ROLE OF MICROBIAL BIOREMEDIATION OF HEAVY METAL FROM CONTAMINATED SOILS: AN UPDATE

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ABSTRACT

Heavy metals are natural constituents of the environment, but indiscriminate use for human purposes has altered their geochemical cycles and biochemical balance. The results in excess release of heavy metals such as cadmium, copper, lead, nickel, zinc etc. into natural resources like the soil and aquatic environments. Prolonged exposure and higher accumulation of such heavy metal can have deleterious health effects on human life and aquatic biota. The role of microorganisms and plants in biotransformation of heavy metals into nontoxic forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for bioremediation of metal-contaminated sites. In view of this, the present review investigates the abilities of microorganisms and plants in terms of tolerance and degradation of heavy metals. Also, advances in bioremediation technologies and strategies to explore these immense and valuable biological resources for bioremediation are discussed. An assessment of the current status of technology deployment and suggestions for future bioremediation research has also been included. Finally, there is a discussion of the genetic and molecular basis of metal tolerance in microbes, with special reference to the genomics of heavy metal accumulator plants and the identification of functional genes involved in tolerance and detoxification.

Keywords: Bioremediation, Phytoremediation, Heavy metals, Adsorption, Biosorption

INTRODUCTION

Continuously, increasing the population in world with the growth of industry, there has been a considerable increase in the discharge of industrial Waste to the soil and aquatic environment, chiefly soil and water, which has led to the accumulation of heavy metals, especially in urban areas. Slow in a row down of heavy metals also takes place through leaching, plant uptake, erosion and deflation. The unsystematic release of heavy metals into the soil and waters is a major health hazard concern worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. Many of them are toxic even at very low concentrations; arsenic, cadmium, chromium, copper; lead, mercury, nickel, selenium, silver, zinc etc. are not only cyto toxic but also carcinogenic and mutagenic in nature. Plants in very small amounts or their growth and optimum performance require some metals. However, the increasing concentration of several metals in soil and waters due to industrial revolution has created an alarming situation for human life, aquatic biota soil biota. This is evident from various reports citing harmful effects of heavy metals on human and plants health.

In order to make the environment healthier for human beings, contaminated water bodies and land need to be rectified to make them free from heavy metals and trace elements. There are several techniques to remove these heavy metals, including chemical precipitation, oxidation or reduction, filtration, ion exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment. However, most of these techniques become ineffective when the concentrations of heavy metals are less than 100 mg/L. Most heavy metal salts are water-soluble and get dissolved in wastewater, which means they cannot be separated by physical separation methods. Additionally, physico-chemical methods are ineffective or expensive when the concentration of heavy metals is very low. Alternately, biological methods like biosorption and/or bioaccumulation for removal of heavy metals may be an attractive alternative to physico-chemical methods. Use of microorganisms and plants for remediation purposes is thus a possible solution for heavy metal pollution because it includes sustainable remediation technologies to rectify and re-establish the natural condition of soil. However, introduction of heavy metals into the soil causes considerable modification of the microbial community, despite their vital importance for the growth of microorganisms

at relatively low concentrations. The modification of the microbial make up is mainly brought about by exerting an inhibitory action through blockage of essential functional groups, displacement of essential metal ions or modification of active conformations of biological molecules. The response of microbial communities to heavy metals depends on the concentration and availability of heavy metals and is a complex process, which are controlled by multiple factors, such as type of metal, the nature of the medium and microbial species.

