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Review

Microbial-Based Heavy Metal Bioremediation: Toxicity and Eco-Friendly Approaches to Heavy Metal Decontamination

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Abstract: There are several industrial processes in which heavy metals are used, including but not limited to chrome plating and tanning. Amongst the most toxic heavy metals to human health are arsenic, cadmium, chromium, lead, copper, nickel, and mercury. The aforementioned toxic metals possess the ability to cause contamination upon their release into the environment. Humans and aquatic and terrestrial animals are at risk from heavy metals in water and soil. Heavy metal toxicity has the potential to result in several health complications, such as renal and hepatic impairment, dermatological afflictions, cognitive lethargy, and potentially oncogenic manifestations. The removal of heavy metals from wastewater and soil can be accomplished using a variety of conventional methods, such as membrane filtration, reverse osmosis, chemical reduction, and adsorption. These methods have several disadvantages, such as generating an abundance of secondary pollutants, and entail significantly higher costs in comparison to biological methods. Conversely, eco-friendly techniques based on microbes have numerous advantages. This review provides a comprehensive overview of biological processes that remove heavy metal ions, both metabolically dependent and metabolically independent. Additionally, we also focused on the source and toxicity of these heavy metals. This study is expected to be particularly beneficial for the development of biological heavy metal treatment systems for soil and water.

Keywords: microorganism; heavy metals; bioremediation; eco-friendly methods

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1. Introduction

The dissemination of heavy metal contamination on a global scale has emerged as a primary concern due to the potential threats it poses to the environment. Heavy metals occur naturally within the Earth's crust [1]. Nonetheless, due to their recalcitrant properties, they exhibit resistance towards degradation. These heavy metals have the potential to accumulate in humans and animals from soil and water by entering into the food chain [2]. Numerous natural and anthropogenic processes can introduce heavy metals into the environment. However, contemporary methods of agriculture, which involve an increasing dependence on agrochemicals and inorganic fertilizers, have resulted in the contamination of agricultural lands and have had disastrous consequences for ecosystems and the natural world [3]. In addition, heavy metals are introduced into water and soil by sewage sludge and organic waste manure, industrial byproducts, and wastewater irrigation [4]. Furthermore, the extraction of heavy metals from ores can pose significant environmental risks when certain areas are left unattended, and heavy metals may subsequently be displaced to other locations as a result of flooding or wind [5].

There has been extensive recognition of certain heavy metals that pose a health hazard to humans, such as arsenic (As), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), and cadmium (Cd). However, gold (Au) has not been found to pose such health hazards [6]. It is noteworthy that heavy metals, such as As, Hg, Cr, Cd, and Pb, typically possess densities of greater than 5 g/cm^3. There are four primary pathways through which heavy metal

ions are introduced into the human body, including the consumption of food that has been contaminated with heavy metals, the ingestion of water that has been contaminated with heavy metals, skin contact, and the inhalation of air that has been contaminated with heavy metals [6,7]. Heavy metal compounds are accountable for covalent bonds, which are known to be extremely hazardous in metalloid compounds. The propensity of heavy metals to covalently attach to organic groups is well documented, leading to the formation of lipophilic compounds or ions [8]. These metallic compounds are lipophilic, allowing them to effortlessly traverse cell membranes and gain entry into cells [9]. The interaction of these metallic compounds with cell organelles can result in toxic effects. The imperative requirement to eliminate heavy metals from polluted water and soil arises from their elevated toxicity [10].

Several technologies have been created to decrease the existence of detrimental heavy metals in water [11]. For the treatment of heavy metals, the most common approach is physicochemical, including filtration, ion exchange, reverse osmosis, desorption, and precipitation [12]. Precipitation, in particular, is widely acknowledged for its effectiveness in eliminating As, Cu, Cr, Pb, and Cd [12]. This method operates through the alteration of wastewater pH levels. It should be noted, however, that these physicochemical techniques are often associated with high costs and the generation of secondary chemical sludge [12,13]. In light of the limitations outlined above, it is of utmost importance to develop techniques that are both cost-effective and environmentally friendly, as these are essential to the removal of heavy metals from water and soil [14].

As a result of their commendable cost-effectiveness and environmentally sustainable nature, an extensive range of techniques and strategies for bioremediation have been identified, including, but not limited to, biosorption, bioreduction, bioaccumulation, mycoremediation, bacterial bioremediation, and phytoremediation [10]. Specifically, bioremediation has demonstrated an economical and ecologically sound approach to extracting heavy metals from contaminated sites [15]. In addition to rice and wheat husk, activated carbon, lignite, agricultural waste, bananas and citrus peels, and green-synthesized nanoparticles have been used as active biosorbents to effectively remove and eradicate heavy metal pollution [16]. In addition, it has been established through scientific research that living microorganisms, encompassing microalgae as well as bacteria, and plants are significantly efficacious in the role of bioremediation agents [10]. This is primarily attributed to their remarkable attributes of possessing high heavy metal tolerance properties, which have been demonstrated to be key determinants of their effectiveness in this regard [17]. The surface of bacterial cells is replete with a myriad of functional groups that are intimately involved in the binding of heavy metals, which may occur either to the cell membrane or in a reduced state. This intricate interplay effectively serves to mitigate the deleterious effects of heavy metal toxicity [18,19].

This review is focused on heavy metal contamination in water and soil, with natural and anthropogenic sources. Moreover, this review is also focused on the heavy-metal-mediated carcinogenicity and microbial-based techniques for the removal of certain heavy metals such as As, Cr, Cd, Pb, and Cu from contaminated water and soil.

2. Source and Toxicity of Heavy Metal Ions

2.1. Sources of Heavy Metals in the Environment

A vast and diverse array of industrial processes, encompassing a wide range of activities such as leather tanning, the electroplating of materials with Cr, the manufacture of batteries, the operation of glass industries, various agricultural activities, the disposal of domestic waste, and the conduct of pharmaceutical industrial processes, have all been conclusively identified as major contributors to the widespread and pervasive dissemination of heavy metals throughout the environment [14,20]. Toxic heavy metal ions are released into the environment, which poses a significant threat to both human health and the ecosystem. The production of Pb has resulted in the generation of roughly ten million tons of the element [21]. A substantial proportion of the total amount, precisely 85.10%, has been allocated

