

REVIEW

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Microbial bioremediation as a tool for the removal of heavy metals

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Abstract

Background The demand for designing a new technology that can emphasize the complete removal of heavy metals increased as a result of the industrial revolution. Bioremediation was found to have a potent impact on the degradation of organic and inorganic environmental pollutants.

Main body Bioremediation is a multidisciplinary technology that possesses safe, efficient, and low-cost characteristics. Also, one of the important features of bioremediation technology is the in-situ treatment which reduces the possibility of transmitting the contaminants to another site. The application of genetic engineering, to engineer a microorganism to acquire the ability to remove different types of heavy metals at a time or to generate a transgenic plant, is considered one of the new promising bioremediation approaches.

Short conclusion Removal of heavy metal pollution still represents a big challenge for ecologists that's why this review shed some light on bioremediation technology; its importance, mechanism of action, and prospects.

Keywords Bioremediation, Biosorption, Biotransformation, In-situ bioremediation, Ex-situ bioremediation

Background

The world accelerated industrial revolution and the uses of natural resources during metal mining and industry have a great impact on the environment due to heavy metal pollution. Today, one of the most destructive effects facing the world is the contamination with heavy metals, which reaches the air, soil, and water (Asha and Sandeep 2013; Raghunandan et al. 2014, 2018). Although trace concentrations of some metals have a vital effect on the health of living organisms, high levels of heavy metals represent toxic effects too (Ahemad 2019; Ahuti 2015). Also, heavy metals can hardly be degraded in the soil,

so their complete detoxification represents a challenge to scientists. Despite the efforts spent to tackle the environmental pollution issue, the world still suffers from the hazardous effects of heavy metals, and so a new technology should be discovered to contain the disaster of heavy metal contamination, one of which is the bioremediation (Raghunandan et al. 2014, 2018).

Several methods have been accomplished to remediate heavy metals pollution, among them Physico-chemical (conventional) methods such as ion exchange, redox, electrochemical techniques, membrane filtration, and precipitation (Nissim et al. 2018; Qasem et al. 2021). The disadvantages of the conventional methods are the inability of these methods to detoxify heavy metals permanently (Sun et al. 2020), in addition to the cost-effectiveness and the hazardous by-products produced by the elimination process. However, the conventional method is considered effective for large areas contaminated with small amounts of heavy metals and for highly polluted local areas (Huët and Puchooa 2017). Consequently, building a new technology that emphasizes the complete removal of heavy metals represents a challenge for

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scientists. Interestingly, microbial remediation of heavy metal has a far-reaching progressive prospect among the decontamination methods. Microorganisms especially soil microbes can tolerate high levels of heavy metals, some microorganisms need certain types of metals as a micronutrient (i.e., Fe^{3+} is essentially utilized by all bacteria while Fe^{2+} is significant for anaerobic bacteria) to perform their metabolic activities (Ahemad 2019). The bioremediation process could be conducted Ex-situ by transferring the contaminated area to be treated or even in situ by delivering the biological source to the polluted land (Shannon and Unterman 1993; Naz et al. 2005). Most microorganisms follow two common mechanisms in the bioremediation process; metal sequestering or immobilization and enhancement of solubility properties of the metal, other organisms oxidize or reduce the heavy metals to a less toxic form (Donald 2013). The bioremediation process also could be accomplished in aerobic and anaerobic environments; however, the aerobic environment was found to be more efficient and faster than anaerobic conditions.

Main text

Definition of heavy metals

These can be defined as the elements that have a density higher than 5 g/cm^3 , also the metals or metalloids which have an atomic mass greater than 4000 kg m^{-3} or 5 times larger than water are considered heavy metals (Paschoalini and Bazzoli 2021). A lot of elements fall into this class however, only a few metals (arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb),

mercury (Hg), nickel (Ni), tin (Sn), vanadium (V) and zinc (Zn).) commonly existed in the contaminated air, water, and soil. These metals could be found in many forms; insoluble such as carbonate, oxides, silicate, and sulfides, or soluble such as salt forms (Arfala et al. 2018), also, heavy metals when persisted in their ionic state (e.g., Cd^{+2} , Pb^{+2} , Hg^{+2} , As^{+3}) represent the most toxic form as they combined with bio-molecules and for a complex harder to be dissociated (Duruibe et al. 2007). Recently, researchers paid great attention to studying the diffusion phenomenon and mobility through soil layers and in aquifers (Cuevas et al. 2012). According to the European Environment Agency reports, industrial process and product use, energy production and distribution, and energy use in industry are the most contributed sectors in the emission of Cd, Hg, and Pb as represented in Fig. 1. However, road transportation, commercial, institutional, and households have a significant contribution in Pb emission (EEA 2019).

Effect of heavy metals on living organisms

Heavy metals with trace concentrations are considered micronutrients that are essential and have nutritional value for some metabolic processes of living cells (Ray and Ray 2009), however, elevated levels may have an adverse impact on the health of aquatic and terrestrial living organisms and the environment as they cause dangerous morbidity and mortality (Wang et al. 2006; Ray and Ray 2009). Heavy metals could be transported to the living cells through the air, water, and food chains and consequently, they alter the physical and chemical

