

Application of Microorganisms for Bioremediation of Heavy Metals

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Abstract

As a result of rapid industrialization, heavy metal pollution has become a major global concern. Toxicological manifestations occur from the accumulation of heavy metals as a result of anthropogenic activities. When heavy metals enter the food chain at somewhat greater concentrations, they have a detrimental effect on human health. Ion exchange, coagulation, precipitation, reverse osmosis, nanofiltration, ultra-filtration, and other traditional procedures for heavy metal cleanup are employed. Because of the high energy and reagent needs, these approaches are effective yet costly. They also produce a lot of hazardous sludge and byproducts, which contaminate the environment. As a result, it is essential to formulate effective, efficient, cost-effective, and ecologically acceptable ways for reducing heavy metal ion concentrations in the environment from harmful to safe levels. Bioremediation is a technique for cleaning up contaminated environments by using microorganisms or enzymes to convert hazardous heavy metals into less dangerous forms. Bioremediation is a 'green' approach for removing heavy metals from the environment without releasing hazardous byproducts or metabolites. Their short generation time, large surface area and ease of genetic manipulation make them ideal candidates for the bioremediation process. Bioremediation techniques' effectiveness is determined by a variety of factors, both biotic and abiotic, which primarily determine the bioavailability of metal for remediation. The tapering metal ion at various loci or sites is caused by various metal microbe interactions such as sorption, accumulation, mineralization, and transformation. This review focuses on the origins of heavy metals, their toxicological manifestations, and heavy metal bioremediation employing microorganisms.

Keywords: Heavy Metal; Microorganism; Bioremediation; Biomineralization; Biosorption

Introduction

Metal bioavailability to endemic populations has increased as a result of modern globalization and industrialization, influencing the variety and composition of endemic populations in diverse agricultural fields and drinkable water sources. Heavy metals' persistence causes health concerns; a typical example is mercury poisoning in Minamata, Japan [14]. For heavy metal cleaning, ion exchange, coagulation, precipitation, reverse osmosis, nanofiltration, ultra filtration, and other traditional methods are used. These methods are effective but expensive due to the high energy and reagent requirements. They also generate a

significant amount of harmful sludge and pollutants, which pollute the environment. The use of microorganisms in bioremediation is an effective, efficient, and environmentally acceptable way for removing heavy metal. Indigenous microorganisms are often used in polluted site remediation, whereas site treatment necessitates excavation of contaminated soil or water from the site, followed by treatment at a different location, perhaps using genetically modified bacteria. Many metals contaminated places can be remedied using the unique traits of genetically designed organisms as well as indigenous species [4]. This review provides an overview of the occurrence of heavy metals and their toxicity, as well as microbial tolerance responses and various bioremediation strategies.

Heavy metals: Occurrence

Metals or metalloids with an atomic density more than 5 g/cm³ are classified as heavy metal [4]. The impact of these metals on the environment and human health is referred to as “toxic metal.” Heavy metals are naturally found in the environment as a result of biogeochemical cycles, rock weathering, ore leaching, coal mining, or volcanic eruptions. Metals are harmful and long-lasting pollutants that are discharged into the environment as a result of human activities such as industrial, mining, and agricultural operations (repeated application of fertilizers and pesticides), and they cannot be degraded or removed. (See Figure 1). When present at low concentrations, heavy metals such as copper, zinc, and nickel are required for life and play a important role in metabolic processes in living organisms; they are known as microelements or trace elements. Maximum prokaryotic and eukaryotic organisms are hazardous to elevated doses of these metal ions. Mercury, cadmium, and lead, among other non-essential heavy metals, caused catastrophic harm to organisms even at extremely low quantities. They enter the body system through food, air, and water to a minor level, bio-accumulate over time, and create human health issues [6]. The three classes of heavy metals classified the metals as toxic metals (such as Zn, Ni, Cu, Hg, Cr, Pb, Cd, As, Co, Sn etc.), precious metals (such as Au, Pd, Pt, Ag etc.), and radionuclides (such as Th, U, Am, Ra etc.). Heavy metals are stable in nature, but their persistence in the environment causes pollution, and their bioaccumulation or biomagnification in the food chain poses a severe health and environmental concern. The sources of heavy metals permitted limits, and harmful effects of various heavy

metals on living forms are depicted in table 1 [20]. Figure 1 schematic flow chart shows sources of heavy metal contamination and biomagnifications of heavy metals (image created by PP in MS Office 365 ProPlus, PowerPoint).

