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Review

Remediation of heavy metal contaminated soil

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The worldwide awareness of the deleterious effects of heavy metal pollution has resulted in intensive research aiming at understanding metal interactions in soil and their removal in an efficient way. Although, the knowledge and practice of the conventional physio-chemical remedial technologies for degraded soils are age-old, they are not in demonstration these days due to their detrimental impact on various ecosystems. On the other hand, phytoremediation has received much attention as a biological and natural way of treating the polluted lands. In addition, augmentation of essential rhizobacteria to reduce phytotoxicity and remediating metal polluted soils has also gained interest. This paper investigates the plant-microbial interactions in reclaiming the metal contaminated soil with attention to some significant soil biochemical characteristics during the process.

Key words: Heavy metals, bioremediation, phytoremediation, rhizosphere, rhizobacteria, bioaugmentation.

INTRODUCTION

Soil pollution by heavy metals has become one of the chief topics of discussion of all environmental crises today. Heavy metals exist in colloidal, ionic, particulate, and dissolved phases. They are present in soil as free metal ions, soluble metal complexes, exchangeable metal ions, organically bound metals, precipitated or insoluble compounds like oxides, carbonates, and hydroxides, or a part of silicate materials (Leyval et al., 1997).

Metals are natural constituents of soil. They persist in soils and have a very slow leaching rate; hence they tend to accumulate in soils. Trace amount of some heavy metals are required by living beings but in excess they are detrimental. The ecotoxicological risks of metal contamination bears potential harm for plants, animals, humans beings, and microorganisms. Heavy metal pollution can suppress or even kill sensitive parts of plant and soil microbial communities and lead to a shift in their functional diversity and structure. Once they are accumulated in the food chain, their effect gets adverse with tropic levels due to biomagnification. On the other hand, heavy metals like Cu, Fe, Mn, Ni, and Zn are essential for plant growth and are important constituents of many enzymes. In addition, metals like Al, As, Cd, Cr, Hg, Pb, Sb, Se, among others are nonessential and toxic above certain threshold levels (Panda and Choudhury, 2005).

Soils contaminated with heavy metals are poor in nutrients and microbial diversity and contribute to suboptimal plant biomass accumulation as well as impeded rates of remediation (White et al., 2006). They result from anthropogenic activities with lack of awareness of health and environmental effects connected with the production, use, and disposal of hazardous substances into soil (Vidali, 2001). The sources of heavy metals in soil are

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Abbreviations: PCs, Phytochelatins; **MTs,** metallothioneins; **DTPA,** diethylene-triamine-penta-acetic acid; **ED,** ecological dose; PGPR, plant growth promoting rhizobacteria; **AM,** arbuscular mycorrhiza; **VAM,** vesicular-arbuscular mycorrhiza; **GEMs,** genetically engineered microorganisms.

variable but they mostly arise from mining, smelting, industrial effluents, repeated applications of sewage sludge, municipal wastes and animal slurries, impurities in fertilizers, decomposition of air pollutants by burning of fossil fuels, and various other industrial activities (Wang et al., 2003).

A number of biological properties in soil are influenced by heavy metals and changes in these properties may act as sensible indicators of soil quality, since they are more dynamic and often more sensitive than physical or chemical parameters. One of such biological properties is the microbial and soil enzyme activity that is frequently used for determining the influence of various pollutants on the living system.

There has been keen interest in the development of *in situ* strategies for remediation of environmental contaminants over past few years. The prospects of bioremediation to restore contaminated environments are well known these days. Nevertheless, phytoremediation, a strategy that uses plants to degrade, stabilize, and/or remove soil contaminants, has been extensively investigated. Regardless of tremendous prospects, phytoremediation often encounters various challenges at extreme levels of contamination. However, treatment of such soil with organic sludge, organic fertilizer, and impactful microorganisms makes the conditions appropriate for reclamation.

BIOREMEDIATION

Bioremediation is defined as a process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Mueller et al., 1996). This uses living organisms, especially plants and microorganisms, to reduce, eliminate, transform, and detoxify the benign products present in soils, sediments, water, and air. Phytoremediation technology, one of its many approaches, uses plants as filters for accumulating, immobilizing, and transforming the contaminants to less harmful form (Vidali, 2001). More specifically, it is the utilization of vascular plants, algae, and fungi to control, breakdown, remove wastes, or to encourage degradation of contaminants in the rhizosphere (McCutcheon and Schnoor, 2003). Phytoremediation has recently become a tangible alternative to the traditional methodology in restoring the polluted sites (Glass, 2000).

