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Bioremediation of Heavy Metals (Pb and Hg) from Contaminated Sites by Potential Micro-organisms

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Abstract

Contemporary apprehensions about environmental degradation stem from various natural and anthropogenic activities, encompassing rapid industrialization, global economic expansion, increased agrochemical use, and increased hydrocarbon deposition. This multifaceted array of activities contributes to environmental decay, resulting in decreased crop yields and deleterious effects on the biota. Heavy metals emerge as prominent contaminants in environmental pollution, characterized by their nonbiodegradable nature and prolonged persistence in ecosystems. The deleterious repercussions of heavy metal contamination on soil and aquatic organisms are multiple, thereby necessitating vigilant consideration. The adverse consequences of heavy metal exposure extend beyond the environmental impact to include significant implications for human health. Consequently, remedial interventions become imperative to eliminate hazardous organic heavy metals or facilitate their conversion into less harmful inorganic forms. This review synthesizes the existing literature on the intricate interplay between anthropogenic activities, heavy metal contamination, and the resulting environmental and health implications. By scrutinizing these intricate dynamics, this review seeks to illuminate the imperatives of effective remediation strategies in mitigating the adverse consequences of heavy metal pollution, thus fostering sustainable environmental practices and protecting public health.

***Key words*: Bioremediation, Contaminants, Mercury, Lead, Microorganisms**

Significant aspects of life are air, soil, and water, and they are polluted by various natural and anthropogenic activities [1]. Natural activities such as volcanic eruption, erosion, and rock disintegration are the main sources of pollution [2]. Anthropogenic activities, such as active urbanization, increasing global economy and industrialization [1]. Human activities cause degradation in soil quality due to heavy metal contamination; various heavy metals present in the soil such as mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As) and chlorium (Cr) cause toxicity to living organisms [3]. Elements with the highest density of more than 4-5 g/cm^3 present on earth are known as heavy metals and even in low concentrations they pose toxicity, classified as extremely poisonous inorganic chemicals [4]. The heavy metals present in the environment pose a serious threat to living organisms and the ecosystem [5-9]. Heavy metals possess the ability of bioaccumulation and non-biodegradability, which is of great concern to the environment [10-11]. Containment due to heavy metals in the environment poses a serious health hazard such as heart disease, liver damage, neurological diseases, cardiovascular diseases, cancer, hypophosphatemia, and damage to the central nervous system [12]. Heavy metals can accumulate in various cell organelles such as mitochondria, lysosomes, endoplasmic reticulum and the process of protein formation, which has a toxic effect on the biological system [13].

Industrially lead is one of the trace elements that are referred to as toxicant, occupational hazard, environmental pollutant, and biological nutrient [14]. There are various sources of lead discharge in the environment, such as plating, mining, lead smelting, storage batteries, the ceramic and glass industries, and the manufacturing of tetraethyl lead [15]. Since the prehistoric ages, lead has been used in various fields, such as casting, piping, ammunition, and building materials [16]. Along with the use of lead, it can also cause damage to wildlife, as lead is one of the major causes of the destruction of wildlife and causes damage to human health such as anaemia, damage to the nervous system, and kidney diseases [17]. Higher concentrations of lead exposure during pregnancy can lead to various serious problems such as miscarriage and sterility in women, while a high concentration of lead in men affects increased blood lead levels of up to 1.9 µmol/l which destroy semen quality. Therefore, lead is classified as a carcinogen that causes cancer [18]. To prevent such health hazards, eco-friendly and cost-effective techniques should be used to remove toxic heavy metals and their effects.