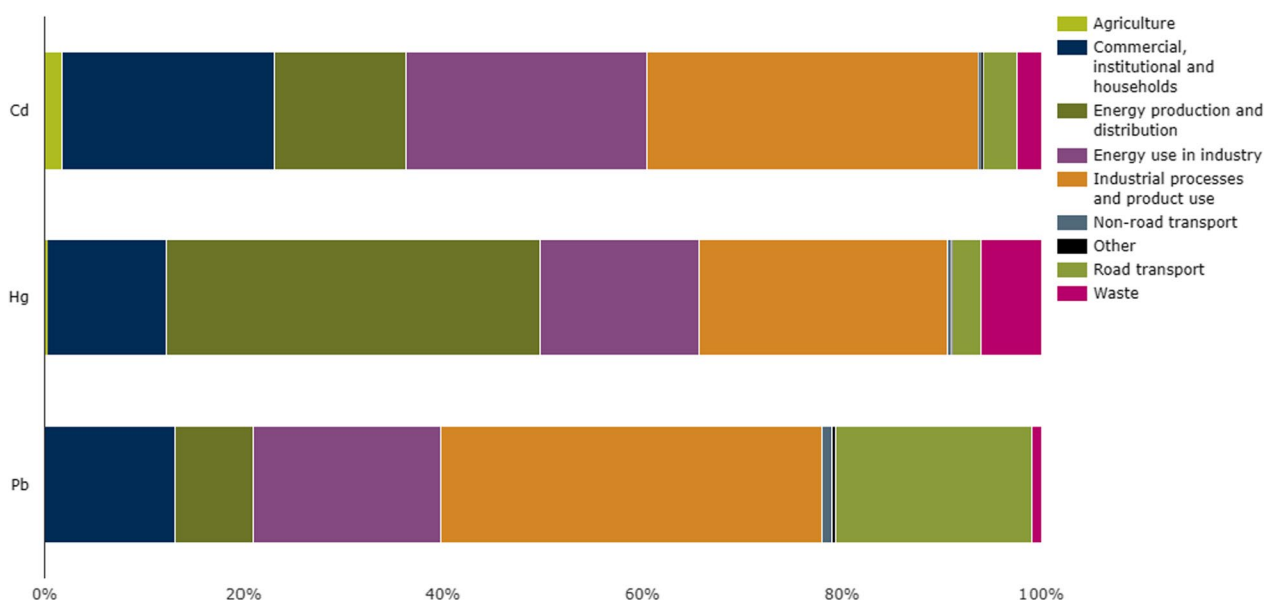


Fig. 1 Effect of different life sectors on the emission of Cd, Hg, and Pb in the environment (EEA, 2019)

properties of the transported material. Heavy metal pollution affects the ecosystem balance by reducing the microbial population of the soil which participates in decomposing the organic material used in crops growing, and so they indirectly affect the food chain of other living organisms, thereby, the world health organization (WHO) and the United States Environmental Protection Agency (USEPA) assigned the acceptable limit for different heavy metals in water as represented in Table 1. Some metals can destruct living cells directly such as mercury, cadmium, lead, and chromium others have indirect effects such as zinc a corrosive material, and arsenic which pollute catalysts (Hogan 2010).

Effect of heavy metals on human

Heavy metals exert their effects by interfering with the function of the organs, however, some of these metals are useful at low concentrations such as arsenic, copper, nickel, iron, etc. (Ray and Ray 2009), however, at a high concentration, these metals become cytotoxic as well as carcinogenic for the cells, especially after long term exposure (Jaishankar et al. 2014; Valko et al. 2016).

Malfunction of human organs is the predominant phenomenon of infected bodies, Zinc for example causes severe gastrointestinal, kidney, brain, respiratory, and heart damage (Hryniewicz and Baum 2014). Cadmium has the same effect in addition to hypophosphatemia and causes damage to the central nervous system (Hryniewicz and Baum 2014). Arsenic and mercury damage the liver, the heart, and the central nervous system and cause hypophosphatemia and cancer (Tamele and Vázquez Loureiro 2020). Lead which is commonly introduced to the environment in different forms such as mining, lead smelting, ceramic and glass industries, ammunition, storage battery, and tetraethyl-lead manufacturing (Held and Don 2000) has a destructive effect on the liver, the heart,

and the central nervous system and cause hypophosphatemia, cancer, and anemia (Koning et al. 2001; Iranzo et al. 2001; Hryniewicz and Baum 2014). A disastrous disease has already emerged due to heavy metal pollution such as “Itai Itai” in Japan as a result of Cd pollution (Gautam et al. 2015), “Arsenecosis” in Bangladesh due to As, and “Minimata” in Japan due to Hg (Volesky 1990).

Effect of heavy metals on plants

Physiological dysfunction and malnutrition are the most important disorders that affect plant growth due to excessive concentration of heavy metal pollution, also the disturbance in the ecological balance between plants and microorganisms has a great impact on crops. Malfunctions of the vital physiological processes such as Photosynthesis, and respiration may lead to the degradation of the major organelles following plant death (Glombitza and Reichel 2013). As a consequence of the excessive intake of heavy metals by plants, human and animal health will be affected (Babak et al. 2013).

Toxicity of heavy metals to the microorganisms

Heavy metals also have a great impact on the growth of microorganisms depending on the type and concentration of the polluted source. Different mechanisms were found to be involved in the toxicity of heavy metals such as dysfunction of enzymatic reactions, production of reactive oxygen species (ROS) which function as soluble electron carries, induction of oxidative damage that may cause changes in DNA and protein formation (Gauthier et al. 2014; Hildebrandt et al. 2007). Also, heavy metal toxicity affects the transcription and translation of DNA by charging the phosphate group negatively using electrostatic interaction which may cause mutagenesis (Genchi et al. 2020), causing acute hurt to the cell membrane and cytoplasmic molecules. Hence, exposure to heavy metals can affect both morphological, biochemical, and physiological properties (Frimmel 2003; Fashola et al. 2016).

