organ in vitro culture methods for AMF inocula production have also been attempted by various workers (Mohammad and Khan, 2002; Fortin *et al*., 2002) 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 (Sylvia and Jarstfer, 1994a; 1994b; Mohammad *et al*., 2000; Mohammad and Khan, 2002). Substrate-free colonized roots produced by these methods can be sheared and used for large-scale inoculation purposes. Mohammad *et al*.(2004) 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.

Arbuscular mycorrhiza-rhizobacteria interactions

The increased microbial activity in the rhizosphere soil affects the plant. A range of stimulated rhizosphere microorganisms such as saprophytes, pathogens, parasites, symbionts, etc., carry out many activities which are important to plant health and growth. Some of these microbes affect plant root morphology and physiology by producing plant growth-regulating hormones and enzymes. Others alter plant nutrient availability and biochemical reactions undertaken by them. AM fungi have differential effects on the bacterial community structure in the mycorrhizosphere (Paulitz and Linderman, 1989; Marschner and Baumann, 2003). PGPR (plant growth promoting rhizobacteria) may also improve plant P-acquisition by solubilizing organic and inorganic P sources through phosphatase synthesis or by lowering pH of the soil (Rodríguez and Fraga, 1999). Garbaye (1994) defined MHB (mycorrhization helper bacteria) as "bacteria associated with mycorrhizal roots and mycorrhizal fungi which collectively promote the establishment of mycorrhizal symbioses". There is growing evidence that diverse microbial populations in the rhizosphere play a significant role in sustainability issues (Barea, 2000; Barea *et al*., 2002), and that the manipulation of AMF and certain rhizobacteria like PGPR and MHB is important. Vivas *et al*.(2003) used a dual AM fungus-bacterium inoculum to study the effect of drought stress induced in lettuce grown in controlled-environment chambers. Their results showed that there was a specific microbe- microbe interaction that modulates the effectivity of AMF on plant physiology. The authors concluded that plants must be mycorrhizal in nutrient- poor soils and that mycorrhizal effects can be improved by co-inoculation with MHB such as *Bacillus* spp. Results of this study by Vivas and co-researchers show that co-inoculation of selected free-living bacteria isolated from adverse environments and AM fungi can improve the formation and function of AM symbiosis, particularly when the plant growth conditions are also adverse. Both AMF and PGPR complement each other in their role in N-fixation, phytohormone production, P-solubilization, and increasing surface absorption. Behl *et al*.(2003) studied the effects of wheat genotype and *Azobacter* survival on AMF and found that the genotype tolerant to abiotic stresses had higher AMF infection and a cumulative effect of plant-AMF-PGPR interaction was found. Similar observations were made by Chaudhry and Khan (2002; 2003), who studied the role of symbiotic AMF and PGPR N-fixing bacterial symbionts in sustainable plant growth on nutrient- poor heavy metal contaminated industrial sites and found that the plants surviving on such sites were associated with N-fixing rhizobacteria and had a higher arbuscular mycorrhizal infection, i.e. a cumulative and synergistic effect. The MHB cannot be ignored when studying mycorrhizal symbioses in their natural ecosystems. They are quite common and, as Garbaye (1994) said, they are found every time they are looked for, and they seem to be closely associated with the mycorrhizal fungi in the symbiotic organs. They are adapted to live in the close vicinity of the AM fungi as high frequencies of MHB populations are isolated from the mycorrhizae. Some MHB isolates also promoted ectomycorrhizae formation in four conifers (Garbaye *et al*., 1992), indicating that the MHB effect is not plant-specific. Various researchers showed that MHB's are fungus selective (Garbaye, 1994). Mosse (1962) showed that cell-wall degrading enzyme producing *Pseudomonas* sp. enhanced the germination of AM fungal spores of *Glomus mosseae* and promoted the establishment of AM on clover