Sources of Heavy Metal in the Environment

Heavy metals occur naturally in the environment from pedogenetic processes of weathering of parent materials and also through anthropogenic sources. The most significant natural sources are weathering of minerals, erosion and volcanic activity, while the anthropogenic sources depend upon human activities such as mining, smelting, electroplating, use of pesticides and phosphate fertilizer discharge, as well biosolids (e.g., livestock manures, composts, and municipal sewage sludge), atmospheric deposition, etc.[1-4]. The disturbance of nature's slowly occurring geochemical cycle of metals by man results in accumulation of one or more of heavy metals in the soil and waters, and above defined levels, this is enough to cause risk to human health, plants, animals and aquatic biota [5]. The heavy metals essentially become contaminants in the soil and water environment because of their excess generation by natural and man-made activities, transfer from mines to other locations where higher exposure to humans occurs, discharge of high concentration of metal waste through industries, and greater bioavailability.
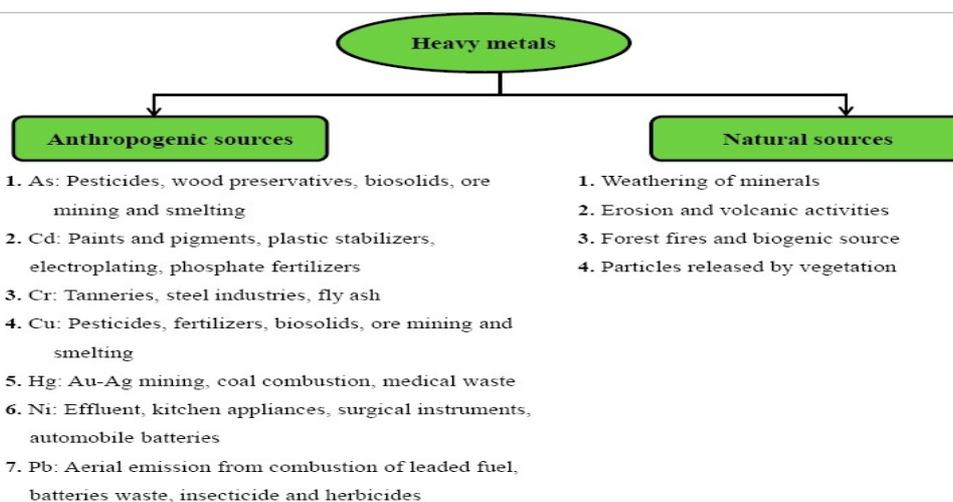

Figure 1: Source of Heavy Metals

Bioremediation: Introducing Microbe Based Clean Up System

Remediation of environment niches such as soil, sediments and water amended with heavy metals can be achieved through biologically encoded changes in the oxidation state. Bioremediation is the microbe-mediated process for clearance or immobilization of the contaminants, including all possible toxins like hydrocarbons, agrochemicals and other organic toxicants. But for inorganic toxic compounds such as heavy metals, microbes are unable to simplify them into harmless compounds, and they should be used according to their specialization for the type of contaminants. Thus the bioremediation strategy for heavy metals depends on the active metabolizing capabilities of microorganisms. Several microorganisms are known to require varying amounts of heavy metals as essential micronutrients for growth and development. For example, $Fe3+$ is essentially required by all bacteria while $Fe2+$ is important for anaerobic bacteria [6]. However, the adsorption capacity depends on microbial total biomass and geochemistry of the system. Some oxyanions of metals do not interact with microbes, and their bioremediation is based on their catalyzed redox conversion to insoluble forms. These reduction or oxidation reactions take place due to enzymatic activity and biomass concentration of microbes. Microorganisms have a great deal of undiscovered and unexplored potential for remediation of soil pollutants and increasing the production of agricultural crops with low input. Selection of rhizospheric microbes should be done based on an understanding of mechanisms involved in the adsorption and mobilization of heavy metals and trace elements in the soil to restore soil health. Microorganisms as metal accumulators possess an inherent novel remediation property for toxic metals in the soil. The study of genetics of such metal accumulator microorganisms can help us to transfer the traits in the microbes that are missing through the development of microarrays, which result in differentially expressed microbe genes.

Mechanisms of Bioremediation

Microorganisms are omnipresent that dominate in heavy metal-contaminated soil and can easily convert to heavy metals into non-toxic forms. In bioremediation processes, microorganisms mineralize the organic contaminants to end-products such as carbon dioxide and water, or to metabolic intermediates which are used as primary substrates for cell growth. Microorganisms are capable of two-way defense *viz.* production of degradative enzymes for the

