Review on Bioremediation as a Strategy to Remove Heavy Metals from Soil and Aqueous Solution

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The footprint of heavy metals resulting mainly from anthropogenic activity has had a hazardous impact on the environment and human health globally. Soil and water polluted by toxic metals adversely affect plant growth and eventually enter the food chain and affect human health. Physical and chemical approaches are commonly applied to remove heavy metals by transforming them into less or non-toxic forms. However, these methods are not economical or environmentally friendly and their effectiveness is questionable. Bioremediation is a biological approach that employs microbes or plants to remediate the accumulation of heavy metals in the environment. This method has significant benefits that can solve this issue in the long-term without requiring a lot of capital. Bacteria, algae and fungi are omnipresent in the environment and have a high adaptability and tolerance to high toxicity and concentrations of heavy metals. Phytoremediation employs plants to perform detoxification of heavy metals which are an ideal media due to their autotropic system and simple management. Additionally, combined bioremediation (applying two or more organisms) can optimize and enhance heavy metal removal by synergizing the metabolic capacity. This paper discusses and reviews the mechanisms, applications, and limitations of bioremediation for future reference and improvement. This study should be highly beneficial to researchers and practitioners that require information on the significance and development of bioremediation to remove heavy metals in the environment.

Keywords: Bioremediation; heavy metal treatment; microbial remediation; phytoremediation

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In the current era of industrialization and urbanization, the impact of heavy metals on the environment has escalated tremendously and raised concerns globally [1]. The adverse effects to the environment and human health are a serious threat due to the high toxicity and hazards, especially at levels exceeding safe limits [2]. Heavy metal pollution covers up to 20 million hectares of land globally [3]. In general, a heavy metal is an element with an atomic number higher than 23 and a density greater than 5 g cm^{-1} [4]. Heavy metals are inorganic compounds that may have negative impacts on human health, the ecosystem, water, soil, air and the food chain [5]. These include cancer in humans, inhibition of seed germination in plants, deactivation of enzymes in microorganisms, etc. The existence of heavy metals tends to be persistent within the ecosystem, and exposure is irreversible. Therefore, it is fair to say that the only allowable mitigation strategy is to degrade and transform these metal pollutants into less harmful substances, rather than completely eliminating them [6]. The severity of the effects are mainly dependent on the availability, dosage absorbed, route and duration of exposure [7]. The occurrence of these elements is somewhat inevitable. Heavy metals originate from natural phenomena like erosion, volcanic eruptions and weathering of minerals [8]. Figure 1 indicates that a major source of heavy metals is human anthropogenic activity. It is clear that the presence of heavy metals and its drawbacks have been known for decades. However, this issue has become a greater concern after urbanization due to the expansion of heavy metal applications in many industries around the globe [9].

Considering all the unfavourable factors and consequences, it is crucial to treat these environmental pollutants and contaminants. There are multiple methods to tackle this issue, including membrane filtration, chemical precipitation, reverse osmosis, and physical adsorption. Although these methods are quite promising, they often have limitations and constraints that make them less effective or costly [12-14]. Landfills are also one way to counter the abundance of heavy metal pollutants; however they also demand a high capital and require extensive monitoring [15]. (See Table 1). For that reason, many studies have used biological approaches by employing microorganisms to assist in minimizing the repercussions of heavy metals on the environment.

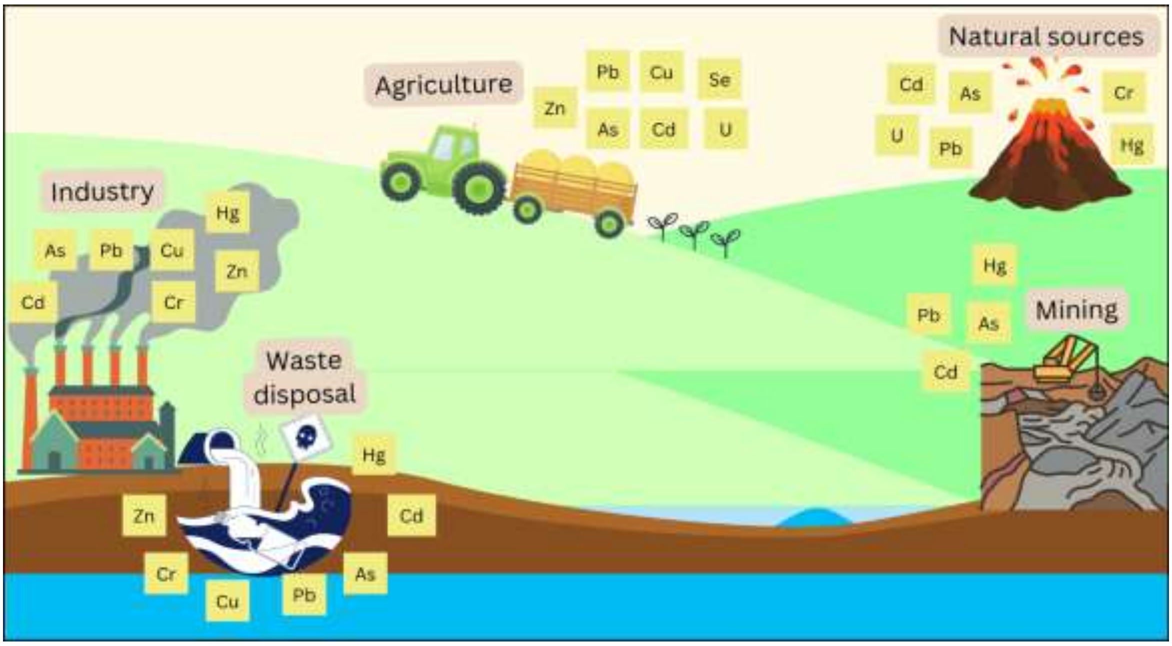

Figure 1. Provenance of heavy metals from natural sources and anthropogenic activities [6, 9-11].

Table 1. Typical methods for reducing heavy metals [9].

Method	Technique	Details	Type of heavy metals and removal efficiency	Limitations	Reference
Physical	1. Membrane separation	Separation of heavy metals by passing through a semipermeable membrane, attained by pressure differences, membrane pore size and the metal's molecular size.	Ni = 95 % Cu = 96 % Zn = 95 %	Not feasible at high temperatures.	[16-18]
	2. Adsorption	Binding between heavy metal ions and the adsorbent's surface to initiate a physical or chemical reaction. A large surface area of adsorbent is required.	Cu = 11.45 mg/g adsorbed Pb = 6.1 mg/g absorbed	Low selectivity of adsorbent.	[19-21]
Chemical	1. Electrolysis	Removal of heavy metals by redox reaction at the cathode and anode by introducing direct current into a cell containing heavy metal ions.	Mn = 48 % Fe = 91 % Zn = 99 %	High cost and low efficiency.	[22-24]
	2. Ion exchange	Exchange of heavy metal ions and ion exchange resins to deplete heavy metal contaminants in wastewater.	Pb = 23 mg/g removal capacity Zn = 50 mg/g removal capacity Cu = 270 mg/g removal capacity	Needs frequent replacement.	[25-27]
	3. Chemical precipitation	Assisted by the addition of chemicals to the polluted media which results in the formation of water-insoluble compounds from the dissolved heavy metals.	Ni, Cu, Cd, and Zn = 95 to 99 %	Hydroxide and sulfide precipitation may generate toxic fumes in acidic media unless paired with chelating agents or other elements.	[13, 27, 28]
Biological	1. Bio-remediation	Utilizes biological elements (plants, bacteria, fungi, algae) to detoxify heavy metals to less or non-toxic forms. Can be done *in situ* or *ex situ*.	Cu = 88 % Hg = 96 % Pb = 90 % Zn = 94 %	Optimum conditions and ideal biological elements are essential. Moreover, the intermediate product might be more harmful than the parent element.	[19, 29-33]

Bioremediation is one of the most viable techniques as it is efficient, eco-friendly and cost-effective, utilizing microorganisms or microbially-derived products to detoxify metals into less harmful substances or inactive forms [34]. Various factors play vital roles in the efficiency of bioremediation methods, including the type of microbe, pH, temperature, nutrient availability, biological factors and the presence of metal ions [35]. The significance behind bioremediation of heavy metals is that microbes and plants utilize heavy metals or other pollutants as their source of nutrients. Bioremediation can take place under two conditions: (1) *in situ*, where the treatment of heavy metal contamination is performed at the site where the metals are located, or (2) *ex situ*, where the contaminants are removed from their original location [15].

There have been extensive studies on the use of bioremediation to solve heavy metal issues in contaminated environments such as soil, plants, river-banks, and wastewater, both *in situ* and *ex situ*. However, a detailed evaluation of bioremediation applications including microbial and plant remediation along with their achievements and limitations is not yet available. Additionally, comparative studies of different types of bioremediations are a research gap that needs to be highlighted. This review aims to emphasize the significance of biological remediation and its ability to tackle heavy metal problems globally. The objectives of this paper are: (1) to highlight the limitations of conventional methods to eliminate heavy metals; (2) to address the factor of heavy metal toxicity; (3) to highlight the appalling effects of heavy metals on the environment and human health; (4) to discuss bioremediation approaches, specifically microbial and phytoremediation; and (5) to explore the findings of new studies in this area that are gaining attention.

1. Heavy Metal Toxicity Factors

Heavy metal contamination is a severe hazard to the environment, including riverbanks and soils. Heavy metals commonly comprise mercury (Hg), lead (Pb), arsenic (As), iron (Fe), cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), thallium (TI), chromium (Cr), strontium (Sr), manganese (Mn), etc., and can be classified into three categories, based on their toxicity. The first group, which can be toxic at low concentrations, consists of metals like Hg, Cd and Pb, while Ti and As belong to the second group which is less hazardous. Zn, Cu and Fe are the examples of essential metals that are only hazardous beyond certain limits [36]. The allowable concentration limit for each metal is governed by the World Health Organization (WHO), and these limits are displayed in Table 2. Naturally, equitable amounts of heavy metals like Zn, Fe and Cu are essential to plant growth and development, while other metals like Cd, Hg and Pb have no beneficial contribution to agriculture [37]. Based on statistics provided by the United States Environmental Protection Agency (EPA), the elements most toxic to the environment are As, Pb, Cd and Hg [38].

