Vetiver grass as an Ideal Phytosymbiont for Glomalian Fungi for Ecological Restoration of Heavy Metal Contaminated Derelict Land

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Abstract: Pollution of the soil environment with toxic materials from fossil burning, mining and smelting of metalliferous ores, disposal of sewage, fertilizers and pesticides, etc. has increased dramatically since the onset of industrial revolution. Various strategies including bioremediation and phytoremediation are employed to remove heavy metals from such soils and making them available for agricultural purposes and urban developments. Role of plants as phytosymbionts and their associated arbuscular Glomalian mycorrhizal fungi as mycosymbionts are discussed as an alternative (mycorrhizo-remediation) strategy for safe and efficient decontamination of such soils. Prospects of using vetiver grass as an ideal phytosymbiont due its fast growth rate and root morphology and Glomalian mycorrhizal fungi as mycosymbionts for enhanced uptake of heavy metals is discussed.
Key words: phytosymbionts, mycosymbionts, arbuscular Glomalian mycorrhiza, heavy metals, vetiver grass, *Vetiveria zizanioides,* mycorrhizo-remediation, phytoremediation.
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1 GLOBAL SOIL CONTAMINATION

Due to ever increasing industrial, agricultural, and mining activities worldwide, heavy metal pollution of land and water is becoming globally important environmental, health, economic, and planning issue. There is an increase in world population, and unpleasant disposal of industrial effluents, especially in the third world countries, is causing soil pollution. Utilization of these lands for agricultural purposes and urban developments requires a safe and efficient decontamination process. With the increasing use of agrochemicals to maintain and improve soil fertility, unwanted elements such as cadmium into soils due to contaminated sources of fertilizers, especially in developing countries, are being introduced into agricultural soils, which, poses a potential threat to the food chain (Chaney and Oliver, 1996). Mining and industrial operations also lead to significant challenges for the management of the natural environments during and after these activities. Increased public awareness of the environmental impact of such activities is demanding an interdisciplinary, inter-organizational, and international effort.

2 REMEDIATION TECHNIQUES

2.1 Physical and Chemical Techniques

Various physical and chemical techniques to de-contaminated soils have been undertaken during the last 25 years (Salomons *et al.* 1995; Wise and Trantolo, 1995; Rao *et al*. 1996; Burns *et al*. 1996) and millions of dollars being spent by governments all over the world on preventive measures. However, all of them are labor intensive and costly, and cannot be applied to thousands of hectares of land contaminated with inorganic heavy metals (Burns *et al.* 1996). These technologies results in rendering the soil biologically dead and useless for plant growth as they remove all flora, fauna and microbes including useful nitrogen fixing bacteria and P-enhancing mycorrhizal fungi.

2.2 Bioremediation Techniques

Microbial bioremediation technology, well known for decontamination of organics (Flathman *et al.*, 1994), is not available for large-scale biodegradation of inorganic heavy metals. The health hazards caused by the accumulation of toxic metals in the environment together with the high cost of removal and replacement of metal-polluted soil have prompted efforts to develop alternative and cheaper techniques to recover the degraded land.

2.3 Phytoremediation

Restoration of derelict land by establishing a plant cover is important before it poses serious health hazard by transferring the trace metals into the surroundings. Current research in this area now includes utilization of plants to remediate polluted soils and to facilitate improvement of soils structure in cases of severe erosion, the innovative technique being known as phytoremediation (Chaudhry *et al.,* 1998; Khan *et al.,* 2000; Brooks, 1997).

Recently Reeves (2003) have reviewed tropical hyperaccumulator of heavy metal plants and concluded that there is a lack of investigation for the occurrence of hyperaccumulator plant species. No botanical or biogeochemical exploration of trace metal tolerant and/or accumulating plant species has yet taken place in many parts of the world. Many plant species, which can accumulate high concentrations of trace elements, have been known for over a century. Renewed interest in the role of these hyper-accumulating plants in phytoremediation has stimulated research in this area (Brooks, 1997). Several plant species or ecotypes, associated with heavy metal enriched soils, accumulate metals in the shoots. These plants can be used to clean up heavy metal contaminated sites by extracting metals from soils and accumulating them in aboveground biomass (Chaudhry *et al.*, 1999; Khan *et al.*, 2000). The metal enriched biomass can be harvested and smelted to recover the metal (phytomining).

