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Rehabilitation of abandoned mine sites: connection to bioprospecting of metal tolerant plants and phytoassisted rhizoremediation

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ABSTRACT

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Contemporary strategies for rehabilitation and remediation of abandoned mine sites and the need for bioprospecting metal tolerant plants have been reviewed with particular emphasis on phytoassisted rhizoremediation. The key processes involved in phytoremediation technology are (i) metal uptake, transport, accumulation and (ii) phytostabilization. Other related applications are: erosion control of mine tailings and metals and use of environmental and industrial crops. Phytoremediation can be *in situ*, growing, harvesting plants on a contaminated site an aesthetically pleasing process, solar-energy driven, and passive technique. This technique is being used along with or, in some cases, in place of expensive conventional chemical and mechanical cleanup methods. Environmental degradation due to Acid Mine Drainage and role of aquatic macrophytes for its rehabilitation are also presented.

Key-words—Metal tolerant plants (MTP), Phytoassisted rhizoremediation, Toxic metals, Accumulators, Excluders, Indicators, Environmental implications, Phytomanagement and Phytotechnologies.

परित्यक्त खदान स्थलों का पुनर्वास : धातु सह्य वनस्पतियों व पादपसहायी राइज़ोउपाय का जैवपूर्वेक्षण से संबंध

एम.एन.वी. प्रसाद, मणि राजकुमार एवं हेलेना फ्रैटाज

सारांश

परित्यक्त खदान स्थलों तथा जैवपूर्वेक्षण धातु सह्य वनस्पतियों हेतु जरूरतों के पुनर्वास एवं उपायों हेतु समकालीन योजना पादपसहायी राइज़ोउपाय पर विशेष जोर के साथ पुनरीक्षित की गई हैं। पादपउपाय प्रौद्योगिकी में आलिप्त मुख्य प्रक्रियाएं हैं ;पद्ध धातु अंतर्ग्रहण, परिवहन, संचयन तथा ;पपद्ध पादपस्थिरीकरण। अन्य संबंधित अनुप्रयोग हैं; खदान पछोड़न एवं धातुओं का अपरदन नियंत्रण तथा पर्यावरणीय एवं औद्योगिक फसलों का उपयोग। पादपउपाय तत्रैव हो सकता है, खड़ी फसलों वाले पौधे संदूषित स्थलों पर उगाना एक सौंदर्यात्मक रूप से सौर ऊर्जा प्रचार तथा निष्क्रिय तकनीक सुखद प्रक्रिया है। यह तकनीक खर्चीले रूढ़ रासायनिक एवं यांत्रिक शोधन विधियों के कुछ मामलों के साथ या उनमें प्रयुक्त होती है अम्ल खान अपवाह तथा जलीय दीर्घफाइटों की वजह से पर्यावरणीय अपक्षय इसके पुनर्वास हेतु भी प्रस्तुत किए गए हैं।

मुख्य शब्द—धातु सह्य वनस्पतियाँ (एम टी पी), पादप सहायी राइज़ोउपाय, विष धातुएं, संचायक, अपवर्जित, सूचक, पर्यावरणीय युगपत अंतर्वृद्धियाँ पादप प्रबंधन एवं पादप प्रौद्योगिकी।

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INTRODUCTION

BIOGEOCHEMICAL cycling of essential and non-essential elements in ecosystem is a complex phenomenon (Adriano, 2001). Al, Ca, Fe, Na, P, K, S, Si, Ti, Mg; bioelements, viz. C, H, N and O, constitute about 99% of the elemental composition of the environment. Elements such as As, Cd, Co, Cu, Cr, Hg, Mo, Mn, Ni, Pb, Se, Zn, etc. constitute about 1% of the total elemental content of the soil, and hence are called trace elements. The term 'heavy metal' for those weighing more than 5 g cm^{-3} such as Zn (7.1), Cr (7.2), Cd (8.6), Ni (8.7), Co (8.9), Cu (8.9), Mo (10.2), Hg (13.5) and Pb (11.4); while Al (2.7) is a light metal; 'As and Sn' half-metals and 'Se' non-metal. Some metals can occur in different valence states, so that one element may be more or less toxic in different states. One example is Cr(III) and the more toxic Cr(VI). A few like Cs, Hg and Ga are liquids at room temperature. Elements, viz. As, B, Cd, Cr, Cu, Hg, Ni, Pb, Se, U, V, and Zn are present naturally in soils in low concentrations but may be elevated because of human activities, fossil fuel combustion, mining, smelting, sludge amendment to soil, fertilizer application, and agrochemical application (Prasad, 2006a-d). At low concentrations some trace elements, e.g. Cu, Cr, Mo, Ni, Se, Zn, etc. are essential for healthy functioning of biota. However, higher concentrations of these essential elements cause toxicity. Some trace elements are also non-essential, e.g. As, Cd, Hg, Pb, etc. are extremely toxic to biota even at very low concentrations.

Metal tolerant plants (Metallophytes) are predominated by members of Brassicaceae, Cyperaceae, Cunoniaceae, Caryophyllaceae, Fabaceae, Flacourtiaceae, Euphorbiaceae, Lamiaceae, Poaceae, Violaceae, etc. Plants that accumulate and hyper accumulate toxic metals would have the following ecological and environmental implications (Baker, 1981; Baker *et al*., 1994a, b; Bashmakov *et al*., 2002, 2006; Brooks, 1998; Prasad, 2006 a-d).

(a) Exhibit elemental allelopathy (elemental defense)

(b) Biogeochemical prospecting of minerals

(c) Occur as endemics, hot spots are mineralized belts

(d) Diversified applications in phytotechnologies

(e) Pose risks to human health through food chain, when leached out and the bioavailability is increased

Phytoremediation (use of green plants to remove, contain, or render environmental contaminants harmless) is an emerging biogeotechnological application based on the "green liver concept". This technology operates on the principles of biogeochemical cycling. Phytoremediation projects have been successful for the cleanup of metal polluted/contaminated soil, surface water, groundwater, air and for ecological restoration of degraded mines. Extensive diversity of native and non-