to the battery industry for utilization. An additional 5.5% has been employed in the development of pigments. The remaining 2.1% has been directed towards miscellaneous industrial processes [4]. Furthermore, Pb is commonly utilized in petrol as tetraethyl and tetramethyl agents, which act as anti-knocking compounds. On the contrary, Cr finds its purpose in the production of steel, wood preservation, chrome plating, pigments, and electroplating [22]. Meanwhile, Cd is primarily employed in the electroplating and battery industry. To tackle the issue of heavy metal discharge in industrial effluent, environmental agencies such as the central pollution control board (CPCB), India, and the state environmental protection administration (SEPA), China, have laid down the maximum permissible limit for heavy metals. The maximum permissible limit of heavy metals in industrial effluent should be 0.2 mg/L (As), 2 mg/L (Cd), 0.1–2 mg/L (Pb), 2 mg/L (Cr), and 3 mg/L (Cu) [23]. The maximum permissible limit of heavy metals in industrial effluent according to the SEPA should be 0.1 mg/L (As), 0.01 mg/L (Cd), 0.05 mg/L (Cr^{6+}), 0.1 mg/L (Pb), and 0.5 mg/L (Ni) [4,24]. Similarly, the United States Environmental Protection Agency (US EPA) has also established the maximum allowable limit of heavy metals present in drinking water as 0.05 mg/L (As), 0.05 mg/L (Cr), 0.1 mg/L (Pb), 0.01 mg/L (Cd), and 0.10 mg/L (Ni) [25]. Any transgressions beyond these permissible limits may result in multiple detrimental effects caused by heavy metals.

2.1.1. Natural Arsenic Sources

There exists a multitude of naturally occurring origins that contribute to the contamination of heavy metals in water and soil. The principal natural sources of these toxic heavy metals are found within geological formations, such as sedimentary deposits, volcanic rocks, and soils, as well as the combustion of vegetation and coal and volcanic eruptions [26]. Geothermal water is a significant contributor of heavy metals to groundwater, surface water, and soil. The concentration of As in the Earth's composition varies, and the average concentration typically ranges from 1.5 to 5 mg/kg. As is commonly found in the sulphide ores of various metals, including silver (Ag), Cu, Au, and Pb. Heavy-metal-containing minerals such as pyrites, realgar, and orpiment have an important role in the groundwater contamination of heavy metals [27]. Phyllite ore has the highest arsenic concentration. Thereafter, some other major natural sources of arsenic are peaty soils and mudstones/marine shale. Higher heavy metal levels in groundwater are probable in sedimentary aquifers [28].

2.1.2. Anthropogenic Arsenic Sources

The anthropogenic sources of heavy metal pollution in soil, surface water, and drinking water have been reported worldwide. This type of heavy metal contamination in the water is related to human intervention, mining and coal processing, and petroleum refineries [29]. Heavy metal contamination through anthropogenic activity may be characterized into several types, such as coal-related and mining-based contamination [30]. The smelting and mining of heavy-metal-containing compounds cause heavy metals to leak into the surface water and groundwater by dumping and discarding these heavy-metal-containing waste materials [30]. The harmful impact of coal-mining-related heavy metal contamination has been extensively recorded and meticulously catalogued in an overwhelming prevalence of approximately 74 countries, spanning the globe [31]. The insidiousness of petroleum-based heavy metal contamination can scarcely be overstated, for it represents one of the most significant sources of heavy metal contamination, with its deleterious effects felt across a swath of approximately 17 countries across the world [32].

2.2. Heavy-Metal-Mediated Toxicity

2.2.1. Exposure to Heavy Metals and Environmental Pollution

ROS are generated in living cells when heavy metals bond to sulfhydryl groups and enter the cell. As a result, macromolecules are inactivated, ROS stress is caused, and glutathione levels are depleted [33]. When toxic metals enter the body, they initiate several processes, including interfering with metabolic pathways or inhibiting them. As a result,

both humans and animals suffer from a variety of toxic effects of heavy metals [21]. A variety of problems may result, including organ dysfunction, metabolic abnormalities, hormone changes, congenital disorders, immune system problems, and cancer [34]. Metals in the environment, food, and water have therefore been subject to standards established by several international organizations. Food and water heavy metals are subjected to risk assessment studies [35]. Approximately 20% of 193 Ayurvedic medicines and famous Indian herbal medicines were found to contain Hg, Pb, and As, according to Saper et al. [36]. Pb, Hg, and As were detected in nearly 21% of samples [36]. The level of acceptable daily metal intake was exceeded by all metal-containing products. This would cause Pb and/or Hg levels to exceed acceptable levels by 100 to 10,000 times [37]. The presence of heavy metals in dietary supplements is a matter of considerable concern, and as such, these potentially toxic substances must be subjected to daily dosage limits as established by regulatory authorities [38]. It is important to note that the establishment of such dosage limits is crucial for ensuring the safety and well-being of individuals who consume such supplements and that any deviation from these limits could result in severe health consequences. Thus, manufacturers and regulatory authorities must work together to ensure that the levels of heavy metals in dietary supplements are strictly controlled and monitored to minimize any potential risks. Ultimately, the establishment of and adherence to daily dosage limits for heavy metals in dietary supplements is a critical component of ensuring public health and safety and should be given the utmost priority by all stakeholders involved in the production and regulation of such products [39]. Manufacturers must assess these standards as well [40]. The phenomenon of environmental pollution has a profound impact on areas that possess an unspoiled quality. This can be observed in the case of Mount Everest, a location that is isolated and remote [38–40]. There have been reports of heavy metal contamination in this area associated with Pb, Cd, Cr, As, and Hg [41]. As and Cd levels in all snow samples collected from Mount Everest have exceeded the drinking water guidelines outlined by the US EPA, according to Yeo and Langley-Turnbaugh [42]. Moreover, a substantial amount of As contamination was detected in soil samples taken from the heavy-metal-contaminated sites located on Mount Everest.

2.2.2. Heavy Metals Binding to the Thiol Group

Upon entering the body, heavy metals form bonds with proteins, nucleic acids, and lipids with a particular tendency to form bonds with lipids and proteins. In proteins, cysteine residues are modified by thiol (-SH) groups when metals bond with enzymes and proteins [43]. It is important to note that such inhibition of protein function can ultimately lead to the disruption of the intracellular redox equilibrium [44]. Consequently, an impaired antioxidant defense mechanism can contribute to the onset of hepatocellular injury. It is worth mentioning that heavy metals can elicit comparable reactions with proteins [44]. In the course of the intricate process of complexation that involves heavy metals and thiol-containing proteins, it is noteworthy that the ligands which participate are none other than amino acids that are characterized by the presence of the -SH functional group [44,45].

