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Bioremediation of Heavy Metals by Use of Bacteria

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ABSTRACT

Heavy metal pollution generally occurs due to socio-economic, industrial, and anthropogenic activities, which may cause an environmentally hazardous and serious severe threat to the survival of the organisms (genotoxic, carcinogenic, and clastogenic effects on it). Many physical and chemical remediation approaches have been proposed to deal with this pollution, but these are very time-consuming and costly. While bioremediation stands out as an inexpensive and efficient approach, the use of bacteria is thought to be a potential and productive organism to prevent this pollution. This review has evaluated the bacterial potential to clean up heavy metals from the environment and elucidated the mechanisms responsible for bioremediation.

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Introduction

Throughout the history of humanity, water and soil sources have played a vital role in human civilization. Consequently, the demand for water and soil resources has increased globally, increasing population and growth and socio-economic activities such as mining, agriculture, energy, and fuel production & consumption, industrial waste and sludge, and leather processing. This situation is of particular importance, especially in areas with insufficient water resources that require the need for wastewater treatment and re-use. Heavy metals contamination has emerged as a global threat from the beginning of the industrial revolution to the present day. Due to the increasing industrialization and other human activities, significant amounts of heavy metals are added to the soil and wastewater every day. Heavy metal polluted industrial wastes are discharged directly to aquatic resources such as lake and river beds, acting with a sense of miraculous destruction in nature. While a small number of heavy metals of about 1 mg. L^{-1}, due to their toxic structure, can cause serious health problems. It is thought-provoking how severe direct and indirect discharge of industrial-scale contamination will cause severe health and environmental problems. Besides, heavy metal-rich wastewater can damage terrestrial ecosystems. If these pollutants enter the food chain, they will cause genotoxic, clastogenic, and even carcinogenic effects in humans and animals (Ferrera and Sánchez, 2016; Jacob et al., 2018; Kumar, 2018; Panwar, 2020)

Many of the physicochemical methods used today to remove heavy metals from industrial wastes, such as electrochemical treatment, ion exchange, and reverse osmosis, are not efficient and highly expensive. For this reason, biological agents such as plants and microorganisms offer more suitable and environmentally friendly ways to remove heavy metals (Jacob et al., 2018). Bioremediation is defined as a promising environmentally friendly technology, consisting of the processes of restoration, rehabilitation, and cleaning of contaminated areas with the help of biological tools that mostly microorganisms, plants and also their enzymes, as a result of the production, storage, transportation, and use of inorganic and organic chemicals (Skinder, Uqab and Ganai, 2020). As biological tools, microorganisms are relatively efficient and inexpensive in terms of withstanding the adverse effects of heavy metals in the habitats contaminated with heavy metal, to survive, and to convert into less toxic forms of heavy metals from contaminated soil. These small and invisible organisms are used to clean polluted areas more effectively (Gouma et al., 2014). Other than that, the efficiency of bioremediation depends on many factors i.e., the hazardous forms and concentration of heavy metals and their forms, the physicochemical characteristics of the contamination sites (type of soil, temperature, pH, nutrients, the presence of electron donors and acceptors), and their availability to the microbial communities. Due to optimizing these factors, bioremediation is a complex approach. In addition to what has been stated, these heavy metals are limited to biodegradable, research is needed to develop for detoxification, the forms of heavy metals may be more persistent or toxic than the metals, and it takes longer than other treatment methods (Kumar et al., 2011; Abatenh et al., 2017). So that, many bioremediation strategies have been developed, considering the metabolic processes of microorganisms.