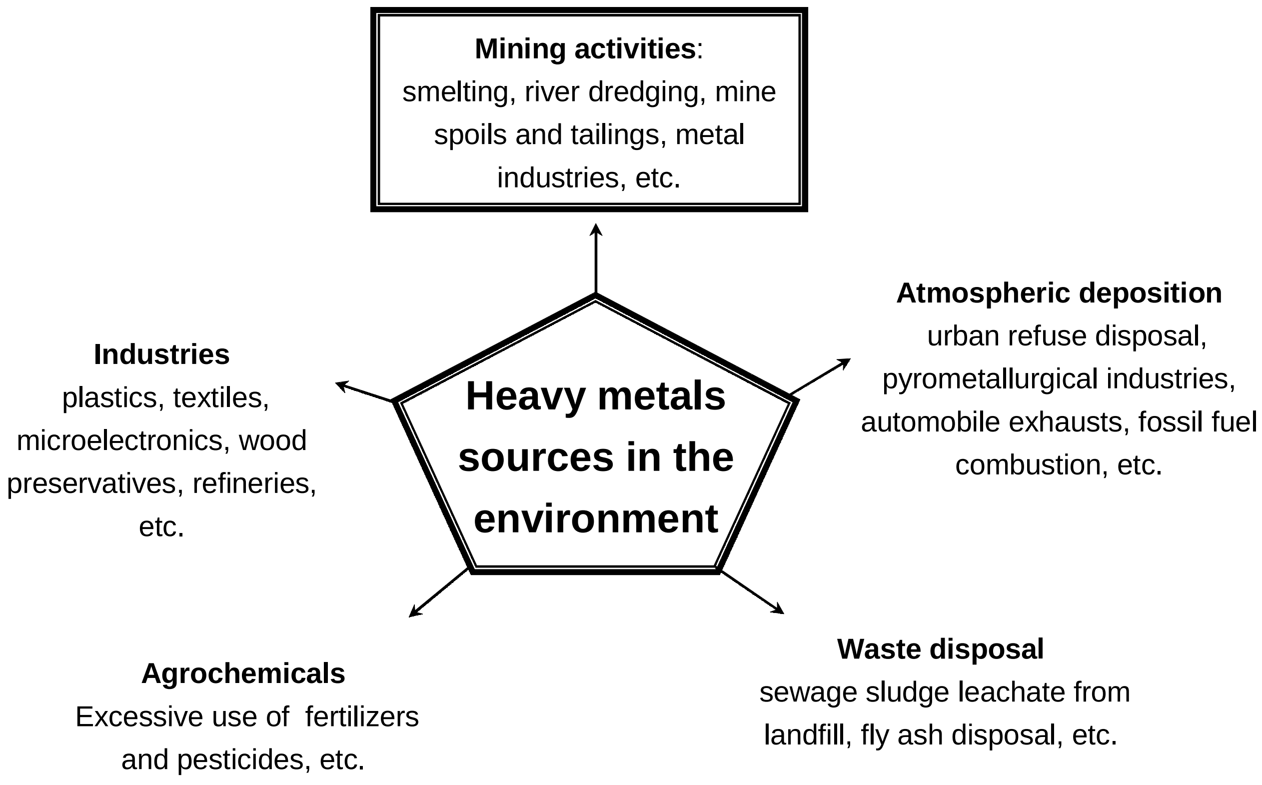

Fig. 1—Environmental exposure of heavy metals due to mining and related technogenic activities.

native plants have been applied in this strategy. This review will focus on the recent advancements in phytoremediation technology emphasizing the need for (i) biodiversity prospecting for success of this strategy, (ii) rhizosphere biotechnology.

STRATEGIES OF ABANDONED MINE RECLAMATION

Different terms have been frequently used in the literature for remediation of the abandoned mines.

(a) Restoration: Replication of site conditions prior to disturbance.

(b) Reclamation: Rendering a site habitable to indigenous organisms.

(c) Rehabilitation: Converting the metal contaminated land to a producive form which is in conformity with a prior to land use plan.

(d) Phytoremediation: It is an environmental cleanup strategy in which selected green plants are employed to remove, contain, or render environmentally toxic inorganic and organic contaminants harmless. This is an emerging biogeotechnological application based on the "green liver concept" and operates on the principles of biogeochemical cycling.

(e) Full restoration: Restoration of a site to its pre-damaged condition.

(f) Partial restoration: Restoration of selected ecological attributes of the site; and Creation of an alternative ecosystem type; the latter though often desirable, is not to be called restoration.

Mining and related technogenic sources of heavy metal resulted in contamination and pollution of the biogeosphere. The failure to rehabilitation resulted in man-made catastrophe, e.g. pyrite mine of Aznalco´llar in SW Spain (Moreno *et al*., 2001). Several strategies have been proposed for variety of mine spoils. Addition of organic matter and calcium amendments are the promising strategies.'Green corridor' or 'Green belt' establishment on technogenically contaminated and polluted sites would depend on successful

