

Review

Effects and Mechanisms of Microbial Remediation of Heavy Metals in Soil: A Critical Review

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Abstract: The use of microbes to change the concentration of heavy metals in soil and improve the ability of plants to deal with elevated metals concentrations has significant economic and ecological benefits. This paper reviews the origins and toxic effects of heavy metal pollution in soil, and describes the heavy metal accumulation mechanisms of microbes, and compares their different bioconcentration abilities. Biosorption, which depends on the special structure of the cell wall, is found to be the primary mechanism. Furthermore, *Escherichia coli* are found to adsorb more heavy metals than other species. Factors influencing microbial treatment of wastewater and soil containing heavy metals include temperature, pH, and different substrates. Finally, problems in the application of microbial treatment of heavy metal contamination are considered, and possible directions for future research are discussed.

Keywords: microbe; soil; heavy metal; contamination; remediation

1. Introduction

Soil heavy metal pollution mainly refers to the deposition of heavy metals, such as mercury, cadmium, lead, chromium, and other bio-toxic significant heavy elements in the soil [1], resulting in concentrations that exceed background values. Metals are not biodegradable, and through biological amplification, their concentrations can be increased thousands of times, with significant effects on human health [2]. In recent years, discharges of large volumes of heavy metals from industrial activity and mining, with final deposition in the soil, have led to increases in soil heavy metal concentrations. Widespread use of pesticides and fertilizers may also have led to an increase in soil heavy metal concentrations [3].

The management of heavy metal pollution in soil relies on two processes: (1) Traditional chemical and chemical restoration methods based on curing and leaching and (2) ecological restoration methods based on adsorption and transfer [4]. Traditional chemical methods usually involve direct reactions between chemical reagents and heavy metal ions, without any other promotion method, such as chelation and redox, while chemical restoration methods are often promoted by other methods, such as electrochemical repair [5]. However, according to Gazso [6], traditional methods are often expensive, complicated, frequently cause secondary pollution, and significantly alter the soil structure, among other limitations and deficiencies. Recently, ecological restoration has become more widely used because of its lower cost and measurable ecological, social, and economic benefits. Traditional ecological restoration is generally phytoremediation, i.e., the use of hyperaccumulators to absorb heavy metals from contaminated soils [7]. Ranieri et al. [8] found that, for two plant species,

Phragmites australis and *Ailanthus altissima*, total removal of Cr^{3+} from water ranged from 55 to 61%. However, the use of microbial remediation has become more common, and it is generally considered promising owing to its many advantages [9], including retention of soil structure, and the fact that the pollutants and microbes can be almost completely removed from the soils, and secondary pollution can be avoided [10]. Microbial remediation presents new techniques for addressing the problem of heavy metal pollution in soil, and it has become a focus of new research and development in bioremediation technology. This paper reviews the sources and hazards of heavy metals in soil, and discusses the techniques and influencing factors for microbial remediation, providing a useful reference for the restoration of soil ecosystem health.

2. Sources and Hazards of Heavy Metal Pollution in Soil

According to Jä Rup [11], in the past several years, both the production of and emissions from heavy metals have increased. Heavy metal compounds are often used in color pigments, batteries, fertilizers, or other industrial products. Eventually, these metal elements enter the atmosphere through evaporation or the soil as sediment, and valence transformation occurs. Then, these deposited elements are bioabsorbed into the biosphere.

The impact of heavy metals in the soil manifests in several ways. First, the heavy metal content affects the respiration and metabolism of microorganisms (metabolic entropy response) [12] and the activity of microorganisms, thereby affecting soil respiration. The microbial metabolic entropy of soil heavily polluted by metals is higher, and the organic carbon content converted to bio-carbon is reduced. Finally, heavy metals can lead to physiological dysfunction and malnutrition in plants as metal contamination can be transferred to plant seeds. Metals can also accumulate in the human body, causing great and irreversible harm to human health. Introduction of Cd^{2+} into the body causes bone pain and brittle bones, and Pb^{2+} pollution can seriously endanger fertility [13].