Heavy Metals

Heavy metals are elements with metallic properties such as ligand specificity, conductivity, etc., whose atomic number is greater than 20, and the density is greater than 5 $g.cm^{-3}$ in the periodic table. Millions of people from different countries are primarily dependent on groundwater resources for their drinking water needs. Likewise, the irrigation needs of agricultural lands of many countries that rely on the farming economy are provided from artificial ponds. Some of these metals are released to the environment due to the wastes discharged generated through geological or industrial processes and the burning of fossil fuels (Soni et al., 2019). Heavy metals are categorized into three classes: toxic metals such as mercury (Hg), cadmium (Cd), chromium (Cr), zinc (Zn), arsenic (As), precious metals such as gold (Au), lead (Pb), platinum (Pt), uranium (U) and radionuclides such as radium (Ra), thorium (Th) (Kumar, 2018). Heavy metals are hazardous pollutants that can cause significant ecological, evolutionary, nutritional, and environmental problems for humans and ecosystems (Yadav, Gupta and Sharma, 2019; Nkhalambayausi-Chirwa et al., 2020). Even the metals which are required in a trace amount to plants, animals, and humans, such as nickel (Ni), copper (Cu), zinc (Zn), and iron (Fe), cause toxic effects (cytotoxic, mutagenic, and carcinogenic) when supplied with the high amount (Verma and Kuila, 2019).

Heavy metal toxicity, which we can define as the ability of a metal to cause harmful effects on the organism, varies depending on the dose of the metal, the route, and the duration of the metal exposure. The increase in dosage in plants causes a decrease in growth and gradually death, and also causes loss of function of essential organs such as the brain, liver, lungs, and increasingly important health problems in animals and humans (Kumar, 2018; Soni et al., 2019). Various heavy metal pollutants in living organisms and their environmental pollution and toxic effects showed in Table 1.

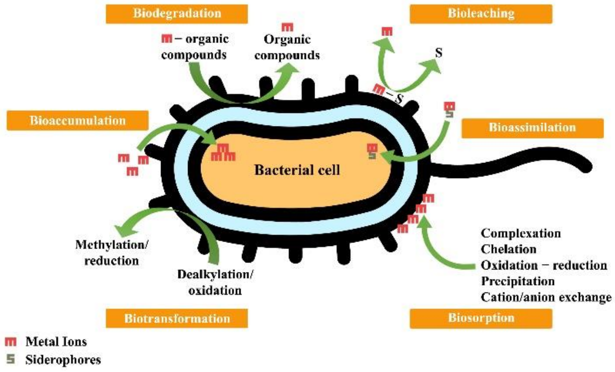

Figure. Mechanism of microbial heavy metal bioremediation from contaminated sites (Modified from Kumar, 2018).

Heavy Metal Accumulation Mechanism in Bacteria

Bioremediation technology using a specific metal accumulator bacterial population with unique metabolic activities for metal accumulation and detoxification is one of the most promising and effective biological tools for removing metals/metalloids from the environment. It has been reported that some of these microorganisms can tolerate heavy metals, so they can either convert toxic metals into less toxic forms or altogether remove them from the contaminated environment (Mustapha and Halimoon, 2015).

In order to convert heavy metals into harmless or less toxic forms, microorganisms have developed several different bioremediation mechanisms such as bioremediation, bioaccumulation, biosorption, biotransformation, bioleaching, and biomineralization (Kumar, 2018; Figure). In addition to the bioremediation process; microorganisms, including bacteria, use an anaerobic process involving the introduction of reactive oxygen species (ROS) into redox reactions mediated by enzymes such as mono and dioxygenases, hydroxylases, oxidative dehalogenases, reductases, laccases, ligninases, or peroxidases, and the oxidative catabolic process of the anoxic electron receptors mediated by the oxidative catabolic anaerobic reactions.

Bioleaching is another process for removing metals by dissolving sulfide minerals and producing less toxic metals directly by discharged bacteria, whose optimal temperature is between 35-40°C (the most included chemolithotrophic gram-negative species is *Thiobacilli* such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* or *Leptospirillum ferrooxidans*) (Gadd, 2000). Sulfate (SO_4^{2-}) occurs as a result of oxidation of reduced or partially reduced sulfur-derived compounds such as sulfur (S) in its elemental form, sulfide (S^{2-}), and thiosulfate ($S_2O_3^{2-}$). In general, bioleaching reactions take place in the pH range of about 1.5 to 3.0. Typical microorganisms of bioleaching are mostly mesophilic bacteria which can acidify the heavy metal-dense environment which can activate H^+-ATPase pump in the plasma membrane, the proton (H^+) motive power that is released as a result of the accumulation of CO_2 accumulation by maintaining the electrical charge balance (Gadd, 2004).