target pollutants as well as resistance to relevant heavy metals. Different mechanisms of bioremediation are known, including biosorption, metal-microbe interactions, bioaccumulation, biomineralisation, biotransformation and bioleaching. Microorganisms remove the heavy metals from soil by using chemicals for their growth and development. They are capable of dissolving metals and reducing or oxidizing transition metals. Different methods by which microbes restore the environment are oxidizing, binding, immobilizing, volatizing and transformation of heavy metals. Bioremediation can be made successful in a particular location by the designer microbe approach, and by understanding the mechanism controlling growth and activity of microorganisms in the contaminated sites, their metabolic capabilities and their response to environmental changes. Many contaminants are organic solvents which disrupt membranes, but cells may develop defense mechanisms including formation of outer cell-membrane-protective material, often hydrophobic or solvent efflux pumps [7]. For instance, plasmid-encoded and energy-dependent metal efflux systems involving ATPases and chemiosmotic ion/proton pumps are reported for As, Cr and Cd resistance in many bacteria [8].

Bioremediation by Adsorption

Heavy metals can be biosorbed by microbes at binding sites present in cellular structure without the involvement of energy. Among the various reactive compounds associated with bacterial cell walls, the extracellular polymeric substances are of particular importance and are well known to have significant effects on acid-base properties and metal adsorption [9]. Studies on the metal binding behavior of extracellular polymeric substances (EPS) revealed a great ability to complex heavy metals through various mechanisms, which include proton exchange and micro-precipitation of metals [10,11]. Recent studies have characterized and quantified the proton and adsorbed metals on bacterial cells and EPS free cells in order to determine the relative importance of EPS molecules in metal removal [12].

Bioremediation by Physio-Bio-Chemical Mechanism

Biosorption is the process which involves higher affinity of a biosorbent towards sorbate (metal ions), continued until equilibrium is established between the two components [13]. *Saccharomyces cerevisiae* acts as a biosorbent for the removal of Zn (II) and Cd (II) through the ion exchange mechanism [14,15]. *Cunninghamella elegans* emerged as a promising sorbent against heavy

metals released by textile wastewater [16]. Heavy metal degradation involves energy for the cell metabolic cycle. The combined active and passive modes of toxic metal bioremediation can be called bioaccumulation [17]. Fungi have emerged as potential biocatalysts to access heavy metals and transform them into less toxic compounds [18].

Some fungi such as *Klebsiella oxytoca*, *Allescheriella* sp., *Stachybotrys* sp., *Phlebia* sp. *Pleurotus pulmonarius*, *Botryosphaeria rhodina* have metal binding potential [19]. Pb (II) contaminated soils can be biodegraded by fungal species like *Aspergillus parasitica* and *Cephalosporium aphidicola* with biosorption process [20,21]. Hg resistant fungi (*Hymenoscyphus ericae, Neocosmospora vasinfecta* and *Verticillum terrestre*) were able to biotransform a Hg (II) state to a nontoxic state [22]. Many of the contaminants are hydrophobic, and these substances appear to be taken up by microbes through the secretion of some biosurfactant and direct cell-contaminant association. Biosurfactants form stronger ionic bonds with metals and form complexes before being desorbed from soil matrix to water phase due to low interfacial tension [23].

Bioremediation may also involve aerobic or anaerobic microbial activities. Aerobic degradation often involves introduction of oxygen atoms into the reactions mediated by monooxygenases, dioxygenases, hydroxylases, oxidative dehalogenases, or chemically reactive oxygen atoms generated by enzymes such as ligninases or peroxidases. Anaerobic degradations of contaminants involve initial activation reactions followed by oxidative catabolism mediated by anoxic electron acceptors. The technique used to reduce the mobilization of heavy metals from contaminated sites by changing the physical or chemical state of the toxic metals is called immobilization. Solidification treatment involves mixing of chemical agents at the contaminated sites or precipitation of hydroxides [24]. Microbes mobilize the heavy metals from the contaminated sites by leaching, chelation, methylation and redox tansformation of toxic metals. Heavy metals can never be destroyed completely, but the process transforms their oxidation state or organic complex, so that they become water-soluble, less toxic and precipitated [25]. Microorganisms use heavy metals and trace elements as terminal electron acceptors or reduce them through the detoxification mechanism, used for the removal of metals from the contaminated environment. Microorganisms remove heavy metals through the mechanisms which they employ

to derive energy from metals redox reactions, to deal with toxic metal through enzymatic and non-enzymatic processes.