Table 1 Upper concentration limits for heavy metals in water according to WHO and USEPA:

Metal	Symbol	WHO ($\mu\text{g L}^{-1}$)	USEPA ($\mu\text{g L}^{-1}$)
Cadmium	Cd	3	5
Lead	Pb	10	15
Chromium	Cr	50	100
Mercury	Hg	1	2
Zinc	Zn	1000	1000
Copper	Cu	2000	1300
Nickel	Ni	20	–
Aluminum	Al	200	200
Manganese	Mn	100	50
Iron	Fe	300	300
Arsenic	As	10	10

Principles of the bioremediation process

Bioremediation can be defined as the use of biological diversity, directly or indirectly, to convert toxic pollutants into a harmless form (Asha and Sandeep 2013), so bioremediation is a holistic approach that includes plant, fungi, bacteria, actinomycetes, and algae all of them could be used as a biological agent to detoxify heavy metals. Two different strategies are utilized to remediate toxic pollutants; in-situ, where the process of decontamination occurred at the contaminated place itself by bringing the biological agent to the site of contamination or promoting the indigenous organisms to deal with contaminants by facilitating the suitable condition for their

propagation. The second one is *ex-situ*, by which the contaminated place is transferred away to another site to be processed (Kumar et al. 2011a, b; Kumar et al. 2016; Raghunandan et al. 2014, 2018). There are many mechanisms by which the organism can manipulate the detoxification process, however, the utilization of the toxic metal by the microorganism as a source of nutrition is the main concept (Sun et al. 2020). So, microbial bioremediation is considered a multidisciplinary field that required more research and investigations.

Types of bioremediations

Bioremediation is classified, according to the site at which the bioremediation process occurred, into two different strategies:

In-situ bioremediation

This strategy corresponded with treating the polluted surfaces where they are located, this strategy depends on detoxifying the dissolved and sorbed pollutants directly by the microorganism, it can be applied in groundwater, unsaturated and saturated soils, also it is considered an efficient method to remediate organic chemicals in contrary to *ex-situ* strategy (Brar et al. 2006), also *in-situ* bioremediation expanded to treat inorganic and toxic metals. Moreover, the application of microorganisms that have a chemotactic ability to facilitate moving into the contaminated areas and hence the degradation of harmful compounds will be safer (Kulshreshtha et al. 2014). Furthermore, stimulating the reduction of heavy metals at the place minimizes the chance of contaminant transportation downgradient. A challenging issue facing *in-situ* bioremediation is the selection of one organism or a consortium of organisms that has the potential ability to detoxify the targeted metals. In lab-scale, it was found that Fe^{3+} and sulfate-reducing microorganisms have the enzymatic ability to biodegrade some heavy metals such as U(VI), Tc (VIII), Cr (VI), and Co (III) (Gorby et al. 1998; Tebo and Obraztsova 1998; Lloyd et al. 2000). Also, species of Geobacteraceae were found to be a dominant group during the stimulation process for reducing Fe^{3+} , also, the members of this group were detected in the stimulation process to reduce U(VI) of contaminated Aquifer. So, the Geobacteraceae group was considered to play an important role in stabilizing contaminants and reducing metals within subsurface environments (He et al. 2019). The following are some techniques used for “*in-situ*” bioremediation:

Biosparging

Biosparging system is Constructed by injecting the air through a pipe below the water table which enhances the growth of indigenous microbes due to elevated

oxygen concentration (Jain et al. 2012). Also, it differs from bioventing in mixing the soil and the groundwater by injecting the air in the saturated area, which allows the movement of volatile organic compounds upward to the unsaturated area this process is affected by the biodegradability of the contaminants and soil characteristics. This system possesses low construction cost and flexibility in adapting the design (Atlas and Philp 2005).

Bioventing

Bioventing is a system that stimulates the existing soil microorganisms to degrade the source of pollution via injecting a limited amount of oxygen that sustains microbial activity (Jain et al. 2012). Injection of air is conducted in the unsaturated area in addition to supplementing it with nutrients and moisture (Philp and Atlas 2005). Bioventing could be more efficient in anaerobic biodegradation, also mixing nitrogen with oxygen will increase the potency of chlorinating remediation (Mihopoulos et al. 2000, 2002; Shah et al. 2001).

Bioaugmentation

Bioaugmentation is the application of outsourcing microbial strains that naturally occurred or are genetically engineered to decontaminate polluted soil or water. Treatment usually utilizes a consortium of microorganisms that produce all the required enzymes and degradative pathways. Bioaugmentation is used to treat municipal wastewater, soil, and groundwater polluted with chlorinated ethenes which are degraded to nontoxic ethylene and chloride (Jain et al. 2012).

Intrinsic bioremediation

Intrinsic bioremediation is defined as the stimulation of naturally occurring organisms by providing nutritional materials and oxygen to remediate heavy metals without attribution of any engineering steps (Riseh et al. 2022).

Engineered bioremediation

Engineered bioremediation is the adaptation of physico-chemical conditions to enhance the propagation of introduced microorganisms to accelerate the bioremediation process.

Advantage of *in-situ* bioremediation

- Cost-effectiveness of *in-situ* bioremediation
- It can be used to treat large contaminated areas which could reach inaccessible regions.
- Treating a wide variety of wastes, it may be used the decontaminate organic and inorganic wastes.
- *In-situ* bioremediation is faster than the pump-and-treat method.

Challenges facing in-situ bioremediations

- Limitations in depending on indigenous microorganisms as their metabolic activity could be inhibited by high levels of heavy metals.
- Some pollutants may be bio-transformed due to microbial metabolic activity to an intermediate which could be more toxic and mobile than the original form.
- In-situ bioremediation could be inappropriate in treating some contaminants such as recalcitrant.
- In-situ bioremediation is most suitable for low-level scenarios of pollution (Kulshreshtha et al. 2014).