Biosorption can be defined as the removal of toxic heavy metals from the solution through biological materials. This process is managed by physicochemical factors that do not require ATP and is used effectively, especially in wastewater treatment. Heavy metal pollutants are directed to cellular structures through absorption from contaminated aquatic and soil areas. Radical groups such as carboxyl (−COOH), amine, sulfonate, and −OH are the main groups responsible for binding metal ions in bacterial cell walls. There are three main mechanisms for bacterial cell walls such as *(i)* ion-exchange reactions with teichoic acid and peptidoglycan, *(ii)* precipitation under the nucleation reactions, and *(iii)* complexing with oxygen and nitrogen ligands (Gadd, 2000). The secretion of extracellular polymeric substances, i.e., proteins and exopolysaccharides, increase biofilm formation in the surface; because of that, it gives higher biosorption capacity due to the negatively charged sites in the EPS. Besides that, pH, ionic exchange and strength, binding site composition, cell physiology, biosorbent surface area, etc., factors also affect biosorption capacity. Bacteria areshown to consume many heavy metals, i.e., Ag, Au, Cd, Cu, Hg, Pb, Pd, and Pt (Kuippers et al., 2021).

Biomineralization is based on the precipitation of secondary metabolites produced by microorganisms through metabolic processes and the complexing of the polluting heavy metals in the environment. Mineral formation and geochemical precipitates occur as a result of these processes (White and Gadd, 2000). Bacterial species with enormous diversity are capable of metabolizing hydrogen in aerobic and anaerobic environments, nitrifying or denitrifying, reducing sulfate (*Thiobacilli* and *Metallogenium* species), or oxidizing sulfur (*Desulfovibrio* or *Desulfomonas*) or utilizing iron (*Geobacter metallireducens* and *Shewanella putrefaciens*) in either their oxidized or their reduced forms to obtain energy (Skinner and Ehrlich, 2014).

Bioaccumulation is a substrate-specific process involving transport mechanisms such as passive diffusion, facilitated diffusion, and active transport of heavy metals through metal transporters such as metal transporting ATPases, ABC transporters, etc., requiring external energy (e.g., ATP, NADH etc) to transport and accumulate metals in the cells. Since this method is based on the uptake of heavy metals by living microorganisms in the cell, it is a slower process compared to biosorption. In contrast, some of the active transport systems that play a role in the transport of heavy metal ions are selective. For example, the system that carries cadmium ions also transports zinc ions (Mikulewicz, Chojnacka and Szynkowska, 2014; Wang, 2016). The most important bacterial species that have the capability of bioaccumulation processes are as follows; *Staphylococcus*, *Bacillus*, *Corynebacterium*, *Escherichia*, *Enterobacter*, *Aeromonas*, *Klebsiella*, *Pseudomonas*, and *Thiobacillus* (Ibrahim et al., 2021).

Biotransformation is the process of converting the toxic and environmentally hazardous forms of heavy metals (Cr^{6+}, Se^{6+}, V^{5+}, Au^{3+}, Pd^{2+}, U^{6+}, As^{5+}) into more volatile or less soluble forms (Cr^{3+}, Se^{0}, V^{3+}, Au^{0}, Pd^{0}, U^{4+}). As a result, less toxic heavy metal forms are obtained (Lloyd, 2003).

Table 1. Toxicity of heavy metals to microorganisms (Modified from Tiwari and Lata, 2018)