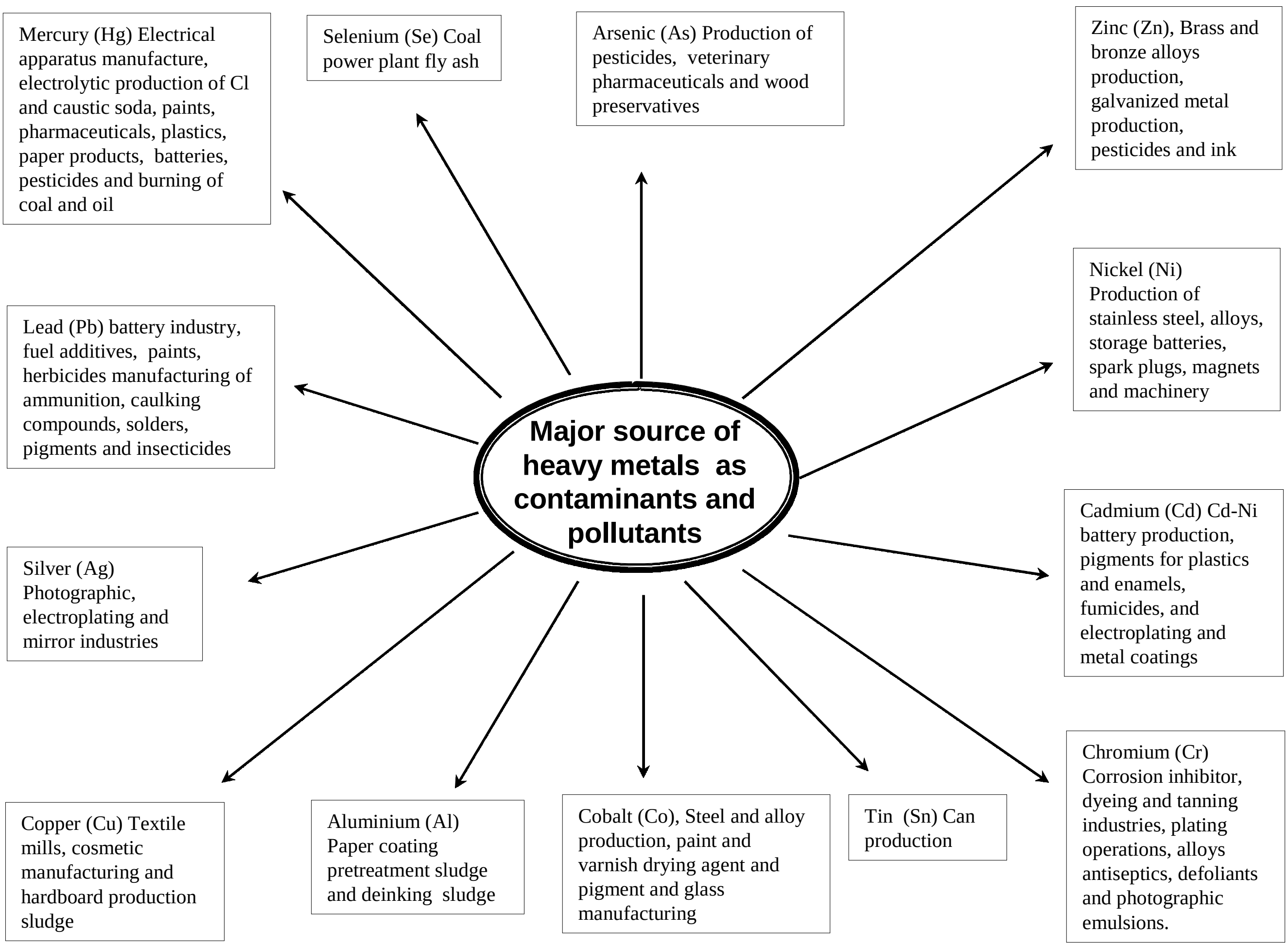

Fig. 2—Plant availability of toxic trace elements.

immobilisation of trace elements in the heavy metal laden substrates.

The restoration of a dense vegetation cover is the most useful and widespread method to physically stabilise the mine wastes and to reduce metal pollution effects (Bargagli, 1998). Different plant species that are well adapted to the local conditions, capable of excluding and accumulating heavy metals without showing toxic symptoms are the ideal species that should be considered for early stages of revegetation of the 'green corridor' or establishment of 'green belt'. Several of the grasses, legumes and trees can be a suitable material for this purpose (McLaughlin *et al*., 2000; Prasad, 2006d). Smith *et al*. (1998) suggested the Bermuda grass (*Cynodon dactylon*), for stabilising metalliferous soils.

METAL TOLERANT PLANTS FOR REHABILITATION OF MINE SITES

Serpentine soils (ultrabasic rocks) "hotspots" of metallophyte endemics are the rich source of toxic trace elements. Serpentinized rocks are distributed all over the world.

Serpentine soils contain very high nickel (~10 mg per gram soil); cobalt and chromium, both of which are present at lower levels compared to nickel. These soils are also rich in iron and magnesium mixed with silica and low nutrient levels. Soil scientists call this condition ultramafic ("ma" stands for magnesium and "f" for ferrum, or iron). The weathering processes in serpentine soils are highly dependent on variations in climatic regime, age, topography, chemical composition of the parent material and biological activity. Hyperaccumulator plants are geographically distributed and are found throughout the plant kingdom (Brooks, 1998). For latest information about such plants please refer to Demers *et al*., 2008; Dixon *et al*., 2008; Green & Renault, 2008; Lottermoser *et al*., 2008; Trois *et al*., 2007; Prasad, 2004 a-c, 2006 a-d, 2007; Prasad *et al*., 2006 and others. Tree-grass-legume association was found to be the best combination for restoration of mica, copper, tungeston, marble, dolomite, limestone, and mine spoils of Rajasthan and elsewhere in India (Prasad, 2007).

PHYTOASSISTED RHIZOREMEDIATION BY MICROBES

A number of plants which can tolerate and accumulate high concentration of metals were discovered and defined as hyperaccumulators. In general the ideal hyperaccumulators for phytoremediation require the characteristics of rapid growth and a high amount of biomass (Nie *et al*., 2002). But in fact, to date most of the identified metal hyperaccumulators are small and slow growing (Mulligan *et al*., 2001). Moreover, the metals at elevated levels are generally toxic to most plants impairing