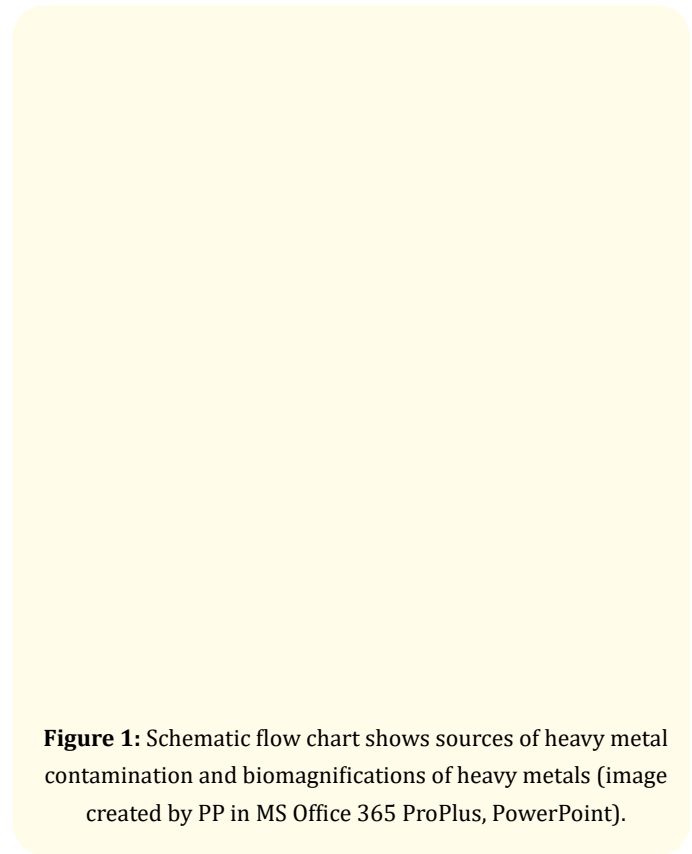


Figure 1: Schematic flow chart shows sources of heavy metal contamination and biomagnifications of heavy metals (image created by PP in MS Office 365 ProPlus, PowerPoint).

Heavy metal	Source	Toxic effect on prokaryotes	Toxic effect on eukaryotes	Accepted range in drinking water (ppb)	Accepted range in soil (mg/kg)	References
Lead	Emission from industry and combustion of blended fuels, Coal burning, Manufacture of batteries and lead based paints	Destroys nucleic acid and proteins Inhibit enzyme activity and transcription	Anemia Liver injuries Low sperm count Miscarriage Loss of memory Nausea Inhibit seed germination, chlorophyll production Inhibition of Calvin cycle enzymes	15	400	[4,18]

Cadmium	Phosphate fertilizer Detergent Refined petroleum Products By product of Zn and Pb refining	Denature protein, Destroy nucleic acid, Hinder cell division and transcription	Fibrosis Emphysema Vitamin-D deficiency Lung cancer Chlorosis Leaf rolls	5	20	[4,18]
Nickel	Metal plating industries Combustion of fossil fuels Nickel mining Electroplating Power plants	Destroy cell membrane Hinder enzyme activities and oxidative stress	Pulmonary fibrosis Renal edema Dermatitis	2,00,000	35	[4,18]
Copper	Electroplating industry Mining Biosolids Smelting Refining	Denature protein, Destroy nucleic acid, Decreased viability, Inhibition of respiration	Abdominal pain anemia Diarrhea Headache Liver and kidney damage Metabolic disorders Nausea and vomiting	1300	2-60	[31]
Cobalt	Coal combustion Fertilizers Mining of lead, iron and silver	Inactivates iron sulfur enzymes	Nausea Deafness Vomiting Nerve problems	>1-2	50	[4,18]
Zinc	Coal and waste combustion Mining Steel processing	Death Decrease in biomass Inhibits growth	Abdominal pain Nausea Diarrhea Anemia Metal fume fever	5000	2-25	[4]
Mercury	Volcano Forest fire Fossil fuels Mining and pulp industries Waste incineration	Denature protein, Inhibits enzyme function, Disrupt cell membrane	Memory loss Insomnia Kidney failure Neuro muscular effect	2	72	[4]

Table 1: Toxicity of various heavy metals and their effect on life forms.