Heavy metals	Environmental pollution and toxicity profile
Nickel (Ni)	The shrunken cell membrane reasoned by nickel causes inhibitory enzyme activities and oxidative stress.
Cadmium (Cd)	Acute Cd exposure causes abdominal pain, burning sensation, nausea, vomiting, muscle cramps, itai-itai disease (combination of osteomalacia and osteoporosis), vertigo, and shock. Coma may develop due to lung, liver, or kidney damage and intoxication. However, chronic exposure to Cd causes a depressive effect on norepinephrine, serotonin, and acetylcholine levels.
Zinc (Zn)	Death, reduction of biomass, growth inhibition.
Chromium (Cr)	It is one of the main raw materials of industries such as metallurgical, chemical, refractory bricks, leather, wood protection, and pigments and paints. Serious health problems such as skin and nose irritation, ulceration, eardrum perforation, lung carcinoma, growth inhibition on microorganisms, prolonged delay phase, and inhibition of oxygen intake could occur.
Silver (Ag)	It causes cell lysis and also inhibits cell transduction and growth.
Copper (Cu)	It inhibits cellular functions and enzyme activities.
Lead (Pb)	It causes disorders such as injury to the organs such as kidneys, livers, hematopoietic system, endocrine system, and reproductive system, headache, poor attention span, irritability, memory loss, loss of consciousness, and dullness, mainly in the central nervous system (CNS). Nucleic acid and protein denaturation also inhibit enzyme effects and transcription.
Arsenic (As)	Arsenic occurs in natural soil and pesticide and wood preservative applications such as volcanic effects and rock erosion and can be found in different oxidation states (in the form of As^{3+} and arsenate (AsO_4^{3-}/H_3AsO_4). As a result of human activities, it is one of the heavy metals that can cause serious environmental problems by leaving permanent effects on humans, animals, and plants. Arsenic (As) causes severe disorders in the cardiovascular systems and central nervous systems, bone marrow depression, hemolysis, polyneuropathy, and encephalopathy. Eventually leads to death. Swallowing can also cause black foot disease, which is only reported in Taiwan. It inhibits enzymes on microorganisms.
Mercury (Hg)	It causes mental retardation, dysarthria, blindness, hearing loss, developmental disorders in humans and animals.

Table 2. Some References of Genetically Modified Bacteria (GMBs) to Possess the Ability to Perform Bioremediation (Modified from Gupta and Singh, 2017 and Tonelli et al., 2021)

Source/Transgene	Genetically Modified Bacteria (GMBs)	Desired Characteristics Presented by the GMBs	References
Yeast and mammalian/CUP1 and HMT-1A metallothioneins genes	Escherichia coli	Improved ability to bind Cd^{2+}	Sousa et al., 1998
Escherichia coli/merA	Deinococcus radiodurans	Hg^{2+} increased accumulation	Brim et al., 2000
Shigella flexneri/Tn21	Pseudomonas strain K-62	The merE protein encoded by transposon Tn21 (broad Hg transporter) roled to transporting Hg (Across the bacterial membrane)	Kiyono et al., 2009
Bacillus Megaterium strain MB1/TnMERI1	Arabidopsis thailana	Mercuric (Hg^{2+}) ion binding protein (MerP)	Hsieh et al., 2009
E. coli/gshI	Indian mustard	γ-Glutamylcysteine synthetase	Zhu et al., 1999
ChrR genes	Methylococcus capsulatus (Bath)	Cr (VI) reductase activity (Cell-associated Cr removal in laboratory conditions)	Hasin et al., 2010
Rhodopseudomonas palustris/arsM	Sphingomonas desiccabilis and Bacillus idriensis strains	Overexpression of encoding bacterial and archaeal homologs of the mammalian Cyt19 As (III) S-adenosylmethionine methytransferase gene at invitro	Liu et al., 2011
Photinus pyralis (firefly)/lucFF	Bacillus subtilis BR151 (pTOO24)	Luminescent recombinant bacterial Cd sensor	Ivask et al., 2011

Transgenic Approaches Utilized in Phytoremediation

Genetically modified bacteria (GMBs) whose genetic material has been manipulated by biotechnological methods could be a new potential candidate for bioremediation of contaminated soils, groundwater or activated sludge environments. The advantages of GMBs are to recover heavy metals rapidly from the polluted sites, high catalytic or utilization capacity with a less amount of biomass, and purifyng contamination areas by neutralizing any harmful effect of heavy metal derivatives. The disadvantage of GMBs are (i) in some cases the cells could not survive for a longer time (ii) at a particular level of contamination, the latency of growth and detoxification of heavy metal and derivatives increases, (iii) seasonable variation and other abiotic factor surge could have severe impact and relationship on microbial activity, and (iv) sometimes introduced transgene to the main system could be non-functional and can cause immeasurable adverse effects on the functional and structural bacteria composition (Abatenh et al., 2017). Table 2 shows some examples of GMBs used in bioremediation processes.