Two main mechanisms for development of resistance in bacteria are detoxification (transformation of the toxic metal state and making it unavailable) and active efflux pumping of the toxic metal from cells [26]. The basic redox (oxidation and reduction) reaction takes place in the soil between toxic metals and microorganisms; microorganisms act as an oxidizing agent for heavy metals and cause them to lose electrons, which are accepted by alternative electron acceptors (nitrate, sulphate and ferric oxides). In aerobic conditions, oxygen acts as an electron acceptor, while in anaerobic conditions microbes oxidize organic contaminants by reducing electron acceptors. The microorganism takes energy for growth by oxidizing the organic compound with Fe (III) or Mn (IV) as an electron acceptor [27]. Anaerobic degradation of organic contamination is stimulated with the higher availability of Fe (III) for microbial reduction [28,29]. Metals being used as terminal electron acceptors is called dissimilatory metal reduction [30]. Biodegradation of chlorines from contaminants takes place through reductive de-chlorination, where contaminants as chlorinated solvents acts as

an electron acceptors in respiration. Microorganisms reduce the state of metals and change their solubility, like the *Geobaccter* species, and reduce the Uranium soluble state (U6+) to insoluble state (U4+) [31].

Different defense systems (exclusion, compartmentalization, complex formation and synthesis of binding protein and peptides) reduce the stress developed by toxic metals [32]. Heavy metal accumulation by microorganisms can be studied by the expression of metal binding protein and peptides (phytochelatins and metallothionein) [33]. These metal binding protein transcription factors are known to mediate in hormone and redox signaling process in the context of toxic metal (Cd, Zn, Hg, Cu, Au, Ag, Co, Ni and Bi) exposure [34]. *Synechococcus* sp. (cynobacterial strains) has been reported with the expression of the smtA gene and production of metal-binding protein [35]. *Ralstonia eutropha* has been genetically modified to express mouse metallothionein on the cell surface and decrease the toxic effect of the Cd (II) in the contaminated sites [36]. Expression of different proteins and peptides by the *Escherichia coli* regulates the range of accumulation of cadmium [37]. Co-expression of precursor glutathione (GSH) along with phytochelatins (PC) resulted in the

10 fold increase in PC that finally increased cadmium accumulation twofold [38]. Natural resistant pathways for heavy metals (Hg and Ar) in microorganisms have been regulated by metalloregulatory protein [39].

Molecular Mechanisms Involved in Bioremediation Process

Various mechanisms involved in the removal of heavy metals by microorganisms are known. In a genetically engineered bacterium *Deinococcus geothemalis*, Hg reduction has been reported at high temperatures due to the expression of mer operon from *E. coli* coded for Hg2+ reduction [40]. Mercury resistant bacteria *Cupriavidus metallidurans* strain MSR33 was modified genetically by introducing a pTP6 plasmid that provided genes (merB and merG) regulating Hg biodegradation along with the synthesis of organomercurial lyase protein (MerB) and mercuric reductase (MerA) [41]. Modification of *Pseudomonas* strain with the pMR68 plasmid with novel genes (*mer*) made that strain resistant to mercury [42]. Two different mechanisms for Hg degradation by bacteria (*Klebsiella pneumonia* M426) are mercury volatilization by reduction of Hg (II) to Hg (0) and mercury precipitation as insoluble Hg due to volatile thiol (H2S) [43]. Genetic engineering of *Deinococcus radiodurans* (radiation resistant bacterium)

which naturally reduces Cr (IV) to Cr (III) has been done for complete toluene (fuel hydrocarbon) degradation by cloned genes of *tod* and *xyl* operons of *Pseudomonas putida* [44]. Microbial metabolites like metal bound coenzymes and siderophores mainly involved the degradation pathway [45].