Conventional methods

Physical and chemical methods for removing heavy metals are commonly used, but instead of completely destroying them, they only transform them from toxic to less toxic forms. Ion exchange, reverse osmosis, electrochemical treatment, electro dialysis, ultra filtration, solvent extraction, and chemical precipitation are some of the common methods for removing heavy metals from the environment. These processes are costly because they take a lot of energy and reagents, and they produce a lot of toxic sludge and byproducts, which harm the environment [9].

Metal-microbe interaction

Biosorption

Biosorption is one of the bioremediation methods. Biosorption is a passive method that does not include microbial metabolism [9]. Both dead and living biomass can be employed in biosorption. Even in much diluted metal solutions, this approach can be applied [33]. Gram-positive bacteria have multiple layers of peptidoglycan, which include teichoic acid, amino acids (alanine and glutamate), and meso-diaminopimelic acids, whereas gram-negative bacteria only have one layer [21]. This layer contains enzymes, glycoprotein, lipopolysaccharides, and phospholipids, which act as ligands and provide active sites for metal binding [7]. In the cell wall, complex

carbohydrates, lipids, nucleic acids, and proteins combine to produce an additional polymer material (EPS). EPS has a strong metal-binding ability to complex heavy metals, preventing metal access into the microbial intracellular environment. As a result, they shield bacteria from metal toxicity [30].

Bioaccumulation

The process of bioaccumulation is a metabolically active one [9]. Heavy metal ions are transferred through the lipid bilayer into the cytoplasm or intracellular regions by transporter proteins in bioaccumulation [30]. There are two stages to the bioaccumulation process. Metal ions are adsorbed onto cells in the first stage, which is fast and similar to biosorption. The second stage is more time-consuming and involves active transport of metal species within cells [16]. Metal ion sequestration is supported by metal-binding entities such as proteins and peptide ligands [30]. This metal can be found in particulate form, insoluble forms, and by products after sequestration [19,22]. Endocytosis, ion channels, carrier-mediated transport, complex permeation, and lipid permeation are just a few of the mechanisms that allow heavy metals to accumulate in the bacterial membrane [8].

Biotransformation

The structure of a chemical compound is changed during biotransformation, resulting in the production of a molecule with a higher polarity [2]. As a result of this metal-microbe interaction, metal and organic molecules are changed from harmful to less toxic ones. Microorganisms have used a variety of enzymatic modification methods to change metals and reduce their toxicity, including oxidation, reduction, alkylation, and methylation [32]. Microbes have several characteristics that make them excellent for biotransformation, such as a high surface-to-volume ratio, rapid growth, high metabolic activity, and the ability to maintain sterility. Microbial transformation is used to biotransform a variety of contaminants, including hydrocarbons, pharmaceuticals, and metals [17]. The mobility of heavy metal may be reduced indirectly through the formation of biosurfactants, pigments, or siderophores.

Bioleaching

Microbes such as bacteria and fungus are used in bioleaching. They're found in nature and help to dissolve metal sulphides and oxides from ores and secondary wastes [30]. In bioleaching, there are primarily two mechanisms. There are two types of bioleaching: contact and non-contact. Microbes and mineral sulphide make physical contact in the contact mechanism. Some processes are

catalysed by enzymes and result in electron transfer from the mineral surface, resulting in sulphide oxidation to sulphate [29]. There is no physical contact between the mineral surface and the microorganisms in non-contact methods. There is a secretion of low molecular weight organic acids that are the outcome of microbial metabolism in non-contact bioleaching. Heavy metals and soil particles containing heavy metal minerals can be dissolved by these organic acids [15]. Solubilised metal can be purified via adsorption, ion exchange, membrane separation, and selective precipitation processes.

Biomining

Biomining is the process by which living bacteria convert aqueous metal ions into crystalline precipitates. Metals are removed from the solution as a result of this reaction, providing a way of detoxification as well as bio recovery [24]. Mineral formation is aided by the presence of a cell wall and additional organic layers (EPS and S-layer), which are highly variable and reactive interface with varied hydrocarbon composition and structure [30]. Organic ligands such as amine, carboxyl, hydroxyl, phosphoryl, and sulphur deprotonate as pH rises, leaving the microbial surface with a net negative charge. Because our hazardous metals have positive charges, they precipitate into more solid and compact mineral forms in a consistent manner [23,35]. Microbes precipitate biomining such as oxides, sulphides, oxalate, sulphates, and phosphates [24].