Microorganisms such as bacteria, fungi, yeasts, algae, and cyanobacteria are used to remove heavy metals from the polluted sites. The use of organisms such as bacteria, fungi, or algae, being environmentally friendly and cost-effective, makes these methods more attractive. Moreover, they have a small genome size and are pretty simple in terms of cell structures. In addition, their ability to replicate genomic material in a short time, to evolve rapidly, and to adapt to changing environmental conditions, makes bacteria more suitable biological candidates for the bioremediation of environmental pollution both *in situ* and *ex situ* (Dvořák et al., 2017).

Understanding the biological structure of the bacterial cell walls and membranes that are in contact with the external environment is essential to understand the relationship of these organisms with heavy metals. Gram-positive bacteria are N-cross-linked with the majority of the short peptides. In contrast, gram-negative bacteria have a multi-layer outer membrane structure (about 20% in the periplasmic space) consisting of phospholipids, a lipopolysaccharide layer (LPS), and a small layer of peptidoglycans. It has an outer membrane consisting of acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) units and a peptidoglycan layer containing teichoic acid. Because they are converted into a dense negative charge from the carboxyl, hydroxyl, and phosphate groups in the peptidoglycan layer; they allow them to interact with heavy metals that are positively charged by nature. Bacteria, especially of these characteristics, can convert toxic chemicals to the environment into more harmless derivatives in aerobic and anaerobic conditions (Vollmer, 2015; Prabhakaran, Ashraf and Aqma, 2016). Table 3 shows the metal-resistant bacteria collected from different sources.

There are five different mechanisms in the microorganisms that play a role in metal toxicity. The first is that the metal-ligand binding, cutting, or cleavage of the target molecules revealed in their biological functions occurs when another metal ion is exchanged in the binding region of the specific biomolecules. Second, covalent and ionic redox is the reaction of heavy metal ions with thiol groups present in microbial cells (R-SH), and the formation of Pinter-type reactions (thiols produced by metal oxidation such as selenium (SeO_4^{2-}, SeO_3^{2-}) oxyanions) that produce highly reactive oxygen-derived compounds that can oxidize any biological macromolecules. The third is Fenton-type reactions, which generate ROS and include transition metals such as copper (Cu), nickel (Ni), and iron (Fe). The fourth is that membrane transport systems cause inhibition of specific membrane transporters, with toxic heavy metals entering the binding sites and interrupting the conserved membrane strength for substrates. Finally, by siphoning electrons by thiol-disulfide oxidoreductase in the respiratory chain, it destroys the protein motive force occurring in the cell membrane (Prabhakaran, Ashraf and Aqma, 2016).

Table 3. List of some important metal-resistant bacteria isolated from different sources with their removal capacities.