As high contaminant levels inhibit plant and microbial activity, therefore an effective phytoremediation is realized where contaminants are present at low to medium levels. Recent research have shown stunning effects with the amendments of biofertilizer and biosludge to the contaminated lands, plants were able to grow and survive in extreme higher levels of heavy metal contamination (Juwarkar et al., 2008). Nanda and Abraham (2011) have evaluated the effect of As, Cr, Mg and Cu on some essential soil bacteria such as *Azotobacter*, *Pseudomonas* and *Rhizobium*. They found As to be the most toxic of all followed by Cr, Mg and Cu. The interaction between microorganisms, plant roots, and amendment might have a greater impact on both the increase of nutrient uptake and migration of metal uptake (Smith, 1994). As a result of plant root microbial interaction, the migration of contaminants to ground water are reduced by immobilization. Establishment of a vege-tative cover on contaminated sites can retain contami-nants in place, thus reducing their loss via erosion and percolation into the soil profile (Pulford and Watson, 2003). When re-vegetation of contaminated soil is com-bined with soil amendments, such as organic matter, the mobility of contaminants in the soil can be further reduced (Mench et al., 2000).

The microorganisms act in synergism with the plants for effective phytoremediation. This synergistic relationship promotes the exchange of water and nutrients established between plant roots and specialized soil fungi and mycorrhizae thus, enhancing the plant growth. The application of microorganisms in phytoremediation helps to improve plant growth and survival rate. The microbial activity in the contaminated site acts as an indicator for the plant growth and bioremediation (Kumar et al., 2008).

PHYTOREMEDIATION APPROACHES

Often exuded enzymes are capable of detoxifying organic compounds without microbial assistance, through phytodegradation or phytotransformation (McCutcheon and Schnoon, 2003). The process of holding contaminated soil in place with vegetation, minimizing disturbance of contaminants bound to soil particles, and preventing their movement is referred to as phytostabilization. By this, mobility of contaminants is reduced by accumulation within plants, absorption onto roots, or conversion to immobile species within the root zone (Vangronsveld et al., 1995). The process where heavy metal contaminants in water are absorbed or precipitated onto/into plant roots is referred to as rhizofiltration (McCutcheon and Schnoon, 2003).

Plant processes promote the removal of contaminants from the soil and water either directly or indirectly. Direct processes include plant uptake into roots or shoots and transformation, storage, or transpiration of the contaminants by microbial, soil, and root interactions within the rhizosphere (Hutchinson et al., 2003). Plants transform certain contaminants through oxidation and reduction reactions, conjugation phase (where foreign compounds are conjugated together by plant sugar amino acid, thisol, or glutathione molecule), and deposition of conjugates into vacuoles and cell walls (Subramanian and Shanks, 2003). The availability of the contaminant for uptake and transformation is also dependant on age of the contaminant and the plant species. The process of breaking down of contaminants by plant metabolic activity also occurs outside the plant with the release of extracellular enzymes resulting in its transformation. Depending on the plant type and contaminant, direct uptake of contaminants can be considered either a passive and/or active process (Chiou, 2002).

For effective phytoremediation, the plant should be non-edible and can be grown abundantly on wastelands. It has been established that certain wild and crop plant species have the ability to accumulate elevated amount of toxic heavy metals (Blaylock and Huang, 2000). A variety of plant species including vegetable crops and grasses are known to accumulate or immobilize heavy metals. For instance, *Thlaspi* (Pennycress), *Silene vulgaris* (Bladder campion) (Ernst et al., 2000), *Phaseolus vulgaris* L. cv. Contender (Bush bean plant) (Barcelo et al., 1986), *Larrea tridentata* (Creosote bush) (Gardea-Torresdey et al., 1996), *Sutera* aka Bacopa, *Convolvulus arvensis* L. (Field bindweed) (Gardea-Torresdey et al., 2004), *Dactylis glomerata* (Orchard grass) (Ortiz and Alcañiz, 2006), *Lotus purshianus* (Spanish lotus) (Lin and Wu, 1994), *Jatropha curcas* (Barbados nut) (Juwarkar et al., 2008; Kumar et al., 2008), *Jatropha multifida* (Psysic nut) (Nanda and Abraham, 2011) and a few others have been found to be effective plants in phytoremediation. *J. curcas* has gained much importance in phytoremediation as it can withstand environmental stress. A novel betaine aldehyde dehydrogenase gene (*JcBD1*) in *J. curcas* produces JcBD1 protein that helps it to survive in environmental stress like drought, heat, and salt (Zhang et al., 2008). The expression of this novel gene into *Escherichia coli*, results in expression of JcBD1 enzyme that makes it resistance to abiotic stressors like salt. It adds value for being a petroleum-substitute biodiesel crop, a renewable resource that would serve the increasing demand of the exhausting fossil fuels.

