

## Heavy Metal Bioremediation and Metallo Bacterias - A Review

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### Abstract

Heavy metals pollution of the soils is the most challenging problem for the different ecological and environmental conditions. All the biological and organic compounds are degradable naturally but heavy metals cannot, so it leads to accumulation in the environment, which further causes several problems. Heavy metals are eliminated from the environment by plants (phytoremediation) and microbes (bioremediations). Microbes use different mechanisms for converting the toxic metals into the less toxic using their Enzymes or several types of adaptation techniques. Microbes can develop different kinds of heavy metals resistance via biosorption, precipitation, efflux mechanism, entrapment in extracellular, and reduction of heavy metals into less toxic forms. The present review article provides you with an overview of the principles of bioremediation, microorganisms of bioremediation, and strategies of bioremediation along with the future perspectives of heavy metals bioremediation by bacteria's.

**Keywords:** Bioremediation; Heavy Metals; Microbes; *Ex-situ*; *In-situ*; Bioremediation Strategies

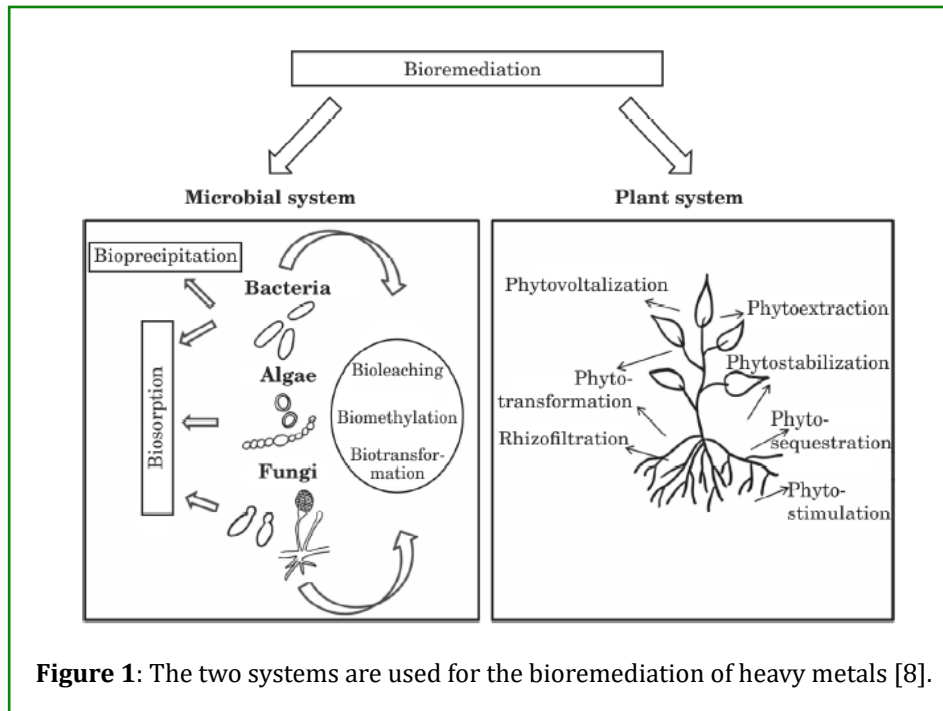
### Introduction

Since the industrial revolution, heavy metal waste has been increased faster. The toxic metals are mostly used in industrial activities and fossil fuels consumption and they are accumulated by many ways like the food chain, leading to always harmful for the ecosystem as well as for human health, so we have to handle these types of challenges are very carefully and appropriately so that any new problems are not created [1]. Bioremediation includes all those processes and actions that take place to return the natural environment altered by contaminants to its original condition [2] primarily microbes, to clean up contaminated soils, aquifers, sludges, residues, and air, known as "bioremediation", is a rapidly changing and expanding area of environmental biotechnology, that offers a potentially more effective and economical clean-up technique than conventional physicochemical methods. Although it is certain that up to now the technologies employed are not technically complex,