Mercury is a very harmful metal, and methyl mercury causes a wide range of health hazards such as neurological damage, paralysis, blindness, chromosome damage, irritability, insanity, and birth defects [19-20]. Mostly environmental mercury is present in two forms such as organomercuric salt

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and inorganic mercury, the most common species of mercuric salt are HgS, $HgCl_2$, CH_3HgCl and CH_3HgOH [21]. Most organomercuric compounds are derived from methyl mercury, which is one of the very toxic species because it accumulates quickly in tissues of biological systems [22]. Mercury is released from various sources, natural and anthropogenic activities, and some are reemission-originating [23-24]. Various anthropogenic activities of mercury emission are the mining, various agricultural materials, combustion, and industrial discharge, which annually releases about 2000 to 2200 tons of mercury [25-27].

Various physicochemical methods have been used to remove and treat environmental contaminants, such as heavy metals. Methods include evaporation, sorption, precipitation, ion exchange, reverse osmosis, and electrochemical treatment [28-29]. However, these physicochemical methods have their disadvantages such as high reagent, partial removal of metals, higher use of energy, production of toxic waste or by-products [30]. Therefore, to overcome such problems, cost-effective and environmentally friendly alternative tools are necessary [31]. Utilizing naturally occurring materials such as clays, zeolites, activated carbon, and biochar as sorbents or adsorbents can effectively remove contaminants from water and soil. These materials are abundant, cost-effective, and often exhibit high selectivity and efficiency in pollutant removal.

Bioremediation is one of the processes that uses various microbes such as bacteria, fungi, algae, and higher plants to treat heavy metal pollution [32]. Bioremediation tools are used to convert toxic heavy metals into their less toxic form [33-35]. The presence of excessive heavy metals in the environment causes microorganisms to evolve several mechanisms such as adsorption, chemical reduction, and complexation to survive in a polluted environment and to clean up the polluted environment [36-37].

Industrial sources and effects of heavy metals (Hg and Pb)

Metals are naturally present in the environment, but some natural as well as anthropogenic activities lead to excess emission of heavy metals, worldwide lead emission in the atmosphere is 12.0 thousand tons per year naturally, while 332.0 thousand tons per year through anthropogenic activities [38]. The main sources of lead emission are coal burning, mining, automobile emissions, smoking, paint and sources of mercury emissions are batteries, the paper industry, and pesticides [31]. The effect of heavy metals on human health shown in (Table 1).

Table 1 Effect of heavy metal mercury and lead on human health and plants

Heavy metal	Effects on human health	Effects on plant	References
Mercury	Loss of memory, kidney damage, lung cancer, autoimmune disease, insomnia, fatigue, depression.	Inhibit mitochondrial activity and generate oxidative stress leading to the formation of reactive oxygen species (ROS), interfere with cellular metabolism	[39-40]
Lead	Cardiovascular diseases, short-term memory loss, problems in learning and coordination.	In plants, it reduces germination, decreases cell division, and impairs mineral nutrition.	[41-43]

Microbial bioremediation of Hg and Pb

Microorganisms can alter the drastically changing environment and sustain themselves in the presence of inorganic heavy metals. To adapt to changing environment microorganisms, use various mechanisms such as the production of exopolysaccharide (EPS), exclusion, enzyme use, biotransformation and metallothionein production to survive the toxicity of metals [44-45]. The mechanism evolved by microorganisms for resistance to various heavy metals and detoxification of heavy metals includes redox processes, ion exchange, precipitation, and surface complexation [46]. The most common mechanisms used by microbes to resist heavy metals are the production of biosurfactant, metallothionein, metal oxidation, metal-ligand degradation, methylation, demethylation, metal efflux pump, extracellular and intracellular sequestration of metals, exclusion of the permeability barrier, and enzymatic decrease [47]. Microorganisms carry a negative charge on the surface of the cell, and this anionic charge helps the microorganism bind to the metal cation, which facilitates the adsorption mechanism [48].

The absorption of heavy metals by various microorganisms occurs via two mechanisms such as bioaccumulation, which is an active process, whereas adsorption is a passive transport. Various microorganisms, such as bacteria, algae, plants, and fungi, are used to decontaminate or clean up the heavy metal-polluted environment [49-50].