2.2.3. Heavy-Metal-Mediated Carcinogenicity

The complex and ambiguous nature surrounding the etiology of carcinogenicity in heavy metals has been widely postulated [46]. It has been put forth as a proposition, with a high degree of specificity, that the carcinogenic properties of certain heavy metals are attributable to their binding to regulatory proteins that perform an exceedingly crucial function in the regulation of the cell cycle and DNA synthesis and repair mechanisms, as well as the highly intricate process of apoptosis [46,47]. As a result of toxicogenomic investigations, gene expressions following metal toxicity have been characterized. Activator protein 1 (AP-1) and nuclear factor-kappa B (NF-KB) are two transcription factors that are targeted by Cd and As [48]. Uncontrolled cell growth and division are the result of failure to control protective gene expression. Several studies have examined the carcinogenic effects of heavy metals and mutations in Ras proteins [48,49]. Human prostate epithelial cells

exposed to As exhibited the overexpression of Ras, as demonstrated by Ngalame et al. [49]. Furthermore, in vitro analysis has uncovered that Cd triggers the augmentation of the extracellular signal-regulated kinase 1/2 (ERK 1/2) level, as well as the jun and fos transcription factors. Correspondingly, Cr (VI) has been discovered to instigate the overexpression of jun in cultured cells [49]. Consequently, the mutated Ras protein loses its ability to undergo inactivation, and the kinase cascade remains unturned. Furthermore, the heightened jun and fos or activated ERK 1/2 sustains the gene expression, resulting in the activation of an incessantly active signaling pathway, which ultimately culminates in the continuous activation of proliferation and the increased formation of tumors [50,51].

3. Heavy Metal Removal Approaches

The notion of utilizing biological means to remove heavy metals is exceedingly appealing when juxtaposed with other conventional techniques because biological procedures are characterized by their cost-effectiveness, eco-friendly nature, and their exceptional efficiency when it comes to dealing with low concentrations of heavy metal ions in wastewater [52]. A wide range of heterogeneous biological entities, including various substances such as plant biomass, agricultural detritus, microbial biomass, nanoparticles synthesized through green methods, residual fruit matter, and biopolymers, have been effectively employed in the removal of heavy metals [53]. These aforementioned agents have demonstrated their efficacy in this role, and their use has been widely documented in the literature [54]. It is significant to acknowledge that the application of these biological agents for heavy metal elimination holds immense importance within the realm of environmental science. This is because it presents a sustainable and environmentally conscious approach towards tackling the problem of heavy metal pollution [52,54]. Thus, further research into the application of these agents in heavy metal removal is warranted and is likely to yield valuable insights and contributions to the field. Living organisms, which are vital constituents of the biosphere, including bacteria, algae, and fungi, have been identified as playing significant and emerging roles in the process of heavy metal removal from the environment [55]. Recent advances in biotechnology have enhanced the understanding of the mechanisms through which these organisms mediate the detoxification of heavy metals. These mechanisms include biosorption, biomineralization, bioaccumulation, and bioremediation and are crucial in the removal of heavy metals from ecosystems [10,56]. Therefore, it is evident that the utilization of living organisms in the removal of heavy metals is a promising approach towards achieving sustainable environmental management. Various types of microorganisms, including fungi, bacteria, and algae, can absorb heavy metals from their surrounding environment and transport them into their cells [57]. It has been documented and reported in numerous studies that a diverse range of microbial species possesses the unique capability to convert the extremely hazardous Cr (VI) to a less toxic form of Cr (III). Microbial cells possess the ability to effortlessly internalize Cr (III) owing to their limited solubility in aqueous solutions and negligible toxicological characteristics [58,59]. Numerous techniques for heavy metal bioremediation have been proposed in the past, including biosorption, phytoremediation, bioreduction, and bioaccumulation [58]. Biosorption involves the use of dead biomass, while phytoremediation necessitates the removal of heavy metals through plant mediation. On the contrary, it is imperative to note that bioreduction is a highly intricate process that encompasses the transformation of the oxidation states of heavy metal ions, which is a highly significant phenomenon in the field of environmental chemistry [60]. Conversely, bioaccumulation is a process that is characterized by the absorption and uptake of heavy metal ions into the intracellular space, which is a crucial mechanism that is fundamental to the understanding of the behavior of heavy metals in biological systems. These techniques have been extensively researched and have shown promise in the bioremediation of heavy metals [61]. The process of remediating heavy metals can be achieved through the utilization of two distinct mechanisms: metabolically independent mechanisms, which entail the use of inorganic, lifeless materials, and metabolically dependent mechanisms, which involve the use of living cells of bacteria,

fungi, and algae that are capable of carrying out the necessary metabolic processes required to transform the heavy metal contaminants into less harmful forms [4,62]. In the context of this current evaluation, the esteemed authors have decidedly and intently directed their concentration towards the intricate and multifaceted process of the bioremediation of heavy metals, which is undeniably and inextricably linked to the intricate and complex workings of metabolic pathways and processes.

3.1. Microbial Mechanisms Involved in Heavy Metal Bioremediation

Bioremediation is a biological technique that is employed to extract heavy metal ions from sites that have been contaminated with heavy metals [63]. In bioremediation, absorption/adsorption, bioaccumulation, biotransformation, and bioleaching are included in the remediation of toxic metal ions. There are several natural components, such as bark, coconut husk, rice husk, wheat husk, seaweeds, seeds, aquatic plants, agro-waste, and microorganisms, which are utilized to reduce toxic heavy metal concentrations in polluted sites. In these materials, microorganisms play a significant role in bioremediation [64]. The phenomenon of microorganisms altering the ionic state of heavy metals is a significant process that has far-reaching implications for their solubility, bioavailability, and movement in both soil and aquatic environments [65]. The mobilization and immobilization of heavy metals is a crucial aspect of microbial remediation, a complex process that involves a multitude of intricate mechanisms including, but not limited to, oxidation–reduction, chelation, modification of the metallic complex, and biomethylation [63]. It should be emphasized that microbial enzymatic catalysis plays a pivotal role in the solubilization of metals with higher oxidation states to lower oxidation states. For example, the enzymatic oxidation of uranium by *Thiobacillus ferrooxidans* and *T. thiooxidans* serves as a prime illustration of this phenomenon [66]. Although the isolation of microorganisms responsible for the degradation of heavy metals from both aerobic and anaerobic environments is feasible, aerobic microbes are considered to be more suitable for bioremediation when compared to anaerobic microorganisms [67]. Furthermore, microorganisms utilize membrane-linked transport mechanisms to transport heavy metals and convert them into non-hazardous forms, which is of utmost importance for their survival in a metal-polluted environment [68]. In this regard, microorganisms employ a range of processes, such as biosorption, bioaccumulation, biotransformation, and bioleaching, to counteract the detrimental effects of heavy metals and ensure their sustainability [68,69].