Certain plants accumulate essential and non-essential metals in their roots and shoots in higher concentrations than the levels present in soil (Raskin et al., 1994). Plants that can absorb high levels of contaminants by concentrating them in roots and shoots are called hyperaccululators. The *Brassicaceae* family contains a large number of hyperaccumulating species with widest range of metals that include 87 species from 11 genera (Baker and Brooks, 1989). Plants have developed mechanisms of chelating and sequestering metal ions by a particular class of metal binding legands dominating phytochelatins (PCs) and metallothioneins (MTs) (Cobbett and Goldsbrough, 2002). For long-term bioremediation, metal tolerant species are used for revegetation of degraded lands (Lan et al., 1997). *In situ* phytoremediation strategy exploits natural or genetically engineered plant species to accumulate toxic substances (heavy metals, radioactive compounds, organic pollutants, etc.) directly from the soil (Juhanson et al., 2007).

Organic and inorganic fertilizers are used primarily to increase nutrient availability to plants; however, they can affect population, composition, and function of soil microorganisms (Marschner et al., 2003). Organic fertilizers usually increase soil microbial biomass (Masto et al., 2006), CO_2 evolution (Ajwa and Tabatabai, 1994), and enzyme activities (Crecchio et al., 2001). Inorganic fertilizer has relatively less effect on soil microbial biomass and activity than the organic ones (Plaza et al., 2004). The balanced fertilization of major elements (N, P, and K) for plant nutrients could be beneficial for the growth of plants (Chu et al., 2007). Moreover, the amendment of biofertilizer, especially *Azotobacter* has been found successful in treating contaminated soil as a consortium with phytoremediation (Juwarkar et al., 2008; Kumar et al., 2008).

BIOCHEMISTRY OF SOIL DURING PHYTOREMEDIATION

In general, the physical and chemical parameters of soil are less useful in studying its properties as they change only when the soil undergoes a radical variation. On the contrary, biological parameters are sensitive to slight modifications as the soil quality may alter in the presence of any degrading agent (Yakovchenko et al., 1996). Regarding the selection of properties for use as indicators, Doran and Parkin (1996) considered a 'minimum data set' for use in soil quality evaluation, which includes physical (for example, texture, rooting depth, infiltration rate, bulk density, water retention capacity), chemical (for example, pH, total C, electrical conductivity, nutrient level), and biological (for example, microbial biomass carbon and nitrogen, potentially mineralizable nitrogen, soil respiration) properties. Among the general parameters, the microbial biomass carbon is considered the most reliable (41% of authors), followed by dehydrogenase activity (28%), and nitrogen mineralization capacity (16%) (Gil-Sotres et al., 2005). Phosphatase (28%), β-glucosidase (16%), and urease (11%) activities are the most frequently used among the specific biochemical parameters and appropriately represent C, N, and P cycles. The biochemical properties of soil have been widely used to evaluate soil quality, both individually and in combination, in simple indexes, and in more complex ones, which states the fact that the scientific community recognizes their potential value (Gil-Sotres et al., 2005).

Microbial activity in soil is highly influenced by soil pH and water availability. The optimum pH ranges between 5 and 10. Low pH is optimal for metal availability but is adverse to the vegetation (Hutchinson et al., 2003). Likely to pH, microbial activity is enhanced when 60% of soil pores are filled with water. In heavy metal cation (Cd, Cu, Hg, Ni, Pb and Zn) studies, solubility has been shown to increase with decreasing pH. When organic sludge is added to soil, a threshold is reached followed by a decrease in soil pH and increase in metal solubility (Sanders and Adams, 1987). The higher the sludge-metal concentration, the higher is the threshold pH point of decreasing metal solubility. High metal concentration in applied sludge results in increased metal solubility, hence increased plant uptake at higher soil pH values is noticed. Electrical conductivity measures soil salinity, a property referring to the amount of soluble salts and total soluble ions in soil.