The toxicity effects of heavy metals can be influenced by several factors. In general, the toxicity of heavy metals may vary based on pH level, temperature, organic matter content and nutrient deficiency. Heavy metals may impact more severely under acidic conditions (lower pH levels) compared to basic conditions of the medium. This is due to the fact that the bioavailability and solubility of heavy metals vary with the pH of the medium. For instance, at low pH levels, there are a higher number of protons available to saturate metal binding sites due to the free ionic species formed from heavy metals.

Table 2. Allowable limits for each heavy metal as given by the WHO [10], and its effects on human health [19].

Metal	Allowable limit of heavy metal in water and soil (mg/L)	Effects on human health
Cu	2.0	Irritation, headache, stomach ache, dizziness, diarrhoea, and organ failure, including liver and kidney.
Zn	3.0	Often associated with “metal fume fever” in welders exposed to Zn.
Cd	0.003	Carcinogenic to the breast, liver, gastric, colon, lungs, testicles, gall bladder, blood, pancreas and brain.
Cr	0.05	May indirectly cause cancer and inflammatory skin diseases.
As	0.01	Nausea, abdominal pain, fatigue, diarrhoea, carcinogenic effects, organ failure, skin hyperpigmentation, reproductive difficulties, neurological complications.
Hg	0.001	Is corrosive; can cause skin diseases like dermatitis, and severe muscle pain.
Ni	0.02	Carcinogenic effects, difficulty in breathing causing bronchitis and pneumonia.
Pb	0.01	Diarrhoea, abnormal pain in muscles and joints, anaemia

This leads to the increased bioavailability of heavy metals, thus increasing environmental toxicity. On the other hand, at higher pH values or alkaline conditions, metal ions tend to replace protons to form other species like hydroxo-metal complexes [39, 40]. Based on a recent study, a comparison of the uptake of Mn, Cu and Zn in the ground and root parts of the soil under three different pH conditions were observed. It was found that the concentrations of Mn, Cu and Zn at pH 4.7 were higher than at pH 6 and pH 8.5 in both the ground and root parts of the basil plant [41]. The solubility of these complexes varies depending on the type of heavy metal. The Cd, Ni and Zn complexes are soluble in water, but the Cr and Fe complexes are not [42, 43]. Moreover, in bodies of water, the solubility of the metal depends solely on the pH of the water. Subsequent to the release of heavy metals into water, the acidity of the water increases before the deposition of heavy metal precipitates on the bed [44].

The toxicity of heavy metals may also be altered by soil composition, such as organic matter content. Soil that contains low organic matter content commonly tends to absorb, accumulate, and precipitate the heavy metal, resulting in elevated toxicity. Organic matter plays a vital role in the cation exchange capacity, buffer capacity and retention of heavy metals. This implies that heavy metals in organic soils will have lower toxicity than metals in mineral soils without organic compounds [45]. Further, contamination of heavy metals in soil affects soil enzymes which are responsible to nurture soil ecology and health [46].

In addition, toxicity of heavy metals is also highly dependent on the temperature of the media (i.e., soil). The rate of adsorbate diffusion increases heavy metal adsorption, especially at elevated temperatures [47]. Thermal stress from climate change may also amplify the toxicity of heavy metals to freshwater organisms, as discussed in detail by Nin and Rodgher [48]. A comprehensive review has been done to analyse the effect of temperature on the toxicity of heavy metals in freshwater containing aquatic organisms. It was established that both temperature increases and the presence of pollutants are viewed as stressors that affect aquatic organisms. Therefore, when both factors are applied to the species simultaneously, it causes negative impacts including physiological damage in fish, a decline in the diversity of plankton, and fatalities [48]. A study by Khan et al. reported that an increase in temperature from 20 °C to 27 °C resulted in an increase of 7 to 12 % in the toxicity of Zn, Cu and Cd to crayfish [49]. Further, some researchers have reported that the interactions between temperature, heavy metals and organisms are subjective and complex to analyse because in some cases, a temperature rise can promote the degradation of metals, thus reducing their toxicity, which is contradictory to the results of earlier literature [50]. One study utilized SEM (Structural Equation Modelling) to form a hypothesis for the correlation between a few influential factors. The results indicated that temperature had adverse effects on soils containing calcium. Also, soils with Ca content had a large effect on soil pH. Finally, the toxicity and bioavailability of heavy metals were influenced by both soil Ca content and soil pH [51].

2. Impact of Heavy Metals

The accretion of heavy metals globally has caused tremendous hazardous effects on the environment and human health. The effects are alarming as the extensive amount of heavy metal pollutants have resulted in reduced soil quality, and caused fatalities in plants growth, chronic diseases and even human fatalities. These negative impacts are thoroughly explained in the next section to strongly emphasize the importance of remediation efforts to eliminate these pollutants.

2.1. Impact of Heavy Metals on the Environment

Researchers have opined that waste is the dominant source of heavy metals contaminating the environment. The implications are worse for developing countries as their waste management technologies may not be up to par. However, developed countries are also affected due to the rapid and advanced technologies for urbanization and industrial applications that release more effluent from its wastewater and solid waste treatments [52]. Polluted soils with high concentrations of heavy metals are often related to deficiencies in nutrients and necessary organic matter, low water retention capacity and limited cation exchange ability. Generally, these persistent toxic contaminants indirectly attack soil biota by disrupting microbial processes, decreasing microorganism numbers and activity, and limiting soil enzyme activity [53]. Naturally, the ecosystem of the soil detects foreign elements like heavy metals as a threat which causes physical stress.

There are a number of studies that have observed how Cd and Hg in soils have impacted the metabolic functions of organisms responsible for soil fertility, structure, drainage, and aeration [54-56]. Cd is well known for its high mobility that results in its fast translocation from soil to groundwater [55]. According to a study by Wen et al., Cd levels in soil at a mining area in Jinding, China, have exceeded 531 mg/kg, which is highly toxic [57]. On the other hand, Zn inhibits soil activity by reducing the macronutrients needed by soils like phosphorus [52]. However, in the right ratio, Zn and phosphorus are able to mutually synergize and maintain soil as a source of nutrients [58]. Excessive amounts of Pb in the soil cause numerous problems such as lowering soil productivity [59], obstructing the activity of soil enzymes, including urease, dehydrogenase and phosphatase, that act as indicators of soil biological function [60], and causing anomalies in metabolic functions of organisms within the soil that affect plant growth [61]. The uptake of up to 2,100 mg/kg of Pb by tea plants resulted in yellowed and wilted leaves with smaller buds after 3 years of exposure. However, the plants did not die due to their high tolerance towards Pb [62]. Soil pollution resulting from heavy metals are of concern to the agricultural industry globally. This is because the quality and health of plants and crop yields have changed drastically due to the effects of heavy metals on the soil. Thus, there is a high likelihood of heavy metals contaminating the food chain and consequently threatening food safety and human health [63]. Generally, different heavy metals have different impacts on plants.

The accumulation of Cd in plants results in unwanted phenomena including a decline in photo-synthesis and reduction in uptake of water and nutrients. This leads to inhibition of plant growth and hence, death. [64]. In plants, Cd toxicity effects usually consist of the depletion of chlorophyll content, the development of oxidative stress in ROS and is the cause of plant membrane damage [37]. Moreover, the occurrence of Zn within the soil may result in phytotoxicity, which delays germination and causes stunted growth in plants [65]. Zn pollution may affect the development of the shoots and roots of plants and promote chlorosis, which is when plants or leaves produce inadequate chlorophyll and causes yellowing [34]. The accumulation of Cu that is predominantly sourced from the mining industry and sewage sludge stimulates soil stress, resulting in plant damage. This hinders plant growth and causes chlorosis [66].

Apart from soil and plants, water is also greatly affected by heavy metal toxicity. Heavy metals enter lakes, rivers, groundwater, and numerous water sources that subsequently impact living organisms, such as aquatic organisms that are infected by their surroundings and humans that are infected through their consumption of water. The intake of heavy metals from polluted water by the human body is described in Figure 2. Typically, heavy metals that enter the water will penetrate and disperse through the water column, precipitate in sediments, or be absorbed by aggregation [67]. Heavy metals have a high flexibility that contributes to their elementary bioaccumulation in aquatic creatures [68]. Heavy metals cause vigorous neurotoxins in fish that can be deadly. They are introduced into fish either through their gills, body surface or digestive tract. The communication of fish with their surroundings may be inhibited due to the restrictions resulting from the reaction between toxic metals and biochemical inducement. One of the common unwanted diseases that has spread to aquaculture is Minamata disease which was caused by heavy metal pollution due to organic mercury poisoning [68]. Further, although the growth of fish larvae is usually rapid, the growth of newly hatched fish is somewhat hindered due to the high levels of Pb [69]. In an experiment, 100 ppb of Pb was exposed to fertilized zebrafish eggs and significant abnormal growth was reported [70]. The reproductive system of fish can be affected by the accumulation of Cu in terms of fertility and embryo maturity [71]. Zn and Cr also caused negative impacts on fish by damaging their gills and respiration that consequently resulted in hypoxia [72]. Moreover, abnormalities may occur in fish at high concentrations of Cd, Ni and Hg. It was observed that underdeveloped organs, e.g., the liver and gills, shortened fins and damaged fin function are typical abnormalities caused by heavy metal toxicity [73]. The aquatic environment has been found to be polluted with high levels of Cd due to a number of sources including absorption, industrial waste, and surface runoff through soil and sediments [74]. The impacts of other metals on human health are listed in Table 2.