3. Remediation of Heavy Metal Contaminated Soil

Traditional methods of remediating heavy metal contamination in soils include engineering repair and physical and chemical restoration [14]. These methods generally involve high energy consumption and high cost. The cost of vegetative remediation is low, but this method requires a long repair cycle. Besides, it is not always applicable for the toxicity of the heavy metals. The percentage of the extraction could be changed from the concentration in the soil. For high concentration the plant can be dead. Soil washing and soil flushing are available in the remediation process. Soil washing involves three main methods: physical separation, chemical separation, and integrated processes. Among these processes, both chemical and physical separation have limitations: Physical separation is primarily applicable to particulate forms of metal, and the removal rates of chemical separation are highly dependent on soil geochemistry [14]. Compared with other methods, soil washing is permanent and relatively thorough. However, there are several disadvantages of soil washing, including a need to excavate contaminated soil. In these cases, soil flushing can be an alternative method. Soil flushing involves the direct injection of a leaching solution into the soil, which avoids the need for excavation [15]. Both soil washing and soil flushing have their advantages, the metals obtained from soil can be recycled and reused again [15,16]. Thus, we prefer this method. Both single chemical remediation and microbial remediation have advantages and deficiencies (Table 1). Chemical remediation is not environmentally friendly but is thorough and relatively easy to carry out, while microbial remediation is environmentally friendly but complicated to implement. It is possible to use microbial remediation to improve the soil quality after chemical treatment. Trellu et al. [17] found that soil washing and soil flushing processes using biosurfactants showed promising results.

4. Microbial Remediation of Heavy Metal-Contaminated Soil

4.1. Remediation Mechanisms

4.1.1. Biosorption

Microbes can accumulate heavy metals by either adsorption or absorption, which are two main ways to increase metal ions in soil [18]. The process of adsorption differs from absorption, in that a fluid (the absorbate) is dissolved by or permeates a liquid or solid (the absorbent) [19]. Thus, adsorption is a surface phenomenon, while absorption involves the entire volume of material. Table 2 describes the overall sorption mechanisms, including precipitation, chemical adsorption and ion exchange, surface precipitation, the formation of stable complexes with organic ligands, and redox reaction. However, due to the limitations of current analytical techniques and complexity of soil matrix, the exact immobilization mechanisms have not been clarified [20]. Adsorption involves complexation of heavy metals on the cell surface, from which they can be absorbed into the cell [21]. Because of the cell surface structure—principally the cell wall and mucus layer—heavy metals can be adsorbed and absorbed relatively easily. Many ions in the cell surface functional groups, such as nitrogen, oxygen, sulfur, and phosphorus (Figure 1, [22]), can be complexed with metal ions as coordination atoms. In addition, phosphoric acid anions and carboxyl anionic groups on the surface of the microbial cell wall are negatively charged, and most heavy metal surfaces carry a cationic group that interacts with the cell wall and allows the metal ions to bind or pass through the cell membrane (Figure 1, [23]).

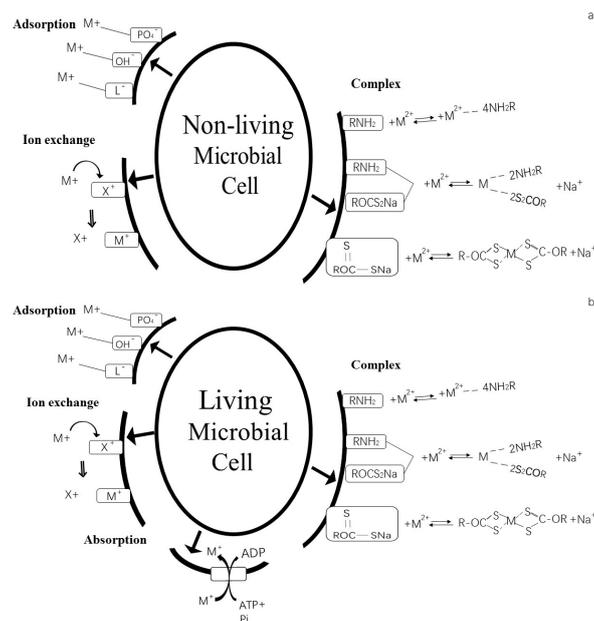


Figure 1. Biosorption mechanisms of microorganisms.

Wang et al. [24] showed that the primary mechanism by which microbes accumulate heavy metal ions is adsorption, which normally does not depend on energy metabolism, rather than absorption, which depends on energy metabolism and almost exclusively occurs in living cells. Generally, microbes adsorb large amounts of heavy metal ions rapidly. It has been found that at pH 7.2, *Bacillus* can adsorb 60% of its Cu^{2+} capacity within the first minute and reach adsorption equilibrium within 10 min [25]. In contrast, absorption is time-consuming and relatively inefficient; although, the removal rates can be improved by 26.3 to 31.5% through the addition of agents such as lemon oil or ethylenediaminetetraacetic acid (EDTA), respectively [26]. However, the intake of essential nutrients can reduce the ability of a cell to absorb heavy metal ions.

Table 1. Advantages and disadvantages of different remediation methods.