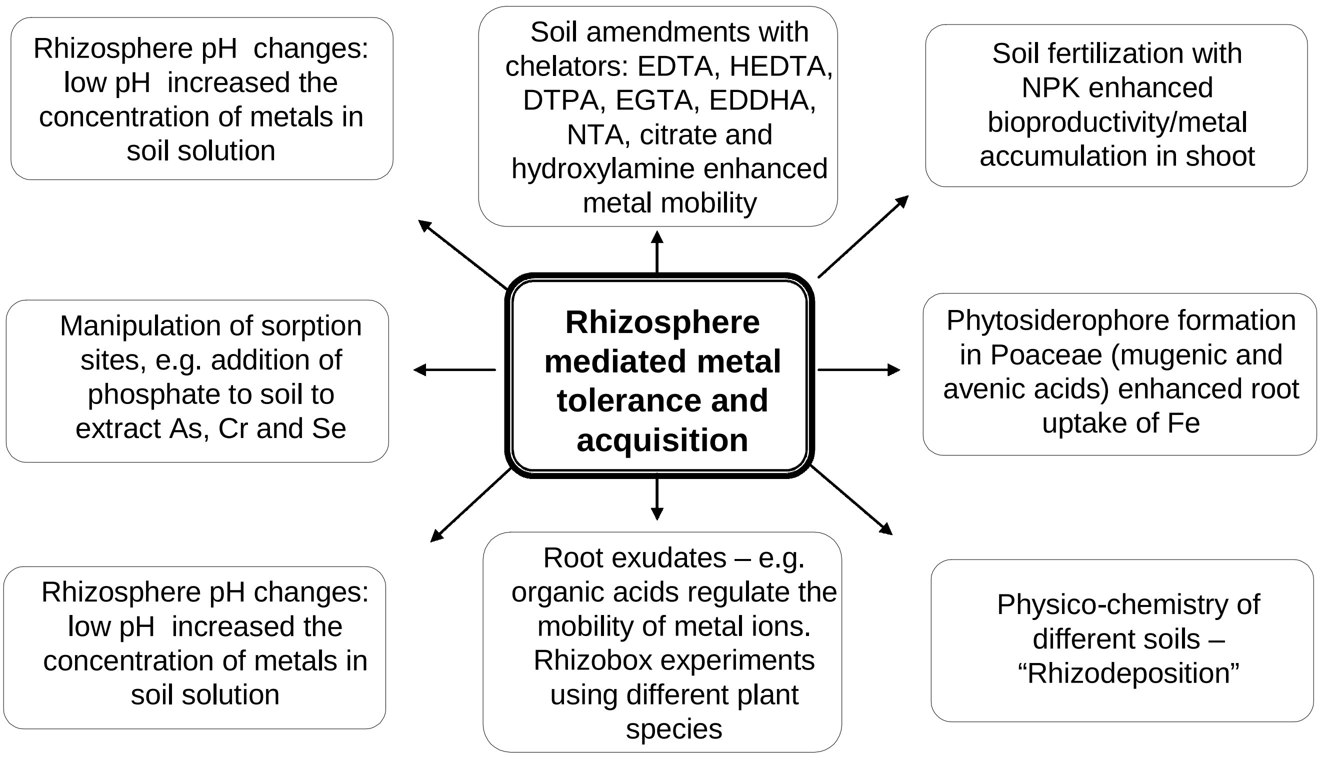

Fig. 3—Rhizosphere mediated metal tolerance and acquisition mechanisms.

their metabolism and reducing plant growth. These properties have an adverse impact on the potential for metal phytoextraction and restrict the employment of this technology. In this regard, studies on the interactions among metals, rhizosphere microbes and plants have attracted attention because of the biotechnological potential of microorganisms for plant growth promotion in metal rich soils and metal removal directly from soils or the possible transfer of accumulated metals to higher plants (Zaidi *et al*., 2006; Dell'Amico *et al*., 2008; Rajkumar & Freitas, 2008a). Further, rhizosphere microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests (Elsgaard *et al*., 2001; Filip, 2002). On the other hand, the plant root exudates provide nutrition to rhizosphere microbes, thus increasing microbiological activity in the rhizosphere, which in turn stimulate plant growth and reduce the metal toxicity in plants. Among the rhizosphere microorganisms involved in plant interactions with the soil milieu, the plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) deserve special attention.

PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

Rhizosphere microorganisms, which are closely associated with roots, have been termed plant growth promoting rhizobacteria (PGPR) (Glick, 1995). Several metal-tolerant rhizosphere bacteria have been isolated from metal contaminated soils, which can be useful for reclamation of such metal contaminated soils as they are found to be associated with a large number of plant species in metal rich soil. The bacterial flora associated with rhizosphere of various hyperaccumulators have been isolated and characterized from metal contaminated soils (Idris *et al*., 2004; Mengoni *et al*., 2001; Aboudrar *et al*., 2007). Abou-Shanab *et al*. (2007) collected forty-five bacterial strains from Oregon serpentine soil (thirty from the rhizosphere of *Alyssum murale* and fifteen from Ni rich soil) and tested for their ability to tolerate arsenate, cadmium, chromium, zinc, mercury, lead, cobalt, copper, and nickel in their growth medium. The authors observed a large number of the strains were resistant to Ni (100%), Pb (100%), Zn (100%), Cu (98%), and Co (93%). Five of the strains (about 11.2% of the total), specifically *Arthrobacter rhombi*, *Clavibacter xyli*, *Microbacterium arabinogalactanolyticum*, *Rhizobium mongolense* and *Variovorax paradoxus* were tolerant to nine different metals. Similarly, Mengoni *et al*. (2001) characterized the heterotrophic nickel-resistant bacteria from three different serpentine outcrops in central Italy populated by the nickel-hyperaccumulating plant *Alyssum bertolonii*. These authors found that serpentine bacterial communities tolerated spiking of metals, such as nickel, more than those collected from unpolluted soils and that the presence of *A. bertolonii* led to an increase in metal-resistant bacteria proportion in the soil samples collected near the plants.

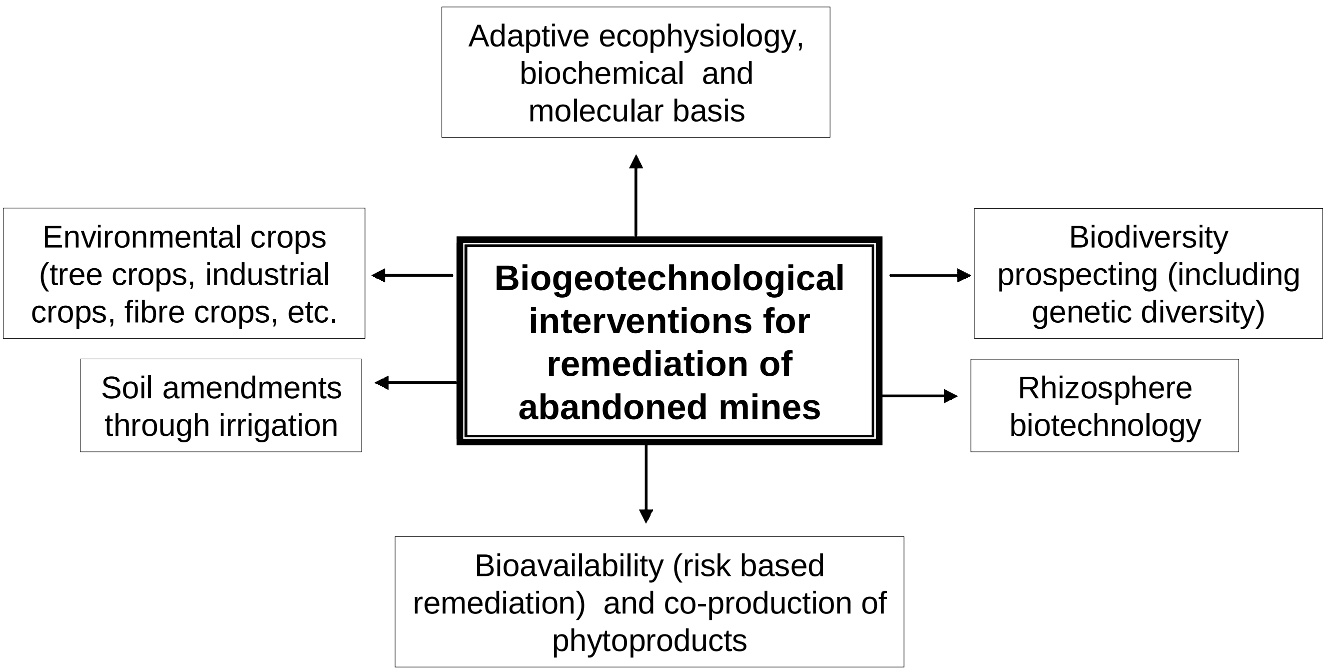

Fig. 4—Metal-ion bioavailability and interactions between phases is the key factor for remediation of abandoned mine spoils.