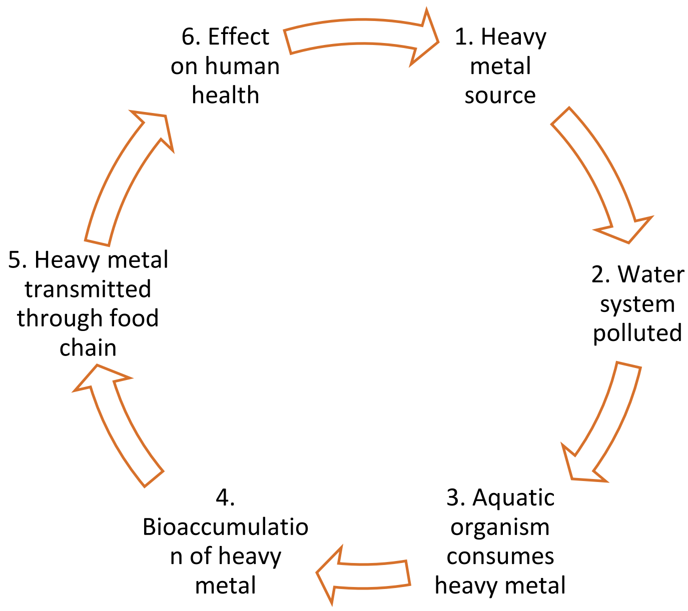

Figure 2. Intake of heavy metals through the food chain [68].

2.2. Impact of Heavy Metals on Human Health

Heavy metal exposure in humans is irreversible, hence prevention is advised at all costs. Heavy metals can have both acute and chronic detrimental effects on human health [75]. The toxicity of heavy metals can cause cancer, problems in the kidneys and other organs, immune system breakdown, birth defects and nervous system failure [76]. The severity of these effects may be influenced by two factors: (1) the amount of heavy metals (exposure to two or more heavy metals at the same time leads to a higher risk) and (2) the duration of exposure (long exposure will cause greater accumulation of heavy metals) [77]. The accumulation of heavy metals in the food chain that can affect human health may be caused by biomagnification, also known as "nature's karma" (Figure 3) [78]. Additionally, heavy metals can be considered as human carcinogens. Examples of heavy metals that have a high risk of causing cancer when exposed beyond their allowable limits are Pb and Cr [79].

As has been identified as the "King of Poisons" due to its toxicity. In general, As^{3+} imparts a higher toxicity than As^{5+} due to its high mobility across the cell membrane [80]. The main route of exposure of As is through digestion and in the small intestines. However, inhalation and skin contact are also possible paths for As exposure [75]. The source of As varies by location. For instance, in Brazil, As contamination is commonly linked to the mining industry and usage of wood preservatives like chromate, copper, and arsenic, while in countries like Bangladesh, As can be present naturally in drinking water [77]. According to the WHO, the maximum safe limit of As in drinking water is 10 μg/L [81]. Acute effects of As include nausea, abdominal pain, fatigue and diarrhoea. As also causes serious chronic effects like cancer, organ failure, skin hyperpigmentation, reproductive difficulties and neurological complications [82]. Oxidative stress caused by the effects of As may lead to type 2 diabetes [83]. In addition, As poisoning is commonly related to darkening of the skin, which is a known symptom of As contamination in the blood that often leads to death. This usually occurs due to the rupture of blood vessels because of the accumulation of blood within the interstitial space [77].

Pb commonly exists in small amounts in the environment and is mostly released through the production, consumption and disposal of batteries, paints, and electronics. The abundance of Pb has increased tremendously due to several industries including mining, manufacturing and burning of fossil fuels [84]. The potential threat of Pb to human health is correlated with the initial concentration of Pb within the soil that can have negative effects depending on soil properties such as pH, phosphorus and organic matter content, cation exchange ability and texture [85]. Exposure to Pb beyond hazardous limits can be poisonous and exert harmful effects on human organs which cause problems like severe diarrhoea and abdominal pain [86]. Smokers often have elevated levels of Pb within their system, which indicate a high risk of cancer [87].

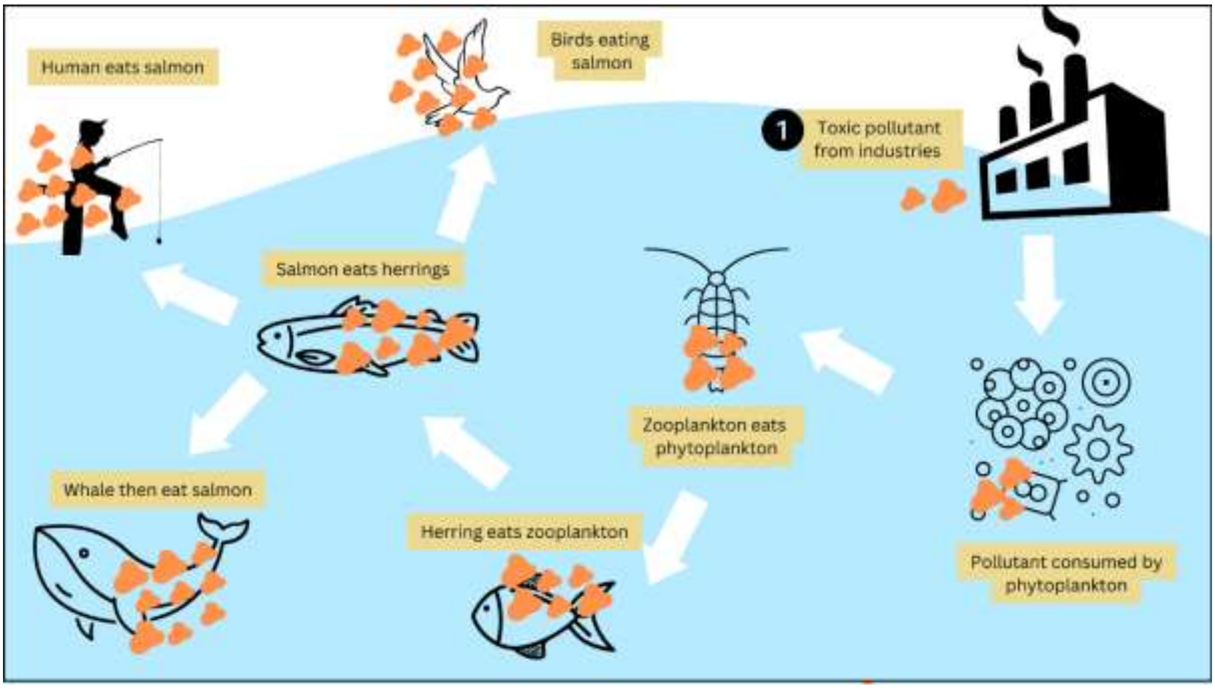

Figure 3. Representation of how biomagnification occurs and its pathway to humans.

In humans, the toxic effects of Cd are exhibited in the kidneys, liver, and bones, as well as in the resistance towards the absorption of calcium to the body [88]. Humans are mainly exposed to Cd by inhalation, smoking and the consumption of contaminated food and water. There are numerous studies that have reported excessive levels of Cd in landfills [89]. Therefore, it can be presumed that in general, Cd enters the human body through the consumption of contaminated food. Chronic illnesses like cancer are regularly associated with exposure to Cd at high concentrations. These cancers can occur in the breast, liver, gastric, colon, lung, testicles, gallstones, blood, pancreas, and brain [46, 90]. Cr occurs in the environment in two different oxidation states that have different properties and effects: trivalent chromium (Cr III) and hexavalent chromium (Cr VI). Trivalent chromium (Cr III) is an essential nutrient for humans and is safe and eco-friendly, even at elevated concentrations. On the other hand, hexavalent chromium (Cr VI) is toxic, and carcinogenic [91]. Alidadi et al. reported on heavy metals in the drinking water in Iran, and found that a hazard index of up to 71 % leads to hazardous carcinogenic effects [92].

3. Bioremediation Approach

Upon evaluating the dangerous impact of heavy metals on the environment and human beings, efforts to abate these toxic pollutants are increasingly necessary. Heavy metal exposure from various sources have substantial effects on soil quality, crop yield productivity and indirectly disrupts the food chain, which consequently affects human health. Many researchers have discovered that by implementing bioremediation, heavy metals can be decontaminated efficiently, without jeopardizing the environment or investing too much capital, significant advantages when compared to the conventional method of remediation. Bioremediation is defined as a technique or strategy that employs biological organisms to convert toxic pollutants such as heavy metals into less toxic forms and degrade organic substances into carbon dioxide, water, nitrogen gas etc. [93] The notable mechanisms used in bioremediation that eliminate heavy metals are precipitation, biosorption by sequestration to intracellular metal binding proteins and conversion of toxic metals to less or non-toxic forms [94].

Although both *in situ* and *ex situ* bioremediation processes have their respective advantages, certain factors should be taken into consideration before selecting the best modus operandi. *In situ* techniques can be implemented to utilize the existing indigenous organisms or by introducing new engineered micro-organisms to stimulate the bioremediation process without disrupting the structure of the soil. Soil features like soil type and geochemistry along with site location and degree of contaminants have a great influence on bioremediation activity [95]. The limitations of the *in situ* method are uncertain uniformity and difficulty in corroborating treatment effectiveness [96]. On the other hand, an *ex situ* method is more complex and requires excavation to transport the contaminated medium to the treatment site. The process is similar to an *in situ* method, but the additional factor of cost needs to be taken into account. The reason is because *ex situ* methods are very expensive as they require a shorter time and are easier to control [96]. Predominantly, microbial remediation and phytoremediation are two primary approaches for bioremediation strategies [97].

The challenges faced by bioremediation that limit its effectiveness include insufficient nutrients, competitiveness between microorganisms and in-consistent efficacy, which can be influenced by the type and concentration of the metal pollutant [98]. In the next part of this paper, microbial remediation, phytoremediation and combined bioremediation will be discussed in terms of their applications and limitations.