Type	Method	Application	Advantages (+) and Disadvantages (-)
Soil washing	Physical Separation Chemical Extraction Integrated process	Large area (a large volume of soil)	(+) Thorough and permanent [5] (+) Both soil and metal can be recycled [5] (+) Fine-grained soil may be treated [16] (-) Inconvenient (need large equipment) [16] (-) High cost (use of large equipment and chemical agents) [5] (-) Treatment of soil or sludges rich in metal can be difficult [5]
Soil flushing	Direct injection of a leaching solution	Large area (a large volume of soil)	(+) In situ [15] (+) Less disruption to the environment [27] (+) Reduces worker exposure to hazardous materials [27] (-) Efficiencies depend on other factors (nature of soil contaminants) [27]
Engineering management	Change soil	Small area (a small volume of soil) of heavily polluted soil	(+) Thorough [28] (+) Stable [28] (-) Implementation requires substantial engineering [29] (-) High investment cost [29] (-) Destroys the soil structure, reducing soil fertility [29] (-) Pollution in removed soil is still problematic [30]
Chemical repair	Add chemical modifier	Wide range of applications	(+) In situ [31] (+) Easy [31] (-) Temporary repair measure: The heavy metal remains in soil (i.e., elemental heavy metals are chemically bonded) and is easily re-activated [28]
Phytoremediation	Introduce plant life	Wide range of applications (especially suitable for mining reclamation)	(+) Low cost [32] (+) Protects topsoil [32] (+) Reduces soil erosion [30] (+) Generates less waste [32] (+) Recovers heavy metal [32] (-) Long repair cycle [33] (-) The percentage of the extraction could be changed from the concentration in the soil [32]

Table 1. *Cont.*

Type	Method	Application	Advantages (+) and Disadvantages (-)
Physical chemistry repair	Electro-chemical methods	Low permeability clay and silt soil	(+) Economically feasible [28] (+) Does not stir the soil [22] (+) Shortens repair time [34] (-) Poor conductivity of high permeability sandy soil reduces effectiveness [31]
	Electro-thermal methods	Volatile heavy metals (e.g., Hg)	(+) Efficient [30] (+) Can fundamentally eliminate soil heavy metal pollution [31,34] (-) Easy to destroy organic matter and water in soil [31] (-) Consumes a large amount of energy [31]
	Soil leaching	Small area of severely polluted soil	(-) Can cause some leaching and precipitation of some nutrients [31]

Table 2. Comparison of overall mechanisms of sorption.

Type	Condition	Mechanism	Example
Surface precipitation	Edge charges on adsorbent	Reagent residue enhances metal ion stability in soil solid phase components, reduces migration and bioavailability	<ol style="list-style-type: none"> 1. Pb precipitates in phosphogypsum and red gypsum minerals to form stable lead sulfate minerals [35]. 2. Phosphate formed by phosphate precipitated on the surface of soil plant roots passivated Pb [36].
Ion exchange	Other metal ions existing	The metal ions bound by the cell material are combined by other metal ions with stronger binding ability.	<ol style="list-style-type: none"> 1. Inactive <i>Agrobacterium rhizogenes</i> adsorbs Pb, Cu and releases Ca, Mg [37]. 2. <i>S. cerevisiae</i> adsorbs Ag and releases H [37].
Ligand exchange	There are organic functional groups, such as R-COOH, R-SH	Metal ions and ligands are bonded to the surface of the adsorbent by covalent or ionic bonding	<ol style="list-style-type: none"> 1. Cr is reduced by organic matter or iron reducing substances [37]. 2. <i>Arbuscular mycorrhizal</i> production of polysaccharide substance ligands chelated with heavy metals to form stable complexes [38].
Diffusion and chemical modification of adsorbent surfaces	Manganese oxide Iron oxide Zeolite	Reduction of toxicity of heavy metal ions by chemical transfer between heavy metal ions and chemical modification	<ol style="list-style-type: none"> 1. Cr is reduced by organic matter or iron reducing substances [35]. 2. Sulfate bacteria reduce sulfate to sulfide and combine with heavy metal elements to form a precipitate [39].

Heavy metal ions bind to the surface of the cell not only by electrostatic interaction and complexation but also by ion exchange to the cell surface; for example, the non-living brown algae (*Ascophyllum nodosum*) exchanges the original cell wall adsorption of K^+ , Ca^{2+} and Mg^{2+} to adsorb Co^{2+} (Figure 1, [40]). Brady and Duncan [22] showed that yeast releases approximately 70% of K^+ rapidly and 60% of Mg^{2+} slowly in the process of adsorbing Cu^{2+} . Furthermore, studies have shown that ion exchange can occur with complexation [41,42]. However, other studies have shown that ion exchange is not the main mechanism of microbe remediation, because the amounts of released cations (Ca^{2+} and Mg^{2+}) are always less than those of heavy metal ions [22].