2.3.1 Plants for Phytoremediation:

Plants that are used to extract heavy metals from contaminated soils have to be the most suitable for the purpose, i.e. tolerant to specific heavy metal, adapted to soil and climate, capable of high uptake of heavy metal(s), etc. Plants either take up one or two specific metals in high concentrations into their tissues (hyperaccumulators) with low biomass (Chaudhry *et al.*, 1998)), or extract low to average heavy metal (not metal specific) concentrations in their shoots with high biomass. Low biomass hyperaccumulators, generally, have a restricted root system (Ernst, 1996). In contrast, non-accumulators, high biomass producing and tolerant plants have physiological adaptation mechanisms, which allow them to grow in contaminated soils better than others (Palazzo and Lee, 1997). The tolerance and specific behaviour at the root level must be taken into consideration while selecting plants for phytoremediation (Keller *et al.*, 2003). Root system morphology allows some plants to be more efficient than others in nutrient uptake in infertile soil or stressed soil conditions (Fitter and Stickland, 1991).

2.3.2 Vetiver grass as an ideal plant for phytoremediation:

Recently vetiver grass, due to its ecofriendly nature, found a new use for phytoremediation of contaminated sites. Vetiver grass is both a xerophyte and a hydrophyte and, once established, is not affected by droughts or floods (Greenfield, 1988). It is highly tolerant to droughts and water logging, frost, heat, extreme soil pH, sodicity, salinity, Al and Mn toxicity (Truong and Claridge, 1996). It is also highly tolerant to a range of trace elements such as As, Cd, Cu, Cr, and Ni (Truong and Claridge, 1996). It is suitable for the stabilization and rehabilitation and reclaiming of acid sulfate and trace metals contaminated soils, i.e. phytoremediation. In Australia vetiver has been successfully used to stabilize mining overburden and highly saline, sodic, magnesic, and alkaline or acidic tailings of coal and gold mines (Truong, 1999). This grass has been extensively used for land protection by mitigating soil erosion and water conservation, especially on very steep slopes, due to its faster root growth, i.e. root length may reach up to 3 m just in one year (Lavania and Lavania, 2000; Walle and Sims, 1998). Vetiver grass is regarded as a tool for environmental engineering (Mucciarelli *et al.*, 1998) and as one of the most versatile crops of the third millennium (Maffei, 2002). Truong (1999) furnished field observations relating to high tolerance levels of vetiver grass to adverse soil conditions, trace metal toxicities, and agrochemicals. Chen *et al.* (2000) made a comparative study of the effects of chemical methods on the growth and uptake of trace elements by many plants including vetiver grass and found this perennial grass having a greater ability to remove Cd, Pb, and Zn from soil, the values of Cd accumulation close to those of hyperaccumulator *Thlaspi caerulescenes*. The authors discussed the effectiveness of phytoremediation with this grass with great biomass and concluded that 'VGT is an effective, low-cost, and environmentally friendly technology to clean Cd contaminated soils'. The authors suggested to develop a genetically modified vetiver grass incorporating genes of hypoaccumulator. Recently Shu *et al.* (2002) reported enhanced trace metal extraction in field experiments using vetiver grasses for re-vegetation of Pb / Zn mine tailing. VGT is emerging as an alternative technology for rehabilitation of degraded, saline, or trace metal contaminated soils, and for purification of water polluted with trace elements, agrochemicals, and industrial- effluent disposals.

The success of phytoremedial efforts is not only dependant upon the choice of plant species but also their method of establishment (Bradshaw, 1987). Among the plants involved in phytoremedial measures, Vetiver grass {*Vetiveria zizanioides* L. (Nash)}, should receive special attention. It is a densely tufted, awnless, wiry and glabrous plant occurring in large clumps as hydrophyte or xerophyte on vertisols through to red alfisols. It can grow on both acidic (pH 3) and alkaline (pH 11) soils, and is tolerant to high levels of various trace metals such as arsenic, cadmium, copper, chromium and nickel (Truong and Claridge, 1996; Truong and Baker, 1998; Truong, 1999). It is one of those few plants which possess both economical and ecological capabilities, i.e. volatile essential oil distilled from its roots in over 70 countries (Akhila and Rani, 2002) and its conservation properties, such as up to 2 m high plant with a strong dense and mainly vertical root system often measuring > 3 m, useful in soil erosion control (Greenfield, 1988, 1989, 1993, 1995; Truong, 2002). It is propagated vegetatively and is non-invasive (National Research Council, USA, 1993)). It is extremely resistant to insect pests and diseases (Zisong, 1991) and is widely used worldwide for soil and moisture conservation and soil restoration. It is immune to flooding, grazing, fires, and other hazards (Grimshaw and Helfer, 1995). Maffei (2002) stated that this plant is one of the most versatile crops of the third millennium. On the request of the World Bank, the US Agency for International Development, and the US Conservation Services, the National Research Council of USA, evaluated the usefulness of the vetiver system and its implications. China is among the nations most active in studying vetiver system and massive projects have been started in several-conservation areas, as soil and moisture conservation are China's national priorities. Vetiver was introduced in China in 1950's as a source of aromatic oil and when the oil prices dropped, the plant was abandoned. It was only in 1988, that its importance in soil and moisture conservation was realized and massive vetiver trial projects started by China's Ministry of Water Resources in many provinces (Vetiver Information Network, 1993). Chinese scientists and researchers also started their own vetiver networks to exchange their experiences and results of vetiver trials. Hill and Peart (1999) reviewed the methodology of vetiver trials and results of these trials in Hong Kong and Guizhou, China, for soil erosion control.