3.1. Microbial Bioremediation

Microbial bioremediation generally takes advantage of the properties and mechanisms of living micro-organisms to diminish the concentration of heavy metals and their hazardous effects. Commonly, microbes including bacteria, fungi and algae are appointed as biological agents of bioremediation. The utilization of microbes have interested researchers recently, but applications have not been well developed [34]. Generally, microbes are employed to break down heavy metals into less toxic forms using enzymes. According to a review by Alvarez et al., microbes commonly have two methods of defence, producing enzymes or defying pollutants [99]. In addition, Sharma reported that microbes have a significant characteristic that contributes to the process of removing toxic pollutants, which is their high level of adaptability. This is because microbes can grow at extreme high and low temperatures with the aid of carbon to facilitate its microbial activity [29].

There is also significant comparison between anaerobic and aerobic bioremediation. Generally, anaerobic bioremediation is the condition in which oxygen is deficient upon applying microbes to remove heavy metals. These microbes usually utilize other molecules to survive and hence, break down the contaminant. Most anaerobic bioremediation takes place *in situ* rather than *ex situ*. Microbes that are able to live and survive in the absence of oxygen have potential advantages here [29]. On the other hand, aerobic bioremediation requires oxygen to transform heavy metals into less toxic forms. According to a previous study, the rate of bioremediation in aerobic conditions was higher than in anaerobic conditions when tested on two polluted crude oil samples with *Pseudomonas aeruginosa*. Anaerobic bacteria reduced the biological oxygen demand (BOD) of the polluted site up to 95.9 %, while the result was 99.8 % for aerobic bacteria [100]. For heavy metal removal, aerobic bioremediation gave slightly better results than anaerobic. This is because anaerobic bioremediation is usually used for highly halogenated contaminants [101].

There are several factors that may greatly influence the effectiveness of microbial bioremediation on heavy metals in the environment [102, 103]. These include:

(1) temperature – affects microorganisms' metabolism and survival rate. The value should be within the optimum range of 20 °C to 30 °C.

(2) humidity or surrounding properties – influences toxicity effects and remediation rate

(3) nutrient supplementation – ensures growth of microorganisms

(4) characteristics of the polluted site – includes pH, redox potential

(5) concentration of the heavy metal – relates to the bioavailability of the pollutant

Microbial bioremediation necessitates three basic components that are directly interconnected: microorganisms, food and the environment. This is known as the bioremediation triangle, as depicted in Figure 4. The effectiveness of bioremediation can be optimized by promoting the synergy and interaction between these elements [104]. Elevated quantities of toxic metals may disrupt the growth of microbes and result in the microorganisms' demise. Therefore, optimum condition and factors are crucial to guarantee the growth and development of bacteria to ensure successful heavy metal removal. It is important to properly handle and manage the bioremediation process to make sure the expulsion of heavy metals within the environment is accomplished [103]. The dominant microbial bioremediation strategies that been practiced globally are bioaugmentation and biostimulation. Mainly, bioaugmentation enhances the degradation of pollutants by incorporating microbes that are cultured externally to support the original microbes within the site. This method facilitates in stimulating bioremediation because typically the native microbes at the site are insufficient to counter the pollutants effectively. On the contrary, biostimulation also promotes contaminant degradation by adding nutrients to the site that assist in the growth and survival of the microbes and hence, the successful removal of the pollutants [105].

The presence of extra-cellular polymeric substances (EPS) such as polysaccharides, proteins, and lipids in a biomass enables the microorganism to undergo proton exchange or micro-precipitation of heavy metals that assist in the degradation. Other mechanisms like redox processes, adsorption, complexation, and electrostatic attraction also help microorganisms to detoxify such metals. The transformation of insoluble and stationary forms of toxic metals to soluble and mobile phases is one of the methods used by microbes to perform bioremediation [93]. Some examples are: 1. Hg(II) is converted to Hg(0) that is more volatile [106]; 2. Fe(III) is reduced to Fe(II) [107]; 3. As(V) is reduced to As(III) [108].

To truly understand microbial bioremediation, the application of bacteria, fungi and algae in this approach are explored in the next section. Previous studies involving microbial bioremediation are listed in Table 3.

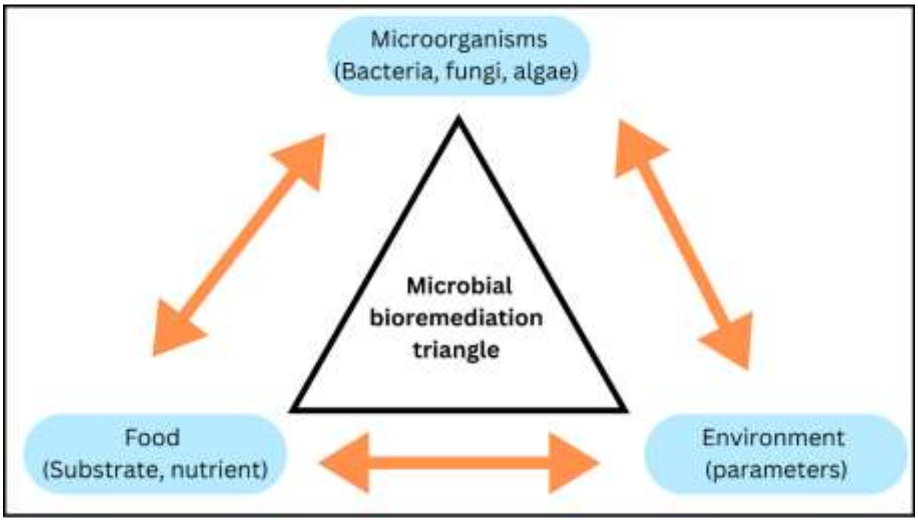

Figure 4. Microbial bioremediation triangle [104].

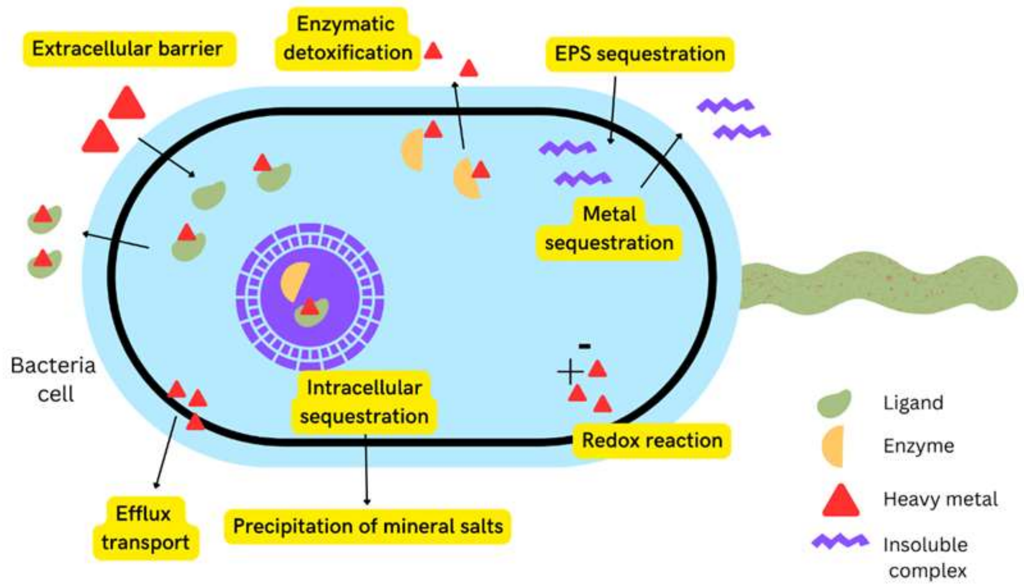

Figure 5. Mechanisms employed by bacteria in removing heavy metals [113-115].

3.1.1. Bacteria

Bacteria are classified based on their common shapes, spherical (cocci), rod (bacilli) and spiral (spirilla) [109]. The most prevalent organisms used in microbial bioremediation for heavy metals are bacteria. This is mainly due to the attributes of bacteria which include the participation of metals in the metabolic processes of bacteria, the adaptability of bacteria that are able to survive and grow under extreme conditions, and the ubiquitousness of bacteria in nature [110]. There are a number of procedures used by bacteria to endure the toxicity of heavy metals: biotransformation, extrusion, enzyme application, generation of exopolysaccharides (EPS) and metallothioneins [111]. The initial physical contact between heavy metal ions and a bacterial biomass takes place at the bacteria cell wall. Anionic functional groups in bacteria complement the metal-binding capability on the cell wall by creating an overall negative charge. Examples of functional groups are amine, hydroxyl, and phosphate, which occur in both Gram-positive and Gram-negative bacteria [93]. It is crucial to have a good comprehension of the mechanisms involved to ensure accurate selection of bacteria type. A contaminated medium with one type of heavy metal is easier and simpler to remediate by bacteria compared to a medium with multiple heavy metals. Optimizing physiochemical parameters within the environment can also contribute to better bio-remediation results [112].

In bacteria, there are five major mechanisms of resistance to heavy metals: (1) Extracellular barriers that act as first prevention or resistance for the entrance of metal ions to the cell; (2) Efflux, where the toxic metal is transported from the cytoplasm by the aid of existing proteins; (3) EPS sequestration, the accumulation of metal ions by cell elements at the outer membrane or the complexation process of heavy metals ions; (4) Intracellular sequestration: accumulation of heavy metals in non-bioavailable forms like metallothionein within the cytoplasm, (5) Redox reaction of metal ions and (6) Enzymatic detoxification [113-115] (See Figure 5). The uptake of heavy metals by bacterial cells takes place through two different processes. The first ensures fast transportation of heavy metals into the cells by implementing an ATP-independent mechanism. ATP, which is a high-energy phosphate bond (~P), is the primary chemical energy source. The mechanism engages in the synergy of secondary active transport with the concentration difference in protons across the inner membrane, which is called the chemiosmotic gradient, to enhance metal uptake by bacteria. The second process is an ATP-dependent mechanism that is relatively slower compared to the first [110].