Ex-situ bioremediation

The core concept of this strategy is to treat the contaminated site by the excavation of soil to enhance microbial degradation. Five techniques were used in this strategy.

Slurry-phase

This technique relies on excavating the contaminated soil and mixing it with water and transporting the mixture to a bioreactor, followed by stone and rubble removal. The amount of water depends on the pollutant's type and concentration, the soil's nature, and the biodegradation rate. This process is followed by the separation of the soil by filtration or centrifugation, the soil is dried and retransferred to its original location, and the fluids are submitted to a further treatment step (EPA 2003).

Solid-phase

This technique involves three steps: excavation of the soil, followed by putting the soil into piles, the soil may contain municipal, agricultural, and organic wastes, followed by stimulation of the biodegradation process by supplying oxygen through a network of pipes to enhance microbial respiration and subsequently microbial activity. Solid-phase bioremediation requires a large space and a long time to be completed (Hyman and Dupont 2001).

Landfarming

This technique relies on the stimulation of indigenous organisms spread over the surface by supplementing the excavated soil with suitable nutrients and minerals, the excavated soil should be periodically tilled to stimulate the biodegradation process.

Soil biopiles

This technique is almost similar to landfarming bioremediation except in using above-ground piles and perforated pipes to inject air through the soil (Verma 2022). Application of this technique is interestingly valuable

because of its low cost and full control of nutritional feed, aeration, and temperature (Whelan et al. 2015), also it's the technique of choice in treating contaminated sites of extreme environments and in treating low molecular weight compounds by limiting volatilization (Gomez and Sartaj 2014).

Composting bioremediation

Composting bioremediation is quite similar to landfarming bioremediation in excavating the contaminated soil to the surface and stimulating the indigenous microorganisms through feeding of nutrients and injecting air but differs in supplementing the soil with a bulk of additives such as corncobs, straw, and hay, this additive helps in oxygen distribution through the soil, maintaining the moisture content constant and turning frequency, however, application of composting process for biodegradation of volatile pollutants is not favorable because of the periodic turning during the process (Hobson et al. 2005).

Advantage of ex-situ bioremediation

- Adequate control of the biodegradation process.
- Suitability to detoxify a wide variety of contaminants.
- Reduction of time required to complete the treatment process.

Challenges facing ex-situ bioremediation

- Limitation of ex-situ bioremediation to biodegrade chlorinated hydrocarbons.
- Some types of soils required further processing such as non-permeable soils.

Bioremediation mechanism of action

Due to the ubiquitous nature of microorganisms, they play a crucial role in the bioremediation of heavy metals, they can interact with heavy metals using different mechanisms to survive the toxicity of the metals. The two main concepts by which the organism can deal with contaminants are using the contaminant as a source of nutrition and protecting the organism itself (defense mechanism) from the toxic effect (Alvarez et al. 2017). As illustrated in Fig. 2, the microorganism reacts with the environmental contaminants using direct or indirect mechanisms some of which are biosorption and biotransformation (Tang et al. 2021).

Biosorption

It's a mechanism by which the organism binds with the metal to form a complex that possesses a nontoxic

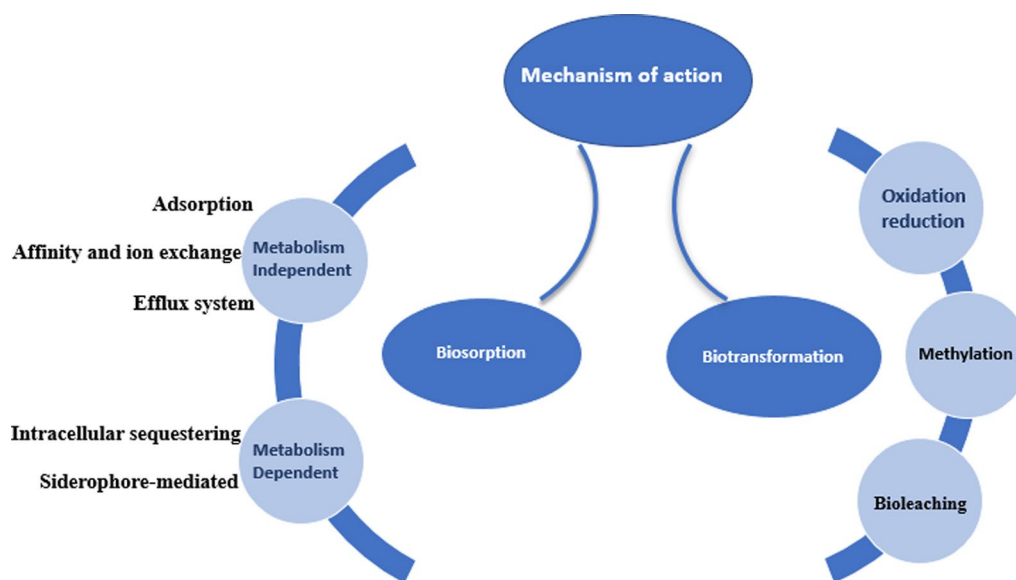


Fig. 2 Diagram showing different mechanisms of bioremediation action

feature. Certain criteria should be considered and investigated to achieve a potential biosorption mechanism; nature of the biosorbent, sorption capacity, kinetics of sorption, regeneration ability of the sorbent, percentage of metal recovery, cost-effectiveness of biosorption process, and separation flexibility of the biosorbent-metal complex (Bae et al. 2001, 2002, 2003). Two main categories are involved in the bioremediation process using the biosorption mechanism.