Bacteria strains	Metal	Metal removal capacity (%)	References
Micrococcus roseus	As	85.61	Shakya et al., 2012
Pseudomonas stutzeri ASP3		82.97	Shakya and Pradhan, 2013
Bacillus anthracis		84.00	Shakoori et al., 2010
Exiguobacterium sp.		99.00	Pandey and Bhatt, 2015
Bacillus megaterium		92.00	Ghodsi et al., 2011
Brevibacillus reuszeri		96.67	Neeratanaphan et al., 2016
Rhodococcus sp.		94.17	
Pseudomonas sp.		78.00	Jebelli et al., 2017
Bacillus flexus As-12		28.00–45.00	Jebeli et al., 2017
B. megaterium		93.00	Miyatake and Hayashi, 2009
Pseudomonas sp. W6	Pb	55,00	Halttunen, Salminen and Tahvonen, 2007
B. longum 46		85.40	Murthy, Bali and Sarangi, 2012
Bacillus cereus		84.62	Bharagava and Mishra, 2018
Cellulosimicrobium sp. KX710177		55.00	Halttunen, Salminen and Tahvonen, 2007
Bacillus firmus	Zn	61.80	Salehizadeh and Shojaosadati, 2003
Pseudomonas sp.		49.80	Kumaran, Sundaramanickam and Subramanian, 2011
Caulobacter crescentus	Cd	99.00	Patel et al., 2010
Exiguobacterium sp.		99.00	Park and Chon, 2016
Klebsiella pneumoniae		82.00	Shamim and Rehman, 2012
Pseudomonas aeruginosa		94.70	Nooghabi et al., 2010
Stenotrophomonas maltophilia		80.00	Chien, Hung and Han, 2007
Lysinibacillus fusiformis		92.30	Mathivanan and Rajaram, 2014
P. aeruginosa		89.00	Sinha and Mukherjee, 2009
Bacillus firmus	Cu	74.90	Salehizadeh and Shojaosadati, 2003
Paenibacillus sp.		59.00	Govarthanan et al., 2016
Alcaligenes faecalis GP06		70.00	De, Ramaiah and Vardanyan, 2008
P. aeruginosa CH07		75.00	
Micrococcus sp.	Ni	55.00	Congeevaram et al., 2007
Pseudomonas sp.		53.00	Kumaran, Sundaramanickam and Subramanian, 2011
Acinetobacter sp. B9		68.94	Bhattacharya and Gupta, 2013
Vibrio fluvialis	Hg	60.00	Saranya et al., 2017
P. putida		90.00	Okino et al., 2000
Pseudomonas sp. B50D		85.00	Giovanella et al., 2017
Vibrio parahaemolyticus PG02		80.00	Jafari et al., 2015
Bacillus circulans MN1	Cr	71.40	Chaturvedi, 2011
Cellulosimicrobium sp.		98.60	Naeem, Batool and Jamil, 2013
Bacillus cereus		81.00	Nayak et al., 2018
Acinetobacter haemolyticus		75.00	Zakaria et al., 2007
Cyanobacteria		93.00–99.50	Pandi, Shashirekha and Swamy, 2009
Staphylococcus sp.		92.00–93.00	Varadhan and Mohan, 2017
Bacillus sp. QC1-2		99.00	Campos, Martinez-Pacheco and Cervantes, 1995
Acinetobacter sp.		87.00	Bhattacharya et al., 2014
Ochrobactrum intermedium		97.10	Batool, Yrjala and Hasnain, 2012
Enterobacter cloacae	Co	8.00	Jafari et al., 2015

Discussion

Rapid urbanization, industrialization, and intensive agricultural activities are the primary cause of heavy metal pollution. Heavy metals can remain in soil and water for centuries before they can be broken down. The permanence of heavy metals can adversely affect the aquatic and terrestrial ecosystem, which can affect agricultural product and water quality and soil and aquatic microorganisms, and indirectly human health (Kidd et al., 2012). In this respect, bioremediation is an innovative technique for treating and disposing of industrial wastewater from the environment. This approach is advantageous compared to traditional physicochemical methods that produce costly, time-consuming, and toxic end products. Bioremediation is also important because it is a potentially cheap and environmentally friendly technology that can use several microorganisms to treat wastewater, soil, and sediment (Gupta et al., 2019). Various microorganisms such as bacteria, fungi, yeasts, algae, and cyanobacteria are used to remove heavy metals. But bacteria can tolerate wider pH, temperature, and oxygen ranges, and manipulations to the bioremediation process can be more manageable. In addition, bacteria exhibit tremendous adaptation to anaerobic conditions and tolerate temperature, pH, and oxygen limitations (Wu and He, 2013). These advantages make bacteria more beneficial than other microorganisms.

Conclusion

Bioremediation approaches are one of the significant cost-effective technologies and are easy to use without complicated procedures. Several bioremediation approaches have been proposed considering the metabolic pathways of bacteria. The ability of bacteria to survive even under extreme conditions makes them more advantageous than other microorganisms such as fungi, yeasts, and algae. However, the precise mechanism of heavy metal bioremediation needs to be elucidated.

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