The metal resistant PGPR include a diverse group of free-living soil bacteria that can improve host plant growth and development in heavy metal contaminated soils by several mechanisms. The best-known mechanism is the utilization of ACC by PGPR. A number of PGPR, which stimulate the growth of different plant species including mustard, castor bean, canola (Ma *et al*., in press; Rajkumar & Freitas, 2008b; Dell'Amico *et al*., 2008), contain the enzyme ACC deaminase, which hydrolyses ACC (the immediate precursor of the plant hormone ethylene. Some of the plant ACC is exuded from roots or seeds and cleaved by ACC deaminase to NH_3 and á-ketobutyrate (Penrose & Glick, 2001). The PGPR utilize the NH_3 evolved from ACC as a source of N and thereby decrease ACC within the plant with the concomitant reduction of plant ethylene (Grichko & Glick, 2001). A recent study by Belimove *et al*. (2005) found that there is a high correlation between the in vitro ACC deaminase activity of the bacteria and their stimulating effect on root elongation suggested that utilization of ACC is an important bacterial trait determining root growth promotion in metal contaminated soils.

It should be mentioned that siderophore production by PGPR is also believed to play an important role in plant growth in metal contaminated soils. In general, the reduction of plant growth in metal contaminated soil is often associated with iron deficiency and reduced uptake of some other essential element (Ma & Nomoto, 1993). However, microbial siderophore-iron complexes can be taken up by plants, and thereby serve as an iron source for plants (Burd *et al*., 2000; Rajkumar *et al*., 2006). For instance, the siderophores produced by PGPR improve Fe uptake by mustard and pumpkin (Sinha & Mukherjee, 2008) and maize (Sharma & Johri, 2003) with the disappearance of the chlorosis phenomenon in mung bean (Tripathi *et al*., 2005). This suggests that the inoculation of siderophore producing PGPR in the rhizosphere/seeds can increase the growth of plants in the presence of heavy metals including nickel, lead and zinc (Idris *et al*., 2004; Tripathi *et al*., 2005).

Besides, the PGPR promotes the plants growth by solubilising insoluble phosphate and producing IAA. The heavy metals at elevated levels in soil interfere with uptake of P and other nutrients and lead to plant growth retardation (Halstead *et al*., 1969). Under such conditions, metal resistant PGPR offer a biological rescue system capable of solubilizing the insoluble P and make it available to the plants (Zaidi *et al*., 2006; Rajkumar & Freitas, 2008b). For instance, *Achromobacter xylosoxidans* isolated from Cu rich mine soil exhibits a potential

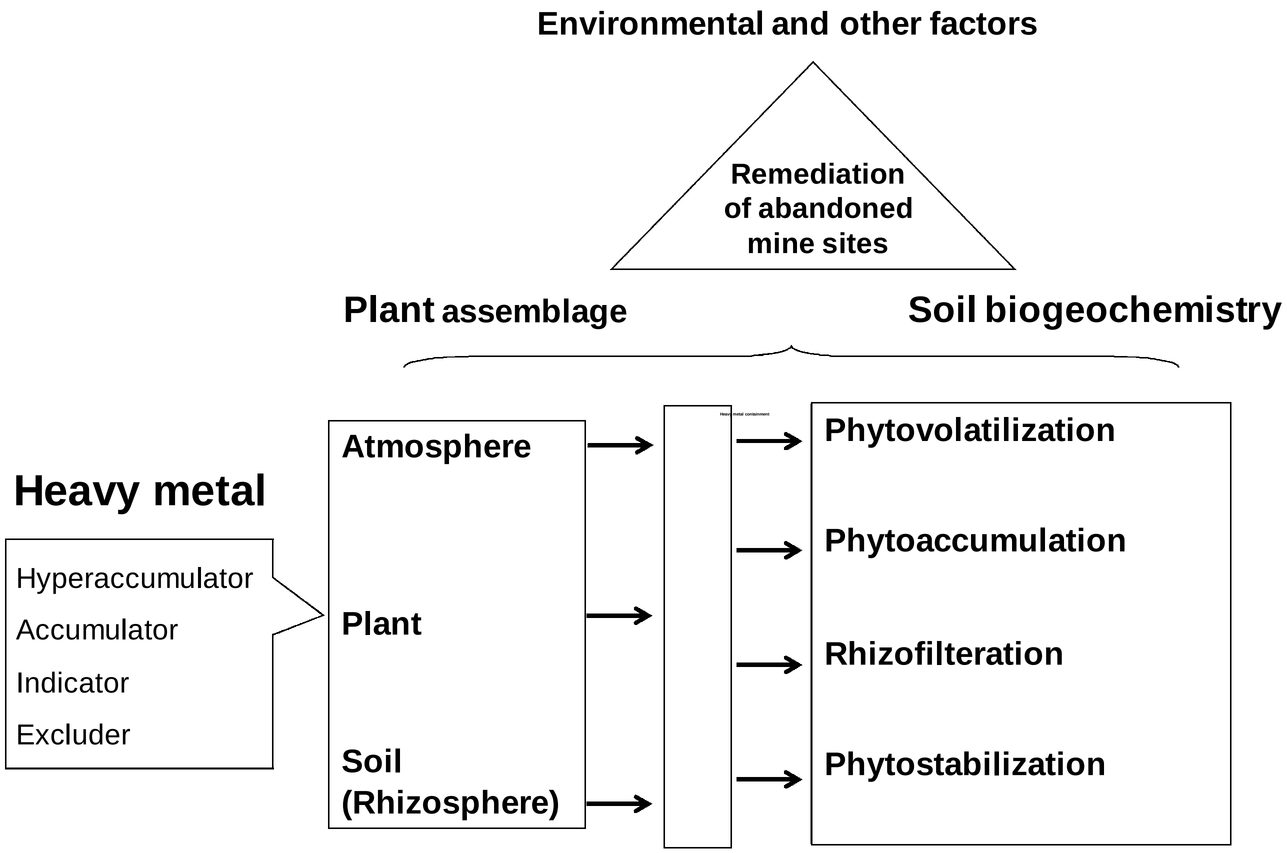

Fig. 5—Biogeotechnological interventions and biodiversity and genetic diversity prospecting for remediation of abandoned mines.