Soil enzyme activity is a key feature of plant nutrients and cycling processes, thus measurement of specific enzyme activities is found useful in determining soil biological activity which in turn is an index of soil fertility (Perucci, 1992). Soil enzymes are believed to be primarily of microbial origin (Ladd, 1978) but also originate from plants and animals (Tabatabai, 1994). The free enzymes form complexes with humic colloids and are stabilized on clay surfaces and organic matter. The rate of hydrolysis of fluorescein diacetate by soil has been considered a suitable index of overall enzyme activity because its hydrolysis is carried out by active cells with a variety of enzymes, including lipases, pro-teases, and esterases (Schnurer and Rosswall, 1982). Soil bacteria and fungi excrete the enzyme cellulase under a variety of environmental conditions, such as high temperature of 30 to 50°C and low pH of 5 to 6 (Doyle et al., 2006). Some of the advanced tools in studying the extracellular enzymes produced by soil microorganisms include genomics, transcriptomics, proteomics, enzyme assays, and soil respiration measurements (Wallenstein and Weintraub, 2008). However, the biggest challenges in bioremediation are to understand how the physical, chemical and biological properties of soil influence microbial enzyme production, diffusion, substrate turn-over and the proportion of the product that is made available to the microbial cells (Burns et al., 2013).

Heavy metals indirectly affect soil enzymatic activities by altering the microbial communities that synthesize enzymes and their mode varies with the enzyme type (Moreno et al., 2003). Soil enzyme inhibition by heavy metals depends on the nature and concentration of the metals, and its extent varies from one enzyme to another and at certain concentration some heavy metals can also stimulate the activity of an enzyme. Metal ions may inhibit enzyme reactions by complexing the substrate, reacting with the protein-active groups of enzymes, enzyme-substrate complex (Mikanova, 2006), and sulphydral group of enzymes (Shaw and Raval, 1961). Different metals show different ability to act as enzyme inhibitors. Some of the trace elements added to soil might form complexes with the organic matter in soil and are not completely available for reaction with the enzyme-active sites. A few also act as co-factors and activators to enhance the enzyme activity. The soil enzyme activity is influenced by certain other factors. Air-drying of field-moist soil significantly decreases cellulase activity while it increases the glucosidases activity, especially β-glucosidase (Eivazi and Tabatabai, 1990). Rhizosphere has a positive impact on soil α- and β-glucosidase and α- and β-galactosidase, respectively. Enzyme activities also vary seasonally and with different cultivation modes (Vepsäläinen et al., 2001). The activities are affected by plants, animals, and microorganisms, substrate availability, feedback, and other inhibition and physical and chemical characteristics of soil. The activities are also sensitive with decrease in water availability (Sardans and Peñuelas, 2005). The activity of soil enzymes and organic carbon are negatively correlated to soil depth, that is, with an increase in soil depth there is a decrease in soil enzyme activity and organic carbon (Tabatabai, 1994).

Agronomic practices, especially tillage and residue management are known to influence the soil physical and chemical properties, organic carbon, microbial, and enzyme activities (particularly four amidohydrolases for example urease, amidase, L-asparaginase, and L-glutaminase) (Deng and Tabatabai, 1996). These prac-tices also have similar effects on the activities of en-zymes involved in carbon and nitrogen cycling. No tillage reduces chances of soil erosion and water evaporation but increases levels of water infiltration and soil organic matter. In combination with mulching, it increases activity of glycosidases (Deng and Tabatabai, 1996). In addition, mulching induces water-holding capacity, carbohydrate level, and cellulase activity along with a rich population of cellulolytic fungi in soil. Deforestation and subsequent tillage results in 50% decrease in organic carbon compared to an undisturbed forest soil.

Dehydrogenase activity is used as an active soil biomass measurement indicator and is related to the overall microbial activity in soil that reflects their total range of oxidative activity (An and Kim, 2009). Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from sub-strates to acceptors (Makoi and Ndakidemi, 2008). Dehydrogenase assay measures the total activity of a soil sample which is due to active microorganisms and enzymes stabilized in the soil matrix (Knight and Dick, 2004). This can also be used as a method to describe the biological activity in thermophilic and mesophilic stages of composting (Barrena et al., 2008). Because it is difficult to extract intact enzymes from soil, activity rather than mass is measured. These processes are a part of respiration pathways of soil microorganisms and are influenced by environmental factors. Their activity not only increases in well-irrigated soil but also on addition of nutrients to soil but decreases with soil depth (Brzezińska et al., 2001). The activity is enhanced only if the rate of sludge addition is limited (Obbard et al., 1994). Dehydro-genase is sensitive to heavy metal pollution; hence it is used to access the side effects of chemicals on microorganisms (Nweke et al., 2007). The activity also varies with soil type and season. Buyanovsky et al. (1982) found higher dehydrogenase activity in the rhizosphere during the dry seasons than in moist winters.

