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Bioremediation of Arsenic and Lead by Plants and Microbes from Contaminated Soil

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Abstract The persistence of heavy metals in the environment may pollute or contaminate soils and aqueous streams as both natural components or as the result of human activity. Bioremediation process in this regards is an option that offers the possibility to destroy or render harmless various contaminants using plants and microbes. Amongst the various bioremediation processes, phytoremediation and bioremediation by microbes are quite effective. Phytoremediation includes the removal of contaminants with the help of green plants, while the microbial bioremediation includes the removal of heavy metals by microorganisms (bacteria, fungi, yeast and algae) as sorbets. Amongst the various heavy metal contaminants arsenic and lead are recognized as the leading toxicants worldwide and having the various toxic effects on human and animal health as well as on the environment. The aim of this article is to give an overview of the arsenic and lead contaminant in soil and also the mechanism of removal of these toxic metals from the contaminated sources by the potent application of plants and microbes.

Keywords: *heavy metals, microbial bioremediation, phytoremediation, soil contaminant*

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1. Introduction

Due to global industrialization, war, and nuclear processes a large amounts of toxic compounds have been released into the biosphere. The heavy metals released through the various industries as effluent, nuclear radiation and releases of heavy metals by other process in the environment may contaminate the soil. The soil contaminate were divided into two major classes i.e. inorganic and organic. Inorganic pollutants comprises of heavy metals such as arsenic, cadmium, mercury, and lead while organic contaminants include petroleum, hydrocarbons, phenolic compounds, fertilizers, herbicides, and pesticides [1]. However, Meena *et al.* [2] found that release of metals contaminants are responsible for specific toxicity symptoms, harmful effects on human and animal health and may also pollute the environment. Under certain environmental conditions, metals may accumulate up to toxic levels and cause ecological damage. Amongst the various metals, mercury, lead, cadmium and chromium are regarded as toxic metals, whereas, copper, nickel, cobalt and zinc are not as toxic at lower concentration but due extensive use their level is increased in the environment which may lead to the serious concern on environment and global population [3].

According to Giller *et al.* [4] metal wastes could possibly contaminate the human and animals through their inhalation, consumption of contaminated food, water and skin contact. Moreover, metals such as mercury, lead and arsenic have shown their toxic effect on kidneys, nervous system, which may lead to the symptoms of mental disorder, and cause weakness, headaches, abdominal cramps, diarrhea, anemia. The chronic exposure to these metals may lead to the permanent damage of organelles. There are several ways to remove the heavy metals from various contaminated sources. The common procedures are chemical precipitation, dialysis, ion exchange, reverse osmosis and solvent extraction. These methods are very costly and low in efficiency. Some time it may change the soil properties and spread contaminants from one to other places after remediation [5].

Bioremediation is a natural process which relies on bacteria, fungi, and plants to alter contaminants as these organisms carry out their normal life functions. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. Thus, bioremediation provides an alternative tool to destroy or render the harmful contaminants through biological activity and this method is also cost effective [6].

Phytoremediation the use of plants to remove or degrade contamination from soils and surface waters, has been proposed as a cheap, sustainable, effective, and environmentally friendly approach alternative to conventional remediation technologies. Plants use solar energy (through photosynthesis) to extract chemicals from the soil and to deposit them in the above-ground part of their bodies, or to convert them to a less toxic form. These plants can then be harvested and treated, removing the pollutants [7] Similarly, the use of microorganisms for the removal of heavy metals from the contaminated sources is another effective method. It was reported that the microorganisms had the ability to utilize the contaminants as nutrient or energy sources [8]. Bioremediation activity through microbe is stimulated by supplementing nutrients (N and P), electron acceptors, and substrates (methane, phenol, and toluene), or by introducing microorganisms with desired catalytic capabilities [9].