4.1.2. Bioleaching

Biomining is a general term that covers both bioleaching, which involves the mobilization of positive heavy metal ions from insoluble ores often by biological dissolution or complexation processes [41,43], and bio-oxidation [44]. Microbial metabolism can produce secretions, such as low molecular weight organic acids, that can dissolve heavy metals and soil particles containing heavy metal minerals (Figure 2). Chanmugathas et al. [45] showed that microbes can effectively use nutrients and energy to secrete organic acids and promote the leaching of Cd in nutritious conditions. For example, the leaching rate in the absence of nutrients was found to be 9%, and that with the addition of glucose and other nutrients was 36% [45]. There have also been studies that showed that some microbes, for example, *Citrobacter*, could generate free inorganic phosphate, leading to the formation of an insoluble metal phosphate coat that can entrap a large volume of toxic metals (Figure 2, [46]).

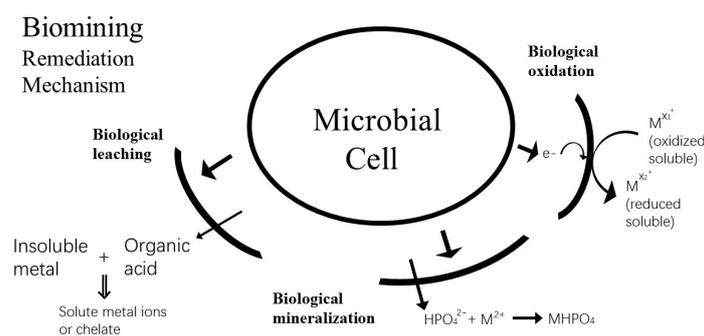


Figure 2. Biomining mechanism of microbes.

Microorganisms, which are mostly prokaryotic, participate in redox reactions and change the valence of heavy metals (Figure 2), thereby changing their activity, which can affect their mobility or toxicity [47]. For example, Hg^{2+} , once reduced by aerobic bacteria to Hg^0 , can evaporate, and tobacco smoke can reduce Hg^{2+} to Hg^0 [21]. Toxic and water-soluble Cr^{6+} can be reduced by *Coryne bacterium* and other microbes into toxic and poorly water-soluble Cr^{3+} , and dead *Bacillus licheniformis* R08 can reduce Pb^{2+} to Pb^0 [48].

4.1.3. Plant–Microbial Remediation

Many microorganisms, including mycorrhizal fungi and other organisms in the rhizosphere, can enhance the ability of plants to absorb or adsorb heavy metals [34]. Joner and Leyval [49] showed that when the concentration of Cd^{2+} in soil is 1, 10 and 100 mg/kg, the uptake of Cd by mycorrhizal plants was 90%, 127% and 131% higher than that of non-mycorrhizal plants, respectively. Mycorrhizal fungi, for example, have mycelia that extend into the soil and effectively increase the surface area of plant roots [17]. Bissonnette et al. [50] showed that, after inoculation of mycorrhiza, the ability to absorb Cu^{2+} , Cd^{2+} and Zn^{2+} is improved. Endophytic mycorrhiza can help host plants develop resistance to heavy metal ions. Plant-endophytic mycorrhiza synergize mainly through

acidification, production of chelating agents, iron carriers, organic acids, and activation of metal phosphates. When the content of heavy metals in the soil reaches toxic levels, the mucus secreted by the fungal cell wall can combine with the polyphosphate and organic acid ions in the fungal tissue to combine the heavy metal ions and reduce the mobility. Chen et al. [51] showed that the adsorption capacity of arbuscular mycorrhizal fungi on Mn^{2+} , Zn^{2+} and Cd^{2+} was equivalent to 1.6%, 2.8% and 13.3% of their dry weights, respectively. Moreover, the number and composition of root exudates change after the fungi infect the plant roots, thereby affecting the oxidation of heavy metals in the vicinity of the rhizosphere [52]. In addition, plant mycorrhizae have a protective mechanism that binds heavy metals to cell walls and prevents them from transferring to the plants [53].