It is of great importance to note that the utilization of the aforementioned techniques has proven to be efficacious in facilitating the process of environmental clean-up, thereby emphasizing the substantial capacity possessed by microorganisms in ameliorating the deleterious impact of heavy metals on the ecosystem [70].

3.2. Biosorption

The phenomenon of biosorption, which is frequently defined as the process of adsorption of heavy metal ions by biomass, is considered to be metabolically independent heavy metal adsorption [71]. This captivating process is generally executed by a diverse range of deceased biomass, encompassing but not limited to plant waste, microalgae, agro-waste, microbial biomass, and agriculture residue [72]. In the course of biosorption, it is widely acknowledged that heavy metal ions tend to adhere to the surface of the biomass using a series of functional groups that are present on said surface. It is, however, worth noting that biosorption can also be executed by live cell biomass. Specifically, living microbial cells possess the exceptional capacity to attach heavy metal ions to their cell surface through the process of passive adsorption and complexation [73].

Biosorption, an intriguing phenomenon for researchers in the field, entails a multifaceted process whereby heavy metal ions adhere to surface functional groups, namely amino, amide, imidazole, sulfonate, and carboxyl. It is noteworthy to emphasize that the pKa value of the medium in which biosorption takes place exerts a substantial impact on the adsorption of heavy metal ions and the binding propensity of functional groups. It

is believed that hydroxyl groups have a pKa value between 9.5 and 13.21. As a result, carboxylate groups are mostly responsible for the negative charges on biomass between these Pka values. Aqueous media requires the deprotonation of carboxylic groups to generate negative charges and H+. As a result, different metal ions can be adsorbed [74]. One of the most crucial and indelible aspects of biosorption is the judicious selection of a suitable material to employ. This material should be easily obtainable, non-hazardous, and economically viable, which represents an important consideration for researchers in the field [75].

The efficacy evaluation of a biosorbent is contingent upon the array of surface groups that are present. For a biosorbent to be deemed optimal, the concentration of surface functional groups must be augmented [25]. The surface morphology of an adsorbent represents a pivotal characteristic in the heavy metal biosorption process. Surfaces that are rough and porous provide a larger surface area, thereby conferring an advantage for the binding of heavy metal ions onto the biosorbent surface, as per reference [76]. It is crucial to carefully scrutinize the surface morphology and functional groups of a biosorbent. A myriad of techniques, including Fourier Transformation Infra-Red (FTIR), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDX), Nuclear Magnetic Resonance (NMR), and X-ray Diffraction (XRD) are readily available to characterize a biosorbent, as per reference [77]. As such, it is imperative to employ these aforementioned techniques judiciously to obtain a comprehensive understanding of the intricate details of the biosorbent's surface morphology and functional groups [76,77].

The biosorption process, which involves the use of biomass to remove metal ions from aqueous solutions, is influenced by various factors that impact its efficacy. These factors encompass the morphology and composition of the biomass, the presence of multiple metal ions in the media, the ambient temperature, and the pH of the medium [78]. It is commonly observed that a decrease in pH results in competition between cationic heavy metal ions. Conversely, an increase in pH leads to the deprotonation of the biosorbent surface, which causes the exposure of surface functional groups [79]. Additionally, desorption, which involves releasing adsorbed heavy metals from the biosorbent, can be employed to regenerate the biosorbent. The recovery of metal ions can be achieved by manipulating the pH of the medium, which is a critical factor in the process [80]. Moreover, the biosorption mechanism of Cr (VI) is a complex phenomenon in which the Cr (VI) ion attaches to the biosorbent surface and is subsequently reduced to less toxic Cr (III) [80].

The process of reducing and adsorbing Cr (VI) proceeds in a sequence of three distinct stages, which have been observed and studied extensively. The initial stage involves the binding of chromate ions, which are negatively charged, to positively charged functional groups [81]. This binding process is an essential step in the overall reduction-cum-adsorption process, as it enables the subsequent stages to take place. In the second stage, Cr (VI) is reduced to Cr (III) by functional groups, which donate electrons to facilitate the reduction process [82]. This stage is crucial in the sense that it marks a significant transformation in the chemical composition of the system, as Cr (VI) is converted to Cr (III). The ultimate stage in the process entails the discharge of Cr (III) ions into the solution, while the residual Cr (III) ions are retained by the adsorbent surface, which is adorned with negatively charged functional groups. This phase is a fundamental requisite in guaranteeing the consummation of the adsorption process and the total occupation of the Cr (III) ions on the adsorbent surface [83]. It is noteworthy that this process represents a complex interplay of various chemical and physical phenomena, which require further investigation and elucidation [84]. Table 1, which is effectively and efficiently utilized as a visual representation of data, illustrates, in a comprehensive manner, the inherent capabilities of different biosorbents to uptake heavy metal ions.

Table 1. The heavy metals removal by microbial-based biomasses and heavy metal biosorption capacity.

Biosorbent	Heavy Metal Ion	Heavy Metal Biosorption Capacity (mg/g)	References
Phytolacca americana L. biomass	Pb (II)	10.83	[85]
Pseudomonas aeruginosa PU21 (Rip64)	Cu (II)	23	[86]
Pseudomonas aeruginosa PU21 (Rip64)	Pb (II)	70	[86]
Pseudomonas aeruginosa PU21 (Rip64)	Cd (II)	58	[86]
Halomonas BVR 1	Cd (II)	12.023	[87]
Mix bacterial biomass of *Enterobacter ludwigii*, *Zoogloea ramigerais*, and *Comamonas testosteroni*	Cu (II)	6.52	[88]
Staphylococus xylosus biomass	As (III)	54.35	[89]
Staphylococus xylosus biomass	As (V)	61.34	[89]
Bacillus strain MRS-2 (ATCC 55674)	Pb (II)	206.5	[90]
Bacillus subtilis	Pb (II)	57	[91]

The microbial biomasses, as previously mentioned, have been identified as remarkably efficient biosorbents when it comes to removing heavy metal ions. Additionally, it is noteworthy that the biosorption phenomenon can be conducted on immobilized biomass. This includes bacterial biomass that exists on a solid surface, fungal biomass located on a supportive medium, or deceased biomass that has been immobilized on another supportive surface [92]. Immobilization serves to not only augment the stiffness, potency, and longevity of the biosorbent but also ultimately to enrich the biosorption capacity. The existence of a diverse array of matrices has made immobilization possible, with matrices such as alginate and polyurethane being extensively employed for this purpose [92,93]. Moreover, it is noteworthy to emphasize that a multitude of microbial and plant biomasses have been employed for immobilization on the aforementioned matrices [91,94]. To illustrate, the biomass of *Chlorella homosphaera* has been successfully immobilized on matrices containing sodium alginate [95].