Small amount of extracellular enzymes of microbial, plant, or animal origin are stabilized on soil colloids which maintain their activity for extended periods of time (Nannipieri et al., 1996; Burns, 1982). Burns (1982) described 10 categories of soil enzyme locations. They included '*abiontic*' enzymes, a term coined by Skujins (1976) to describe enzymes of biological origin no longer associated to living cells. These may be excreted to soil solution or immobilized enzymes of microbial origin absorbed in clay or humic colloids. This stabilization of extracellular enzymes might not reflect variations in microbial biomass, which instead might correlate to internal enzyme activities for example dehydrogenase or to measurements of overall activity (respiration) (Simona et al., 2004). However, respiration per unit of biomass might increase depending on the type of pollutant and/or stress present (van Beelen and Doelman, 1997). Determining the specific enzyme activities (for example, phosphatase, urease, amylase, among others) or other parameters (for example, ATP content, respiration, adenylate enzyme charge among others) together with general soil parameters helps in determining the soil microbial activity and for understanding its response to compost amendments, cultivation practices, and environmental factors.

Microbial biomass represents the living component of the organic matter of soil, excluding plant roots and animals (Gil-Sotres et al., 2005). The changes in biomass carbon are much faster and greater than total soil organic carbon. Biomass carbon as percentage of soil organic carbon decreases with an increase in heavy metal concentration (Barajas-Aceves, 2005). The link between biomass carbon and total soil organic carbon constitutes a form of 'internal control' within soils of similar type and under similar management. Thus, if the ratio of biomass carbon and soil organic carbon changes under these conditions, this could indicate damage to the soil ecosystems by heavy metals (Barajas-Aceves, 2005). Microbial biomass carbon and nitrogen mineralization capacity have primarily been used to estimate changes in soil quality prior to management and use while dehydrogenase activity, as a general measure of viable microbial activity, has also been employed in degraded soils for studying the degree of contamination and effective diagnosis for recovery (Gil-Sotres et al., 2005). The nitrogen mineralization capacity refers to the capability of soil to transform organic nitrogen compounds into ammonia or nitrate under optimum moisture and temperature conditions over a given period of time (Gil-Sotres et al., 2005). Perucci (1990) analysed the biochemical properties of soil amended with municipal reuse and reported that the amendment improved the microflora. In addition, Perucci (1992) reported a significant increase in biomass carbon in soil by the compost addition which varied as per the dosage of application. In most studies, organic carbon usually decreases with soil depth (Deng and Tabatabai, 1996).

Diethylene-triamine-penta-acetic acid (DTPA) has been widely used to estimate the bioavailability of metals in soil and sludge due to its capacity to chelate a wide range of metallic elements (Halim et al., 2003). It can also be used to restore the degraded soil (Francis, 1999). A number of authors have agreed that the presence of organic matter increases DTPA extractability of metals. The bioavailability of many metals depends on the quality of soil organic compounds than on their quantity (Ortiz and Alcañiz, 2006). Thus, the bioavailability of metals increase when they are associated with labile or soluble organic compounds and decrease when they are found associated with stable organic compounds, such as humic acids (Halim et al., 2003). Soil conditions like pH, redox potential, cation exchange capacity, and organic matter highly influence the metal assimilation by plant roots by affecting root growth and mobility of pollutants. Chemical amelioration using acids, lime, and organic matter alter soil chemical conditions and the concentration of chelating and complexing agents in the soil solution and solid phase (Iskandar and Adriano, 1997).

Speir et al. (1995, 1999) and Haanstra and Doelman (1991) have quantified the effect of heavy metals on various soil enzyme activities by determining the ecological dose 50% (ED_{50}). The concept of ED was introduced by Babich et al. (1983), in the study of effect of heavy metals on microbial processes in the soil ecosystem. ED is the concentration of heavy metals at which the enzyme, or other biological activity, is reduced to 50% of the uninhibited value (Moreno et al., 2003). The addition of sewage sludge to soil, changes the inhibitory effect of heavy metals particularly Cd and Ni on enzymatic activities by increasing the ED values, thereby indicating diminished toxicity. The negative effect of heavy metals on the enzymatic activities might be masked by the positive effect of sewage sludge (Moreno et al., 1999). In addition, a metal fraction might be absorbed on the organic colloids added with the sludge. This prevents the heavy metals to interact directly with the active sites of enzymes, thus affecting their activity (Doelman and Haanstra, 1986).