Oziegbe et al. has analyzed the potential of *Pseudomonas aeruginosa*, *Klebsiella edwardsii* and *Enterobacter cloacae* that were isolated from landfills, to remediate heavy metals by applying an *ex situ* method. It was found that *Pseudomonas aeruginosa* exhibited the highest removal result, at 58 % in 50 mg of Cd contamination. It was also reported that enhancement of the process could be made by altering the pH to 6 and adding more carbon sources (i.e., peptone) which could increase the remediation process up to 92.4 % by *Klebsiella edwardsii* genus [116]. The removal of Cd and Pb by applying *Lactobacillus plantarum* MF042018 are disregarded at pH levels greater than 5 and temperatures over 30 °C. It was found that Pb remediation by the selected bacteria were concentration-dependent. A higher metal removal efficiency was recorded at 10 ppm of Pb compared to 50 ppm, due to the limitation in metal absorption caused by oversaturation of the adsorption site and inadequate free binding sites [117]. In addition, Touahir et al. investigated the resistance of 118 bacteria species isolated from coastal waters to Zn, Cu, Hg, Pb and Cd. The total resistance value was calculated by comparing the number of bacteria growing in media with and without heavy metals [118]. Thermophilic *Bacillus cereus* exhibited significant tolerance and bioaccumulation of heavy metals. It was discovered that B. cereus had a higher tolerance to Mn and Cu in a solid medium compared to a liquid medium. There was good growth of bacteria at Mn and Cu concentrations of 2.5 mg/L but this may change at higher concentrations [119].

The drawbacks of using bacteria for heavy metal remediation are the slow process and long time period required to complete the removal operation. This is because bacteria require time to adjust and adapt to a new environment. There is also a possibility that the biodegradation product may be more toxic than the native compound, due to the likelihood of bacteria to magnify toxicity. Unpredictability and irregularity are also some of the disadvantages encountered when utilizing bacteria. In addition, the performance of bacteria in remediating the pollutant might be impossible to evaluate when there is no acceptable endpoint [120, 121].

Table 3. Previous studies of microbial remediation approaches to heavy metals pollutants and their performance.

Type of microbe	Microorganism	Type of HM reduced	Reduction efficiency	Reference
Bacteria	*Acidiphilium cryptum*	Cu, Zn, Cd, Hg, Pb, Ni, Cr	35-45 % with enhancement by addition of glucose.	[122]
	Pantoea. agglomerans	Pb, Fe, Cu	Pb and Fe is reduced by 99 %, and Cu by 60 % within 8 hours contact time.	[123]
	Acinetobacter junii	Cr	Removed a total of 83 % of Cr and reduced 98 % of Cr^{6+} to Cr^{3+} at 10 ppm Cr concentration.	[124]
	Bacillus cereus	1. Cu, Cr, Pb, Zn, Fe, Mn, Mg (Batch culture method – *ex-situ*)	91 %, 79 %, 97 %, 77 %, 81 %, 62 %, and 60 % of heavy metals were removed, respectively.	[125]
		2. Pb, Cu, Cr (*in-situ*)	71 %, 53 %, and 41 % of heavy metals were removed, respectively.	
	Sporosarcina pasteurii	Zn	70% removed in contaminated solution.	[126]
	Pseudomonas monteilii and *Bacillus infantis*	As	Oxidized As3+ to As5+ by 92 % and 96 % respectively.	[127]
Fungi	*Aspergillus penicillioides*	Pb	73 % optimized bio-adsorption at pH 8.85, 30 °C and 5.7 hours by using an extracellular polymeric substance (EPS).	[128]
	Aspergillus sp.	Fe, Co	Able to remove 100 % of a 500 mg/L concentration of Fe.	[129]
	Sterigmatomyces halophilus	Cd, Cu, Fe, Mn, Pb, Zn	90 %, 82 %, 70 %, 78 %, 48 %, 82 % removal, respectively. Potato broth was used for promoting growth of fungi.	[130]
	Penicillium simplicissimum	Cu	225 mmol/kg was bio-sorbed from 250 mg/L of heavy metal contamination.	[131]
	Beauveria bassiana	Cu, Ni, Cd, Zn, Cr	74 %, 75 %, 63 %, 67 % and 61 % of metals were removed, respectively.	[132]
Algae	*Chlorella sorokiniana*	Cd	Removed 65 % and accumulated up to 11,000 mg/kg of Cd.	[133]
	Parachlorella kessleri	Cd and Cr	Cd and Cr accumulation of 89 % and 96 % in 192 hours of incubation.	[134]
	Chlorella coloniales	Cr, Cd, Co, Fe, As	Response surface methodology (RSM) was used. 96 - 98 % of heavy metals were removed.	[135]
	Chlorella vulgaris	Fe	Removed 91 % of Fe from a contaminated river. However, the value was still above WHO-recommended limits	[136]

3.1.2. Fungi

Filamentous fungi are examples of typical saprophytic microorganisms and eukaryotic organisms that act as decomposers and are important in nutrient cycling. Mainly, fungi are characterized into different classes, which include Ascomycota, Zygomycota, Basidiomycota and Deuteromycetes. The common types of fungi that contribute to bioremediation efficacy are Ascomycota and Basidiomycota, which consist of fungal strains that have great degradation capabilities [137]. Both living or dead cells of fungi can be applied as candidates for bioremediation to remove heavy metals, a process typically known as mycoremediation. The high adaptability of fungi to grow and survive in extreme conditions involving high temperatures, inconsistent pH and unavailability of nutrients, has attracted researchers to explore its potential in bioremediation [138]. The tolerance of fungi to heavy metals may vary according to the sites of isolation as indigenous fungi have greater tolerance compared to non-indigenous fungi [139].

There are a few alterations and adjustments that can be made to promote the adsorption capacity of fungi towards heavy metals. These modifications mainly facilitate the degradation process by increasing the surface area and enhancing the amount of cationic groups [140]. The modifications include heat treatment (removal of moisture), acid treatment (replacement of cations) and alkali treatment (protection of cell stability) [138]. Fungal bioremediation starts with biosorption which initiates the entrapment of metal ions in the cell wall. The distinctive factor of this process is the rigid cell wall of fungi along with the presence of glycoproteins and polysaccharides, which is promoted by the formation of functional groups that absorb the toxic pollutants [137]. Upon biosorption, a few mechanisms such as bioaccumulation, ion exchange, complexation, and precipitation are believed to occur during the binding of metal ions to the fungi. In the complexation and precipitation process, a few components are formed by fungi such as organic acids, polymers and anions like sulfides and phosphates that promote the development of insoluble metal complexes [138]. These mechanisms are as displayed in Figure 6.

According to a study carried out by Talukdar et. al., it was found that at pH 5 and an incubation time of 120 hours, Cd and Cr were successfully removed at a maximum of 72 % and 68 % respectively. *Aspergillus fumigatus* and *Aspergillus flavus*, from the Ascomycetes division, were isolated from the heavy metal contaminated site as the potential agents to remove 987 mg/L of Cr and 162.71 mg/L of Cd [141]. *Rhizopus stolonifera*, under the Zygomycetes division, was found to effectively eliminate Pb by 44 % after 96 hours. It was reported that this species was also able to reduce Ni metal contamination by 16 % [142]. By comparison, it can be deduced that the *Aspergillus* species might have a higher tolerance and adaptability to heavy metals compared to the latter. Das et. al. published a study on the utilization of *Alternaria alternata* as manglicolous fungi from a mangrove forest in India to tackle Pb and Cd pollution by modifying several conditions like pH, temperature and contact time to obtain the optimum outcome. It was indicated that for Pb, the optimum conditions were pH 6, contact time of 72 hours and 30 °C temperature to abolish by the fungi by up to 98.3 %. On the other hand, Cd removal was slightly less compared to Pb, as the maximum removal was 80 % under the same conditions as the former [143]. A study by El-Bondkly and El-Gendy analyzed the morphology differences of *Penicillium* sp. in the absence and presence of heavy metal Fe and Co. They demonstrated that the regular fungal shape of *Penicillium* sp. was severely deformed after the introduction of Fe and Co for remediation. However, the fungi successfully removed 100 % of the Fe and Co collected from various source of wastewater [129].

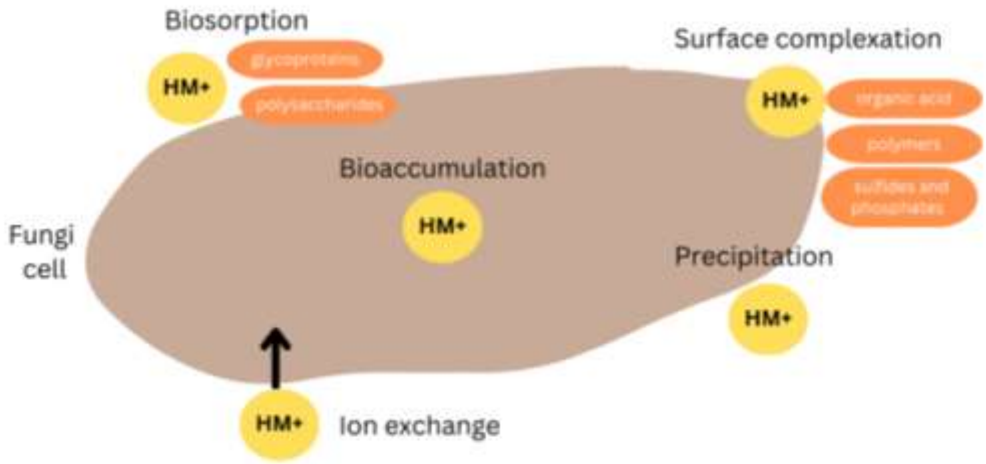

Figure 6. Mechanisms employed by fungi in heavy metal uptake [138].