considerable experience and expertise is required to design and implement a successful bioremediation program. As a matter of fact, and since bioremediation frequently addresses multiphasic, heterogenous environments (i.e., soils. It primarily uses microorganisms, fungi, green plants, or their enzymes to degrade and transform environmental contaminants into harmless or less toxic forms. It uses relatively low-cost, low-technology techniques, which generally have a high public acceptance [3]. Several metals are used as micronutrients in our body and they are used as enzymes and cofactors. Many metals are toxic to microbial cells like mercury, cadmium, arsenic, silver, etc. This is more common that many metals are resistant to some microbial which has to be studied by plasmid technology. A lot of bacterias contain genes that are responsible for the development of resistance for any specific heavy metals which are shown by engineering technology [4,5]. There are several protection mechanisms of heavy metal resistance by microbial cells [5]. These mechanisms are an extracellular

barrier, extracellular sequestration, and active transport of metal ions (efflux), intracellular sequestration, and reduction of metal ions [6,7]. We know that heavy metals are harmful to our health so we have to solve this problem using microorganisms because they can tolerate these toxic metals by several mechanisms.

Conventional technologies like precipitation, reduction or oxidation, ion exchange, evaporation, and membrane filtration. These technologies can be used to eliminate these toxic metals from the environment, but these technologies lack efficiency and cost-effectiveness so we have to replace them by developing an effective new modern technology, which has cost-effective, easy, and more efficient [1].



**Figure 1:** The two systems are used for the bioremediation of heavy metals [8].

In the current situation, we have two options one is plants (phytoremediation) and the other is microbes (bioremediation), both methods are efficiently eliminated heavy metals toxins from soil and water, Figure 1. These biological agents are eco-friendly and harmless to the environment because they can efficiently accumulate heavy metals from contaminated sources, hence reducing the pollutant content to a safe level [9].

### Principles of Bioremediation

Bioremediation is the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state or levels below concentration limits established by regulatory authorities [10]. By definition, it is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. Three essential components are needed for bioremediation. These three components are microorganisms, food, and nutrients. These three main components are known as the bioremediation triangle. Microorganisms are found almost everywhere on earth and nutrients are usually the missing

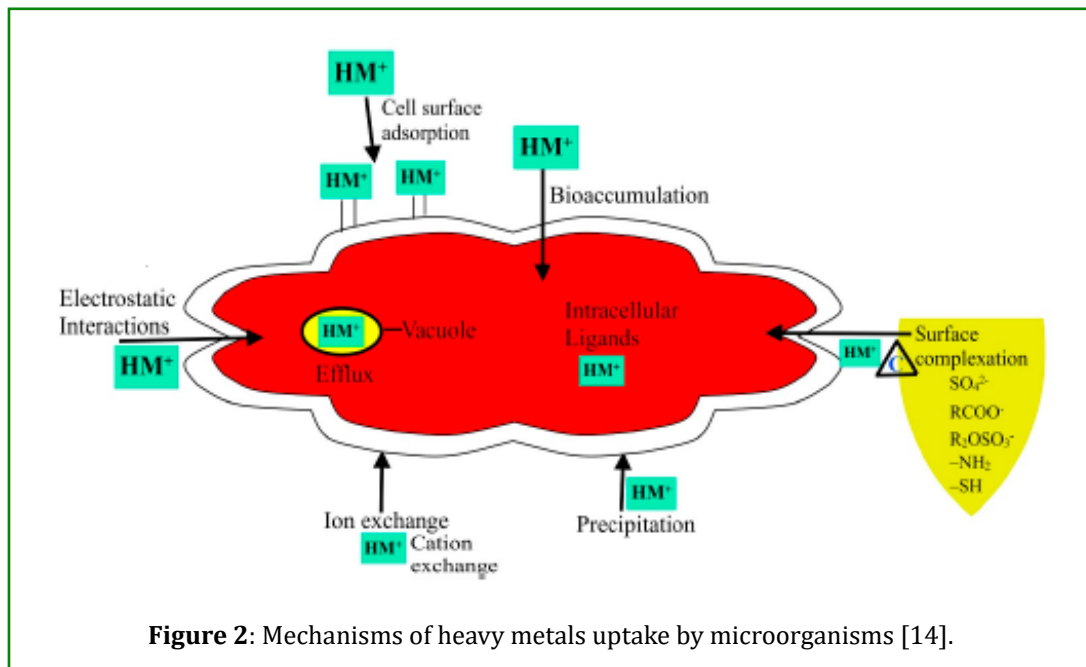
ingredients that prevent successful bioremediation. We can find out the microorganisms in water or soil where a sufficient amount of food is available. However, if a contaminant is present it can become an additional food source for the microorganisms [11].