The sewage sludge is considered valuable fertilizer due to its plant nutrient content and humus-forming effect but its long-term application may affect the soil features (Zaman et al., 2004). Its frequent use leads to deterioration of soil ecosystem due to the accumulation and persistence of heavy metals in soil. It is used as an organic amendment to soil, particularly to the soil that lacks organic matter (Moreno et al., 2003). Application of sewage sludge to soil improves the physical and chemical characteristics of soil, especially organic matter content, and plant nutrient, particularly nitrogen and phosphorous. It contains numerous components required for microbial growth and increases the activity of soil microorganisms, including rhizobial growth (Kinkle et al., 1987). Corey et al. (1987) concluded that specific metal adsorption on sludge surfaces would normally be the controlling factor in metal phytoavailability in soil-sludge mixtures. Sludge with higher metal concentration causes higher metal uptake by plants. Application of waste water containing organic matter and nutrients has been found to increase soil microbial biomass, bacterial, fungal, and actinomycetes community in field experiments (Goyal et al., 1995). Amendment of effluent treatment plant sludge along with biofertlizer has been successfully used to improve the coal mine spoil dump (Juwarkar and Jambhulkar, 2008). The effect of sludge on soil dehydrogenase, urease, and phosphatase activities (that is, stimulation and inhibition) is related to sludge origin and levels of sludge contamination with organic and inorganic pollutants as well as the kind of enzymes under study (Kucharski et al., 2000).

Degradation of organic matter from the organic amendments used in the remediation of metal contaminated soils leads to changes in soil chemical properties shortly after their addition, which may affect the soil metal distribution (Clemente et al., 2006). Despite high levels of nitrogen, phosphorous, and potassium in soil, dairy sludge is found to improve the poor physical properties and microbial activities of contaminated soils (Kumar et al., 2008). Moreover, effective growth of *J. curcas* in heavy metal contaminated soil with amendments of biosludge, dairy sludge, and biofertilizer has been reported (Juwarkar et al., 2008; Kumar et al., 2008).

BIOAUGMENTATION

The use of microorganisms in association with plants in clean-up approach of heavy metal contaminants from soil is gaining momentum. The microorganisms employed for the purpose may be indigenous or exogenous to the contaminated area. The process of importing microorganisms to the contaminated site is called bioaugmentation which enhances the metabolic capacities of the indigenous microbiota in order to boost bioremediation (El Fantroussi and Agathos, 2005). The process is often acelerated by the extracellular enzymes secreted by the microbial cells that cleave to the complex forms of the contaminants to the forms easily assimilated by their cells where they are metabolised (Mueller, 2006). The indigenous soil organisms are often the indicators of ecological harm caused by heavy metal contamination until the heavy metals are removed or immobilized (Frey et al., 2006). In many contaminated soils, the microorganisms are exposed to various chemicals directly with the resultant effects being additive, synergistic, or antagonistic (Chaperon and Sauvé, 2008). Additive effects refer to zero interactions between soil components and toxicity greater than the additive effect is described as synergistic and lower to it as antagonistic effects, respectively.

A phenomenon called biosorption appears to be critical in bioremediation. It is defined as the uptake of organic and inorganic metal species, both soluble and insoluble, by physicochemical mechanisms, such as adsorption. In microbial cells, metabolic activities may also influence this process because of changes in pH, conductivity, organic and inorganic nutrients, and metabolites. Biosorption provides nucleation sites for the formation of stable minerals by sorption to cellular surfaces or accumulation within the cells via membrane transport mechanisms. Inside the cells, metal species may be bound, precipitated, localized within intracellular structures or organelles or translocated to specific structures depending on nature of the metal and the organism (Gadd, 1996). The biomineralization process by microorganisms offers an efficient way to sequester inorganic pollutants and heavy metals within relatively stable solid phases (Li et al., 2013). The remediation mechanisms by microorganisms include extracellular complexation, oxidation-reduction reaction, precipitation and intracellular accumulation (Yao et al., 2012).

Plants take up most mineral nutrients through the rhizosphere where microorganisms interact with plant products in root exudates that consists of a complex mixture of organic acid anions, phytosiderophores, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions, gaseous molecules, enzymes, and root border cells (Dakora and Phillips, 2001). The rate of exudation is increased by the presence of microorganisms in the rhizosphere (Gardner et al., 1983) and promoted by the uptake and assimilation of certain nutrients. Some root exudates act as metal chelators and increase the availability of metallic soil micronutrients. Metal chelators form complexes with soil metals, thus releasing metals that are bound to soil particles and increasing metal solubility and mobility. Immobilization of heavy metals by cysteine rich peptides is a major mechanism employed by plants for counteracting heavy metal toxicity. Phytochelatins have shown to bind heavy metals with high affinity (Dakora and Phillips, 2002). In addition, bacterial and plant siderophores also act as chelating agents that solubilize the Fe bound to soil particles. After solubilization, Fe is taken up by the living cell through specific membrane carriers and is metabolized.