4.2. Comparison of Microbial Removal Ability

4.2.1. Microbial Remediation Potential

Microbial remediation of heavy metal pollution of the soil has definite advantages including low cost and maintenance of the soil structure [19]. Numerous microbial species, including bacteria and fungi from *Bacillus* [54], *Pseudomonas* [34,55], *Streptomyces* [56], *Aspergillus* [57,58], *Rhizopus* [1] and *Penicillium* [23], have significant removal ability (Table 3). At present, we have found that a variety of bacteria can absorb soil heavy metals. Among them, *Escherichia coli* K-12 can absorb the widest variety of metal ions; the outer membrane of this strain can absorb more than 30 different kinds of metal ions [16,59]. *Rhizopus* can absorb Zn, Cu, Cd, Pb, and other heavy metal ions [1], and *Thiobacillus* can absorb heavy metal ions as well as inorganic ions, such as S, which combines with the metal ions to form a precipitate that can be separated from the soil [60].

Table 3. Metals that can be removed by different microbes.

Microbe	Metals Which Can Be Easily Removed
<i>Escherichia coli</i> K-12	Hg, Cd, Pb, Cu, Ni, Zn etc.
<i>Rhizopus arrhizus</i>	Zn, Cu, Cd, Th
<i>Aspergillus niger</i>	Zn, Cu, Cr, Pb, Th, Co, Mn, Ni
<i>Saccharomyces cerevisiae</i>	Cu, Cd, Pb, Ag
<i>Thiobacillus thiooxidans</i>	Cu, Pb, Zn, Cd

4.2.2. Adsorption Equilibria

Equilibrium sorption isotherms are used to describe the capacity of an adsorbent, where their values express the affinity and surface properties of the adsorbent. Recently, the most widely applied adsorption isotherm is the Langmuir isotherm, which assumes that adsorption occurs at specific homogenous sites within the adsorbent [61]. The saturated monolayer isotherm can be represented as:

$$Q_{eq} = (Q_{max} \times bC_{eq}) / (1 + bC_{eq}),$$

where Q_{eq} (mg/g biomass) indicates the quantity of metal ions adsorbed by the bacteria, b (L/mg) is the adsorption constant, which is related to the affinity of the binding sites, and C_{eq} (mg/L) is the concentration of metal ions remaining in the equilibrium solution [34,47].

Usually, the Langmuir isotherm is valid for the following simple cases: The adsorbing site surface is a perfectly flat, homogenous plane with no corrugations, and all sites are equivalent [62]. The shortcoming of this isotherm is that it fails to account for the surface roughness of the adsorbent, and, thus, deviates significantly in many cases. In addition, the model overlooks the interaction with adsorbate, which affects the adsorption of other adsorbate molecules [63].

Compared with Langmuir isotherm, the Freundlich isotherm is a multisite adsorption isotherm for rough surfaces [61] that can be represented as:

$$Q_{eq} = K_F C_{eq}^{1/N},$$

where the two parameters K_F and N are the Freundlich constants related to the adsorption capacity and adsorption intensity of the adsorbent, respectively. Because the Freundlich isotherm has two parameters while the Langmuir isotherm only has one, it is more flexible and is able to fit data on rough surfaces better than Langmuir's equations [64].

4.2.3. Kinetics of Adsorption

Numerous sorption systems have been investigated during the past several years, and most have been reported as pseudo-first-order. However, pertaining to the microbial adsorption of heavy metal ions in contaminated soil, most fixed models are different, and whether a model is appropriate depends on several factors, including the type of metal ions, the type of microbe, and whether other metal ions exist. Rahman et al. compared four different models of metal ions biosorption by *Kappaphycus* sp., and found that most fixed models were second-order, while the kinetic data did not follow the Elovich model [44]. Goyal et al. [48] also found similar results. However, Omorogie et al. [64] showed that for *Nauclea diderrichii*, most fixed models were first-order, and Jiang et al. [31] showed that for *Bacillus Subtilius* second-order effects were better fitted. Recent comparison of sorption mechanisms is summarized in Table 4.

Table 4. Comparison of mechanisms of sorption.

Microbial	Metal	Best-Fitted Model	Source
<i>Bacillus Subtilius</i>	Hg ²⁺	Second-order	[31]
<i>Nauclea diderrichii</i>	Cd ²⁺ Hg ²⁺	Second-order	[65]
Rice husk	Pb ²⁺	Second-order	[66]
<i>Kappaphycus</i> sp.	Pb ²⁺ Cu ²⁺ Fe ²⁺ Zn ²⁺	Second-order	[44]
<i>Helix pomentia</i>	Fe ²⁺ Cr ³⁺	First-order	[59]
<i>Helix pomentia</i>	Cd ²⁺ Pb ²⁺	Second-order	[59]