The main impediment in the application of fungi towards the remediation of heavy metals is the slow process and long development cycle involved. In addition, some fungi may encounter obstacles or limitations in nitrogen requirement to ensure their survival or growth. In order to overcome this, researchers are exploring methods to promote microbial growth and development along with enhancement of nitrogen sources [144].

3.1.3. Algae

Phycoremediation is a type of bioremediation that exploits algae that abundantly exist in the marine ecosystem to destroy heavy metals in the environment. Generally, algae can be classified into two types, microalgae and macroalgae. Microalgae are photo-synthetic organisms and microscopic single cells while macroalgae are multi-cellular algae that are visible with the naked eye, and mostly known as seaweed [145]. There are a number of factors that favour the application of algae in bioremediation techniques, which include the capability of algae to regenerate, the high adsorption of the contaminants by algae, the low requirement of nutrients, the extensive generation of biomass, the unlikeliness of producing any toxic metabolites and the inexpensive capital [146, 147]. Ultimately, the ability of algae to remove heavy metals surpasses that of bacteria and fungi by up to 84.6 % [148]. Both living and dead algae are able to extract heavy metals from the environment but living algae have a higher efficiency, resulting better remediation. On the other hand, dead algae can counter heavy metals at higher concentrations and also have great industrial and development prospects [149]. Salama et al. investigated the abiotic factors that were capable of influencing algal performance in removing heavy metals in an ecosystem such as pH, temperature, ionic strength, contact time and presence of counter ions [150, 151]. These parameters should be taken into account to optimize the phycoremediation process and hasten the removal procedure.

Algae are in fact severely affected by high concentrations of heavy metals, which can eventually cause fatalities. In order to survive in heavily polluted ecosystems, algae have evolved defence mechanisms [152]. There are two key stages utilized by algae in bioremediation, which are biosorption and bioaccumulation. The first stage is biosorption which is composed of the adsorption of heavy metal ions to the cell wall that results in the attraction of the positive and negative charged ions that lead to the abatement of the heavy metal. One of the contributing factors is the existence of functional groups like carboxylic acids, amides and hydroxyls within the cell wall. The second stage occurs when heavy metal ions are transferred steadily into the cell well, which is called bioaccumulation. At this stage, there are a few processes transpiring such as metal detoxification and efflux transport, as shown in Figure 7. These are self-protection mechanisms of algae that ensure its survival while neutralising toxic metals [146].

Henriques et. al. performed a heavy metal removal study using *Ulva lactuva*, a living seaweed, and demonstrated the reduction of heavy metals including As, Cd, Pb, Cu, Cr, Hg, Mn and Ni in contaminated waters. They discovered that by using 6.0 g L^{-1} of the algae in fresh water, the remediation efficiency for As was up to 48 %, while for Hg it was 98 % [153]. Another macro-green algae, *Enteromorpha intestinalis,* was introduced as novel way to eliminate Cr ion and malachite green dye contamination simultaneously in water. Cr was successfully removed by up to 94 % with a pH of 9.92 and a reaction time of 38.5 minutes [154]. There are other common types of algae that have been employed extensively for the bioremediation of contaminated water, such as *Phormidium* spp., *Fucus vesiculosus, Spirulina platensis, Chlorella vulgaris* and *Oedogonium westi* [155-158].

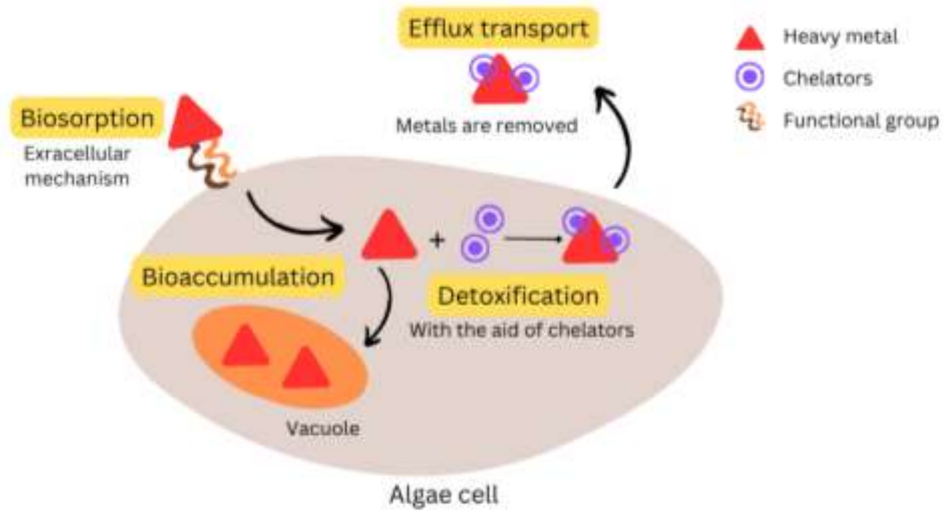

Figure 7. Self-defence mechanisms of algae in the presence of heavy metals [146].

It is advisable that living algae should only be used in conditions where heavy metal levels are less than 10 mg/L because the high toxicity can disrupt its metabolic functions and lower its toxicity tolerance. Hence, it can be quite a challenge to absorb heavy metals efficiently. Bacillariophyta species is recorded to be the least feasible algae to detoxify heavy metals due to the recalcitrant features in its structural characteristics [151]. Although algae bioremediation seems promising, there are a few challenges that need to be addressed to improve and refine this technique. The constraints involve difficulty in harvesting algae, unstructured downstream processing and requirements for specific nutrients. It is difficult to employ algae on a large scale due to the complex technology and high capital required [159].

3.2. Phytoremediation

Phytoremediation is a dynamic method that has been verified to reduce heavy metals in contaminated soil by up to 98.2 % [160]. Phytoremediation is an *in situ* remediation that employs plants to absorb, transform, immobilize, extract, and hence, deplete the heavy metal contaminants in soil. The efficiency of phytoremediation is due to the presence of certain enzymes that facilitate in accumulating metals and metalloids in soil and precipitating them on the surface and biomass of the soil [161]. Moreover, phytoremediation has gained the interest of researchers and users because of its simplistic application by using inherent hyperaccumulating plants, whether native or genetically modified species [162]. A range of plant species can be utilized in cleaning up heavy metal contamination, including ornamental plants, flowering species and also grass species [162]. Basically, plants employ two different approaches to tackle heavy metals: avoidance and tolerance. Avoidance acts as the first defence mechanism, and involves the reaction between metals and ions resulting from root sorption to form complex elements that assist in degrading the toxicity and availability of heavy metals. The latter approach is mainly triggered when heavy metal ions enter the cytosol and are adsorbed by complexation with transport proteins [163]. Some major factors play an important part in the effectiveness of phytoremediation, especially the plant species, the bioavailability of the heavy metal and the soil condition (i.e., pH, moisture, organic matter and oxygen content) [164]. The application of phytoremediation methods by previous researchers and their performance are listed in Table 4.

There are different strategies implemented by plants to absorb heavy metals in the soil. Some examples of their adaptability include producing chelating agents which increase the solubility of metal cations in plant growth media, or triggering pH modification [165]. The ability of plants to uptake and accumulate metals in plant tissues and cells is measured by applying a bioconcentration factor (BF) that can be calculated using Equation 1:

$$BF = C_p/C_{so} \quad (1)$$

where C_p is the concentration of metal in the plant while C_{so} is the concentration of metal in the soil [105].

In addition, one of the characteristics of phytoremediation is the capability of plants to translocate elements that are not essential but have the properties of nutrients. Equation 2 is commonly applied to identify the transfer of metals using the translocating factor (TF):

$$TF = C_s/C_r \quad (2)$$

where C_s is the concentration of metal in the shoots of the plant while C_r is the concentration of metal in the roots of the plant.

Plants with TF >1 are highly preferable for phytoremediation as these plants are capable of translocating metals efficiently from the roots to the shoots. On the other hand, plants with TF < 1 will accumulate metals within their roots specifically, so transfer through aerial parts such as branches, leaves, fruits or seeds would not be feasible [105].

According to a comprehensive study by Sarker, there are a number of techniques performed in phytoremediation which are considered the underlying mechanisms that comprise several inherent steps. These mechanisms are analysed and assessed as demonstrated in Figure 7, and include phytoextraction, phytovolatilization, phytostabilization and phytodegradation [162]. However, for heavy metals in particular, phytodegradation is not applicable as it is generally feasible and favourable to organic pollutants.

3.2.1. Phytoextraction

The orthodox process of phytoremediation is known as phytoextraction or phytoaccumulation, phytoabsorption and phytosequestration [166]. This process generally takes up metals through plant roots before translocating and transporting them to the shoots along with the plant biomass without affecting soil properties [162]. The main aim of this mechanism is to accumulate a large amount of heavy metals in the plant and store it for a certain time before utilizing it for harvesting or disposal [167]. Typically, the plants that are selected for phytoremediation have identical properties which include a strong root architecture, robust biomass structure, and a good tolerance and adaptability to high levels of toxic heavy metals at the polluted site [168]. These qualities are crucial to the effectiveness of phytoextraction. There are two routes for extracted heavy metals to penetrate into plants: the pathway of soil to plant and the pathway of air to plant. The former is mainly the extraction of heavy metals near the roots of the plants which are then absorbed in the shoots, leaves, and other parts of the plants with the aid of xylem vessels. The latter pathway is when air particles containing heavy metals are deposited or precipitated on the surface of the plants [169].