Plant roots can regulate the microbial activities in the rhizosphere, encourage beneficial symbiosis, influence the physical and chemical properties of soil, and inhibit the growth of competitive plant species. The plant growth promoting rhizobacteria (PGPR) are considered to promote plant growth directly or indirectly by producing plant growth promoters (auxin, gibberellin, cytokinin, indole a-cetic acid, to mention but a few), phytohormones, siderophores, chelating agents, antibiotics (Shanahan et al., 1992), cyanide (Flaishman et al., 1996), asymbiotic N-fixation (Boddey and Dobereiner, 1995), solubilising mineral phosphate and nutrients (de Freitas et al., 1997), and serving a few other functions (Joseph et al., 2007; Kamnev and van der Lelie, 2000). Some of such essential PGPR include *Pseudomonas*, *Achromobacter*, *Azotobacter*, *Azospirillum*, *Acetobacter,* and *Rhizobium*. A large array of bacteria including species of *Klebsiella*, *Enterobacter*, *Gluconacetobacter,* *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Seratia* have also been reported to enhance plant growth (Saravanan et al., 2008; Glick, 1995; Okon and Labandera-Gonzalez, 1994). Several microalgae have also been studied for their combined lipid production and heavy metal removal from leachate, a few of which are *Nanochloropsis*, *Pavlova lutheri*, *Tetraselmis chuii* and *Chaetoceros muelleri* (Richards and Mullins, 2013). Microbial cells remain functionally active in soil by producing and sensing certain chemical signals through biofilm production by a phenomenon called quorum sensing. This quorum sensing, with other regulatory system, expand the range of environmental signals that target gene expression beyond population density (Daniels et al., 2004). The use of PGPR in phytoremediation technologies is considered to play an important role as their amendment can aid plant growth on contaminated sites (Burd et al., 2000) and enhance detoxification of soil (Mayak et al., 2004). Nevertheless, pairing PGPR with arbuscular mycorrhizal (AM) fungi serves a good way in increasing the efficiency of phytoremediation.

Among soil microorganisms, mycorrhizal fungi are most efficient in enhancing heavy metal tolerance in plants through symbiosis. These symbiotic fungi increase nutrient and water uptake, alleviate cultural and environmental stress, and enhance disease resistance and plant health (Filion et al., 1999). Among the AM fungi, genus *Glomus*, *Scutellospora*, *Acaulospora*, and *Gigaspora* are of much importance in phytoremediation (Khan, 2001). Bioleaching processes involving *Thiobacillus* spp. and *Aspergillus niger*, biosorption of low concentration of metals in water by algal or bacterial cells, bio-oxidation or bio-reduction of metal contaminants by *Bacillus subtilis* and sulfate-reducing bacteria, and biomethylation of metals, such as As, Cd, Hg, and Pb have shown some promises in treating degraded soil (Mulligan et al., 2001). Like the AM fungi and phosphate solubilizing bacteria that function as phosphorous solubilizers, *Gluconacetobacter diazotrophicus* is a rhizobacterium that aid in zinc solubilization for easy uptake by plants (Saravanan et al., 2007). While fungi and bacteria are responsible for the major chemical transformation during organic waste decomposition and nutrient release, the soil fauna, especially earthworms can stimulate microbial action by increasing surface area for microbial colonization and enzymatic action through physical breakdown of organic residues into smaller particles (Kızılkaya, 2008).

Mycorrhiza provoke many positive impacts on plant physiology, nutrient availability, and microbial composition that determines a successful outcome of phytoremediation attempt. Beyond the rhizosphere, mycorrhizal hyphae act as roots and form dense networks within the root zone of plants which increases the extent of rhizosphere into the bulk soil by creating a new interface of soil-plant interactions which is termed ‘hyposphere’. Studies on mycorrhizae fungus have focused on their ability to provide the host plant with nutrients in nutrient deficit soil. The vesicular-arbuscular mycorrhizal (VAM) fungi are known to solubilize phosphate complexes in the soil and make it available to plants. In some studies they are also found to degrade organic pollutants. Plants with mycorrhizal associations appear to be protected from the phytoxic affect of heavy metals. Heavy metals are believed to be bound by carboxyl groups in hemicelluloses of the interfacial matrices between the host cell and the fungus. An ericoid mycorrhizae fungus *Hymenoscyphus ericae* was able to metabolize some phytotoxic compounds *in vitro*. This ability of the fungus provides the host plants with added protection against toxins and allows the plants to grow in areas otherwise hazardous to plants.