3.3. Bioaccumulation

Bioaccumulation is an intricate biological phenomenon that involves the accumulation of heavy metal ions within microbial cells. It is a process that occurs at a slower rate in comparison to biosorption, as it entails the active participation of several metabolic pathways [61]. In contrast to the phenomenon of biosorption, characterized by the adsorption of metal ions onto the exterior surface of microbial cells, the process of bioaccumulation entails the uptake and subsequent retention of said metal ions by living microbial cells suspended within a medium [96]. Furthermore, the bioaccumulation process necessitates the persistent monitoring and observation of said cells. Owing to the multifaceted nature of this process, a thorough understanding of the underlying mechanisms is crucial to comprehend the impact of bioaccumulation on the environment and its potential applications in bioremediation [96]. The process of bioaccumulation presents an advantage in reducing various stages of biosorbent preparation, such as biomass collection, drying, preparation through washing and crushing, and storage. However, it is subject to significant influence by experimental conditions, as reported in [75]. The presence of pollutants in wastewater poses a substantial challenge to microbial growth and results in competition with heavy metals selected for bioaccumulation. Furthermore, these pollutants can bind to the bacterial outer surface and disrupt cell heavy metal accumulation, leading to further challenges in the bioaccumulation process [97]. It is noteworthy that numerous species of fungi, bacteria, algae, and plants have been identified as playing an essential role in the elimination of heavy metals, thus reinforcing the importance of considering biotic factors when assessing the effectiveness of bioaccumulation [98,99].

Among the various living systems that exist, it has been observed that microbes exhibit a noteworthy capacity for both effectively removing heavy metals as well as demonstrating resistance towards them [63,100]. Bacteria, which are single-celled microorganisms, have been observed to exhibit the extraordinary ability to selectively assimilate heavy metal ions through the utilization of cell surface receptors, which are specialized proteins that are embedded within the outer membrane of the cell. Following the initial binding of these heavy metals to the receptors, the bacteria have been observed to efficiently accumulate and store them within their cellular structures, thereby enabling them to maintain their physiological functions under adverse environmental conditions [101]. Notably, certain bacterial cells, such as those belonging to the Pseudomonas, Klebsiella, and Microbacterium species, have exhibited a remarkable ability to resist heavy metal ions that have been extracted from diverse contaminated sites. It has been observed that bacteria that have been isolated from areas that have been contaminated with heavy metals have generally developed a resistance to these ions [102]. Furthermore, numerous researchers have successfully identified strains of Microbacterium that exhibit heavy metal resistance within such contaminated regions. Henson and colleagues [103] conducted the isolation of *Microbacterium* sp. (Cr-K29), which exhibited the remarkable ability to reduce up to 88% of Cr (VI) from water that has been contaminated with 2 mM of Cr (VI). In the interim, Pattanapipitpaisal and colleagues [104] discovered that *Microbacterium liquefaciens*, when anchored to solid surfaces, has the capability of eliminating 90–95% from a 50 µM Cr (VI) solution. It is of utmost importance to take note that bacteria have various mechanisms at their disposal to rid heavy metals from their surroundings, as these metals are detrimental to living organisms [105]. These microorganisms employ heavy metal ions to facilitate their metabolic activities, or they employ soluble enzymes that are generated by the bacterial cell in order to detoxify heavy metal ions [106]. The complete bioremediation process of Cr (VI) by bacteria is depicted in Figure 1.

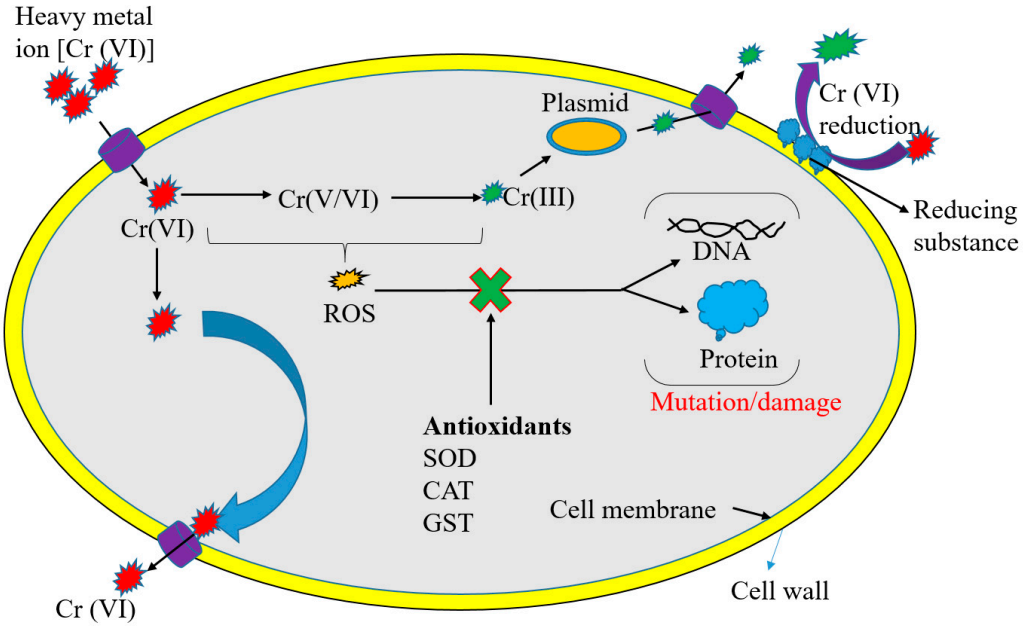


Figure 1. This study focuses on the remediation of heavy metals using bacteria and the mechanism of heavy metal reduction in bacterial cells [14].

Reactive oxygen species (ROS) are known to be generated in bacterial cells upon exposure to toxic heavy metals. Such ROS have the potential to inflict damage upon the cell organelles or cause the disruption of multiple metabolic functions, ultimately leading to aberrations in normal cell functions [107]. As a consequence, the antioxidant system within the microbial cells, including enzymes like superoxide dismutase (SOD), glutathione-S-transferase (GST), and catalase (CAT), plays an active role in combating the stress induced

by ROS generation in bacterial cells [108]. Researchers have made a noteworthy discovery that several bacterial species possess the capability to exhibit resistance towards heavy metals [109]. In Table 2, the bioremediation efficacy of heavy-metal-tolerant bacterial strains has been meticulously outlined.