Phytoremediation

Phytoremediation basically refers to the use of plants and associated microorganisms to partially or completely remediate selected contaminants from soil, sludge, sediments, wastewater and ground water. It can be used for removal of radionuclides, organic pollutants as well as heavy metals [46]. Phytoremediation utilizes a variety of plant processes and the physical characteristics of plants to aid in remediation of contaminated sites. Over the recent years, a special emphasis has been placed on phytoremediation since this property can be exploited for remediation of heavy metal polluted soils [47]. It is a cost-effective, efficient and eco-friendly *in situ* remediation technology driven by solar energy. The technique of phytoremediation includes a number of different processes such as phytoextraction, phytofiltration, phytostabilization, phytovolatilization and phytodegradation [48] The initial step of phytoremediation is phytoextraction, the

uptake of contaminants from soil or water by plant roots and their translocation to and accumulation in biomass, *i.e.*, shoots [49]. Translocation of metals to shoots is an important biochemical process and is desirable in an effective phytoextraction. The next important process of phytoremediation is phytofiltration, which includes rhizofiltration (use of plant roots), blastofiltration (use of seedlings) or caulofiltration (use of excised plant shoots) [50]. In this, the metals are absorbed or adsorbed and thus their movement in underground water is minimized. In addition to the above process, phytostabilization or phytoimmobilization occurs, which reduces the mobility and bioavailability of metals in the environment and thus prevents their migration into groundwater or the food chain [51]. Plants perform the immobilization of heavy metals in soils by sorption through roots, precipitation, complex formation or metal valence reduction in the rhizosphere [52]. Organic pollutants taken by plants are metabolized by enzymes such as dehalogenase and oxygenase, which are not dependent on rhizospheric microorganisms [53]. However, several heavy metals absorbed by plants get converted into volatile forms and subsequently released into the atmosphere by the process called

phytovolatilization. This process has been used for removal of some volatile heavy metals like Hg and Se from polluted soils [54]. However, this is limited by the fact that it does not remove the metals completely but rather transfers those from one medium (soil or water) to another (atmosphere) from which they can re enter in soil and water.

Biotechnological Intervention/Genetic Engineering in Bioremediation Processes
01. Designer Microbes Approach

Genetically engineered microorganisms (GEM) are organisms whose genetic material has been altered using recombinant DNA technology to generate a character-specific efficient strain for bioremediation of soil, water and activated sludge by exhibiting enhanced degrading capabilities against a wide range of chemical contaminants [55]. It offers the advantage of constructing microbial strains which can withstand adverse stressful situations and can be used as a bioremediators under various and complex environmental conditions. Genetic engineering has led to the development of "microbial biosensors" to measure the degree of contamination in contaminated sites quickly and accurately. Various biosensors have been designed to evaluate heavy metal concentrations like mercury (Hg), cadmium (Cd), nickel (Ni), copper (Cu) and arsenic (As) [56,57]. Genetic

engineering of endophytes and rhizospheric bacteria for plant-associated degradation of pollutants in soil is considered to be one of the most promising new technologies for remediation of metal contaminated sites [58]. Bacteria like *Escherichia coli* and *Moreaxella* sp. expressing phytochelatin 20 on the cell surface have been shown to accumulate 25 times more Cd or Hg than the wild-type strains [59,60]. However, one major obstacle for utilizing these GEMs in hostile field conditions is sustaining the recombinant bacteria population in soil, with various environmental conditions and competition from native bacterial populations [61]. Further, the molecular approaches have been applied to only limited bacterial strains like *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis etc*. This means other microorganisms need to be explored for their application in heavy metal bioremediation through molecular intervention.

02. Designer Plant Approach

Recent advances in omics technologies such as genomics, proteomics, transcriptomics and metabolomics play important roles in identifying traits that maximize the benefits of field remediation technologies. The main constraint of phytoremediation technology is the accumulation of pollutants or their metabolites in plant tissues, which shortens

plant life and releases contaminants into the atmosphere via volatilization. This problem can be minimized by manipulation of metal tolerance, accumulation and degradation potential of plants against various inorganic pollutants. Using the designer plant approach, the bacterial genes responsible for metal degradation can be introduced in plant tissues to allow degradation of metals within the plant tissues [62]. Application of genetically engineered (GE) plant-based bioremediation for various heavy metals pollutants is in the forefront due to its eco-friendliness and reduced health hazards compared to physico-chemical based strategies, which are less eco-friendly and more hazardous to human health [63]. Various microbial genes can be harnessed in the transgenic plant for detoxification and accumulation of inorganic contaminants [64]. The metal-detoxifying chelators such as metallothineins and phytochetains can confer resistance to the plant by enhancing uptake, transport and accumulation of various heavy metals [65]. Similarly, transgenic plants carrying bacterial reductase can enhance the volatilization of Hg and Se while accumulating the arsenic in plant shoots. Fast-growing as well as high-biomass-yielding plants like poplar, willow and *Jatropa* could be used for both phytoremediation and energy production.