Metabolism-independent biosorption

This type of biosorption depends on the physical and chemical properties of the cell whether it was a live cell or a dead cell, this category involves the following:

Adsorption, also called extracellular sequestration, relies on the affinity between cellular components of the periplasm and the metal ion. Extracellular polymeric substance (EPS) associated with bacterial cell wall plays a significant role in metal adsorption. EPS is composed of polysaccharides, mucopolysaccharides, and proteins. It also contains a lot of functional groups (hydroxyl, carboxyl, amine, and phosphoric groups) that facilitate heavy metal sequestering (Guine et al. 2006).

Affinity and ion exchange by which the biosorbent (cellular component) binds with the metal ion, Cunninghamella were found to have a promising binding ability to heavy metals released textile wastewater (Tigini et al. 2010), also *Saccharomyces cerevisiae* can degrade Cd(II) and Zn(II) using the ion-exchange method.

Efflux system as a type of extracellular sequestration is one of the most important methods by which the

organism can defend against the toxic effect of heavy metals by forming an outer protective material and ejecting the metal ion out of the cytoplasm to the periplasmic region (Dixit et al. 2015). Ma et al. (2016) reported that transformation and efflux are the basic methods usually used in bacterial resistance to heavy metals.

Metabolism-dependent biosorption

This mechanism is associated with the metabolic activity of a viable microorganism contrary to metabolism-independent biosorption.

Intracellular sequestering (Bioaccumulation), is a process by which the complex form of cell-metal occurred inside the cytoplasm (Ramasamy and Banu 2007), as reported by (Abo-Alkasem et al. 2022a) and illustrated in Fig. 3, examination of *Salipaludibacillus agaradhaerens* strain NRC-R cells using the Transmission Electron Microscope (TEM) showed the accumulation of chromium inside the cell which also confirmed by EDX analysis, accumulation of metals conducted by attaching with the cell surface follows slow penetration to periplasm to the cell cytoplasm by a process that looks like nutrient uptake (Mishra and Malik 2013), it was reported that cysteine-rich protein plays an important role in sequestering Zn, Cd, and Cu in cadmium-tolerant *Pseudomonas putida*, also, glutathione helps in sequestering Cd by *Rhizobium leguminosarum*. Fungi also play a vital role in inorganic metal elimination using their rigid cell wall which works as a ligand in the decontamination process.

Siderophore-mediated biosorption, also called a chelating agent, in aerobic soils some microorganisms produce

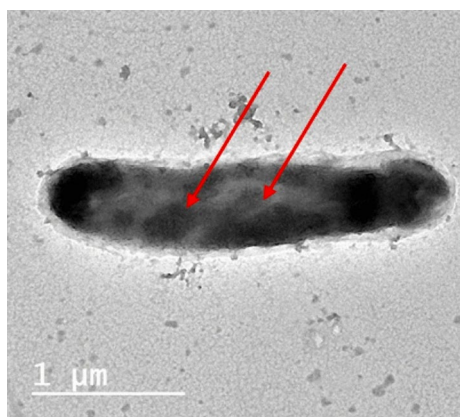


Fig. 3 TEM image of the cells grown in the presence of Cr (VI) (Abo-Alkasem et al. 2022a)

siderophores that mediate the ability of the microorganisms to utilize low water-soluble metals using an energy-dependent process (John et al. 2001). *Microbacterium flavescens* was found to use siderophore to uptake their nutritional requirements of iron, also the organism uses the siderophore desferrioxamine-(DF) to bind with uranium, plutonium, and iron.

Biotransformation

Biotransformation relies on the cellular metabolic activity of the microorganism through the redox mechanism, reduction of metals by changing the oxidation number of the metal is common in nature, such as the reduction of chromium (Abo-Alkasem et al. 2022a, b), selenium (Lloyd et al. 2001), uranium (Chang et al. 2001) and mercury (Brim et al. 2000).

Oxidation and reduction mechanisms

The mechanism by which the microorganism works as an oxidizing agent by releasing electrons that react with the anions in the contaminated soil is the same mechanism utilized to decontaminate organic compounds under anaerobic conditions (Lovley and Phillips 1988). However, it was found that the presence of iron (III) stimulates the degradation process (Spormann and Widdel 2000). The reduction could be occurred directly using a bioreactor, (pump and treat) or after the excavation of soils, inoculated with the appropriate microbial consortium, or indirectly using sulfate-reducing bacteria which plays an important role in the ecological balance directly by sulfate reduction or indirectly by the formation of biofilms (Abo Elsoud and Abo-Alkasem 2022). The indirect mechanism is more favorable due to its cost-effectiveness and eco-friendly method (Asha and Sandeep 2013). Decontamination of uranium by *Desulfosporosinus* spp.

And *Closteridium* spp is an applicable example of utilizing sulfate-reducing bacteria (Prasad and Freitas 2003).

Methylation of metals (volatilization)

Volatilization of metal by microbial methylation plays a significant role in metal remediation, for instance, some *Pseudomonas* spp., *Escherichia* spp., *Clostridium* spp., and *Bacillus* spp. can convert Hg (II), Se, As, and Pb to a gaseous methylated form (Ramasamy and Banu 2007).

Bioleaching

Bioleaching is the secretion of low molecular weight compounds that aid the transformation of a toxic form of metals to a nontoxic form by dissolution or precipitation mechanisms, (Chanmugathas and Bollag 1988) reported that leaching of Cd is promoted by the secretion of organic acids by some microorganisms, also the production of inorganic phosphate by *Citrobacter* organism leads to precipitation of metal phosphate coat.