In the present scenario, use of biotechnological approaches in controlling or removing metal pollution has been paid much attention and gradually becomes hot topic of discussion due to its potent application [3]. Dua *et al.* [10] explained that most important parameters for bioremediation are i) nature of pollutants, ii) soil texture, pH, moisture contents and hydrogeology, iii) nutritional state and microbial diversity iv) temperature and redox-potential. The aim of this article is to give an overview of the arsenic and lead contaminant in soil and also the mechanism of removal of these toxic metals from the contaminated sources by the potent application of plants and microbes.

2. Bioremediation of Heavy Metals

Bioremediation is the naturally occurring process in which microorganisms or plants either immobilize or transform environmental contaminants to innocuous state end products [11]. During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and through redox-potential they can metabolize the target contaminant into usable energy for microbes. Although multitudes of reactions are adopted by microbes to degrade and transform pollutants but all the energy yielding reactions are oxidation-reduction reactions and the typical electron acceptors are oxygen, nitrates, sulfate and carbon dioxide.

For bioremediation, it is important that effective, microorganisms and plants may degrade the pollutants in to harmless products by various enzymatic actions [7]. The microbes can’t degrade heavy metals directly but they can change the valence states of metals which may convert them into immobile or less toxic forms. Collins and Stotzky [12] stated that several metals are essential for biological systems and present in a range of certain concentration. The metals always have been associated with metalloproteins and enzymes as co-factors. Its low concentrations lead to decrease in metabolic activity while high concentrations could act in a deleterious way by blocking essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecules. Besides, they are toxic for both higher organisms and microorganisms.

The progressive accumulation of metals may inhibit the degradation of organic pollutants or humic substances in the environment. This problem can be solved by an increase of the heavy metal resistance of the bioremediating system. Heavy metals are present in soils and aqueous streams as both natural components or as a result of human activity (i.e., metal-rich mine tailings, metal smelting, electroplating, gas exhaust, energy and fuel production, downwash from power lines, intensive agriculture, sludge dumping [13].

2.1. Bioremediation of Heavy Metals by Plants

Phytoremediation is the use of green plants to clean-up hazardous waste from the contaminated sites [14]. Phytoremediation can be used either as an *in-situ* or *ex-situ* application. The *in-situ* applications are frequently considered because it can minimize the disturbance of the soil and surrounding environment and also reduce the spread of contamination via air and water borne wastes. Thus, it is also known as green technology and proper implementation make it eco-friendly and aesthetically pleasing to the public [15].

Phytoremediation does not require expensive equipment or highly-specialized personnel, thus, it is relatively easy to implement. It is capable of permanently treating a wide range of contaminants in a wide range of environments. There are various types of phytoremediation processes, which may cover a large numbers of organic and inorganic compounds discussed below.

2.2. Mechanisms of Bioremediation by Plants

2.2.1. Phytoextraction

Phytoextraction is primarily used for the treatment of contaminated soils. In this method plants absorb the concentrated metals and after precipitated from contaminated soils these metals accumulate were into the above ground parts of plants. There are few plants species known for higher accumulator and show their potential towards the removal of metals from contaminated soils. Brennan and Shelley [16] found that plants have the capability to extract large concentrations of heavy metals into their roots, translocate them into the surface and produce a large quantity of plant biomass. Beside this there are several limiting factors for phytoextraction.

- Metal bioavailability within the rhizosphere
- Rate of metal uptake by roots
- Proportion of metal “fixed” within the roots
- Rate of xylem loading/translocation to shoots
- Cellular tolerance to toxic metals.

2.2.2. Phytostabilization

Phytostabilization is the method used for the remediation of soil, sediment, and sludges. In this method the use of plant roots may limit the contaminant in the soil through mobility and bioavailability process. The plants decrease the amount of water percolating through the soil matrix, which may act as a barrier and prevent direct contact with the contaminated soil. It may also prevent soil erosion and distribution of the toxic metal to other areas [14]. Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction. It is helpful in the treatment of contaminated land areas affected by mining activities and Superfund sites [14]. Phytostabilization is commonly used to treat the metals (arsenic, cadmium, chromium, copper and zinc) contaminants [17] . It has also several disadvantages.