for phosphate solubilization observed in higher shoot and root length and biomass with inoculated plants (Ma *et al*., in press). Another phosphate solubilising PGPR *Pseudomonas* sp. NBRI 4014 was shown to stimulate the growth of soybeans in a soil contaminated with Ni, Cd and Cr (Gupta *et al*., 2002) and the mixed inoculam of N-fixing bacteria (*Azotobacter chroococcum* HKN-5), P solubilizers (*Bacillus megaterium* HKP-1), and K solubilizers (*Bacillus mucilaginosus* HKK-1) increased the growth of mustard in metal contaminated soil by increasing the content of N and S in mustard (Wu *et al*., 2006). These authors established that nutrients play an important role in the detoxification of heavy metals while heavy metals inhibit the assimilation of these elements by plants. Further, the production of IAA by PGPR is believed to play an important role in plant-bacterial interactions and plant growth in metal contaminated soils (Vivas *et al*., 2003; Sheng & Xia, 2006; Dell'Amico *et al*., 2008). Root elongation of mung bean has been shown to be stimulated by IAA synthesized by *Pseudomonas* strain GRP3 (Sharma *et al*., 2003) as well as *P. auriginosa* MHRh3 on the black gram roots (Ganesan, 2008). Concentrations of IAA are different from one microorganism to another: 39 ng/ml with *Pseudomonas asplenii* AC (Reed *et al*., 2005) and 55 mg/ml with *Bacillus subtilis* SJ-101 (Zaidi *et al*., 2006). According to the IAA level, root elongation changes qualitatively. A low level of IAA produced by rhizosphere bacteria promotes primary root elongation whereas a high level of IAA stimulates lateral and adventitious root formation but inhibit primary root growth (Xie *et al*., 1996). Thus PGPR can facilitate plant growth by altering the plant hormonal balance. The metal resistant PGPR belonging to different genera such as *Pseudomonas, Mycobacterium, Bacillus, Agrobacterium* and *Achromobacter* were found to have plant growth-promoting features that can potentially promote plants growth and reduce heavy metals stress symptoms in plants (Dell'Amico *et al.*, 2008; Zaidi *et al*., 2006; Rajkumar *et al*., 2005; Ma *et al*., in press).

The process of metal uptake and accumulation by different plants depends on the concentration of available metals in soils, solubility sequences and the plant species (Gupta & Sinha, 2006). In addition to plant growth promoting

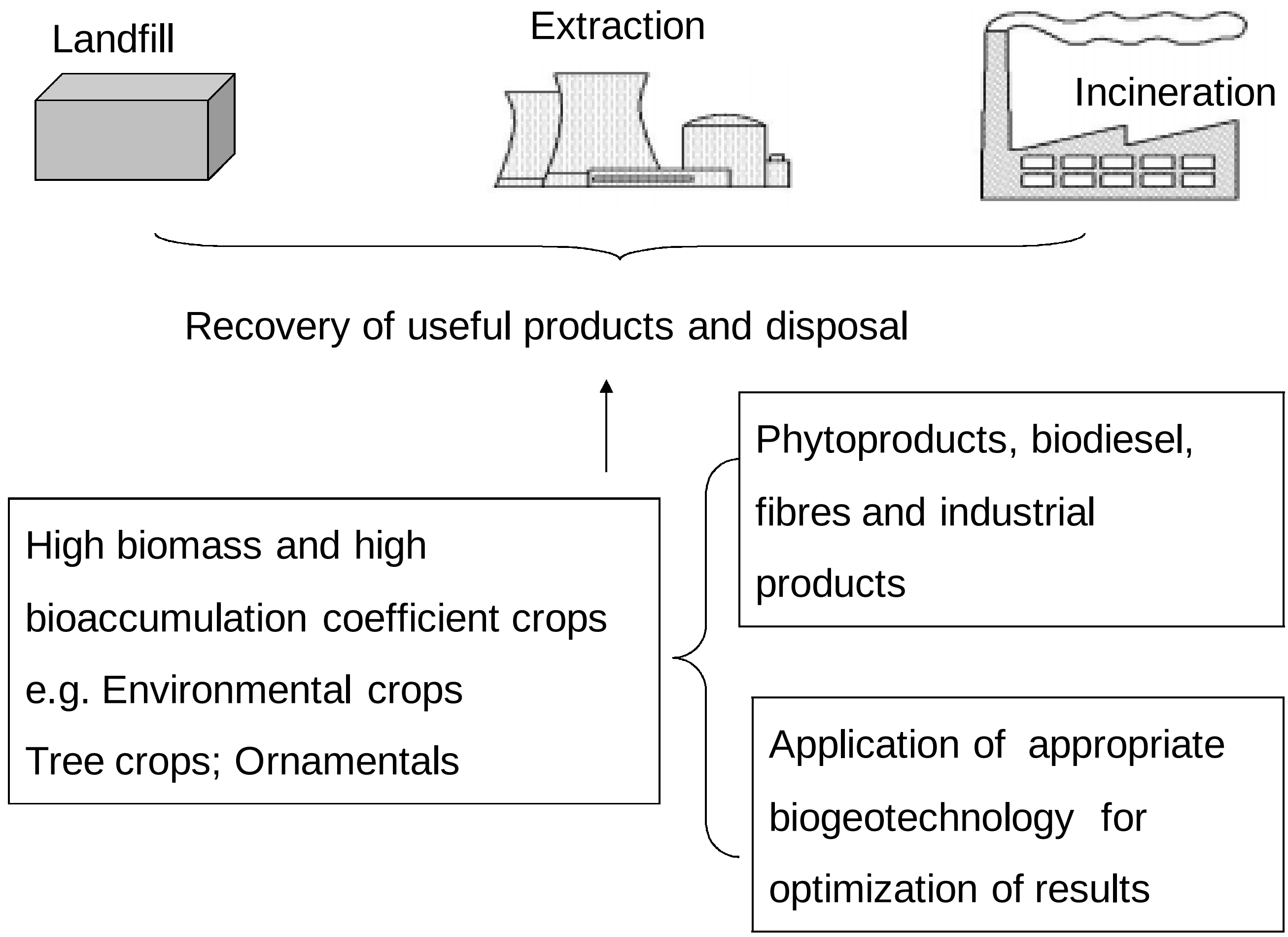

Fig. 6—Recovery of useful phytoproducts and disposal from plants growing on metalliferous substrates.