roots under aseptic conditions. These observations were later supported by other workers (Mayo *et al.*, 1986; Linderman and Paulitz, 1990). Enriched soil microbial communities in the mycorrhizosphere are often organized in biofilms and probably horizontal gene transfer (HGT) between co-inhabiting microbial species and between plant-to-microbe occurs (van Elsas *et al.*, 2003). Many plant associated *Pseudomonas* rhizobacteria produce signal molecules for quorum sensing regulation, which were absent from soil-borne strains (Elasri *et al.*, 2001). This indicates that quorum sensing systems exist and are required in the mycorrhizosphere. Microbial colonies on the root surfaces consist of many populations or strains and positive and negative inter-population signaling on the plant root occur (Pierson *et al.*, 2002), which may play an important role in the efficiency of the use of biofertilizers. In addition to the above described interactions between AM fungi and rhizobacteria, certain bacteria- like organisms (BLOs) reside in the AM fungal cytoplasm, first described by Mosse (1962). Khan (1971) illustrated AMF spores, collected from the semi-arid areas of Pakistan, containing one to ten small spherical 'endospores' without any subtending hyphae of their own. Ultra structural observations clearly revealed their presence in many field-collected AM fungal isolates. Minerdi *et al.*(2002) reported the presence of intracellular and endosymbiotic bacteria belonging to the genus *Burkholderia* (a genus known to fix N) in fungal hyphae of many species of *Gigasporaceae*.

The authors used genetic approaches to investigate the presence of N-fixing genes and their expression in this endosymbiont and found *nif*HDK genes in the endosymbiont *Burkholderia* and their RNA messengers demonstrating that they possess a molecular basis for N-fixation. This discovery, as stated by Minerdi *et al.*(2002), indicates that a fungus which improves P-uptake, might also fix N through specialized endobacteria. Endosymbiont *Bukholderia* may have an impact on AMF-PGPR-MHB-Plant associations and metabolism. This finding suggests a new application scenario worth pursuing. Recent methodological developments in molecular and microscopical techniques together with those in genomes, bioinformatics, remote-sensing, proteomics, etc. will be of help in understanding the complexity of interactions existing between diverse plants, microbes, climates, and soil.

Conclusion

AM are ubiquitous and most plants are colonized by AMF in nature, i.e., mycorrhizosphere is the rule, not the exception. Thus if we are to understand the rhizosphere reactions and interactions, we must understand the mycorrhizosphere. Mycorrhization helper bacteria (MHB) might be exploited to improve mycorrhization, and AMF to improve nodulation and stimulate PGPR. More extensive field investigations on this multi-agent biofertilizer will make this a popular technology among field workers in agriculture, forestry and horticulture. Manipulation of microorganisms in the mycorrhizosphere for the benefit of plant growth requires research at the field level (Khan, 1975; 2002b). In order to exploit microbes as biofertilizers, biostimulants and bioprotectants against pathogens and heavy metals, ecological complexity of microbes in the mycorrhizosphere needs to be taken into consideration and optimization of rhizosphere/mycorrhizosphere systems need to be tailored. Smith (2002) stressed the need to better integrate information on root and soil microbe distribution dynamics and activities with known spatial and physiochemical properties of soil. This, as pointed out by Smith (2002), should be achieved through greater collaborative efforts between biologists, soil chemists and physicists.

References

Allen MF. 1991. The Ecology of Mycorrhiza. Cambridge Uni. Press, Cambridge, 184 P.

Allen MF. 1992. Mycorrhizal Functioning: An Integrative Plant-Fungal Process. Chapman & Hall Inc., Routledge, NY, 534 P.

Azcon-Aguilar C, Jaizme-Vega MC, Calvet C. 2002. The Contribution of Arbuscular Mycorrhizal Fungi to the Control of Soil-borne Plant Pathogens. In: Gianinazzi, S., Schuepp, H. (Eds.), Mycorrhizal Technology: from Genes to Bioproducts–Achievements and Hurdles in Arbuscular Mycorrhiza Research. Birkhauser, Basel, 187-198 P.

Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements a review of their distribution, ecology and phytochemistry. Biorecovery **1,** 81–126.

Barber DA, Lee RB. 1974. The effect of micro-organisms on the absorption of manganese by plants. New Phytologist **73,** 97–106.

Barea JM. 2000. Rhizosphere and Mycorrhizae of Field Crops. In: Toutant, P., Balazs, E., Galante, E.,

Lynch JM, Shepers JS, Werner D, Werry PA. (Eds.), Biological Resource Management, Connecting Science and Policy (OECD). INRA and Springer, Berlin, Heidelberg, New York.