However, transfer of the metals from soil or water does not solve the problem, and burning metal-contaminated plant material for energy production will release the metals into the atmosphere, moving the problem from soil or water to the air. Thus such metal-accumulating biomasses should be stored or disposed of appropriately so that they do not pose any hazard to the environment.
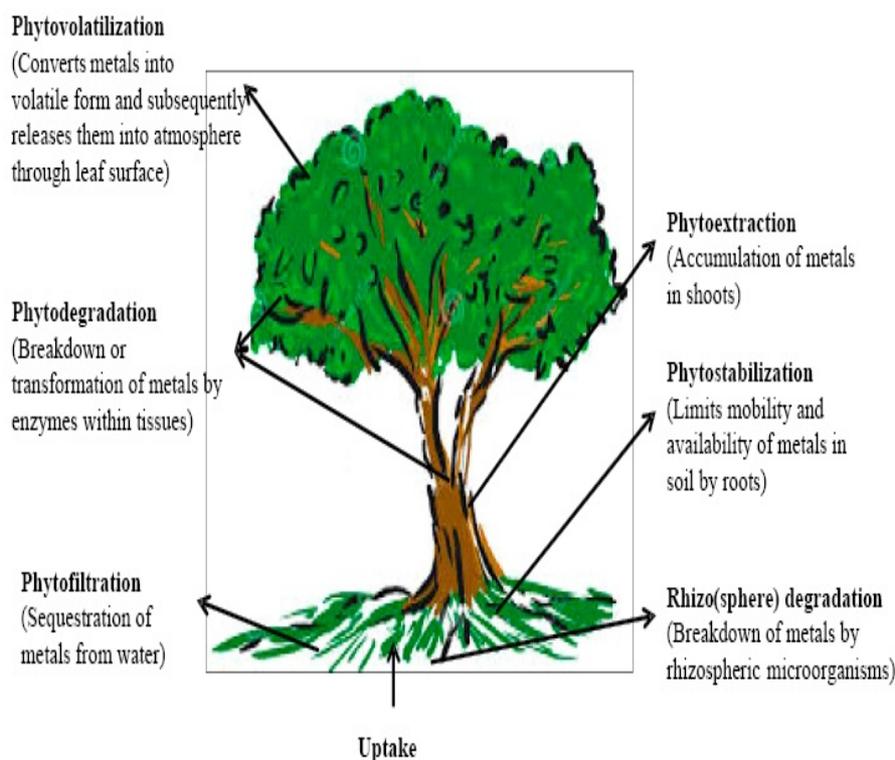
Figure 2: Techniques of Phytoremediation

CONCLUSION AND FUTURE PROSPECTS

Rapid industrialization and technology development have adverse side effects like soil contamination and degrading soil health. Due to the complexity involved in the conventional methods for remediation of soil, the use of microbes has arisen as a time-saver for bioremediation. However, bioremediation technology has limitations; several microorganisms cannot break toxic metals into harmless metabolites, and these have inhibitory effects on microbial activity. Modification in the outer membrane proteins of bacteria with potential bioremediation properties for improving metal binding abilities is the likely way to enhance their capacity for biotransformation of toxic metals. Future studies should focus on the factors involved in improving *in situ*

bioremediation strategies using genetically engineered microorganisms (GEM) and also the applicability and adaptability of these GEMs in all the possible adverse/stress conditions and multiple-heavy-metal-polluted conditions. The reluctance among the public to accept GEM for bioremediation also needs to be considered in future studies, and they must proved non-toxic to the environment.

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