potential, the metal resistant PGPR have been shown to possess several traits that can alter heavy metal mobility and availability to the plant (Rajkumar *et al*., 2005; Chen *et al*., 2005; Wu *et al*., 2006), through acidification, or by producing iron chelators, siderophores, organic acids and/or mobilizing the metal phosphates (Zaidi *et al*., 2006; Abou-Shanab *et al*., 2003; Tripathi *et al*., 2005). Abou-Shanab *et al*. (2006) observed that the inoculation of *Microbacterium oxydans, Rhizobium galegae, Clavibacter xyli, Acidovorax avenae, M. arabinogalactanolyticum* and *M. oxydans* to serpentine soils significantly increased the bioavailable Ni concentration thus enhancing the availability of Ni to *Alyssum murale*. They indicated that rhizobacteria facilitated the release of Ni from the non-soluble phases in the soil as a result of organic acids or siderophore production and phosphate solubilisation. Similar observations have also reported by Rajkumar and Freitas (2008b) who found that the inoculation of *Pseudomonas* sp. PsM6 or *P. jessenii* PjM15 to surface sterilized roots of *Ricinus communis* increased the Zn concentration in shoots compared with non-inoculated controls. The increased accumulation of metals in presence of PGPR might be due to more metal uptake under acidic soil conditions, which develops as a result of P solubilisation and/or excretion of organic acids in soil (Chen *et al*., 2005). Effects of pH on the solubility and speciation of metals are well documented (Gadd, 2001; Gadd & Sayer, 2000). Sheng and Xia (2006) reported that the addition of Cd-resistant bacterial strains to *Brassica napus* grown in metal contaminated soil significantly increased the plant uptake of Cd when compared with the non-inoculated controls as a result of pH reduction. From these studies, it can be concluded that by inoculating the seeds/rhizosphere with selected PGPR with beneficial features (plant growth promoting and metal mobilizing potential), it should be possible to improve the phytoextraction efficiency of hyperaccumulator plants in metal rich mine soils.

In contrast, Madhaiyan *et al*. (2007) reported that the inoculation *Bradyrhizobium* sp. CBMB20 and CBMB40 to metal contaminated soils reduced Ni/Cd availability in soil and also reduced the metal uptake in roots and shoots of tomato. Similarly, Ganesan (2008) reported the inoculation with plant growth promoting bacterium *Pseudomonas aeruginosa* MKRh3 increased the plant growth and reduced the Cd uptake in black gram and it was probably due to bacterial immobilization of Cd in rhizosphere. *Mesorhizobium huakuii* (Ike *et al*., 2007) and the bacterial strains isolated from water hyacinth (So *et al*., 2003) protect the plants through heavy metal immobilization. Furthermore, inoculation of plants with PGPR strains may also contribute in reducing the phytotoxic effects of the metals by sharing the metal load due to their ability of biosorption and bioaccumulation (Vivas *et al*., 2003; Zaidi *et al*., 2006; Rajkumar & Freitas, 2008b).

ARBUSCULAR MYCORRHIZAL FUNGI (AMF)

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that establish mutual symbioses with the majority of the roots of higher plants, providing a direct physical link between soil and plant roots (Smith & Read, 1997). They occur in almost all habitats and climates including disturbed soils such as those derived from mine activities, but soil disestablished usually produce changes in the diversity and abundance of AMF population (Jeffries & Barea, 2001). On the other hand, the occurrence of AMF in metal contaminated soils has been reported for several plant species, suggesting that AMF colonisation could be a critical strategy for plant survival in these areas (Mikus *et al*., 2005; Thangaswamy *et al*., 2005; Tonin *et al*., 2001). Leung *et al*., (2007) conducted a survey for AMF components in five mining sites with higher concentrations of heavy metals such as Pb, Zn, Cu, Cd at Chenzhou City, Hunan Province, southern China. They found three components of mycorrhizal colonization (arbuscules, vesicles and coiled hyphae) in the roots of *Cynodon dactylon* and *Pteris vittata* growing in mining site. It was apparent that AM fungi were associated with a majority of the plants in the mining sites and supported plant survival in metal affected soils.

It is well known that arbuscular mycorrhizal (AM) colonisation can improve plant nutrition as well as protect plants under heavy metal stress. In Portuguese ultramafic soils the occurrence of AM has been reported for several herbaceous species, suggesting that AM colonisation could be a critical strategy for plant survival in these areas (Gonçalves *et al*., 1995). Gonçalves *et al.* (2001) investigated the seasonal trends in the AM colonization of *Festuca brigantina*, an endemic grass and typical serpentinophyte, and suggested that AM colonisation confers enhanced phosphorus nutritional status, enriching its pool for reproductive period during which the demand will be quite high (Fig. 3). The biotechnological interventions for remediation of abandoned mines and criteria of applicability and monitoring are depicted in Fig. 3.