3 SOIL MICROBES AND PLANT RELATIONSHIPS

3.1 Soil Microbes

Soil contains a great variety of microbial populations, the properties and behavior of which affect the function of the soil ecosystem in many crucial ways, especially microbial re- mediation of C, N, S, P, Fe, Mn, Hg and Se (Khan, 2002a). Microorganisms in soil are not only affected by the physical and chemical properties of the soil in general, but also by the moisture, temperature, pH and organic matter released from the roots in the rhizospheres of plants, all of which alter the microbial diversity and activity (Lynch, 1982).

Nevertheless, relatively few studies have focused on the effects of rhizosphere microorganisms on the soil erosion and metal remediation efforts, despite the important role that these microorganisms play in plant interactions with soil environment (for toxic metals in particular). Added to this, the effects of phytoremediation practices on microbial communities to the remediated sites have also been largely ignored, and these microbes may be essential for re-vegetation efforts following the removal of excessive soil metals (Pawlowska *et al.*, 2000; Khan 2003b).

3.2 Arbuscular Mycorrhizal Fungi

Among the rhizosphere microorganisms involved in plant interactions with soil, the arbuscular mycorrhizal (AM) fungi, which belong to Family Glomales, Class Zygomycetes, should receive special attention. This is because over 80 % of the world's plant species are mycorrhizal and potentially benefit from AM fungus-mediated mineral nutrition (Smith and Read, 1997). Recently, plants capable of forming association with AM fungi have been shown to accumulate a considerable amount of trace metals (Burke *et al.*, 2000; Karagiannidis and Nikolaou, 2000). Unfortunately, the role that AM fungi play in plant interactions with soil metals is not fully understood (Pawlowska *et al.*, 2000; Weissenhorn and Leyval, 1996), and little is known about mycorrhiza functioning under conditions imposed by trace metal remediation protocols and also the effects of phytoextraction on AM fungi. Arbuscular mycorrhizal fungi (AMF) should be considered as an essential component of soil microflora and as a potential tool for re-establishment of plant cover and population diversity during ecosystem restoration (Turnau and Haselwandter, 2002). The rate of reclaiming derelict land may be increased by AMF inoculation of plants used for re-vegetation, i.e. mycorrhizo-remediation (Jamal *et al.*, 2002).

Arbuscular mycorrhizal (AM) fungi, which coevolved with plant roots, form symbiotic associations with around 82 % of angiosperms (Brundrett, 2002). These fungi are well known to improve plant growth on nutrient-poor soils and enhance the uptake of P, Cu, Ni, Pb, and Zn (Khan *et al.,* 2000). Despite the important role that AM fungi play in plant interactions with the soil environment in general, and trace elements in particular, relatively few studies have focused on their effect on the metal remediation efforts. Early phytoremediation studies have focused on the predominantly non-mycorrhizal plant families, e.g. Brassicaceae or Caryophyllaceae, so AM have not been considered as important component of phytoremediation practices (Pawlowska *et al.,* 2000).

Term 'mycorrhizo-remediation' is coined by Jamal *et al.* (2002) to use mycorrhizal plants in phytoremediation of heavy metal contaminated soils. The interaction between arbuscular mycorrhizae (AM) and minerals other than phosphorus, particularly trace elements, has been the subject of many recent studies because of the possibility of the beneficial effect of mycorrhizae in improving the tolerance of plants against toxicity (Khan *et al.*, 2000). The uptake of trace elements by mycorrhizal plants depends on several factors such as physicochemical properties of the soil, particularly its fertility level, pH, the host plants and the fungi involved, and above all, the concentration of the trace elements in soil. Under deficiency conditions, most studies point to an increase in trace element uptake by mycorrhizal plants. For example, increased Zn uptake by maize inoculated with mycorrhizal fungus, *Glomus mosseae,* has been reported compared to the non-mycorrhizal plants at low Zn levels (Kothari *et al.*, 1991). Another study revealed an increase in Cu uptake by white clover inoculated with *G. mosseae* under low level of Cu - a limiting factor (Li *et al.,* 1991). Jamal *et al.* (2002) found AM fungi enhancing the uptake of zinc and nickel from contaminated soils by soybean and lentils. Using indigenous AM fungal populations as mycorrhizal treatment, it has been observed that there is a decrease in foliar concentration of Pb in mycorrhizal *Anthyllis cytisoides* plants growing in Pb polluted soil (Diaz *et al.,*1996). However, when the soil contained potentially toxic amounts of trace elements, mycorrhizal formation usually induces lower concentrations of these elements in the aerial part of the host plant, thus consequently lead to a beneficial effect on plant growth. This has been reported for Zn when growing white clover in Zn contaminated soils (Zhu *et al.*, 2001). Reduction in both Zn and Pb contents in shoot when growing *Lygeum spartum* and *Anthyllis cytisoides,* inoculated with AMF in different concentrations of Pb and Zn amended soils, has also been observed (Diaz *et al.*, 1996). Davies *et al.* (2002) reported higher chromium accumulation in sunflower plants inoculated with AM fungus *Glomus intraradices* as compared to the non-mycorrhizal plants. The authors showed that the mycorrhizal fungi helped to partially alleviate Cr toxicity and enhanced plant growth. They found greatest Cr concentrations in the roots, intermediate in the shoots and lowest in leaves. Khan (2001) studied the relationships between Cr III magnification ratio, accumulation factor, and AM in three tree species growing on tannery effluent-polluted soil, reported differing capacity of Cr III uptake, and found AM encouraged mineral nutrition, including Cr.