Microorganisms- the key players in bioremediation

Bioremediation techniques that use microorganisms are divided into bioaccumulation, biosorption, biotransformation, and biomining, whereas phytoremediation techniques use plants and plant components in a metal-containing environment [27]. Biosorption is a beneficial technology that plays a significant role in the elimination of heavy metals. Biosorption is an emerging technology and a passive absorption technique involving adsorption on the cell surfaces of biological materials that are mainly reversible and metabolism-independent. Heavy metals accumulate inside cellular components through a complex process known as bioaccumulation. Microbial biosorption has several advantages, including a low operating cost, a simple, highly effective, and ecologically acceptable approach [11,27]. Heavy metals including Cu, Zn, Ni, Cr, Co, Mo, Fe, and Mn are necessary micronutrients for microbes, plants, and animals [11]. Metals, on the other hand, had a variety of physiological, biochemical, and genotoxic impacts on all types of living things when their concentrations were beyond their

threshold levels. Heavy metal concentrations have a significant impact on microbial communities in two ways: (i) metabolic function suppression and (ii) genetic material regulation [12].

Due to their metal sequestering capabilities, algae, bacteria, fungi, and yeast have proven to be potential metal bio-sorbents. Metal removal using intact microbial cells and cell-bound EPS has been successful in both ambient and industrial wastewater sources [9,26]. The use of metal-resistant strains in a single, consortium, and immobilized form for heavy metal removal has achieved promising benefits. The biosorption capability of any bio-sorbent, on the other hand, is determined by its prehistory, pretreatment, and experimental settings. The bio-sorbent should be inexpensive, efficient, and simple to cultivate and harvest. To improve biosorption, the organism should be adaptable to changes in bioreactor configuration, as well as physical and chemical conditions [10].

Bioremediation by bacteria

Bacteria are the most common bacteria on the planet, and they can survive in a variety of environments. Bacteria have been frequently utilized to clean up heavy metal contaminants from the environment due to their advantages such as small size, rapid growth rate, and ease of cultivation. Through functional groups like carboxyl, amino, phosphate, and sulphate, heavy metal ions can be adsorbed on bacteria's polysaccharide slime layers. Heavy metal ions can attach to these groups and accumulate in significant amounts. Bacterial absorption capabilities for heavy metal ions typically range from 1 mg/g to 500 mg/g [34].

Bacillus sp. has been recognized as having a high potential for metal sequestration and has been exploited in the production of commercial biosorbents. Using *Bacillus subtilis* and *Bacillus megaterium*, Above and his colleagues examined the biosorption of lead (Pb), chromium (Cr), and cadmium (Cd) in tannery effluent [1,22]. *Staphylococcus saprophyticus* removes chromium (Cr), lead (Pb), and copper (Cu) ions from industrial wastewaters at optimal pH values of 2.0, 4.5, and 3.5, respectively. A large amount of lead (Pb) was removed from a synthetic medium using *Micrococcus luteus*. Bacteria thrive in mixed cultures because they are more stable and survive longer. As a result, consortia of cultures are metabolically superior for metal biosorption and are more suited for field use [34]. De Jaysankar et al. used a bacterium consortium of *Acinetobacter sp.* and *Arthrobacter sp.* to reduce chromium (Cr)

by 78 % utilizing a 16 mg/L metal ion concentration [5]. As a type of mercury-resistant strain, *Pseudomonas aeruginosa* can selectively adsorb mercury ions with a maximal absorption capability of about 180 mg/g. These mercury ions are accumulated by cysteine-rich transport proteins, which have a high affinity for mercury ions and are enriched in sulfhydryl groups. In an aqueous solution, both dead and living *Arthrobacter viscosus* biomass can convert Cr(VI) to Cr(III). When Cr(VI) concentrations are less than 100 mg/L, 100 % Cr(VI) can be removed from the aqueous solution by using an acidic condition (pH 2). From a 100 mg/L Cu(II) containing solution, *Eichhornia spp.* biomass obtained from Chandola Lake can remove 85.0 % copper (Cu). The bacteria *Rhodobacter capsulatus* can absorb Zn(II) with a maximum absorption capability of 164 mg/g. The bacteria *Bacillus cereus* RC-1 has Cd(II) biosorption capacity in both living and dead cells, with living cells having 24.01 mg/g and dead cells having 31.95 mg/g biosorption capacity [34].