4.2.4. Methods for Microbial Remediation

A clear measurement methodology for microbial remediation of heavy metals in soil has not yet been proposed. Generally, the microbial culture is established by injecting a specific microorganism into heavily polluted soil. Bojórquez et al. adapted stains that were grown in metal free and metal contaminated soil leachate [34]. After a period of time, the residual heavy metal concentration is measured by a spectrophotometer and compared to pre-injection conditions to evaluate the microbial restoration ability [22]. Sarada used an atomic absorption spectrophotometer to identify the concentration of unadsorbed metal ions [14]. Today, Q_{max} is generally used to describe the microbial adsorption capacity for heavy metal ions. Generally, this capacity varies significantly among species. Thus, there is not a preferred microorganism. However, in meta-analyses of similar subjects, $Q_{max} = 0$, indicating that metal ions cannot be adsorped; thus, this is viewed as a baseline. Thus, the higher the Q_{max} , the better the adsorption ability [67]. Q_{max} is the maximum amount of metal ions adsorped by bacteria per unit of dry weight and is determined using the Langmuir model discussed above.

$$Q_{max} = (Q_{eq} + bC_{eq}^2) / (bC_{eq})$$

We found that those organisms that have spores or genes that degrade heavy metals on the plasmid such as *Bacillus* and *Pseudomonas* often perform better than others. However, for any given organism, both the value of Q_{max} and the removal rate vary for different heavy metals (Table 5, [34]). Mullen et al. [10] showed that the enrichment ability of *Bacillus subtilius* for Cu²⁺ is much greater than

that of *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. The amount of metal adsorbed by *Bacillus subtilis* in $\mu\text{mol/g}$ at an equilibrium concentration of 1 g was 4.150 μmol , whereas the absorption capacities of the other three bacteria were only 2.188, 2.576 and 2.560 $\mu\text{mol/g}$, respectively. However, the enrichment capacity of *Bacillus subtilis* for Cd is weak (only 0.147 $\mu\text{mol/g}$), while that of *Escherichia coli* reached 1.067 $\mu\text{mol/g}$ [57]. Zouboulis et al. [68] found that *Bacillus licheniformis* and *Bacillus laterosporus* had a much better effect on the adsorption of Cd and Cr than other bacteria; thus, the bacterial species showing the highest enrichment varies with heavy metal species.

Table 5. Q_{max} of different microbes.

Microbe	Q_{max}			
	Cd	Cr	Zn	Pb
<i>Bacillus subtilis</i>	101		137	
<i>Pseudomonas aeruginosa</i>	57.37		13.7	79.5
<i>Streptomyces noursei</i>	3.4		1.6	99
<i>Bacillus licheniformis</i>	142.7	62		
<i>Bacillus laterosporus</i>	159.2	72.6		
<i>Rhizopus arrhizus</i>	26.8	4.5	55.6	

The contents of the table are derived from [6,16,41,56].

Current studies have shown that soil fungi, which form mycorrhizal plants through symbiotic relationships, are effective at removing heavy metals from soil. The heavy metal removal ability of the same fungi varies for different heavy metal elements, and for different fungi on the same element. For example, inoculation of *Glomus intraradices* increased the accumulation of Cu in the subsurface part of plants but had no significant effect on the accumulation of Zn [41]. Inoculation of ectomycorrhizal fungi could reduce the contents of heavy metals in soils under Cu^{2+} and Cd^{2+} conditions by 2.64–11.79 and 1.49–7.56 times, respectively [69]. The effectiveness of *Actinomycetes* and other microorganisms on the removal of heavy metals from soil is less studied.

5. Microbial Remediation of Heavy Metal Pollution in Soil and Water

5.1. Microbial Living Environments

5.1.1. pH

The pH plays a crucial role in microbial biosorption, and optimum pH is often different for different microorganisms. An unsuitable pH presents many adverse effects on microbial growth for several reasons [70]. First, the pH affects the activity of enzymes in microorganisms, thereby affecting the rate of microbial metabolism of heavy metals [71]. Second, the pH affects the surface charge of the microorganism, thereby affecting its adsorption of heavy metal ions [72]. Moreover, pH affects the hydration and mobility of many metal ions in the soil [16]. Studies by both Rodríguez-Tirado et al. [73] and Wierzba [54] showed that the removal rate of heavy metals by microorganisms increases with an increase in the pH over a limited range, but the removal rate begins to decrease after the pH rises to a certain limit. Precisely speaking, for Pb^{2+} and Zn^{2+} with pH values from 2.0–5.5, the removal rate continued rising. The adsorption capacity (70 mg/g, 20 mg/g) at a pH of 5.5 is seven times higher versus two times higher at a pH of 2.0 (10 mg/g), respectively. However, at pH values higher than 5.5, the removal rate decreases to the same level as a pH of 2.0. Wang et al. [24] showed that the optimum pH range for most bacteria is 5.5–6.5, but there are exceptions. For example, Rodríguez-Tirado et al. [73] showed that the optimum pH for *Bacillus jeotgali* is 7. This may be the case because some metal ions form hydroxide precipitates and are less susceptible to microbial adsorption when the pH is increased

above a certain value [74]. In addition, the optimum pH for aerobic microorganisms may differ from that for anaerobic microorganisms.