Table 2. Heavy metal uptake capacity of bacterial strains.

Bacteria	Heavy Metals	Initial Heavy Metal Concentration (mg/L)	Heavy Metal Removal Capacity (%)	References
Pseudomonas aeruginosa PU21 (Rip64)	Pb (II)	50	98	[86]
Pseudomonas aeruginosa PU21 (Rip64)	Cu (II)	50	98	[86]
Pseudomonas aeruginosa PU21 (Rip64)	Cd (II)	50	98	[86]
Enterobacter ludwigii, Zoogloea ramigerais, and *Comamonas testosterone* mix culture	Pb (II)	10	72.6	[88]
Enterobacter ludwigii, Zoogloea ramigerais, and *Comamonas testosterone* mix culture	Cu (II)	2.5	83.9	[88]
Pseudomonas aeruginosa	As (III and V)	10	98	[110]
Pseudomonas strain As-11	As (III)	130	48	[111]
Pseudomonas strain As-11	As (V)	312	78	[111]
Microbacterium paraoxydans VSVM IIT (BHU)	Cr (VI)	50	91.62	[9]
Microbacterium paraoxydans VSVM IIT (BHU)	Cd (II)	50	89.29	[9]
Microbacterium paraoxydans VSVM IIT (BHU)	Pb (II)	50	83.29	[9]

3.4. Bioleaching

Bioleaching is an exceedingly multifarious and intricate process that involves the utilization of an extensive range of microbial organisms, with a special emphasis on acidophiles. These remarkable chemolithotrophic microorganisms exhibit an exceptional ability to oxidize Fe (II) to Fe (III) and/or reduce sulfur to sulfuric acid, which endows them with the capacity to thrive in environments with low pH levels typically ranging at or below 2.0 [112,113]. The solubilization of metal sulfides and oxides from ores, thereby enabling the extraction of metal through the segregation of metals in the solid phase from the more water-soluble phase, is accounted for by the production of ferric ions and protons resulting from sulfuric acid [114]. Bioleaching, which involves the reduction of metal ions by microorganisms, plays an indispensable role in the extraction and recovery of heavy metals [115]. The ability of microorganisms to convert solid chemicals within contaminated soil into a soluble substance that can be removed and recovered significantly affects the efficacy of the recovery process, especially given the finite and non-renewable nature of metal resources [116]. In light of this, scholars have proposed bioremediation as a viable means of recovering raw materials from effluent. For instance, an absorbent based on Annona squamosa was utilized to achieve a substantial recovery rate of up to 98.7% for Cd (II) through 0.1 M HCl [117]. Likewise, it is noteworthy to mention that the application of *Pseudomonas aeruginosa* biomass, in conjunction with a highly concentrated solution of hydrochloric acid (0.1 M HCl), has been observed to yield a remarkably substantial rate of retrieval for the elusive heavy metal known as cadmium (II), with recovery rates reaching an impressive 82% [118]. Additionally, a noteworthy 100% recovery rate of Cu (II) was reported by utilizing volcanic rock matrix-immobilized *P. putida* cells that possessed surface-displayed cyanobacterial metallothioneins at a pH of 2.35. Analogously, 100% recovery of Cu (II) was obtained from activated sludge at a pH of 1.0 [119]. It is a matter of significance to highlight that the incorporation of the autochthonous variant *Enterobacter* sp. J1 brought about a remarkable increase in the recuperation percentage, exceeding 90% for both copper and lead ions when the pH level was at 2 [120].

3.5. Biotransformation

Biotransformation, a crucial process in the field of chemistry, refers to the alteration of a molecule's chemical structure, ultimately leading to the creation of a comparatively more polar molecule [121]. To put it differently, the interaction between metals and microorganisms induces a change in toxic metals and organic compounds, converting them into a less hazardous form. This mechanism plays a significant role in facilitating heavy metal adaptation by microorganisms [97]. It is worth noting that microbial transformations can be accomplished through a variety of methods, including carbon bond formation, isomerization, functional group introduction, oxidation, reduction, condensation, hydrolysis, methylation, and demethylation, among others [122]. The process of altering metals utilizing microbial organisms has been extensively recorded. The investigation has brought to light the fact that *Micrococcus* sp. and *Acinetobacter* sp. possess the remarkable capability to expedite the oxidation of the most toxic As (III) into compounds such as arsenic acid and arsenates that are comparatively less soluble and non-poisonous As (V), consequently mitigating its detrimental impact [123]. According to the findings of the study carried out by Thatoi et al. [116], the deployment of NADH-dependent reductase by Cr (VI)-tolerant *Bacillus* sp. SFC 500-1E has been observed to be highly effective in reducing the hazardous Cr (VI) to less toxic Cr (III) [124].

3.6. Some Other Advanced Microbial-Based Approaches for Heavy Metal Removal

3.6.1. Rhizoremediation

The process of rhizoremediation, which is a method employed to eliminate heavy metals from the soil, involves the utilization of microorganisms that are found in the rhizosphere of plants. Rhizoremediation is a technique that combines phytoremediation and bioaugmentation, which are two techniques used to cleanse contaminated substrates [125]. Rhizoremediation is a highly innovative and cutting-edge approach that has been developed to address the pressing issue of organic pollutants and heavy metal contaminants that are present in our environment [126]. This method is based on the use of plants and rhizospheric microorganisms, which have proven to be highly effective in the process of restoring polluted environments. Indeed, the success of rhizoremediation cannot be overstated, as it is an eco-friendly, cost-effective, and highly efficient means of dealing with the problem of pollution [126]. However, it is important to note that the success of rhizoremediation at any given site is dependent on a wide range of factors, including the level of soil contamination and the quantity of metal contaminant present in the soil, as well as the ability of plants to aggressively take up metals from the soil. Therefore, these factors must be carefully considered and taken into account when implementing rhizoremediation to ensure that the process is as effective as possible [127]. Ultimately, the use of rhizoremediation represents a crucial step forward in the fight against pollution, and we must continue to explore and develop this innovative approach to safeguard our environment for future generations [68].

Plants that are employed in the process of rhizoremediation can be categorized into two distinct groups, namely the hyperaccumulators and non-hyperaccumulators. The former group comprises plants that possess an exceedingly high ability to accumulate heavy metals, albeit at the expense of biomass efficiency [128]. Conversely, the latter group has a comparatively lower capacity for extraction of heavy metals when compared to hyperaccumulators, but their total biomass yield is considerably higher. Moreover, non-hyperaccumulators exhibit rapid growth characteristics [129]. In the context of removing heavy metals from contaminated soils, certain plants utilize a variety of processes, which can be observed in Figure 2.