4 VETIVER; AM MYCORRHIZAE & PHYTOREMEDIATION

4.1 Historical Background to Vetiver Grass

Vetiver grass (*Vetiveria zizanioides* (L.) Nash*)* has been known to be a useful plant for thousands of years and has been cultivated for the production of scented oil produced by its roots as well as for its ability to retain soil and prevent erosion (Maffei, 2002). It is a perennial grass belonging to the family Poaceae (Gramineae), originally from India, growing wild or cultivated. The name derives from the Tamil ‘vetti’ (khus-khus) and ‘ver’ (root), referring to aromatic roots. It is mentioned in the ancient Sanskrit writings and it is a part of Hindu mythology. Since late last century, it is being used by the sugar industry in West Indies, Fiji, and eastern African islands like Mauritius for its soil conservation properties.

4.2 Historical Background to Glomalian Fungi

Glomales are one of the oldest group of fungi, older than land plants. First land plants, Bryophytes, appeared 476-430 million years ago in early Silurian periods. No fossil records are available for the rootless fresh water Charophycean alage, which were the probable ancestors of land plants, to show if they were mycorrhizal. Fossil evidence of Glomales in the rhizomes of early vascular plants like Sphenophytes, Lycopodophytes, and Pteridophytes, suggests that the origin of vascular terrestrial plants is likely from their Bryophyte-like ancestors(Edwards *et al.* 1998). Both living and Triassic fossil cycads had glomalian arbuscular mycorrhizal fungi in their roots. AM associations are ubiquous in the living angiosperms which probably arose in the early Cretaceous (Khan, 2003). The phylogenetic relationship between origin and diversification of AM fungi and coincidence with vascular land plants was investigated by Simon *et al.* (1993) by sequencing rDNA genes as a molecular clock to infer dates. The authors estimated the origin of AM-like fungi of 353-462 Myr ago, which is consistent with the hypothesis that AM were instrumental in the colonization of land by ancient plants. This hypothesis is also supported by the observation that AM can now be found worldwide in the angiosperms, gymnosperms as well as ferns, suggesting that its nature is ancestral (Brundrett, 2002).

4.3 Vetiver as Phytosymbiont and Glomalian Fungi as Mycosymbionts for Mycorrhizo-remediation of Heavy Metal Contaminated Land

Although vetiver grass is regarded to be suitable candidate for phytoremediation (Chen *et al.* 2000), no record of its mycorrhizal status exists in literature. *V. zizanioides* showed good growth performance on Pb/Zn mine tailings in Shu et al's (2002) revegetation field experiment. Again no mention of its mycorrhizal status was made by the authors. Vietmeyer (2002), while discussing VGT beyond the hedge against soil erosion and essential oil secretions, identified many areas requiring investigations including symbiosis of vetiver grass roots with mycorrhizal fungi and N-fixing bacteria. Wong (2003) found the roots of vetiver grass, growing in the South China Botanical Gardens in Guangzhou, RPC, in soil containing moderate amounts of basic nutrients and trace elements, to be mycorrhizal. As far as we are aware of, this is the first published record of occurrence of AM in vetiver grass. McGee *et al.* (2003) studied the role of mycorrhizae in revegetation of a waste disposal area south of Sydney, Australia, and reported establishment of seedlings of native plants and mycorrhizal fungi in these extremely disturbed conditions. Thus it is possible to return the degraded site to the original condition, providing we know the nature of plants and their mycorrhizal associates (McGee *et al.*, 2003). The authors also found that mycorrhizal fungi were associated with stabilization of the soil, especially between widely spaced plants. Soil stabilization, in turn, will reduce erosion and assist in revegetation extremely impoverished soils.

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