The contaminant serves two useful purposes for the microbes. First, the contaminant provides a source of carbon needed for growth. Second, the microbes obtain energy by breaking chemical bonds and transferring electrons away from the contaminant. This is known as an oxidation-reduction reaction. The contaminant that loses electrons is oxidized and the chemical that gains the electrons (electron acceptor) is reduced. The energy gained from the electron transfer is used along with the carbon and some electrons to produce more cells [9]. Microbes generally use oxygen as an electron acceptor but sulfate, nitrate, iron, and  $\text{CO}_2$  are also commonly used. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules [11].

## Microorganisms Used in Bioremediation

Microorganisms and plants are usually used for the removal of heavy metals. All the metals are toxic, but some of these are useful in low concentrations. This metal toxicity causes serious morbidity and mortality [12]. The bioavailability of metals is increased using the nutrients to the soil like manure, bio solids, compost, which provides suitable conditions to

the soil and increases its fertility [13]. Microorganisms that carry out biodegradation in many different environments are identified as active members of microbial bioremediations, Figure 2. Bioremediation especially occurs on its own (natural attenuation or intrinsic bioremediation) or is often spurred on via the addition of fertilizers to extend the bioavailability within the medium (bio stimulation) [9].



New advancements have also proven successful by the addition of matched microbe strains to the medium to reinforce the resident microbe population's ability to interrupt down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediation (Bioaugmentation), they include species of Xanthofacter, Penicillium, Bacillus, Pseudomonas, Flavobacterium, Mycobacterium, and Nitrosomonas [9]. Metallic pollutants are not degraded while composting but they may be converted into other forms like organic combinations which have less bioavailability than mineral combinations of the metals [15].

Many microorganisms can produce iron complexing molecules, named siderophores. These molecules are synthesized in the case of iron deficiency. Some of these siderophores also have high affinities for heavy metals, synthesis can also be induced by heavy metals even in the presence of high iron concentrations. A comparison between negative and constitutive siderophores mutants leads to the conclusion that siderophores or, more generally metallophones, can play a role in metal solubilisation [9].

The bacterium can solubilize metals (or increase their bioavailability) via the production of siderophores and adsorb the metals in their biomass, on metal-induced outer membrane proteins, and by precipitation. The difficult point is to find an easy way to separate the biomass, loaded with metals, from the soil matrix. The bacterium was able to improve the settling of the soil by the production of some extracellular polymers. In that way, biomass and soil could be separated more easily, for example, through settling or flotation. The heavy metal resistance, precipitation capacity, and improved soil flocculation lead to the development of a bioremediation method for heavy metal contaminated soils [16].

## Bioremediation Strategies

Based on the removal of wastes for treatment there are generally two types of strategies that are available for bioremediation are in situ, ex-situ, and bio adsorption bioremediation. We summaries all the bioremediations strategies with benefits and limitations in the Table 1.

Technology	Example	Benefits	Limitations
In situ	In situ bioremediation	Most cost effective	Longer treatment period
	Biosparging	Relatively passive	Monitoring difficulty
	Bioventing	Non-invasive	Environment restriction
	Bioaugmentation	Natural attenuation process	
	Bio stimulation		
Ex situ	Ex situ bioremediation	Cheap	Bioavailability limitation
	Composting	Cost efficient	Mass transfer problems
	Biopiles	Easily can be done	Space requirements
	Bioreactor	Better rate and extent	Time-taking process
	Land farming		
Bio adsorption	Bio adsorption	Very economical	Complex mechanism
	Natural	Most potent	limited uses
	Biological	Not expansive	Not widely used
	Derived from waste	High surface area	