5.1.2. Ambient Temperature

Ambient temperature mainly affects the rate of heavy metal absorption by affecting the growth and proliferation of microorganisms [75]. The optimum temperature for different microorganisms is often different [76]; *Thiobacillus ferrooxidans*, *Thiobacillus acidophilus* and *Thiobacillus tepidarius*, are medium-temperature bacteria; *Sulfolobus solfa-tataricus* and *Acidianus brierleyi* are highly thermophilic bacteria. The absorption of Cd^{2+} , Cr^{2+} and Zn^{2+} by *Bacillus licheniformis* and *Bacillus jeotgali* U3 were studied by Zouboulis et al. [67] and Rodríguez-Tirado et al. [73], respectively, and their results showed that the optimum temperature for the same microbes on different heavy metals is different (Table 6). However, the most suitable temperatures generally range from 25 to 35 °C (i.e., the range is not significant) [26,48,74]. The absorption of Cd^{2+} by *Bacillus jeotgali* U3 is highest when the temperature is 35 °C; however, for Zn^{2+} , the optimum temperature is 30 °C. *Bacillus licheniformis* shows similar results, but the influence of temperature on it is less significant [45].

Table 6. Comparison of Q_{\max} for different bacteria at different temperatures.

Bacteria	Temperature	Q_{\max}		
		Cd	Zn	Cr
<i>Bacillus jeotgali</i> U3	25 °C	37.3	105.22	
	30 °C	47.5	222.2	
	35 °C	57.9	128.2	
<i>Bacillus licheniformis</i>	25 °C	142.73		62.02
	37 °C	140.41		63.98

The contents of the table are derived from [49,68].

5.1.3. Substrate Species

There are three relevant factors to consider in understanding substrate species: soil type, heavy metal ions, and soil additives. The adsorption characteristics of heavy metals on different soils often vary significantly. Hu [51] showed that the soil adsorption capacity of beach tidal soil (Freundlich adsorption constant $K = 93.79$) is higher than that of black soil ($K = 16.41$), which in turn is higher than that of yellow mud ($K = 1.17$), and that the mean desorption rate of soil in ascending order is Lithic Ochri-Aquic Cambosols (0.67%) < Endogleyic Fe-accumulic Stagnic Anthrosols (3.62%) < Fe-accumulic Gleyic Stagnic Anthrosols (35.85%). Obviously, the adsorption rate of soil and its retention of heavy metal ions (i.e., its low desorption rate) result in low heavy metal ion mobility and render the removal of these ions by microbial adsorption difficult [74].

Heavy metal ion species affect the removal of heavy metals by influencing the generation time of microorganisms. *Thiobacillus ferrooxidans* with sulfur as the substrate has a generation time of approximately 10–25 h, far greater than the substrate generation time on Fe, which is approximately 6.5–15 h. The solubility of different heavy metals is often different; elements such as Zn, Ni, and Cu are easily dissolved, whereas Pb^{2+} and Cr are less soluble. Furthermore, the presence of metal ions in the soil or the presence of several metal ions also affect the enrichment of microorganisms [57]. Park et al. [77] showed that the individual bioavailability of Cd^{2+} , Pb^{2+} and Zn^{2+} in the soil is often greater than that of multiple metal ions. For Cd^{2+} alone, the adsorption is 11.2 mg/g. However, in the presence of Zn^{2+} and Pb^{2+} , its adsorption is reduced to 3.15 mg/g, with similar results observed for Zn^{2+} and Pb^{2+} , which is reduced from 19.5 and 2.25 mg/g to 8.08 and 0.915 mg/g, respectively. Obviously, this effect is greatest for Cd and least for Zn [77].

Soil additives can significantly increase the removal of heavy metals by microorganisms, and the concentration of additives can have varying effects on the leaching rate of heavy metal ions.

Tyagi et al. [78] showed that the addition of 20 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ increased the leaching rate of Zn and Cu by factors of 2 and 1.9, respectively, but the leaching rate did not increase when the concentration was greater than 20 g/L. With this same additive, Zn and Cu removal rates were increased to 85% and 93%, respectively, 6 and 3.2 times greater than the control. Research indicates that the use of more than one additive, such as a combination of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, results in a higher removal rate than that achieved when these additives are used individually [5].