- Contaminant remaining in soil
- Application of extensive fertilization or soil amendments

- Mandatory monitoring is required

2.2.3. Rhizofiltration

Rhizofiltration is primarily used to remediate extracted groundwater, surface water, and waste water with low concentrations of contaminant. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn, and Cr which are primarily retained within the roots. Camagro *et al*. [18] stated that sunflower, indian mustard, tobacco, rye, spinach, and corn have been removed lead from water or soil and soil but the sunflower significantly reduced lead concentrations with in 1hr after treatment.

The rhizofiltration is useful for both terrestrial and aquatic plants for *in-situ* or *ex-situ* purposes. In this method the contaminants don't translocated to the shoots. The terrestrial plants preferred for rhizofiltration because they have a fibrous and elongated root system. The main limitation of this method is to adjust the pH at regular intervals.

2.2.4. Phytovolatilization

Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms into the atmosphere transpiration. It is basically used for mercury contaminated soil. In this method the growing trees and other plants may take up the contaminants with water and the contaminants may pass through the xylem vessels towards the leaves and converted into non-toxic forms and it may finally volatilize into the atmosphere [19].

2.3. Bioremediation of Heavy Metals by Microbes

Soil, sediment and water sources were contaminated by hazardous heavy metals through industrial activities, such as mining, refining, and electroplating. Mercury, arsenic, lead, and chromium are often prevalent at contaminated sites. White *et al*. [20] reported that bacterial remediation is the process of using metal reducing bacteria to break down the contaminants. The metal-reducing bacteria are able to reduce very toxic soluble forms into less toxic forms. Macaskie *et al*. [21] found that sulfate-reducing bacteria successfully treat the metal leachates generated by sulfuric-acid producing *Thiobacillus* sp. However, Meysami and Baheri [22] reported that white rot fungi have the ability to transform the pollutants from the contaminants in soil through ligninolytic enzymes. Moreover, Lang *et al*. [23] have successfully used the production and activity of ligninolytic enzymes in the bioremediation of contaminated from under field conditions. In natural soil a wide range of saprophytic microorganisms are pre-exist but the introduction of white rot fungi requires effective growth and competition with these native populations. Additionally, the fungi used for the bioremediation proposes should also secrete the degrading enzymes into the soil matrix to enhance degradation of pesticide that they are not able to incorporate across cell walls.

2.4. Mechanisms of Bioremediation by Microorganisms

Microbes could be isolated from almost all types of environmental conditions and also had a wide range of adaptability. It can survive from zero to extremely high and desert conditions. In water, it can survive in presence and absence of oxygen and also in presence of hazardous compounds or waste stream [24]. Bacteria, fungi, yeast and algae can remove heavy metals and radionuclide from aqueous solution in substantial quantities. To survive under metal stressed condition, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions.

These mechanisms of removal of heavy metals includes the efflux of metal ions outside the cell, accumulation and forms complex of the metal ions inside the cell and later reduce the toxic metal ions to a non-toxic state. The microorganisms involved in this process may belong to bacteria, fungi, yeast and algae [24]. Potent metal biosorbents under the class of bacteria include the genera of *Bacillus*, *Pseudomonas*, *Streptomyces* and *P. aeroginosa* [25,26,27].

Boricha and Fulekar [28] found that *P. plecoglossicida* as a novel organism for the bioremediation of cypermethrin while *P. aeruginosa*, *Bacillus* sp., *Streptomyces* sp., *P. fluorescens* were effective against chromium [29]. Similarly, fungal biosorbents includes *Aspergillus* and *Penicillium* spp., while the algal biosorbents include red, green and brown sea weeds [30].