Barea JM, Gryndler M, Lemananceau P, Schuepp H, Azcon R. 2002. The Rhizosphere of Mycorrhizal Plants. In: Gianinazzi, S., Schuepp, H., Barea, J.M., Haselwandter, K. (Eds.), Mycorrhizal Technology in Agriculture: from Genes to Bioproducts. Birkhauser, Basel.

Behl RK, Sharma H, Kumar V, Narula N. 2003. Interactions among mycorrhiza, Azobacter chroococcum and root characteristics of wheat varieties. J. Agron. & Crop Khan / J Zhejiang Univ Science **189(3),** 151-155. http://dx.doi.org/10.1046/j.1439-037X.2003.00026.x

Bethlenfalvay GJ, Linderman, RG. 1992. Mycorrhizae in Sustainable Agriculture. The American Phytopathological Society, Special Publication No. 54, St Paul, Minnesota, USA, 124 P.

Briat JF, Fobis-Loisy I, Grignon, N, Lobreaux S, Pasca,l N, Savino G, Thoiron S, Von Wiren N, Van Wuytswinkel O. 1995. Cellular and molecular aspects of iron metabolism in plants. Biology Cell. **84,** 69–81.

Chaudhry TM, Khan AG. 2002. Role of Symbiotic Organisms in Sustainable Plant Growth on Heavy Metal Contaminated Industrial Sites. In: Rajak, R.C. (Ed.), Biotechnology of Microbes and Sustainable Utilization. Scientific Publishers, Jodhpur, India, 270-279 P.

Chaudhry TM, Khan AG. 2003. Plants Growing on Abandoned Mine Site and Their Root Symbionts. In: Gorban, G.R., Lepp, N. (Eds.), Proceedings 7th International Conference on the Biogeochemistry of Trace Elements. Swedish University of Agricultural Sciences, Uppsala, Sweden, 134-135 P.

Clark RB, Zeto SK. 2000. Mineral acquisition by arbuscular mycorrhizal plants. Journal of Plant Nutrition. **23,** 867-902.

Clarkson DT, Luttge U. 1989. Mineral nutrition: divalent cations, transport and compartmentalization. Prog Botany **51,** 93–112.

Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ. 1991. Mechanisms of iron acquisition from siderophores by microorganisms and plants. Plant Soil, **130,** 179–198.

Doyle RJ, Lee NC. 1986. Microbes, warfare, religion and human institutions. Canadian Journal of Microbiology **32,** 193-200.

Eide D, Broderius M, Fett J, Guerinot ML. 1996. A novel ironregulated metal transporter from plants identified by functional expression in yeast. Proceedings of the National Academy of Sciences **93,** 5624–5628.

Elasri M, Delorme S, Lemanceau P, Stewart G, Laue B, Glickmann E, Oger PM, Dessaux Y. 2001. Acyle-homoserine lactone production is more common among plant-associated Pseudomonas spp. than among soilborne Pseudomonas. Applied and Environmental Microbiology **67(3),** 1198-1209. http://dx.doi.org/10.1128/AEM.67.3.1198-1209. 2001

Fitter AH. 1985. Functioning of vesicular-arbuscular mycorrhiza on growth and water relations of plants. New Phytologist. **89,** 599-608.

Fortin A, Bécard G, Declerck S, Dalpé Y, St-Arnaud M, Coughlan AP, Piché Y. 2002. Arbuscular mycorrhiza on root-organ cultures. Canadian Journal of Botany. **80(1),** 1-20. http://dx.doi.org/10.1139/b01-139

Garbaye J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist., **128(2),** 197-210. http://dx.doi.org/10.1111/j.14698137.1994.tb04003.x

Garbaye J, Churin JL, Duponnois R. 1992. Effects of substrate disinfection, fungicide treatments and mycorrhization helper bacteria on ectomycorrhizal formation of pedunculate oak (Quercus robur) inoculated with Laccaria laccata in two bare-root nurseries. Biology and Fertility of Soils, **13(1),** 55-57. http://dx.doi.org/10.1007/BF00337239

Giovannetti M, Avio L, 2002. Biotechnology of Arbuscular Mycorrhizae. In: Khachatourians, G.G., Arora, D.K. (Eds.), Applied Mycology and Biotechnology **2,** Agriculture and Food Production. Elsevier, Amsterdam, 275-310 P.