Plant-microbial remediation

Rhizoremediation is the association of microorganisms with plants to improve the potential of the bioremediation process and it now plays a crucial role in environmental bioremediation due to cost-effectiveness and outstanding efficiency (Nie et al. 2011; Marihal and Jagadeesh 2013; Prabha et al. 2017).

The capability of microorganisms to develop a symbiotic relationship enhances the biodegradability of different types of contaminants (Kumar et al. 2017). The predominant type of organisms associated with the plant-microorganism relationships is mycorrhizal fungi which can bio-sorb heavy metals (Bojorquez and Voltolina 2016). The potentiality of rhizoremediation was reported by Joner and Leyva (1997) who found that mycorrhizal plants when subjected to soil contaminated with Cd^{2+} 1, 10, and 100 mg/kg, Cd uptake of mycorrhizal was higher than non-mycorrhizal plants by 90%, 127%, and 131% respectively. The mechanisms utilized in rhizoremediation are mainly through the activation of metal phosphates, acidification, production of organic acids, chelating agents, and ion carriers.

Microorganisms responsible for bioremediation

In nature, the presence of microorganisms guarantees the retrieval of ecological balance and the removal of contaminants that hinder biological life. The use of microorganisms for the removal of contaminants from the environment is described as "Bioremediation". The concept of environmental remediation using microorganisms was first registered as a patent in 1981 for the degradation of petroleum oil by *Pseudomonas putida* (Prescott et al. 2002; Glazer and Nikaido 2007). Bioremediation

aims to stimulate microbial metabolic activity, with nutrients or other chemical agents, to be able to remove, destroy, or neutralize the effect of these contaminants. The microorganisms used for bioremediation should not only be able to tolerate a wide concentration range of the contaminant(s) but also be physiologically active. Once favorable conditions are obtained, the metabolic activity and growth rate of these microorganisms reach alarming levels as well as the bioremediation process. Many theories have been illustrated for the mechanism of microbial tolerance to heavy metals. These theories include the accumulation and formation of non-toxic complexes with the metal ions inside the cells, the efflux of toxic metals outside the cell, biotransformation of the toxic metal into a less toxic form, or methylation and/or de-methylation.

In nature, the type of micro-flora (microbial consortium) is a significant factor affecting the tolerance and rate of heavy metal bioremediation depending on the gene and metabolic diversity (Juwarkar et al. 2010). Two types of microorganisms are used for heavy metal bioremediation based on their sources: indigenous (microorganisms present in the site of contamination and have bioremediation capability) and extraneous (microorganisms introduced into the site of contamination and have bioremediation capability), Table 2 summarizes some of the organisms used in the bioremediation process and their target pollutants. The utilization of indigenous microorganisms excludes the need for continuous monitoring according to Asha and Sandeep (2013). After the bioremediation process, the soil and/or water retrieve their ability to be reused in various activities.

It was reported that many microorganisms including bacteria, Actinomycetes, fungi, yeast, and algae can remediate heavy metals from soil and water:

Bacteria

Endophytic bacteria and Plant Growth Promoting Rhizobacteria (PGPR) are the most common bacterial strains associated with heavy metal bioremediation.

The endophytic bacteria colonize the sub-epidermal layer of the plant tissues (Schulz and Boyle 2006). The presence of endophytic bacteria helps the protection of the plant cells from heavy metals stress conditions (Ryan et al. 2008). They diminish or remove the phytotoxicity of the heavy metals by altering their phyto-availability (Weyens et al. 2009; Ma et al. 2011) such as some species of *Pseudomonas*, *Bacillus*, and *Rahnella* that showed high resistance to Pb, Mn, and Cd (Luo et al. 2012; Yuan et al. 2014; Babu et al. 2015).

On the other hand, PGPR comprises a group of free-living, symbiotic, or endophytic bacteria (Glick 2012). For example, *Bacillus*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Gluconacetobacter*, and *Pseudomonas*

(Nadeem et al. 2010) can mitigate the toxicity of heavy metals, improve plant growth in heavy metal-contaminated soils (Seth 2012) and produce phytohormones and siderophores and help phosphate solubilization (Ullah et al. 2015).

Actinobacteria

In addition to their well-known ability to utilize complex organic matter as a carbon and energy source (Kieser et al. 2000), Actinobacteria, such as *Amycolatopsis*, *Corynebacterium*, *Rhodococcus*, and *Streptomyces*, can tolerate and remediate heavy metals, such as Hg(II), Co(II), Cd(II), Cr(VI), Zn(II) and Ni(II) (Oyetibo et al. 2010; Alvarez et al. 2017).

Fungi

Some fungal strains have been reported to possess metal chelating and sequestering systems that increase their heavy metal tolerance and biotransformation into a less toxic form such as *Allescheriella*, *Pleurotus*, *Phlebia*, and *Stachybotrys* (D'Annibale et al. 2007). The hyphal and high biomass growth adds an advantage to this type of microorganism as it allows simple harvest along with the attached heavy metals (Aly et al. 2011). *Aspergillus*, *Penicillium*, *Cephalosporium*, and *Rhizopus* are the most studied fungal genera for their potential activity in the removal of heavy metals, such as Pb²⁺ and Zn²⁺, from aqueous solutions and soils (Volesky and Holan 1995; Huang and Huang 1996; Tunali et al. 2006; Akar et al. 2007).

Factors affecting the bioremediation process

To confine the biodegradation potential on selecting the most appropriate method, mechanism, and technique without paying attention to the factors that may affect the utilized application, limit the efficiency of the bioremediation process. A lot of factors could exhibit significant effects on the bioremediation process, for instance, metal ion concentration, valance state and chemical forms of the metal, the bioavailability of the metal, redox potential, availability of low molecular weight organic acids, and environmental factors such as temperature and pH (Bandowe et al. 2014).