An increase in metal concentration influences the soil microbial properties, especially respiration and enzymatic activity which serve good indicators for metal pollution (Szili-Kovács et al., 1999). In the aftermath of heavy metal pollutions, the role of heavy metal bounding or leaching increases that determines their bioavailability and toxicity. Several studies have shown a negative relationship between heavy metal concentration and microbial activities, such as respiration, mineralization (van Beelen and Doelman, 1997), nitrification (Yeates et al., 1994), intracellular and extracellular enzymatic activities (Yeates et al., 1994; Haanstra and Doelman, 1991), and microbial community biomass and structure (Kelly and Tate, 1998). Earlier reports suggest that heavy metals inhibit the growth of specific microbial groups, particularly nitrifiers and nitrogen fixers; however, there are certain conditions in which no correlation has been found between microbial parameters and heavy metal contamination (Kelly and Tate, 1998). Apart from the above confrontations, applications of beneficial microbial isolates (natural or engineered) often show positive feedback in bioremediation.

PERSPECTIVES

Effective phytoremediation is always encountered by challenges of overcoming plant and microbial stress in fields. The control and optimization of bioremediation processes is a complex system of many factors, such as the existence of microbial population capable of degradation, availability of contaminants to the microbial population, environmental factors (for example, soil type, temperature, pH, presence of oxygen, or other electron acceptors), and nutrients (Vidali, 2001). Moreover, the non-indigenous microorganisms applied for the purpose of bioaugmentation most often compete with the indigenous microbial population for nutrition and space which results is an antagonistic effect and either one population is critically hampered by the other. Furthermore, when pure biosorptive metal removal is not viable, application of a consortium of metal-resistant microorganisms can ensure enhanced metal removal through bioprecipitation, biosorption and continuous metabolic uptake of metals after physical adsorption (Malik, 2004).

It is a known fact today that contaminated lands are a potential threat to mankind and there is a need of international concern to search for remedies as a response to combat the adverse effects and reclaim the soil for reuse. With the current trends in environmental biotechnology, there have been certain promises in using genetically engineered microorganisms (GEMs) in bioremediation but the regulatory and bioethical issues hinder their application (Gerhardt et al., 2009). Additionally, GEMs often fail to compete with normal microbiota in the rhizosphere, and their quantity often dwindles to levels that cannot effectively support bioremediation (Gilbertson et al., 2007). Transgenic plants and GEMs often do not survive due to competition and might undergo mutation during stress conditions which may turn hazardous. This consequences lead to a disturbance in the native biodiversity of the site. Chances cannot be ignored as mutated GEMs can act in antagonism to the host plants and hamper the desired process. In rare cases, GEMs migrate from the site of contamination to neighbouring locations and might create potential ecological risk (Pilon-Smits, 2005). Application of GEMs also requires a good stage management. After introduction of GEMs to the desired soil sites, they form clusters at few particular locations rather than spreading uniformly which often leads to the process inefficiency. Their use mostly suffers non-acceptance due to public opinions and government policies. However, to reduce the ecological risk from non-native (transgenic or non-transgenic) phytoremediation species, it is often necessary to employ a biological containment system (Gressel and Al-Ahmad, 2005). Genes are introduced to prevent propagation, or to render a species overly sensitive to abiotic stressors, such as temperature changes or chemicals. Ideally, multiple transgenes are employed to prevent gene flow between the indigenous and exogenenous species. To reinforce the containment system, mitigator genes linked to the primary transgene are added (Gerhardt et al., 2009). The phytoremediation species are conferred with non-deleterious traits from the mitigator genes unless a gene transfer occurs which may turn harmful to the related species. Instead, the phytoremediation species can be prevented from competing outside the contaminated site.

The long treatment time for reclamation and limiting environmental factors frequently keeps phytoremediation under criticism. A great drawback in phytoremediation is that it encounters many stressors in the field trial than those encountered in laboratory and greenhouse systems. Some of them are variation in temperature, nutrients, precipitation, hervivory, plant pathogens, weeds, and adverse effects of pesticide and weedicide applications whereas, a complete controlled condition is maintained in *ex situ* methods (Gerhardt et al., 2009). In addition, root structure, soil texture and quality, bioavailability of nutrient, among others can change over time and take an undesirable turn in the process. Despite, some limitations, phytoremediation is universally accepted for being a natural way of heavy metal remediation from the environment. A few causes for this are its *in situ* strategy of bioaugmentation which adds more value to the process; mitigation of soil erosion and global warming; and the production of biofuels and natural gas from plant biomass as an alternative to fossil fuels. Plantation helps debase the contaminants and also contribute to a clean and green environment.

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