Various microorganisms have different biosorption abilities that differ among a wide range of microbe biomass, and these microorganisms adjust to altered physical and chemical environments to increase biosorption activity [51]. Bacteria are one of the important biosorbents with the ability to survive in extreme conditions and to resist heavy [52-53].

Marine bacteria are extensively resistant to the concentration of 25 ppm mercury, which is one of the highly toxic heavy metals, marine mercury-resistant bacteria are capable of cleaning up not only mercury but also cadmium and lead. These marine bacteria identified using 16s rRNA are *Bacillus pumilus*, *Brevibacterium iodinium*, *Pseudomonas aeruginosa*, and *Bacillus spp*. Within 96 hours. These bacteria remove about 98% of Pb which shows a good detoxification efficiency and can be used in the implementation of these bacteria for the bioremediation of heavy metals [54]. The study shows Vibrio fluvialis has the capacity to remove mercury with an efficient reduction of 60% mercury ions at a concentration of 250 µg/ml. The strain was isolated from the discharge of effluent from the SIPCOT industrial area and identified using 16s r RNA. *V. fluvialis* shows great bioremediation activity and minimal antibiotic resistance. Therefore, mercury was successfully removed in an environmentally friendly way [55].

Various microorganisms such as bacteria, yeast and protozoa isolated from the tannery effluent were *Bacillus licheniformis*, *Candida parapsilosis*, and *Tetrahymena rostrata*. They showed mercury-resistant activity was identified using 16 s r RNA and 18 s r RNA. These strains successfully demonstrated mercury removal individually and in consortia. When these strains were processed individually, *Bacillus licheniformis*, *Candida parapsilosis*, and *Tetrahymena rostrata* showed a capacity of mercury removal of 73%, 80%, and 40%, respectively, when strains used in combination such as *Bacillus licheniformis* and *Candida parapsilosis* minimize the concentration to 85%, the combination of *C. parapsilosis* and *T. rostrata* reduced 77% mercury, while 73% mercury removed when *B. licheniformis* and *T. rostrata* were used. The consortia of these 3 microorganisms successfully removed 88% mercury after 96 h of incubation [56]. The two locally isolated strains

Klebsiella pneumoniae and *Pseudomonas aeruginosa* showed mercury biosorption activity with significant pH. *K. pneumoniae* showed the optimal biosorption activity at pH 5 with 15% efficiency, while *Pseudomonas aeruginosa* showed the highest biosorption activity at pH 5.8 with almost 25% efficiency [57]. The tannery industries are the main source of heavy metal emissions such as lead (Pb), chlorium (Cr), and cadmium (Cd), three strains were screened *Gamella sp*., *Micrococcus sp*. and *Hafnia sp*. shown degradation potential in which Gamella effectively showed a reduction in Pb of 55.16 ± 0.06% and 36.55 ± 0.01% shown by *Micrococcus sp*. [1]. One of the studies deals with the removal of Pb with various heavy metals by *Bacillus firmus*, various parameters checked during the study were the initial concentration of metal ions, the concentration of polysaccharides, the pH of the solution based on the optimal pH parameter 98.3% of the maximum amount of Pb was removed by Bacillus [58]. During the textile industry dye effluent study, various strains of bacteria such as *B. licheniformis*, *P. fluorescens*, *E. coli*, and *S. typhi* were isolated and studied on three heavy metals such as Cadmium, Lead and zinc in which *Pseudomonas fluorescens* removed the maximum lead 94.32%, 92.15% by *Salmonella typhi*, 89.45% by *Bacillus licheniformis* and 86.73% by *E. coli* [59]. The degradation capacity efficacy in percentage shown in (Table 2).