Phytostabilization is a process which involves the immobilization of heavy metals in soil by the adsorption or precipitation of heavy metals. The term phytoextraction is related to the translocation of heavy metal ions from the soil and their accumulation in plants (root, stem, and leaves). In phytovolatilization, heavy metals are absorbed by plant roots and pushed up through the xylem stream. The heavy metal ions transfer to the upper

parts of the plant, such as the leaves and stem, then convert into a volatile form and are released into the atmosphere [130,131]. This scientific approach has been integrated with several other techniques to create a comprehensive method for soil renewal. Currently, the application of plants and plant-growth-promoting bacteria (PGPB) is being evaluated as an effective and environmentally acceptable method for soil renewal [132]. PGPB in the soil that are close to the roots of plants play a vital role in facilitating the absorption or adsorption of heavy metals by plants. In addition to enhancing remediation abilities, PGPB act as a plant growth stimulant by secreting hormones and metabolites. This stimulates growth, solubilizes minerals, fixes nitrogen, and protects against pathogens. Other biotic and abiotic stresses that plants face can also be relieved by PGPB. It is important to note that the process of rhizoremediation is not only environmentally friendly but also enhances soil quality and promotes the growth of healthy plants [133,134].

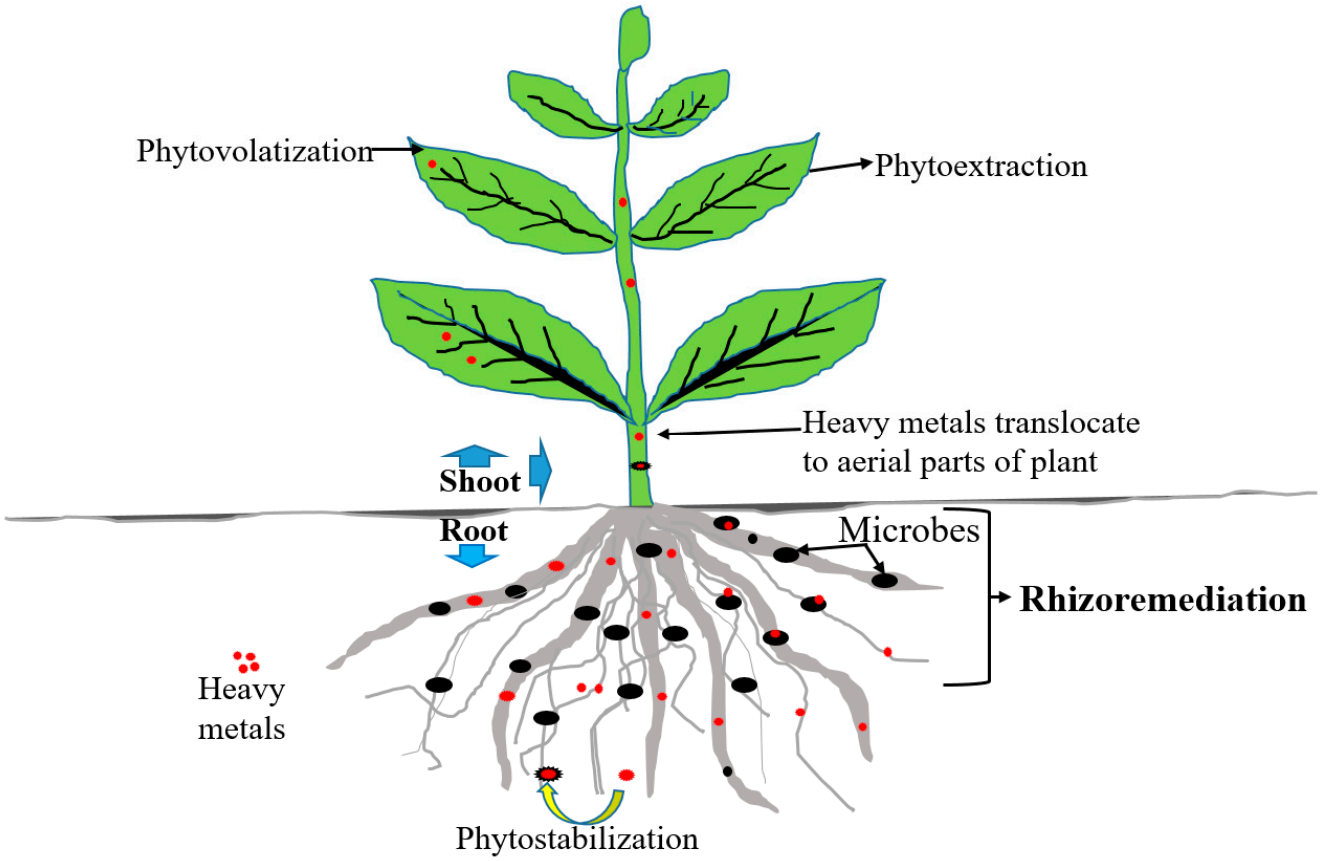


Figure 2. Processes involved in rhizoremediation for heavy metals.

3.6.2. Genetically Engineered Organisms

The utilization of microorganisms for bioremediation has proven to be an efficacious technique for alleviating soil and water contamination caused by heavy metals. Microorganisms possess the capacity to decompose intricate substances into simpler chemicals, thereby offering a long-lasting solution for the mitigation of heavy metal contamination [63,66]. Recent advances in the field of genetic engineering have facilitated the creation of genetically engineered microorganisms or biocatalysts, which have been demonstrated to outperform natural microbes in terms of their aptitude for eliminating persistent compounds from natural surroundings [135]. Various genetic and metabolic engineering strategies have been utilized to produce highly efficient engineered microorganisms. These strategies consist of single-gene editing and pathway construction, as well as modifications of both coding and controlling sequences of pre-existing genes [136]. The central emphasis of the aforementioned adjustments is primarily on the stages that restrict the rate of metabolic processes. It is noteworthy that the employment of genetically modified bacteria has facilitated the

efficient elimination of heavy metals, including but not limited to Cd, As, Cu, Hg, and Ni [137]. However, it is crucial to acknowledge that the speed at which degradation takes place is contingent upon the catalytic efficacy of enzymes that are either present within the cells or have been stimulated to act on a specific substrate [6].