Amongst the various microorganisms, fungal biomasses were very effective due to presence of high percentage of cell wall material, which may have the excellent metal binding capacity. Many fungi and yeast have excellent biosorption potential includes the genera of *Rhizopus*, *Aspergillus*, *Streptoverticullum* and *Sacchromyces* [31]. For endurance under metal-stressed environment, plant growth promoting rhizobacteria have evolved several mechanisms by which they can immobilize, mobilize or transform metals rendering them inactive to tolerate the uptake of heavy metal ions. These mechanisms include (1) exclusion-the metal ions are kept away from the target sites; (2) extrusion-the metals are pushed out of the cell through chromosomal/plasmid mediated events; (3) accommodation metals form complex with the metal binding proteins or other cell components; (4) bio-transformation-toxic metal is reduced to less toxic forms, and (5) methylation and demethylation. Thus, in general the immobilization and mobilization are the two the main techniques used for the bioremediation of metals by microbes.

2.4.1. Immobilization

Immobilization is a technique used to reduce the mobility of contaminants by altering the physical or chemical characteristics of the contaminant. This remediation approach can utilize microorganisms to immobilize metal contaminants. It is usually accomplished by physically restricting contact between the contaminant or by chemically altering the contaminant [32]. Chemical reagents and bacterial reagents assist with the immobilization of metal contaminants. Most sites contaminated with metals use the solidification and stabilization approach to immobilize metals. Solidification treatment involves mixing or injecting chemical agents to the contaminated soil. The prominent mechanism by which metals are immobilized is by precipitation of hydroxides. The chemical composition of the site, the amount of water present, and the temperatures are all factors important to the successful use of the solidification/ stabilization mechanism [32].

The stabilization and solidification technique is achieved by mixing the contaminated material with appropriate amounts of stabilizer material and water. The mixture forms a solidified matrix with the waste. The stabilization and solidification techniques can occur both in situ or ex situ. *In-situ* is preferred for volatile or semi volatile organics. The *in-situ* process is useful for treating surface or shallow contamination.

2.4.2. Mobilization

Microorganisms can mobilize metals through autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, methylation, and redox transformations. Heterotrophic leaching is when microorganisms can acidify their environment by proton efflux thus leading to the acidification resulting in the release of free metal cations. Autotrophic leaching is when acidophilic bacteria retrieve CO_2 and obtain energy from the oxidation of the ferrous iron or reduced sulfate compounds, which causes solubilization of metals. Siderophores are specific iron chelating legends and are able to bind to other metals, such as magnesium, manganese, chromium, gallium and radionuclide, such as plutonium. Methylation involves methyl groups that are enzymatically transferred to a metal, forming a number of different metalloids. Redox transformations can allow microorganisms to mobilize metals, metalloids, and organo-metallic compounds by reduction and oxidation processes.There are various metal-mobilization techniques that can also occur in nature [33].

2.5. Persistence of Arsenic in the Environment

Arsenic is a toxic metalloid of global concern. It is usually originated geogenically but can be intensified by human activities such as applications of pesticides and wood preservatives, mining and smelting operations, and coal combustion. Exposure to arsenic had direct adverse effects on health. Elevated levels of arsenic in drinking water can seriously impact human health and have been implicated in human diseases and mortality. According to an estimate about 6 million people in West Bengal [34] and more than 46 million people in Bangladesh are at risk from drinking water due to arsenic [35].

Arsenic can appear in inorganic or organic forms and it has neurotoxic effect on both peripheral and central nervous systems. Neurotoxicity usually begins with sensory changes, muscle tenderness followed by progressive weakness from the proximal to distal muscle groups [36]. Acute illnesses from arsenic exposure consist of fever, anorexia, melanosis, cardiac arrhythmia, and eventually cardiovascular failure. A few days after exposure to arsenic, anemia, and granulo cyopenia will appear in the body [36].

2.5.1. Bioremediation of Arsenic by Plants

Fern is the hyper arsenic accumulator plant absorbs arsenic from contaminated soil. It could favor the alkaline soil. Giller *et al.* [4] found fern as efficient arsenic removing plant from soils. It has the ability to accumulate about 200 times concentration of arsenic higher than the concentrations measured in non contaminated soils. The growth of fern plants was much better in the arsenic contaminated soil compared to non-contaminated soil.