Table 2 Heavy metals degradation efficacy of various bacteria

Type of the Microorganisms	Name of microorganisms	Heavy metal	Biosorption efficiency	References
Bacteria	*Bacillus pumilus, Brevibacterium iodinium, Pseudomonas aeruginosa*, and *Bacillus spp.*	Pb	98%	[54]
	Vibrio fluvialis	Hg	60%	[55]
	Bacillus licheniformis	Hg	73%	[56]
	Klebsiella pneumoniae	Hg	15%	[57]
	Pseudomonas aeruginosa	Hg	25%	[57]
Consortia of organisms	*B. licheniformis* and *C. parapsilosis*	Hg	85%	[56]
	C. parapsilosis and *T. rostrata*		77%	
	B. licheniformis and *T. rostrata*		73%	
	B. licheniformis, C. parapsilosis and *T. rostrata*		88%	
Bacteria	*Gamella sp.*	Pb	55.16 ± 0.06%	[1]
	Micrococcus sp.	Pb	36.55 ± 0.01%	[1]
	Bacillus firmus	Pb	98.3%	[58]
	Pseudomonas fluorescens	Pb	94.32%	[59]
	Salmonella typhi	Pb	92.15%	[59]
Bacteria	*Bacillus licheniformis*	Pb	89.45%	[59]
Bacteria	*E. coli*	Pb	86.73%	[59]
Protozoa	*Tetrahymena rostrata*	Hg	40%	[56]

Bioremediation of mercury and lead by algae

Heavy metals can be removed using algae, which has one of the greatest abilities for the sorption of heavy metals [60]. The possible mechanism for the removal of heavy metals was absorption and storage of heavy metals, which differs from the type, anatomy of algae, to the conditions of the growing medium [62]. The best alternative today is the use of algae for bioremediation purposes, and it has a great capacity to produce biodiesel, on the surface of algae heavy metals can be easily adsorbed, in this study *Chlorella vulgaris* was used for the biosorption of lead (Pb^{2+}) and its removal efficiency was 99.4% [7]. During the studies by researchers, *Anabaena sp*., *Trichodesmium sp*., *Oscillatoria sp*., *Cylindrospermopsis sp*. and *Nostoc sp*. removed the maximum lead. Showed a minimum inhibitory concentration to various heavy metals, but Nostoc sp. removed the maximum lead. Up to 99.6%, therefore, Nostoc can be used in future bioremediation [61]. In another study that confirmed the potential for enhanced sorption capacity, the cross-linking of native biomass of A. nodosum with bis(ethenyl)sulfone involved the incorporation of sulfone groups into the biosorbent material. Cross-linked *Ascophyllum nodosum* demonstrated substantial lead uptake, reaching levels as high as 370 P mg/g [62]. The research suggests that the readily accessible and cost-effective green marine algae *C. fascicularis* can serve as an effective biosorbent material for the removal of Pb (II) from wastewater. Achieving a maximum adsorption capacity of 198.5 mg/g at 298 K and pH 5.0 demonstrates its efficiency in this regard [63]. The objective of this study was to investigate the potential utilization of *Cladophora glomerata* biomass as an economical sorbent for the removal of heavy metal Pb (II) ions from aqueous solutions. Through batch experiments, it was evident that *Cladophora glomerata* exhibited a noteworthy capacity to adsorb heavy metal ions from Pb(II). The maximum biosorption capacity for these ions in *Cladophora glomerata* was determined to be 25.5 mg/g. The findings indicated that the biosorbent's uptake capacity was influenced by both the pH and the initial metal concentration. Furthermore, modifications involving acid/heat positively influenced biosorption yield and metal uptake [64]. *Oscillatoria quadripunctulata* demonstrated an effective capacity to remove lead from both sewage and petrochemical industry effluent, ranging from 32% to 100% [65]. The dried biomass of *Spirogyra hyalina* was used as a biosorbent for the removal of cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As) and cobalt (Co) from aqueous solutions, considering various initial concentrations of the heavy metals and contact times. By utilizing Spirogyra hyalina biomass as a biosorbent, this approach offers a sustainable and environmentally friendly solution for heavy metal removal from aqueous solutions, potentially mitigating the adverse effects of metal contamination on ecosystems and human health. The findings revealed that the highest adsorption of Cd, Hg, and as occurred when the initial concentration of these heavy metals was established at 40 mg/l. In the case of Pb, metal uptake demonstrated an upward trend with increasing initial concentration, showing the lowest uptake at 20 mg/l (q=5.495) within 30 minutes of contact time and the highest at 80 mg/l (q=15.471) after 120 minutes [66-67]. Various researchers use algae to clean up the contaminated environment, as shown in the (Table 3).