Substrate concentration

To establish the process of bioremediation, bio-sorbent accumulation features should be quantified, two models could be used; the Langmuir model mainly defines adsorption by assuming an adsorbate behaves of the single-layer (Acar and Malkoc 2004), and the Freundlich model which mainly estimates the adsorption equilibrium (Febrianto et al. 2009). However, the main concept is that the adsorption efficiency increases with

Table 2 The most studied microorganisms for bioremediation of some heavy metals

Toxic chemical		Microorganism(s)	References
Heavy metals	Source		
Cadmium (Cd)	Fuel combustion, zinc smelting, and E-waste	<i>E. coli</i> , <i>Bacillus subtilis</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas aerogenosa</i> , <i>Citrobacter</i> spp., <i>S. cerevisiae</i> , <i>Aspergillus niger</i> , <i>Rhizopus arrhizus</i> , <i>Stereum hirsutum</i> , <i>Phormidium valderium</i> , <i>Klebsiella planticola</i> , <i>Nostoc</i> sp., <i>Chlorella vulgaris</i> , <i>Azospirillum lipoferum</i> , <i>Rhizobium leguminosarum</i> , <i>Alcaligenes faecalis</i>	Favero et al. (1991), Gabriel et al. (1994, 1996), Sharma et al. (2000), Sar and D'Souza (2001), Rajendran et al. (2003), Vinopal et al. (2007), Deng et al. (2007), Wu et al. (2007), Bondarenko et al. (2008), Ivask et al. (2011), Kumaran et al. (2011), Goher et al. (2016), Tan et al. (1994), Gomaa et al. (2012), Gomaa et al. (2016)
Copper (Cu)	Mining, electroplating, and smelting operations	<i>Bacillus</i> spp., <i>Micrococcus</i> sp., <i>Staphylococcus</i> sp., <i>Citrobacter</i> spp., <i>Flavobacterium</i> sp., <i>Desulfovibrio desulfuricans</i> , <i>Alcaligenes faecalis</i> , <i>Pseudomonas aerogenosa</i> , <i>Bacillus firmus</i> , <i>Arthrobacter</i> , <i>Aspergillus versicolor</i> , <i>Aspergillus niger</i> , <i>Aspergillus lentulus</i> , <i>Sphaerotilus natans</i> , <i>Chlorella vulgaris</i> , <i>Zooglea</i> spp., <i>Spirogyra</i> sp., <i>Spirulina</i> sp., <i>Pleurotus ostreatus</i> , <i>Stereum hirsutum</i>	Gabriel et al. (1994, 1996), Philip et al. (2000), Sar and D'Souza (2001), Rajendran et al. (2003), Shipra et al. (2011), Kim et al. (2015), Kumar et al. (2011a, b), Marzan et al. (2017), Salehizadeh and Shojaosadati (2003), Kumaran et al. (2011), De et al. (2008), Pepper et al. (2011), Taghan et al. (2010), Achal et al. (2011), Ashokkumar et al. (2017), Parvathi et al. (2007), Iqbal and Edyvean (2004), Javaid et al. (2011), Mane and Bhosle (2012), Goher et al. (2016)
Chromium (Cr)	Industrial coolants, leather tanning, and mining	<i>Acinetobacter</i> sp., <i>Streptomyces</i> sp., <i>Pseudomonas aerogenosa</i> , <i>Bacillus</i> sp., <i>Staphylococcus</i> sp.	Singh et al. (2013), Nayak et al. (2018), Chaturvedi (2011), Kumar et al. (2011a, b), Seema and Alok (2012), Bhattacharya et al. (2015), Bhattacharya and Gupta (2013), Zakaria et al. (2007), Benazir et al. (2010)
Mercury (Hg)	Electrical appliances and thermal power plants	<i>Pseudomonas aerogenosa</i> , <i>Klebsiella pneumoniae</i> , <i>Vibrio parahaemolyticus</i> , <i>Vibrio fluvialis</i> , <i>Bacillus lichemiformis</i> , <i>Citrobacter</i> spp., <i>Candida parapsilosis</i> , <i>Ganoderma applanatum</i> , <i>Rhizopus arrhizus</i> , <i>Chlorella vulgaris</i> ,	Favero et al. (1991), Gabriel et al. (1994, 1996), Rajendran et al. (2003), Jafari et al. (2015), Al-Garni et al. (2010), Muneer et al. (2013), Saranya et al. (2017)
Zinc (Zn)	Electroplating and smelting	<i>Pseudomonas aerogenosa</i> , <i>Bacillus</i> spp., <i>Chlorella vulgaris</i> , <i>Aspergillus niger</i> , <i>Pleurotus ostreatus</i> , <i>Volvariella volvacea</i> , <i>Nostoc</i> sp., <i>Azospirillum lipoferum</i> , <i>Rhizobium leguminosarum</i>	Gabriel et al. (1994, 1996), Philip et al. (2000), Yan and Viraraghavan (2001), Rajendran et al. (2003), Kumaran et al. (2011), Gomaa et al. (2012)
Lead (Pb)	Coal-based thermal power plants, smelting operations, and E-waste	<i>Staphylococcus</i> spp., <i>Streptomyces</i> spp., <i>Citrobacter</i> spp., <i>Pseudomonas</i> sp., <i>Micrococcus</i> sp., <i>Bacillus firmus</i> , <i>Ganoderma applanatum</i> , <i>Phormidium valderium</i> , <i>Rhizopus arrhizus</i> , <i>Chlorella vulgaris</i> , <i>Volvariella volvacea</i> , <i>Nostoc</i> sp.	Favero et al. (1991), Gabriel et al. (1994, 1996), Yan and Viraraghavan (2001), Rajendran et al. (2003), Kumar et al. (2011a, b), Marzan et al. (2017), Salehizadeh and Shojaosadati (2003), Kumaran et al. (2011)

the increment of heavy metal concentration until a certain value.