Guerinot ML, Yi Y. 1994. Iron: nutritious, noxious, and not readily available. Plant Physiolgy **104,** 815–820. 226 Plant biotechnology.

He ZL, Yang XE, Stoffella PJ. 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology. **19(2-3),** 125-140. http://dx.doi.org/10.1016/j.jtemb. 2005.02.010

Kabata-Pendias A, Pendias H. 1992. Trace Elements in Soils and Plants, 2nd Ed. CRC Press, Boca Raton, FL.

Khan AG. 1971. Occurrence of Endogone spores in West Pakistan soils. Transactions of the British Mycological Society. **56(2),** 217-224.

Khan AG. 1975. Growth Effects of Vesicular-arbuscular Mycorrhiza on Crops in the Field. In: Sanders, F.E., Mosse, B., Tinker, P.B. (Eds.), Endomycorrhizas. Academic Press, New York, 419-439 P.

Khan AG. 2002a. The Significance of Microbes. In: Wong, M.H., Bradshaw, A.D. (Eds.), The Restoration and Management of Derelict Land: Modern Approaches. World Scientific Publishing, Singapore, 80-92 P.

Khan AG. 2002b. The Handling of Microbes. In: Wong, M.H., Bradshaw, A.D. (Eds.), The Restoration and Management of Derelict Land: Modren Approaches. World Scientific Publishing, Singapore, p.149 160.

Kinnersely AM. 1993. The role of phytochelates in plant growth and productivity. Plant Growth Regul, **12,** 207–217.

Kramer U, Cotter-Howells JD, Charnock JM, Baker A, Smith A. 1996. Free histidine as a metal chelator in plants that accumulate nickel. Nature, **379,** 635–638. A demonstration of the possible involvement of histidine in Ni aquisition and transport in an Ni hyperaccumulating plant from the genus Alyssum.

Linderman RG, Paulitz TC. 1990. Mycorrhizal-rhizobacterial Interactions. In: Hornb, D. (Ed.), Biological Control of Soil-borne Plant Pathogens. CAB InKhan / J Zhejiang Univ SCIENCE B 2006 7(7):503-514 513 ternational, Wallingford, 261-283 P.

Lovato PE, Gianinazzi-Pearson V, Trouvelot A, Gianinazzi S. 1996. The state of art mycorrhizas micropropagation. Advances in Agriculture, Sciences., **10,** 46-52.

Lynch JM. 1990. The Rhizosphere. John Wiley & Sons, West Sussex, UK.

Marschner H. 1995 Mineral Nutrition of Higher Plants, edn 2. London, San Diego: Academic Press.

Marschner P, Baumann K. 2003. Changes in bacterial community structure induced by mycorrhizal colonization in split-root maize. Plant and Soil. **251(2),** 279-289. http://dx.doi.org/10.1023/A:1023034825871

Mayo K, Davis R, Motta J. 1986. Stimulation of germination of spores of Glomus versiforme by spore-associated bacteria. Mycologia, **78(3),** 426-431.

Minerdi D, Bianciotto V, Bonfante P. 2002. Endosymbiotic bacteria in mycorrhizal fungi: from their morphology to genome sequences. Plant and Soil. **244(1/2),** 211-219. http://dx.doi.org/10.1023/A:1020211821753

Miransari M, Bahrami HA, Rejali F, Malakouti MJ. 2008. Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (Triticum aestivum L.) growth. Soil Bio Biochem **40,** 1197–1206.

Mohammad A, Khan AG. 2002. Monoxenic in vitro production and colonization potential of AM fungus Glomus intraradices. Indian Journal of Experimental Biology. **40,** 1087-1091.

Mohammad A, Khan AG, Kuek C, 2000. Improved aeroponic culture of inocula of arbuscular mycorrhizal fungi. Mycorrhiza, **9(6),** 337-339. http://dx.doi.org/10.1007/s005720050278

Mohammad A, Mitra B, Khan AG, 2004. Effects of sheared-root inoculum of Glomus intraradices on wheat grown at different phosphorus levels in the field. Agric. Ecosystems & Environment **103(1),** 245-249. http://dx.doi.org/10. 1016/j.agee.2003.09.017

Mosse B. 1962. The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions Journal of general microbiology **27,** 509-520.