**Table 1:** Summary of bioremediation strategies.

### In Situ Bioremediation

In situ bioremediation is a subsurface application of bioremediation compared to ex-situ bioremediation, which includes media above the ground readily accessible (e.g., soil piles in bioreactors or treatment cells). In situ bioremediation may be applied in saturated or unsaturated soil and groundwater [9]. There are several type of In situ bioremediation methods such as Biosparging, Bio stimulation, Bioaugmentation, and Bioventing. In situ bioremediation technology was originally developed as a more effective and less costly alternative to the standard pump-and-treat methods used to clean up aquifers and soils contaminated with organic chemicals (e.g., chlorinated solvents, fuel hydrocarbons), but has since widened to address explosives, toxic metals (e.g., chromium) and inorganics (e.g., nitrates). It is an important method in cleaning contaminated environments since it is cheaper and uses harmless microorganisms to degrade the chemicals. Chemotaxis is useful in the study of in situ bioremediation because microorganisms with chemotactic abilities can migrate into an area containing contaminants. Therefore, by enhancing the cell's chemotactic abilities, in situ bioremediation will become a safer method in degrading toxic compounds [11].

### Ex-Situ Bioremediation

Ex-situ bioremediation is a biological process where excavated soil is placed in a lined above-ground treatment area and aerated following processing to facilitate the degradation of organic contaminants by the indigenous microbial population. This process requires the excavation of contaminated soil or the pumping of groundwater to

enhance microbial degradation [17]. Ex-situ bioremediation involves excavating the contaminated material and its treatment in above-ground facilities located on-site or off-site, whereas in situ bioremediation are undertaken at the site of contamination. Ex-situ methods involve extraction separation, treatment of secondary waste streams, and the proper disposal of solid wastes. The ex-situ treatment processes are better understood; hence, they are relatively easy to implement, monitor, and control. The treatment of radionuclide-contaminated soils, sediments, and wastes involves excavation followed by ex-situ treatment or disposal. The common ex-situ treatment for excavated soils is solidification or stabilization [9].

### Bio Adsorption

Bio adsorbents, also called bio sorbents, have attained considerable attention for their application in cleaning environment pollutants [18]. The biosorption technology has emerged worldwide and extensively used as it is very economical in the processing of bio sorbents [19]. From the last few decades, adsorption technology has been regarded as the most cost-effective & potent process. During recent years, various data on the use of various adsorbents from various sources have been isolated and carried out for the purification of wastewater.

Bio adsorbents are materials derived from different biological sources like agricultural by-products. The most common of them are microorganisms, i.e. fungi, algae, yeasts, bacteria, and [20]. They serve as an effective adsorbent to accumulate elemental and toxic wastes including industrial wastes, heavy metals, fertilizers, pesticides, and atmospheric pollutants from

the environment [21]. Adsorption processes like physical, chemical, and ionic are involved in the sorption of different contaminants [22]. These adsorption processes are effective due to the involvement of different specific mechanisms like absorption, acid-base interactions, adsorption, cation- $\pi$  EDA interactions, chelation, complexation, complexes involving association (cooperative effect of the grid of the polymer network), diffusion, electrostatic attraction, electrostatic interactions, hole-filling, hydrophobic interactions, hydrogen bonding, ion-exchange, instance ion exchange, micro precipitation, nonspecific hydrophobic partition,  $p/\pi$ - $\pi$ , pore-filling, physical shielding, surface sorption, surface complexation, van der Waals forces, van der Waals interactions, Yoshida forces, etc. Also, the presence of high pore volume, large surface area, various functional groups, and ligands groups in bio sorbents make them suitable for the absorption process. Ligands such as amine, phosphate sulfhydryl, carboxyl, etc. are involved in the chelation of different organic and inorganic contaminants [23]. The interaction of adsorbate molecules with the surface ligands of adsorbents expedites the processes of adsorption by forming bonds with the surface molecules and holding them inside the pores. The electrostatic attraction between metal ions and negatively charged reaction site on the sorbent paves the way for the formation of complex molecules. This forms to be the general mechanism of biosorption. The presence of a negative charge on the surface of the bio sorbent also facilitates the adsorption of the positively charged cationic dyes via a similar mechanism. The presence of ligands and other functional groups on the surface also assists the formation of complexes that lead to further adsorption [24].

### Types of Adsorbents

Based on the source of origin, bio sorbent has been classified as natural, biological, and derived from waste, but we discussed here more about natural and biological bio sorbents.

#### Natural Adsorbents

Various naturally occurring materials are present in nature which were having characteristics of an adsorbent and are easily available in the ecosystem. Although, there are various naturally occurring adsorbents, clay, zeolite, and siliceous material have been successfully utilized for the eradication of several contaminants like dyes, heavy metals, pharmaceutical compounds, & other organic contaminants from wastewater or water.