In a study by Zunaidi et al., six plant species were analysed as potential agents for phytoextraction as they possessed superior traits including high germination rates and biomass production with short growing periods. These species included *Amaranthus viridis L., Basella alba L., Brassica chinensis var. Parachinensis, Brassica rapa L., Capsicum frutescens L.,* and *Ocimum tenuiflorum L.* which were used to reduce Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn levels in agricultural soil. It was found that all these plants possessed a TF value greater than 1, which indicated the tremendous potential of these plants. It was also shown that all the plants were able to accumulate and extract heavy metals in different areas (stems, leaves, roots). *Brassica rapa L.*, which is a Pak-choi plant, had up to 357 mg/kg of Al extracted from its roots [170]. The enhancement of phytoextraction may be possible by using additives such as DA-6, EDTA, CA, EDTA + DA-6, and CA + DA-6, which resulted in up to 630 mg/kg of Zn obtained from the leaves of *Pfaffia glomerata* [171].

It is known that phytoextraction is conducive to the phytoremediation method and can clean up metal contamination. However, the suitability of a contaminant site should be studied and evaluated first. This is because phytoextraction at a site with a chronic or high severity of toxic pollutants would be implausible as the phytoaccumulators would not be able to survive in those conditions [172].

3.2.2. Phytovolatilization

Phytovolatilization is a key mechanism in the phytoremediation of heavy metals. It utilizes specific plants to reduce heavy metal pollution by transforming toxic metals into volatile forms by transpiration, which emits less or non-toxic metabolites to the atmosphere [162]. Commonly, this technique prioritizes sites that are contaminated by metals that have high volatility and low toxicity, such as Hg, Se and As [164]. There are two types of phytovolatilization: direct and indirect. Direct phytovolatilization is basically the volatilization of heavy metals by the plant to the air. Indirect phytovolatilization is facilitated by the activities of the plant's roots that boost the underground volatile pollutant flux [167]. This mechanism is mainly influenced by temperature and moisture, so this process works best in the summer season. A higher intensity of phytovolatilization activity is observed in bigger sized plants because of its contribution to the increase in the rate of evaporation and transpiration [173].

Guarino studied the effects of phytovolatilization of As using *Arundo donax L.* with the aid of plant growth promoting bacteria which included the *Stenotrophomonas maltophilia* sp. and *Agrobacterium* sp. strains. It was reported that up to 12 % of active volatilization of As resulted with the aid of the bacteria, with a total of removal efficiency of 57 %. From a total of 20 mg of As that was added to the pot, only 7 mg remained in the soil at the end of the experiment, which indicated that the other 13 mg (65%) was partly volatized, removed or precipitated within the plant [174]. The volatilization process of Se was explored by previous researchers and found to occur in the following order: (1) Se was first converted into organic selenomethionine and selenocysteine seleno amino acid; (2) Biomethylated selenide was then formed before being converted into dimethyl selenide; (3) Dimethyl selenide, which is volatile, was then released to the air [9].

However, this mechanism might increase the potential of human exposure to heavy metal hazards from the released air [167]. The released air may re-contaminate the environment and require remediation all over again. Therefore, this technique is known as a phytoremediation approach with "temporary effects" [175].

3.2.3. Phytostabilization

One of the crucial mechanisms in phytoremediation is called phytostabilization, which is known for its higher stability compared to other mechanisms. The process mainly focusses on the restriction of the transfer and locality of the heavy metals to the soil and environment, by employing qualified plants to reduce the mobility and bioavailability of the contaminants. This mechanism involves the stable sequestration of a toxic pollutant by several factors including rhizospheric reactions and chemistry of root exudates [162]. By definition, the rhizosphere is a prime zone within the root that enhances soil-plant interactions, as well as promotes chemical, biological and physical influences on root growth and activity. On top of that, the rhizosphere is vital for boosting the availability of nutrients, and mitigation of abiotic plant stress, including heavy metal stress [176]. The first phase of phytostabilization typically comprises the following processes which are absorption, adsorption, precipitation, chelation, and redox reaction within the rhizosphere which can be enhanced by exploiting several factors including soil pH, soil organic matter and soil microbial exchange [162, 164].The second phase of this process mainly minimizes the bioavailability of heavy metals and comprises the limitations of water percolation, water erosion and contact with the pollutant facilitated by the developed roots [177]. In addition, this strategy commonly orchestrates organic trait changes to promote the reduction of heavy metals and development of plant growth by adding nutrients and organic matter [178].

To ensure effective phytostabilization, the selection of plants with specific traits is crucial. The features of the plant candidates should be as follows: (1) deep root systems to ensure immobilization of heavy metals while maintaining soil structure; and (2) rapid growth that can produce significant amounts of biomass [179]. Typically, plants with fibrous root systems are utilized for this type of approach, such as herbaceous species, grasses, and wetland species [180].

In a study performed by Lacalle et al., contaminated soils from both the agricultural and mining industries were tested by applying a few species for the phytostabilization of heavy metals. It showed that a combination of *Cynara cardunculus* and *Brassica juncea* Czern. plants gave the most significant results in attenuating metal extraction and enhancing microbial activity, simultaneously [178]. *Helicrysum italicum* was analyzed and found to possess a high metal tolerance, weak phytoextraction capacity, and the ability to accumulate heavy metals in roots that made it a prominent candidate for use in phytostabilization [181]. A drawback of this approach is that it is a slow process which is perceived to be feasible only in low value areas [178].

3.3. Combined Bioremediation

Both microbial and plant remediation have shown promising results as strategies to reduce heavy metals in the environment, as discussed previously. It has been scientifically proven to promote and elevate the remediation outcome. However, bioremediation using one type of organism may confer limitations in terms of adaptability and capability to endure complex contaminants. The issues around contaminants that cause impediments for single bioremediation include multiple pollutants, secondary pollution and micro-organism-self pollution [183] Furthermore, inadequate microbial species, along with adversity in the screening process, are also some of the drawbacks of bioremediation using one type of organism [184]. To overcome this, researchers have analysed the probability of increased potency, or synergy, when two or more organisms work together. It was found that in many cases, combined bioremediation was a dynamic tool and had a unique capacity in remediating pollutants like heavy metals and organic compounds. In addition, there are various studies that investigated environments polluted with multiple types of heavy metal ions, and found that combined bioremediation with two or more organisms were able to optimize heavy metal removal [183]. The symbiosis of the interactions between multiple organisms has many advantages, such as: 1. It increases the metabolism process and activity; 2. It makes the bioremediation process faster; 3. One species can complete the unfinished degradation performed by the other species; 4. It has the ability to control multiple contaminants [183]. Previous studies on combined bioremediation are listed in Table 5.

One of the promising combined bioremediation methods that showed significant results is the symbiosis of algae and bacteria. These generally complement each other because algae release oxygen for photosynthesis while converting inorganic pollutants to organic elements, whereas bacteria consume the released oxygen and organic elements for nutrients while producing carbon dioxide [185]. However, the drawback of this application is the competition of nutrients or exploitation of facilities between these two microorganisms [186]. It has been established that a consortium of mixed microorganisms can demonstrate either a mutualistic, commensalistic, or parasitic relationship in a complex environment,

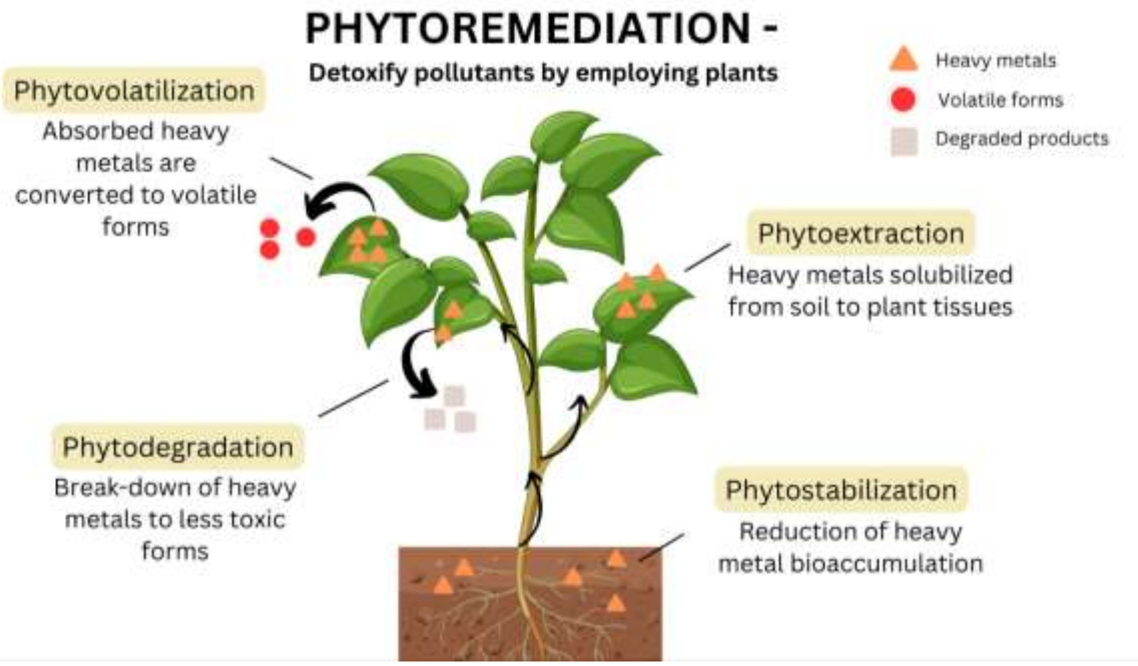

Figure 8. Phytoremediation techniques for soil polluted by heavy metals [10, 105, 182].

which implies varying consequences upon applying combined organisms in resolving heavy metal pollution [187]. A detailed study was performed by Yang et al. to analyse the effectiveness of algal-bacterial granular sludge for the biosorption of Cr(VI) by manipulating various factors such as pH, contact time, dosage and initial concentration of Cr. A total of 89.1 % of Cr was removed from the polluted water at pH 6 in 6 hours. They also found that adding a carbon source like fructose could enhance the removal of Cr by to 91 % because of its nutrient effect on the microorganisms [188]. It was also found that *Cellulosimicrobium* sp. SH8 and *Synechocystis* sp. PCC6803 were able to survive in a carbon-free medium but only when used together. This implied that they enabled and supported each other's growth [189].