Paulitz TC, Linderman RG. 1989. Interactions between fluorescent pseudomonads and VA mycorrhizal fungi. New Phytologist. **113(1),** 37-45. http://dx.doi.org/10.1111/j.1469-8137. 1989.tb02393.x

Pfleger FL, Linderman RG. 1994. Mycorrhizae and Plant Health. Symposium Series, The American Phytopathological Society, St Paul, Minnesota, USA, 344 P.

Pierson LS, Pierson EA, Morello JE, 2002. Positive and Negative Cross-communication among Rhizobacteria. In: Leong, S.A., Allen, C., Triplett, E.W. (Eds.), Biology of Plant-microbe Interactions. International Society for Molecular Plant-microbe Interactions, St Paul, MN, USA, 256-262 P.

Robinson NJ, Tommey AM, Kuske C, Jackson PJ. 1995. Plant metallothioneins. Biochemistry 1993, 295:1–10. Rauser WE: Phytochelatins and related peptides. Plant Physiology **109,** 1411–1419.

Rodríguez H, Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Advances, **17(4-5),** 319-339. http://dx.doi.org/10.1016/S0734-9750(99)00014-2

Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I, Raskin I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Bio-Technology **13,** 468–474.

Salt DE, Prince RC, Pickering IJ, Raskin I. 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiology, **109,** 1427–1433. A characterization of Cd movement, speciation and accumulation in B. juncea.

Senden MHMH, Van Paassen FJM, Van Der Mer AJGM, Wolterbeek Hth. 1992. Cadmium–citric acid–xylem cell wall intractions in tomato plants. Plant Cell and Environment **15,** 71–79.

Shuman LM. 1985. Fractionation method for soil microelements. Soil Science **140(1),** 11-22.

Smith SE. 2002. Phosphate uptake and arbuscular activity in mycorrhiza Allium cepa L. Effects of photon irradiance and phosphate nutrition. Australian Journal of Plant Physiology **17**, 177–188.

Sylvia DM, Jarstfer AG. 1994a. Sheared root inoculum of vesicular arbuscular mycorrhizal fungi. Applied and Environmental Microbiology **58,** 229-232.

Sylvia DM, Jarstfer AG. 1994b. Production of Inoculum and Inoculation with Arbuscular Mycorrhizal Fungi. In: Robson, A.D., Abbott, L.K., Malajczuk, N. (Eds.),Management of Mycorrhizas in Agricture, Horticulture and Forestry. Kluwer, Dordrecht, 231-238 P.

Turnau K, Haselwandter K. 2002. Arbuscular Mycorrhizal Fungi: An Essential Component of Soil Microflora in Ecosystem Restoration. In: Gianinazzi, S., Schuepp, H. (Eds.), Mycorrhizal Technology: from Genes to Bioproducts. Birkhauser, Basel, 137-149 P.

Van Elsas JD, Turner S, Bailey MJ. 2003. Horizontal gene transfer in the phytosphere. New Phytologist., **157(3),** 525-537. http://dx.doi.org/10.1046/j.1469-8137.2003.00697.x

Vivas A, Marulanda A, Ruiz-Lozana JM, Barea JM, Azcon R. 2003. Influence of a Bacillus sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza **13(5),** 249-256. http://dx.doi.org/10.1007/s00572-003-0223-z

Welch RM, Norvell WA, Schaefer SC, Shaff JE, Kochian LV. 1993. Induction of iron (III) reduction in pea (Pisum sativim L.) roots by Fe and Cu status: does the root-cell plasmalemma Fe(III)- chelate reductase perform a general role in regulating cation uptake? Planta, **190,** 555–561.

Xavier IJ, Boyetchko SM. 2002. Arbuscular Mycorrhizal Fungi as Biostimulants and Bioprotectants of Crops. In: Khachatourians, G.G., Arora, D.K. (Eds.), App. Mycol. and Biotechnol. **2,** Agriculture and Food Production. Elsevier, Amsterdam, 311-330 P.