**Clay:** Clay is the most abundant, naturally occurring bio sorbent. Low cost, high adsorption property, high porosity, large surface area, ease of chemical modification, and suitability for remediation makes it an important adsorbent [25,26]. Clay is composed of a layered structure and based on the differences in their structures, they are classified as

(i) Kaolinite (such as serpentine, pyrophyllite, vermiculite, sepiolite) (ii) Micas (such as illite), and (iii) smectites (such as montmorillonite, saponite) [27]. Out of all three types, montmorillonite has the smallest crystallite size; hence, it possesses the largest surface area and has the highest cation exchange capacity.

**Zeolites:** These are crystalline hydrated aluminosilicates having a highly porous 3-D structure with a negatively charged lattice [28]. The framework structure contains pores that are occupied by alkali, alkaline earth cations, and water [29]. Exchangeable cations present in the interstices balance the negative charge. Large surface areas with high ion exchange capacity & cost-effective make zeolite an attractive adsorbent [30]. More than 40 natural species of zeolites are reported, out of which, clinoptilolite is most abundant [31]. Chemical modification such as surface functionalization ion exchange and acid treatment elevates the adsorption capability of zeolites [32]. Natural Chinese zeolite can remove ammonium ions from liquid solution [33].

#### Biological Adsorbents

They are mostly originated from biological sources and microorganisms, i.e. fungi, algae, yeasts, bacteria, and even shells of higher animals. They have a strong affinity towards various contaminants present in the water and soil ecosystems. Different types of biological bio sorbents, like alginate compounds, chitin, and chitosan, etc.

**Alginate Compounds:** They are mostly found in brown seaweeds and composed of linear polysaccharides and are highly soluble in water [34] including contact time, pH, initial concentration of U (VI). Being water-soluble has restricted its application for removing radionuclides and heavy metals. They are used after processing their ion-exchange reaction with multivalent metal ions. After processing, they can be used as an adsorbent or entrapment of various contaminants. They are also converted into hydrogels using calcium and have wide applications in the removal of various heavy metals [35]. They are reported to have a sorption capacity of 70% to 91%. The process of adsorption using alginate compounds is usually endothermic [34].

**Chitin and Chitosan:** Chitin is an important natural biopolymer; is the second most widely used polymer after cellulose. It is a hard, white, inelastic nitrogenous polysaccharide, which forms the supporting material of crustaceans, insects, etc. [36]. Depending on the number of N-acetyl-glucosamine units present in the biopolymer it can be termed as chitin and chitosan with many units less than 50 % and higher than 50 %, respectively [37]. Chitosan and chitin are chiefly isolated from crustaceans (crayfish, krill crab) [38]. Crustaceans' exoskeleton is present in large numbers and is readily available as a metabolite of food processing

(Assam, Silchar). Chitin is a copolymer of N-glucosamine and N-acetyl-glucosamine units. Many studies demonstrated the use of chitosan and chitin-based bio sorbents as efficient materials for adsorption. [39] Reported the use of chitosan for the adsorption of mercury ions from aqueous solutions.

## Conclusion

Heavy metal toxicities are the challenging task because it is harmful to human health via interference of the vital cellular functions of the human's body. Cadmium, mercury, copper, manganese, lead, and selenite's are the metals and metalloids that are widely present in the environment. P-type ATPase system is exported the cytoplasmic ions to the periplasm and efflux transporters that are further exported periplasmic ions to the outside, these are the general mechanism of resistance for heavy metals like Co, Pb, and Cd, etc. Furthermore, in the metal detoxification by sequestration, binding factors will be involved in creating tolerance to heavy metal ions. Bacterias are very important for the bioremediation of heavy metals and more research is required for further improvement of bioremediation of heavy metals by microorganisms.

## Future Perspectives

Bioremediations is a very important strategy for solving the heavy metals ecosystem and environmental pollution using microorganisms. As we know that heavy metals toxicity is the major concern of our health issues so we have to develop a permanent solution for that via new modern technologies. We have to do further detailed studies about heavy metals toxicity and it's a major concern so that we can identify the best way to solve it effectively. Further many types of research are required to understand the whole mechanism of heavy metals bioremediation and metals resistance in microbes.

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