Table 3 Heavy metals degradation efficacy of various algae

Algae	Heavy metal	Biosorption efficiency (%) and maximum uptake capacity (mg/g)	Reference
Chlorella vulgaris	Lead	99.4 %	[10]
Nostoc spp.	Lead	99.6 %	[61]
*Ascophyllum nodosum**	Lead	78%, 72%	[62-63]
Cladophora fascicularis	Pb (II)	198.5 mg/g	[63]
*Cladophora glomerata***	Pb	22.5 mg/g	[64]
Oscillatoria quadripunctulata	Pb	32-100%	[65]
Sargassum natans, Sargassum vulgare	Pb	214 mg/g	[62]
Spirogyra hyaline	Hg, Pb	Hg, (40 mg/g); Pb (80 mg/g)	[67]

*Algal species cross-linked with other chemicals at pH 4.5 and 3.5
**Biosorption capacity at 4.0 pH

Bioremediation of mercury and lead by plants

Phytoremediation is one of the promising eco-friendly and economical techniques derived from plants that has been used as an alternative for bioremediation for many years [68-69]. Phytoremediation is primarily a technique that uses plant and correlated microorganisms to remediate contaminants to clean up the polluted environment, such as wastewater, groundwater, and sludge [44]. Almost the whole plant system can accumulate essential and non-essential metals that play no significant role [70]. A wide variety of wild plants can grow in polluted areas that contain heavy metals that accumulate in the roots and shoots. Heavy metal Pb accumulates in the roots of Brassica, which is transported to the shoot; therefore, Brassica is used to decontaminate the environment, called phytoextraction [71]. Various plants grown in industrial areas can accumulate heavy metals; this study was effectively carried out on Pb, the plants that accumulate Pb are *E. cheiradenia*, *Scariola orientals*, *Centaurea virgata*, *Gundelia tournefortii* and *Eleagnum angustifolia* [72]. In one of the studies, the *X. pensylvanicum* plant was used for the removal of various heavy metals, but the strong activity shown at pH 4 and below after 90 min of shaking time showed that the maximum heavy metal removed was Pb [73]. The more research on bioremediation of mercury and lead by plants shown in (Table 4).

Table 4 Bioremediation of mercury and lead by plants

Plant species	Heavy metal	Reference
Brassica juncea	Pb	[71]
Euphorbia cheiradenia, Scariola orientals, Centaurea virgata, Gundelia tournefortii and *Eleagnum angustifolia*	Pb	[72]
Xanthium pensylvanicum	Pb	[73]
Thlaspi caerulescens, Alyssum lesbiacum	Pb	[74]
Jatropha curcas	Pb	[75]
Populus deltoides, Populus nigra, Populus trichocarpa	Pb	[76]
Populus deltoids	Hg	[77]
Trifolium alexandrinum	Pb	[78]
Zea mays	Pb	[79]
Zea mays, Ambrosia artemisiifolia	Pb	[80]