5.1.4. Substrate Concentration

The concentration of heavy metal ions also affects the adsorption rate of microorganisms. Generally, a proper evaluation should be used to establish the quantification of the accumulative features of a bio-sorbent [53]. The two most frequently used equations to describe the features are the Langmuir model, whose parameters are interpretable and mainly describe the adsorption of a single-layer surface [76], and the Freundlich model, which is mainly applied to the adsorption equilibrium of the adsorption surface equation [61]. Even though the Freundlich model is much simpler, it grows unbounded [53]; therefore, until now the Langmuir model is more widely used than the Freundlich model. Brunetti et al. and Ehrlich [41,56] have used the Langmuir model to study the effect of heavy metal concentration. They showed that the concentrations of heavy metal ions with the highest adsorption rates vary according to the microorganisms and the heavy metal ions studied. However, the trend, which is similar in all cases, indicates that the adsorption increases to a certain value and then remains constant with the increase of the concentration of heavy metal ions (i.e., the equilibrium concentration). In addition, Fenton processes are helpful in the removal of heavy metals. Laurenti et al. [79] showed that the lowest amount of total organic carbon removal is 50%, but removal can reach 100% under certain conditions. Karci et al. [80] showed that in soil and water contaminated by Zn^{2+} and Ni^{3+} , total organic carbon was completely removed for all runs tested in the present study after pH adjustments.

5.2. Composite Reclamation System

The current proposed composite system uses microbial–plant combination and electric–microbial combination technology. These two technologies are combined by using mycorrhizal fungi to enhance metal removal at plant roots and a DC power plant in combination with bacteria to achieve restoration [48,77,81]. In soil washing and soil flushing processes, a composite reclamation system is also applied. Günther showed that microbial–plant remediation technology can enhance microbial removal [82]; the combination of sulfuric acid (0.05 mol/L), *Bacteroides*, and ryegrass joints has shown Cu removal of up to 30% [83]. Studies have shown that results with a composite repair system are better than those using either pure plant or microbial reclamation. Soleimani et al. [84] showed that the removal rate of mycorrhizal fungi and *Festuca* can be 64–72%, whereas the control group showed only 31% of the plant removal rate. The removal rate of mycorrhizal fungi and *Polygonum aviculare* L. was 54–88%, whereas the control groups with only plants or fungi showed removal rates of 24% and 52–76%, respectively [85]. The removal rate with an electric field increased by more than 10% compared to that without the field, and the removal rate increased to 85–88% by using a DC electric field and adding the matrix and a stationary electrode [47,86]. This is mainly because the electric effect can accelerate the movement of microorganisms and metal ions in the environment and the electrode reaction can provide favorable conditions for microbial degradation. Soil washing and soil flushing combine physical and chemical methods. For example, Dermont [16] presented a soil washing method that involved screening, gravity, concentration, hydrocyclone, froth, and floatation technology. Removal reached 50%, and the capacity reached 3 m³/h. Another experiment included vibrating, screen, and magnetic separation technologies. The concentration of heavy metal ions was 52 µg/g initially and only 14 µg/g in the end. However, this composite technology is often affected by a variety of environmental conditions, such as soil moisture, temperature, nutrients, and high cost, preventing it from being widely used [86].

6. Conclusions

In this paper, the mechanisms of microbial degradation of heavy metals in soil are explored, and the comparison of the different abilities of degradation and the reasons for the investigation, as well as the factors that may affect adsorption capacity, are discussed. We conclude the following:

- (1) The mechanisms of microbial degradation of heavy metals are mainly biosorption, biomineralization, and co-metabolism, and biosorption is the main degradation mechanism. Both biosorption and biomineralization can be divided into a variety of physiological processes.
- (2) Microbes have different abilities to degrade heavy metal, and the degradation ability mainly depends on degradative plasmids and spores. Usually, *Escherichia coli* K-12 adsorb the majority of heavy metal ions, and the adsorption capacity of *Pseudomonas* and *Bacillus* are strong.
- (3) The optimum pH ranges of microorganisms are various. Most microorganisms have suitable pH values in 5.5–6.5, except for *Bacillus jeotgali*. Ambient temperature affects the ability of microorganisms to adsorb heavy metals. Although the optimum temperature is related with heavy metal and microbial species, the optimum temperature for most microorganisms is generally between 25 °C and 35 °C.
- (4) The difference in concentrations of six heavy metal ions, and the presence or absence of competitive ions will affect the adsorption capacity of heavy metals for organisms.
- (5) Composite repair systems, such as microbial plant joint repair systems and chemical microbial joint repair systems, can often improve repair efficiency.

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