2.5.2. Bioremediation Arsenic by Microorganisms

The microbial activity could reduce the arsenic through sorption, biomethylation, complexation, and oxidation-reduction processes. Microorganisms have evolved the biochemical mechanisms to exploit arsenic either as an electron acceptor for anaerobic respiration, or as an electron donor to support chemoautotrophic fixation of CO_2 into cell carbon [37]. The key microbes involved are categorized as dissimilatory arsenate-reducing prokaryotes from both bacterial and archaeal domains. It uses arsenate as an electron acceptor and reducing it to arsenite. Chemoautotrophic arsenite oxidizers, microorganisms use CO_2 as a carbon source and use arsenite as an electron source, oxidizing it to arsenate for energy. However, heterotrophic arsenite oxidizers use oxygen as an electron acceptor and oxidize arsenite to arsenate. Arsenate-resistant microbes reduce arsenate into arsenite, which allows the transport of toxic form from the cell [38].

2.6. Persistence of Lead in the Environment

Lead is a ubiquitously distributed in nature in biologically inactive form. The use of lead in batteries, bearing metals, cable covering, gasoline additives, explosives, manufacture of pesticides, antifouling paints and analytical reagents are major source of contamination [39]. Lead may cause the toxic effects on gastrointestinal, muscular, reproductive, neurological systems. It may enter into our body through ingestion of food or through the blood stream. Klassen and Watkins [36] reported that lead impairs communication between cells and modification of neuronal circuitry.

In lead-contaminated soils, biota and vegetation influence the transformations of lead together with environmental characteristics such as soil pH, organic matter content, texture, redox-potential and presence of other elements [40]. According to Bindler *et al*. [41] lead contamination is mainly restricted to surface soil in boreal forests, which are rich in humus contents. However, sometime it is also possible that lead forms a complex with dissolved organic matter and migrate from the surface soil layer to mineral soil and possibly contaminated the underground water.

2.6.1. Bioremediation of Lead by Plants

Phytoremediation technologies have been recognized as quite efficient and cost-effective method for remediating sites contaminated with lead. In this regard plants like *Brassica junce*, *Vetiveria zizanioides*, *Cardaminopsis halleri* play a significant role in the removal of lead from contaminated soil. Plants growing in northern Europe (blueberry, *Vaccinium myrtillus*; cowberry, *Vaccinium vitis-idaea*; crowberry, *Empetrum nigrum*; birch, *Betula pubescens*; willow, *Salix* spp.; pine, *Pinus sylvestris*; and spruce, *Picea abies*) could not accumulate lead into their shoots but when metal chelators like EDTA has been supplied to soils then they can remediate the lead from soil [42].

2.6.2. Bioremediation of Lead by Microorganisms

Soil bacteria could directly or indirectly interact with lead present in the contaminated soil and reduced it into non-toxic forms. Some of bacteria responsible for the bioremediation of lead include *Bacillus* sp. and *P. aeruginosa*. These bacteria may reduce the lead in the contaminated source through biosorption of lead by functional groups on the cell surface or by interaction and complex formation between lead and acidic sites in the cell wall [43].

The possible mechanisms includes exclusion by forming a permeable barrier, intra- and extracellular sequestration, active transport, enzymatic detoxification, dissolution of lead by acid production, chelation of lead, precipitation of lead through the production of organic bases, extracellular metal precipitation, and biotransformation reactions, such as methylation, volatilization, oxidation, and reduction [44].

3. Conclusion

The increase global population, industrialization and urbanization are the some major reasons to contaminate the environment. The release of heavy metals from industries causing the serious health problem to human and other animals and also pollutes the environments due to their persistence and bio-accumulative nature. In this regards, bioremediation process provides and effective innovative measures for treatment of a wide variety of contaminants. Amongst the various known bioremediation process phytoremediation, rhizoremediation and bioremediation by microbes could be efficient methods to reduce the arsenic and lead contaminants of soil.

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