The combined bioremediation of bio-augmentation-assisted phytoremediation (phyto-stabilization) was studied by applying *Bacillus subtilis* (bacteria) and ryegrass, *Lolium multiflorum L.* (plant) to tackle Cd contaminated soil. The minimization of Cd bioavailability in soil and its accumulation in ryegrass increased up to 39.1 % and 27.0 % respectively when a high dosage of inoculated bacteria was added, which indicated the efficacy of the bioremediation enhancement when both mechanisms are in synergy [190]. Sharma's extensive review elucidated the efficient cooperation of bacteria-plant remediation that demonstrated the complementing interactions of both methods to eliminate heavy metals. Selective plants employed for phytoremediation facilitated the growth and activity of bacteria that promoted the removal of heavy metals [182]. Based on Sharma's study, the synergy between the *Phragmites communis* plant and *Bacillus* bacteria enhanced plant growth along with its tolerance under metal stress conditions, and contributed to the reduction of heavy metals in wastewater. The wastewater was polluted with Fe, Cu, Zn, Cd, Mn, Ni, Pb, and As [191].

In addition, the synergy of bacteria with other elements such as biochar may also be able to assist in amplifying the bioremediation process. According to a comprehensive review by Schommer, bioremediation methods utilizing *Bacillus* spp. with immobilized biochar had a high capability to remediate heavy metal contaminants, while improving plant growth and promoting microbial and enzymatic activity within pollutant sites. The reciprocity between the biochar-bacteria and heavy metals occurred through mechanisms such as biomineralization, biosorption, bioreduction, bioaccumulation, and adsorption [98].

Table 4. Previous studies on phytoremediation of heavy metals and their performance.

Plant	Heavy Metal	Process	Reduction Efficiency	Reference
Amaranthus hybridus	Ni, Fe, Sr	Phytostabilization and phytoextraction	Removal rate of 99 %, 87 % and 70 %, respectively, in contaminated water. All TF values more than 1 indicated high potential hyperaccumulators.	[192]
Amaranthus spinosus	Zn, Cu, Pb, Cd	Phytoextraction	24 %, 47 %, 27 % and 48 % removal rates from contaminated mechanic workshop soil, respectively. From polluted refuse dumpsite-soil, the removal rates were 47 %, 52 %,52 % and 72 %.	[193]
Vigna radiata	Cd	Phytostabilization and phytoextraction	Bioaccumulation of 57 % and 21 % at shoots and roots, respectively, at low concentrations of Cd. Not applicable for high levels of Cd.	[194]
Pennisetums inese Roxb	Cd	Phytoextraction	Low Cd phytoextraction of 8 %. Polyaspartic acid was used to enhance remediation, and soil properties were analysed after 2 years of phytoremediation.	[195]
Jatropha curcas	Ni, Zn	Phytostabilization	Removal of 82 % and 25 %, respectively, in municipal solid waste contaminated soil.	[196]
Pongamia pinnata	Ni, Zn	Phytostabilization	Removal of 90 % and 45 %, respectively, in municipal solid waste contaminated soil.	[196]
Not stated	Pb, Cu, Zn	Phytoextraction	Metal concentrations extracted at shoots and roots were 440 and 690 mg/kg (Pb), 210 and 830 mg/kg (Cu) and 160 and 360 mg/kg (Zn).	[197]
Sedum alfredii	Zn, Cd, As, Pb, Cu	Phytoextraction	Zn and Cd had BCF and TF values more than 1, suitable as a hyperaccumulator. Up to 51,800 mg/kg of heavy metal was extracted at the shoots of plants in ultra-highly contaminated soil.	[198]
Arundo donax L.	As	Phytovolatilization	53 % of As was volatilized, while the other 47 % remained in the soil and was extracted by the plants.	[174]

4. Bioremediation Kinetics

To acknowledge the effects of bioremediation on heavy metal contamination, a kinetics study is crucial to obtain information on the removal process. Evaluation of the kinetics study is the best way to analyse and understand a bioremediation method in terms of its efficiency, rate and removal development. According to one study, a consortium of enriched bacteria collected from municipal wastewater was utilized to study the bioremediation kinetics effects on different concentrations of synthetic copper, zinc and nickel. The kinetics study was correlated to the specific growth rate of microorganisms that were evaluated using the experimental data of the concentration of microbes and usage of substrate. It was found that the Luong models with R^2 = 0.923, 0.957 and 0.986 were the best fit to represent the kinetic model of the growth rate of the bacteria by applying a first-order reaction [199]. Based on another study, the capability of *Scenedesmus sp.*, a type of microalgae, to remediate cadmium and lead were investigated and it was found that a pseudo-second-order reaction, along with a Langmuir model were the most optimized and suitable fit for the kinetic modelling [200]. Medjor et al. also concluded that the bioremediation of groundwater contaminated with hydrocarbons and traces of metals by a mixture of pigs, cows and poultry, had a first-order kinetic reaction with a rate constant of 0.002 $hour^{-1}$ with 91.53 % removal efficiency [201].

Additionally, four phases of growth were determined from the kinetics study of the exponential curve of bacterial concentration against time. The first phase was the lag phase, where there was only a slight increment with no notable changes of bacteria concentration observed. This is because of the time taken for the bacteria to adjust and adapt to the newly introduced environment. Subsequently, the curve was observed to increase exponentially, with a significant spike that indicated the log phase. This was when most of the heavy metal substrates were consumed and utilized by the bacteria as food to grow and survive after the adjustment period, which consequently depleted the amount of heavy metals. After some time, the increase stopped, which was the stationary phase where there was no significant activity by the bacteria. Eventually, the curve leads to the death phase where there was no change in concentration at all, and implied the demise of the bacteria [199, 202].

Agarry et al. studied the kinetic modelling of different bioremediation strategies on soil polluted with lead and lubricating motor oil that were explained by applying first-order kinetics. Natural attenuation (no additional microbes), bioaugmentation (addition of mixed *Aeromonas, Micrococcus, and Serratia sp*.) and biostimulation (addition of urea as nutrients) were evaluated with a graph of total petroleum hydrocarbon content against time. The rate constant values of 0.015, 0.030 and 0.033 were calculated for each strategy, respectively. It was deduced that the higher the rate constant, the faster the bioremediation rate [203].

Since most of the bioremediation kinetics studies showed first-order reactions, the common equation that is used for modelling is Equation 3,

$$C_t = C_0 e^{-kt} \quad (3)$$

Table 5. Previous studies of combined bioremediation approaches to heavy metal pollutants and their performance.

Combined Bioremediation	Organisms	Heavy Metal	Reduction Efficiency	Reference
Plant + bacteria	*Chrysopogon zizanioides* + *Bacillus cereus*	Cr, Cd, Pb, Mn	The rate of removal of metals were relatively low (maximum 50 % for Cd and only 7 % for Mn). However, the bacteria enhanced plant growth and improved soil structure.	[204]
Plant + fungi	*Alocasia calidora* + consortium of 13 fungi genes	As, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Successfully removed 77 % to 94 % of heavy metals in contaminated landfill soil. Growth and accumulation of plants were also enhanced.	[205]
Plant + bacteria + algae	*Sorghum bicolor* + *Bacillus sp.* and *Micrococcus sp.* + *Scenedesmus acutus* and *Chlorella pyrenoidosa*	Cd, Pb	99 % removal of Cd and Pb. Combined multi-metal pollution with both Cd and Pb also resulted in 99 % removal.	[206]
Algae + bacteria	*Chlorella* sp., *Scenedesmus* sp., *Stichococcus* sp. and *Phormidium* + *Rhodococcus* sp., *Kibdelosporangium aridum*	Cu, Ni, Zn, Mn, Fe	62 %, 62 %, 90 %, 70 % and 64 % of metals were reduced, respectively. Cell immobilization was demonstrated to improve biomass per unit area and enhance algae harvesting.	[207]

where C_t and C_0 are normally either the concentration of heavy metal in terms of total petroleum hydrocarbon content or biomass concentration. *K* is the rate constant obtained that implied the rate of the overall process relates to its efficiency and speed, while *t* is the time taken for a complete reaction [199].

CONCLUSION

The abundance of heavy metals that is inevitable due to rapid global development has greatly affected the environment. It has been shown that there are tremendous benefits in applying bioremediation to tackle heavy metal contamination issues. Bioremediation can be implemented by using either microbes or plants or a combination of both. The mechanisms used by microbes in their interactions with heavy metals mainly comprise biosorption, ion exchange and detoxification, which can effectively remove or degrade these toxic compounds to less harmful substances. The approaches using bacteria, fungi and algae commonly require optimum parameters to ensure their efficiency while at the same time promoting their growth and survival in toxic environments. The limitations of this process are basically time and the necessity for nutrients. Phytoremediation also has significant advantages, as the utilization of plants are inexpensive and environmentally friendly. Phytoextraction and phytostabilization are the common processes employed to extract and translocate heavy metals as a strategy to reduce toxicity. Phytovolatilization can also be used but is less advisable as it is considered as more of a short-term plan to remove heavy metals. Combined bioremediation on the other hand is an ideal way to improve the degradation process. In conclusion, bioremediation is an efficient approach to resolve pollution from heavy metals and their toxic effects.

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List of abbreviations

Al	Aluminium
As	Arsenic
ATP	Adenosine triphosphate
BF	Bioconcentration factor
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
EPA	Environmental Protection Agency
EPS	Exopolysaccharide
EPS	Extra-cellular polymeric substance
Fe	Iron
HM	Heavy metal
Hg	Mercury
Mn	Manganese
Ni	Nickel
Pb	Lead
ppb	Parts per billion
ROS	Reactive oxygen species
Se	Selenium
SEM	Structural Equation Modelling
Sr	Strontium
TI	Thallium
TF	Translocating factor
U	Uranium
WHO	World Health Organization
Zn	Zinc