Metal-binding is mostly accomplished by the microbial cell wall. Electrostatic forces enable microbial surfaces to bind metal cations due to their anionic nature. Peptidoglycan, teichoic, and teichuronic acids make up the thicker cell wall of Gram-positive bacteria. Teichoic and teichuronic acids are missing in Gram-negative bacteria, and the peptidoglycan layer is thin. Gram-positive bacteria are more efficient in trapping metal ions than Gram-negative bacteria [11]. When the pH is raised, the overall negative charge on the cell's surface grows until the functional groups are deprotonated, which aids electrochemical attraction and, eventually, metal ion absorption [24]. Extracellular polymeric substances (EPS) produce by bacteria, which are made up of nucleic acids, proteins, lipids, and complex polysaccharides, also play a role in heavy metal ion adsorption. By preventing heavy metals from entering the intracellular environment, the EPS on the microbial cell surface can protect microorganisms against toxicity [13]. Heavy metal ions such as mercury (Hg), cobalt (Co), copper (Cu), and cadmium (Cu) can be readily accumulated by the presence of cationic and anionic functional groups on EPS [24]. Heavy metal ions that have been adsorbed can be transferred into living bacterial cells in a metabolism-dependent manner, and their redox state can be modified to lessen their toxicity. The ability of reductase from living bacteria to change the physicochemical circumstances of heavy metal ions after binding by functional groups can decrease their toxicity, which will improve their ability to remediate heavy metal pollutions [11].

Bioremediation by fungi

Fungi can be developed easily, produce a high yield of biomass, and can easily be manipulated genetically as well as morphologically. Fungi show high resistance to a large number of heavy metals and simultaneously can accumulate micronutrients (Cu, Zn, Ni, Co, and Mn) and non-nutrient metals (Cd, Pb, Hg, and Ag). Fungi have exhibited a high take up of heavy metals and therefore they found a broader application to adsorb these metals [13,24]. Through ion exchange and coordination, the chitin-chitosan complex, glucuronic acid, polysaccharides, polyphosphates, proteins, lipids, and inorganic ions in fungal cells play a major role in heavy metal adsorption [11,28]. The adsorption capability and specificity of fungus strains to a heavy metal ion are influenced by different types of ionizable sites and diverse functional groups such as amine, carboxyl, hydroxyl, phosphate, and sulfhydryl groups. With significant biosorption capacities, *Aspergillus niger* has been deemed a potential bio-sorbent for removing Pb(II). The bio-removal of Cr(VI) from mine drainage by a native fungal isolate *Aspergillus fumigatus* has been investigated. The functional groups on the acidic surface of *Termitomyces clypeatus* biomass can absorb Cr(VI). Cu(II) removal from wastewater was examined using unmodified yeast cell biomass of *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* can remove copper (Cu), zinc (Zn), and cadmium (Cd) pollutants in a high-salt environment, and sodium chloride can boost the adsorption ability [13,34].

Algae mediated bioremediation

Algae can grow in both freshwater and the ocean as a photosynthetic organism. Algae are autotrophic, requiring few nutrients and producing a considerable amount of biomass when compared to other microbial biosorbents. They have a high sorption capacity and can be found in considerable quantities [3]. Metal ion binding sites may exist in certain polysaccharides found in algal cell walls. Algae cell walls contain polysaccharides that contain

amide, carboxyl, hydroxyl, and phosphate groups, which function as metal-binding sites, as nitrogen and oxygen atoms capable of forming covalent bonds with metal ions [24]. The accumulation of large amounts of heavy metals by distinct algae species is both reliant and independent of their metabolism. The most promising seaweeds for biosorption are brown and red algae [11].