Type of the substrate

The efficiency of the adsorption mechanism is affected by the type of soil, the type of heavy metal, and the type of soil additives. since the adsorption between the soil and heavy metals may lower the mobility of heavy metals and hence reduce microbial adsorption (Hu et al. 2010). Also, soil additives have a significant effect on heavy metals removal, Tyagi et al. (2014) found that increasing $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ higher than 20 gm/l has an adverse effect on the leaching rate of Cu and Zn.

pH

The potential of hydrogen (pH) plays a vital role in both microbial activity and metal characteristics. Growing of microorganisms in unfavorable pH may affect the enzyme activity thereby lowering the rate of microbial metabolism, also, the charge of the microorganism surface will be changed that affects the binding capacity between the adsorbent and heavy metals (Bandowe et al. 2014; Galiulin and Galiulina 2008). Furthermore, changes in pH value may alter metal mobility and hydration as the metals tend to be free ionic at acidic pH (Bandowe et al. 2014; Dermont et al. 2008). According to (Rodríguez-Tirado et al. 2012; Wierzbna 2015), the adsorption capacity of Pb^{2+} and Zn^{2+} increased by raising the pH value to 5.5, however, an observed decrease in the removal of metals was recorded upon increasing pH value over (5.5).

Temperature

Temperature is an important parameter in adjusting the optimum conditions for microbial growth, metabolism, and enzyme activity (Fang et al. 2011), increasing temperature affects the diffusion of metals across different layers and also, increases the bioavailability of metals. However, the optimum biodegradation temperature differs according to the type of metal, for instance, the biodegradation of Cd^{2+} by *Bacillus jeotgali* was the highest at 35 °C, however, it was 30 °C for Zn^{2+} biodegradation (Chanmugathas and Bollag 1988).

Role of biotechnology in the bioremediation process

Biotechnology is the discipline of using the engineering of scientific principles to improve the efficiency of organisms to serve humans and remediate the environmental toxic substance (McHughen 2016), by using genetic engineering, one of the biotechnology approaches, a single organism can be engineered to produce all the needed enzymes or to utilize all the degradative pathways for bioremediation process (Dangi et al. 2019). The purpose

of utilizing genetic tools is to enhance efficiency and reduce the cost and time of the bioremediation process.

Degradation of Polychlorinated biphenyls (PCBs) is controlled by to group of genes that were found in the genetic material of two different organisms, thereby, using genetic engineering for achieving recombination between *Pseudomonas pseudoalcaligenes* KF707 and *Burkholderia cepacia* LB400 bph genes may enhance the degradation rate of PCBs and stimulate the remediation of toluene and benzene (Seeger et al. 2010), also the application of DNA probes helps in accelerating the process of the isolation and identification of a particular strain from a mixed population (Dua et al. 2002). Another example of using biotechnology is the fusion between metallothionein (MT) isolated from rats, IgA protease protein isolated from *Neisseria gonorrhoeae*, and the fusion vehicle *lpp-ompA* to provide the bacterial cell wall with metal ion-binding polypeptides (Bae et al. 2000 and Valls et al. 2000).

Another discipline of biotechnology involves the use of transgenic plants in the bioremediation process, this could be conducted by transferring a desirable gene from different sources (other plants, microorganisms, or even animals) to improve the ability of the plant to remove the toxic pollutant (Truu et al. 2015) this process of transmission increases the phytoremediation ability of the plant (Dixit et al. 2015).

Immobilized microorganism technology

Immobilization is one utilized technique in bioremediation, it possesses stability of the biological cell, also the immobilized cell did not compete with indigenous organisms, therefore it is considered eco-friendly and has high degradation efficiency (lone et al. 2008).

Advantage of bioremediation

- A natural bioprocess is characterized by a safe effect on the environment which makes it globally accepted as a technique for treating wastes.
- The consumed energy is lower than the technologies.
- Cost-effectiveness is one of the most bioremediation features.
- Several types of pollutants could be eliminated at the same time.
- Minimize the risk of transferring the contamination from one site to another.

Disadvantages of bioremediation

- Several factors could affect the efficiency of the bioremediation process.

- Elimination of toxic metals to be achieved could take a lot of time.
- Limited to those contaminants that can be biodegradable.
- Biodegradation capacity and efficiency cannot be predicted because of dealing with a live organism (Zeyaulah et al. 2009).

Conclusions

Great efforts were spent during the last few decades to address the problem of heavy metal pollution by developing new strategies to fix this issue, however, the application of bioremediation techniques still represents the most favorable strategy due to the cost-effectiveness and safety impacts of bioremediation techniques on the environment and also due to the variability of bioremediation mechanisms which makes these techniques applicable and affordable, this article enumerates different types of bioremediation and the advantage and disadvantage of these types also the suitability of these types to different environments and conditions moreover, the article summarizes some of the mechanisms of action of different bioremediation techniques in addition to the microorganisms that play an important role and the factors that may affect the bioremediation process and how the newly developed technologies can improve the bioremediation techniques to be more efficient.

Abbreviations

DNA	Deoxyribonucleic acid
DF	Desferrioxamine
EPS	Extracellular polymeric substance
PGPR	Plant Growth Promoting Rhizobacteria
PCBs	Polychlorinated biphenyls
ROS	Reactive oxygen species
USEPA	The United States Environmental Protection Agency
WHO	World Health Organization

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