Bioremediation of mercury and lead by fungi

With the wide variety of microorganisms implemented in the biosorption of various heavy metals, fungi have also shown bioaccumulation and biosorption activity in an eco-friendly manner, and today fungi are used as the best alternative to bacteria [81]. Fungi appear to be hyperactive accumulators, as fungi are generally accompanied in areas rich in heavy metals [82]. For the reduction of heavy metal pollution, fungi are one of the best organisms used to monitor studies that can be directly exposed to heavy metals in the environment [83]. The individual and combined effectiveness of microorganisms in the processing of mercury was assessed. When used separately, *Candida parapsilosis* demonstrated the ability to remove 80% of mercury [56]. The research investigated the biosorption of Hg (2+) by Saccharomyces cerevisiae (SC). The experimental findings indicate that SC serves as an effective biosorbent for Hg~(2+). Under conditions where pH is set at 3 and temperature is 15 ° C, using 40g / L biomass of SC as a biosorbent for a 0.5g/L Hg (2+) solution of 0.5g / L of Hg (2+) results in a remarkable 96% removal rate of Hg (2+). The adsorption process is rapid and equilibrium is reached in 15 minutes [84]. A bioremediation method utilizing *Aspergillus niger* was devised to generate mild organic acids, facilitating the leaching of heavy metals from polluted soils. The fungus was cultured on the surfaces of three distinct contaminated soils (clay loam, loam, and sandy clay loam) for 15 days at a temperature of 30 ° C and a pH below 4. In the case of clay loam, the leaching process resulted in a reduction of 85% in mercury levels [85]. In this study, we isolated Aspergillus terreus, a fungal strain with demonstrated tolerance, from the enriched effluent. Subsequent individual testing of these isolates revealed significant biosorption capabilities for metal ions. In particular, the fungal isolate exhibited a remarkable 97.13% biosorption of Pb after 144 hours, underscoring its promising potential for bioremediation [86]. Similarly to the investigation, previous research has documented the biosorption capacity for Pb2 + of the live biomass of *Mucor rouxii* treated with NaOH, revealing a capacity of 36.69 mg/g [87]. The soil in the troughs is regulated to a pH of 5.5. The study reveals varying percentages of Pd2+ removal, ranging from 67% to 82%. Specifically, the removal efficiency reached 82% for 5 ppm, 76% for 10 ppm, 73% for 25 ppm, 67% for 50 ppm and 67% for 100 ppm concentrations [88]. Bioremediation of Pb by *Saprolegnia delica* was influenced by temperature, with optimal performance observed at 20°C, resulting in the elimination of 90% of the initial Pb concentration. As the temperature increased, the bio removal efficiency of *Saprolegnia delica* for Pb followed this order: 25°C > 30°C > 35°C (with removal percentages of 82.5%, 68.8% and 24.6% of the total Pb concentration, respectively). The least bio removal of Pb by *Saprolegnia delica* occurred at 15°C, where only 18.8% of the initial Pb was sequestered [89]. Heavy metals degradation efficacy of various fungi with there percent degradation efficacy was shown in (Table 5).

Table 5 Heavy metals degradation efficacy of various fungi

Name of the microorganisms	Bio remediator	Heavy metal	Biosorption efficiency (%) and maximum uptake capacity (mg/g)	References
Fungi	*Candida parapsilosis*	Hg	80%	[56]
Fungi	*Saccharomyces cerevisiae*	Hg	55.76 (mg/g)	[84]
Fungi	*Aspergillus niger*	Pb	85%	[85]
Fungi	*Aspergillus terrus*	Pb	97.13%	[86]
Fungi	*Mucor rouxii*	Pb	36.69 mg/g	[87]
Fungi	*Saccharomyces cerevisiae*	Pb	67-82%	[88]
Fungi	*Trichoderma viridae, Saprolegnia delica*	Pb (II)	100% and 90%	[89]

CONCLUSION

This review article mainly focuses on the bioremediation of heavy metals lead and mercury. The current state of heavy metal bioremediation, examined in this study, offers great potential for metal detoxification. The cell wall of the biosorbents contains peptidoglycans and polysaccharides that act as active binding sites for increased metal uptake. Faster kinetics, high metal binding over a wide pH range, and temperature are just a few benefits of this technique, which is also environmentally friendly and cost-effective. This review offers a chance to discuss how microbial cells and their metabolites contribute to heavy metal remediation and environmental research. These would make it easier to create more effective methods for heavy metal bioremediation in the environment.

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