In comparison to fungi and bacteria, algae have not been extensively studied as a bio-sorbent. Algal bioaccumulation is aided by a variety of metabolic mechanisms. Biosorption is a surface phenomenon that mainly involves cell surface sequestration. As a result, altering the algal cell wall could have a significant impact on metal ion binding. To improve the metal binding capacity of biomass and understand the intrinsic mechanism, many approaches for modifying cell walls in algae have been used [11]. Dead *Chlorella vulgaris* cells were employed to remove cadmium (Cd), copper (Cu), and lead (Pb) ions from aqueous solution under varied pH, bio-sorbent dosage, and contact time conditions. The results revealed that the biomass of *C. vulgaris* is an exceptionally efficient bio-sorbent for the removal of cadmium (Cd), copper (Cu), and lead (Pb) from a mixed solution containing 50 mg/L of each metal ion at 95.5 %, 97.7%, and 99.4%, respectively [10]. *Cladophora fascicularis* has a maximal adsorption capacity to Pb(II) of 198.5 mg/g at pH 5.0. *Sargassum sp.*, a free and immobilized marine algae, has been demonstrated to have a high ability to remove Cu(II) from aqueous solutions. With a very high adsorption capacity of 160 mg/g at pH 4.5, *Cystoseira crinitophylla* has been examined for Cu(II) biosorption. Cu(II), Cd(II), and Zn(II) removal capabilities in aqueous solutions have been examined in macroalgae *Saccharina japonica* and *Sargassum fusiforme*. *Desmodesmus sp.*, a green microalga, has been used to bioremediate Cu(II) and Ni(II) from wastewater at a high capacity [34]. Metal-binding phytochelatins and polyphosphate bodies are produced by macroalgae and are responsible for their high metal tolerance or metal ion sequestration in storage vacuoles [24,27].

Microbial group	Biosorbents	Target metals	Adsorption capacity (mg/g)	Reference
Bacteria	<i>Pseudomonas aeruginosa</i>	Hg(II)	180	[34]
	<i>Bacillus cereus</i>	Cd(II)	31.9	[34]
	<i>Staphylococcus epidermidis</i>	Cr(VI)	56	[34]
	<i>Bacillus licheniformis</i>	Cr(VI)	62	[11]
	<i>Enterobacter sp.</i>	Cd(II)	46.2	[11]
	<i>Bacillus coagulans, Bacillus megaterium</i>	Cr(VI)	39.9	[11]
	<i>Bacillus firmus</i>	Pb(II), Cu(II), Zn(II)	467, 381, 418	[11]
	<i>Enterobacter cloacae</i>	Pb(II)	2.3	[11]
	<i>Micrococcus luteus</i>	Cu(II), Pb(II)	408, 1965	[11]
	<i>Arthrobacter viscosus</i>	Cr(VI)	1161	[34]

Fungi	<i>Aspergillus niger</i>	Pb(II), Cd(II)	34.4, 11	[11,34]
	<i>Lepiota hystrix</i>	Pb(II), Cu(II)	3.89, 8.50	[11]
	<i>Saccharomyces cerevisiae</i>	Cr(VI), Cu(II)	6.6, 16	[34]
	<i>Mucar rouxii</i>	Ni(II)	0.36	[11]
	<i>Trichoderma</i>	Cd(II)	21.7	[34]
	<i>Penicillium simplicissimum</i>	Pb(II), Co(II)	76.9, 54.6	[34]
Algae	<i>Spirogyra sp.</i>	Cr(VI), Cu(II)	133.30, 49	[11]
	<i>Chlorella sorokiniana</i>	Cd(II)	13.33	[11]
	<i>Chlorella vulgaris</i>	U	14.3	[34]
	<i>Dunaliella sp.</i>	Cr(VI)	58.3	[11]
	<i>Cladophora fascicularis</i>	Pb(II)	198.5	[34]
	<i>Ulva lactuca</i>	Cu(II), Cd(II), Cr(III), Pb(II)	64.5, 62.5, 60.9, 68.9	[11,34]

Table 2: Heavy metal removal by living microorganisms.

Conclusion

The current paper examined the industrial sources of heavy metals, their negative consequences on human health, and the many strategies used to degrade these heavy metals utilising various types of microorganisms. Microorganisms are everywhere; they make up a huge portion of the planet’s living material and play a critical role in ecosystem maintenance. It has demonstrated the use of bioremediation as a superior alternative to conventional procedures for the removal of heavy metals from contaminated sites, which are less effective and expensive due to the quantity of energy required. Biosorption, biotransformation, and bioaccumulation are some of the different pathways for heavy metal breakdown employing microbes, with biosorption being the most common. Some bacteria are resistant to heavy metals and can be utilized to remove them effectively, as they can employ both live cells and the metabolites they create. Large scope for genetic engineering to improve the efficiency of organisms, as well as the combination of all three physical, chemical, and biological approaches, offers up a larger window for heavy metal pollution remediation.

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Conflicts of Interest

We declare that here are no conflicts of interest.

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