The effects of AMF on the plant growth and the uptake of metals by plants have been studied extensively in recent years (Trotta *et al*., 2006; Mikus *et al*., 2005), and it is generally accepted that under high metal concentrations in the soil, AMF may protect the host plants by both improved P nutrition and decreased metal uptake and/or translocation (Chen *et al*., 2007). Most of the reports note a positive effect of AM inoculation on the growth of plants in metal-contaminated soils. This protective benefit may be related to the adsorptive or binding capability for metals of the relatively large fungal biomass associated with the host plant roots, which may physically minimize or exclude the entry of metals into host plant (Cairney & Meharg, 2000). Uptake of metals may be influenced by absorption on hyphal walls as chitin has an important metal-binding capacity. In experiment with *Leucaena* *leucocephala,* Lins *et al*. (2006) reported that a large proportion of increased Cu content of mycorrhizal plants was sequestered in the roots. Further, in the shoots, Cu concentrations were much lower than in the roots and lower in inoculated than in non-inoculated plants. This seems to indicate that mycorrhization benefits plants under conditions of excess of heavy metals, by retention of heavy metals in the roots or even in the mycelium (Huang *et al*., 2005). Trotta *et al*. (2006) reported that the inoculation of *Glomus mosseae* BEG 12 and *G. margarita* BEG34 improved dry weight and leaf area of *Pteris vittata* grown in As contaminated soil and reduced root metal concentration in comparison to non-inoculated control. Similarly, Mikus *et al*. (2006) reported that zinc and cadmium concentrations were lower in the biomass of mycorrhizal *Thlaspi praecox* plant than that of nonmycorrhizal ones, suggesting that *Glomus fasciculatum* decreased zinc and cadmium uptake or its translocation to the shoots. The lower metal concentration in mycorrhizal plants has also been attributed to larger plant biomass resulting in a ‘‘growth dilution’’ effect (Shen *et al*., 2006; Lin *et al*., 2007). AMF reduce metal accumulation in leaves of non-hyperaccumulators (Joner & Leyval, 2001) and it has also been shown that AMF protect the plants against metal toxicity by increasing the concentrations of metal chelating compounds, i.e. cysteine and glutathione (GSH) (Galli *et al*., 1995). Besides, the binding of metals in mycorrhizal structures (vacuoles) and immobilization of metals in the mycorrhizosphere may also contribute to alleviate metal phytotoxicity by lowering their accumulation in plant. AMF can modify Zn/Cd mobility by increasing the pH (less available Zn/Cd in the soil solution and therefore less Zn/Cd in root and shoot) (Shen *et al*., 2006). These examples illustrate AMF could protect the plants from the toxicity of excessive heavy metals by changing the speciation from bioavailable to the non-bioavailable form and reflect that AMF are most suitable for phytostabilization. However, different ecotypes differ in their capacity to protect the plant from metal toxicity by exhibiting differential metal uptake levels into hyphae and plant (Toler *et al*., 2005). Each contaminated site has a specific profile of pollutants for which an appropriate combination of fungal and plant genotypes must be established. Further, other interactions take place in the soil that could positively or negatively affect the expected efficiency of heavy metal stabilization.

AMF can also enhance metal uptake by plants, e.g. Leung *et al*. (2006) reported that the addition of rhizofungi enhanced the uptake and accumulation of As in *Pteris vittata* under the condition of 100 mg As per kg soil, noncolonized plants accumulated 60.4 mg As kg^{-1} while plants colonized by AMF isolated from an As mine accumulated 88.1 g As kg^{-1} and also enhanced plant growth. Similarly, the enhanced accumulation of Zn in shoot by AMF inoculation has been reported for *Solanum nigrum* (Marques *et al*., 2008a, b) and sorghum (Toler *et al*., 2005). These examples illustrate that AMF can lead to increased uptake and subsequent accumulation of metals in above-ground tissues of plants.

The beneficial effects of AMF in combination with bacteria have been reported by a number of workers (Vivas *et al*., 2003, 2005; Rabie & Almadini, 2005). The ability of soil PGPR to stimulate the growth and the activity of AMF has been well documented and most probably involves the production of bioactive stimulatory compounds such as hormones, vitamins, amino acids, organic acids and enzymes (Barea *et al*., 2002; Vivas *et al*., 2006; Kozdroj *et al*., 2007). The inoculation of *Brevibacillus* sp. with a mixer of AMF showed a noticeable increase in plant growth, nitrogen and phosphorus accumulations, and mycorrhizal infection in Pb contaminated soils (Vivas *et al*., 2003). Similarly, Duponnois *et al*. (2006) reported the inoculation of fluorescent pseudomonads increased AMF colonization in the contaminated soil. Several species of bacteria have been identified as mycorrhiza helper bacteria mainly represented by a number of fluorescent pseudomonads (Krupa & Kozdroj, 2007). On the other hands, some reports stated that the presence of AM fungi is known to enhance nodulation and N fixation by legumes (Lin *et al*., 2007). Moreover, AM fungi and N-fixing bacteria often act synergistically on infection rate, mineral nutrition and plant growth.

In conclusion, these examples illustrate that inoculation of metal resistant microbes (PGPR and/or AMF) not only protects plant from metal toxicity but also (mostly the PGPR inoculation) enhances the metal accumulation in plant tissue with concurrent stimulation of plant growth. These beneficial effects caused by inoculation with metal resistant microbes, together with the suggested interrelationship between microbial heavy metal resistance and plant growth promoting efficiency, indicate that inoculation with microbes might have some potential to improve phytoextraction efficiency in metal contaminated soils. However, almost all the previous works on phytoextraction with PGPR and/or AMF were carried out in lab or greenhouse. Hence, extensive research including the interactions among plants, heavy metals and microbes in metal contaminated natural soils are required to implement these microbial-assisted phytoremediation in field level.

ACID MINE DRAINAGE (AMD)

The exposure and oxidation of iron sulphide from coal mining results in acid mine drainage (AMD). AMD significantly impairs the quality of aquatic ecosytems. AMD is a serious problem in the Appalachian region of the United States of America. The US bureau of mines estimated that about 20,000 km of streams or rivers are impaired by the AMD. Therefore, in recent years several low cost preventive and passive technologies are being developed. The technologies rely upon the biological diversity and natural principles of biogeochemical cycling to remediate the contaminated mine waters. For e.g. *Glyceria fluitans* (floating sweetgrass) is an amphibious plant and was found growing in the tailings pond of an abandoned lead-zinc mine. Greenhouse experiments demostrated that *G. fluitans* could grow in sand culture treated with high zinc sulphate solution. *Phragmites australis* and *Typha latifolia* have since been grown on both alkaline and acidic zinc mine tailings in field conditions. In this regards metal tolerant flora in constructed wetlands, anoxic lime stone drains (ALD) and successive alkalinity producing systems (SAPS) have remedial potential and promising results have been obtained in several instances (Prasad, 2001). The vital processes inolved in remediation of mine tailings using by emergent and submerged plants are (a) preventing formation of AMD, (b) removal of metals from AMD, (c) increasing the pH by CO_2 uptake and (d) accelerate sedimentation.

CONCLUSIONS

Revegetation of mine tailings is a challenging task in view of the fact that the metal mine tailings are usually very poor in nutrients, rich in toxic metal content and have low capacity to retain water. Further, wind erosion of mine tailings poses another serious environmental problem. All these problems could be averted if tailings could be revegetated using metal tolerant plants following the strategies of phytoassisted rhizoremediation. Scope and limitations of phytoremediation of abandoned mine sites. “Metallomics” approach pooling disciplines of “-omics” is progressing rapidly and results achieved so far are very promising and opening several opportunities for entrepreneurs. Phytoproducts, e.g. biofuels, from plant assemblages involved in phytoremediation and risk based remediation is a practically feasile solution for protecting mine environment (Fig. 6).

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