The utilization of recombinant DNA technology, a genetic engineering tool, has been implemented to amplify the genome of genetically engineered microorganisms (GEMs) by integrating foreign genes from other species [138]. The development of genetically engineered metabolic pathways in microbial cells through bioremediation processes has been enhanced as a result. The advanced technological nature of GEMs has attracted public attention [139]. The incorporation of metal regulatory genes in bacteria has facilitated the conversion of heavy metals from toxic forms to less toxic ones [140]. Additionally, heavy metal accumulation has been observed to increase in GEMs that express metallothioneins (MT) [141]. Azad et al. [142] conducted a comprehensive evaluation of the application of genetically modified bacteria and plants in the bioremediation of heavy metals and other organic pollutant-contaminated environments [142]. Furthermore, Nascimento and Chartone-Souza [143] have demonstrated that the integration of the mer operon from bacterial resistance to mercury, which encodes for the reduction of Hg^{2+}, into the genetically modified bacterium affords the mer genes the capacity to mitigate Hg pollution at high temperatures [143].

The genes that confer resistance to heavy metals, namely alkB, alkB1, alkB2, alkM, xylE, nidA, and ndoB, have been observed to be harbored on plasmids within n-alkane-degrading microorganisms. These genes, which have been frequently employed as markers for identifying microbial biodegradation, are known to undergo horizontal transfer [144].

Within the realm of environmental bioremediation, the genetic modification of microbial membrane transporters serves as a significant means of enhancing the removal of hazardous metals [145]. The crucial roles of transporters and binding mechanisms in the remediation of such metals cannot be overstated. Typically, transporter systems are categorized into three principal categories, namely channels, secondary carriers, and primary active transporters. Channel transporters, which are a subset of the aforementioned categories, are defined by their ability to facilitate the passage of molecules across the inner lipid membrane [62,146]. Examples of channel transporters include Fps, Mer T/P, and GlpF. Additionally, secondary carriers, such as Hxt7, NixA, and Pho84, can be found in the inner lipid membrane. Finally, primary active transporters, including cdtB/Ip_3327, MntA, TcHMA3, and CopA, are also situated in the inner lipid membrane. It is noteworthy that certain transporters, such as the porin channels, may also be found in the outer lipid membrane [66,147]. This is an important consideration to keep in mind when studying the intricate mechanisms of transporter systems. Once hazardous metals infiltrate the cell, a myriad of phytochelatins, metallothioneins, and polyphosphates work in harmony to sequester the metals [148]. Altering the hazardous metal import–storage systems of microorganisms could potentially augment their proficiency in extracting hazardous metals from water and soil. In the ongoing battle against the deleterious compounds that pervade the environment, it has become increasingly apparent that the utilization of genetically engineered microorganisms constitutes an indispensable tool in expediting the restoration process [149]. Such organisms, endowed with the capacity to efficiently degrade and neutralize the toxic substances present in the environment, have demonstrated their efficacy in mitigating the deleterious impact of these compounds on the ecosystem [150].

Recent research has brought to light recently discovered genetic elements that play a crucial role in the degradation of a broad spectrum of hazardous material contaminants. The elucidation of these genes was made possible through the application of high-throughput and next-generation sequencing techniques [151]. In addition, the advent of innovative technologies such as CRISPR-Cas gene-editing tools has provided an avenue for the optimization of bioremediation. By employing CRISPR technology, heavy metal remediation genes can be transported to plants or bacteria and facilitate heavy metal detoxification. Metallothioneins and phytochelatins can be synthesized more efficiently with CRISPR-

mediated gene expression [152]. The use of CRISPR-Cas9 gene-editing technology has been demonstrated in numerous studies to enhance metal remediation by transferring certain plant and bacterial genes. In Arabidopsis and tobacco plants, the NAS1 gene (encoding the nicotianamine (NA) synthase enzyme) has been shown to improve tolerance to harmful metals, such as Cu, Cd, Ni, Mn, Fe, Mn, and Zn [153]. By genetically modifying microorganisms with genes that are intimately involved in the breakdown of particularly stubborn substances, the bioremediation process can be vastly improved [152,154].

4. Technology Challenges and Future Prospects

The process of remediating heavy metals presents a significant challenge that requires a considerable amount of time and effort [155]. Furthermore, one of the primary concerns is the successful implementation of microbial-based heavy metal removal techniques at a commercial level, specifically in water treatment plants. There exist diverse strategies that can be employed to enhance the removal of heavy metals from aqueous media. These include the manipulation of the immobilization of bacterial strains onto solid substrates and the utilization of composite nanomaterials or biopolymers. Moreover, genetically engineered microorganisms have better heavy metal removal potential in comparison to wild-type microorganisms. It is better to scale up the implementation of these microbes into wastewater treatment plants. It is noteworthy to mention that biological remediation methods have the potential to eliminate other toxic metal ions, including Ni, V, Hg, and Ti, which can be further explored in future research. Moreover, there is a need to implement CRISPR-mediated gene expression in the microbes and plants used in the heavy metal remediation process and their scale-up for wastewater treatment plants. Growth media, optimum growth conditions, and culture maintenance are the major technological changes in microbial-based heavy metal removal.

In addition, phytoremediation is a good method for the removal of heavy metals from contaminated water but there needs to be further research on the safe disposal of the used plant. In addition, advanced technical assessment tools such as the life cycle assessment (LCA) and techno-economic analysis of large-scale wastewater treatment plants can be beneficial in developing cost-effective, energy-saving, and eco-friendly systems in the future. Furthermore, it is of great value to contemplate the molecular mechanisms of microbes underlying the elimination of heavy metals and conduct a comparative transcriptomic analysis in prospective research agendas. As a result, it is imperative to persistently investigate and devise novel strategies to enhance the efficacy of heavy metal elimination from water, all the while taking into account the long-term viability and ecological implications of the remediation process.

5. Conclusions

Various natural and industrial processes release heavy metals, which result in the contamination of both water and soil. The presence of heavy metal ions in these two mediums has detrimental effects on human and animal health. The toxicity of these metals has been linked to a wide range of health issues, including but not limited to liver and kidney damage, skin conditions, mental impairment, and even cancer. This highlights the severity of the matter at hand. Given the observed toxic effects of heavy metals, it is imperative to implement eco-friendly biological methods for their removal. The microbial-based techniques outlined in this review have the potential to effectively decontaminate both soil and water. These methods, including biosorption, bioaccumulation, and rhizoremediation, are deemed to be eco-friendly, efficient, and cost-effective. This study is expected to be particularly beneficial for the development of microbial-based heavy metal removal methods that can significantly contribute to the protection of human and environmental health.

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B.Z., T.Z. and F.W.; writing—review and editing, B.Z. and T.Z.; visualization, B.Z., T.Z. and F.W.; supervision, T.Z. All authors have read and agreed to the published version of the manuscript.

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