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Overview of Bioremediation (Bacteria) of Some Heavy Metals and Metalloids from 2014 to 2024

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ABSTRACT

The continuous growth of humanity across the globe has led to an increased demand for goods that support human life. Consequently, large-scale industrialization occurs to meet these needs by introducing new alternatives. However, large-scale production of such goods has significantly harmed the environment. Industrial effluents contain numerous toxic substances, including heavy metals and metalloids, which are discharged into rivers, lakes, ponds, soil, and the environment during various operations. The results from various researchers have demonstrated that bioremediation is a highly constructive and attractive option that employs microbial activity for the remediation, cleansing, management, and recovery of water and soil contaminated with various heavy metals and metalloids. This method can be beneficial and advantageous for the treatment of wastewater and effluents without any microbial pollution.Based on the review of 54 articles and one thesis in this study, it has been concluded that bioremediation is a technique free from any type of pollution, offering minimal-cost solutions for bioremediation by enhancing natural degradation and biosorption processes. Therefore, with the development of understanding of microbial communities and their responses to natural environments and pollutants, the expansion of knowledge in microbial genetics and genetic engineering to enhance pollutant degradation capabilities, conducting field experiments, and the implementation of new cost-effective bioremediation techniques, it is essential to allocate sites where these opportunities are reserved for long-term research objectives.

Introduction

Just as industrialization has led to the economic development of countries, it has also introduced increasing pollution indices, which have caused serious problems for humans, animals, and even plants, in addition to environmental degradation. Many antibiotics have also become ineffective due to the impact of these harmful elements, which can essentially be classified into two groups: heavy metals and polycyclic aromatic hydrocarbons (PAHs). Undoubtedly, addressing this issue is quite complex, especially considering that, in addition to the aforementioned damages, it poses a significant barrier to the economic growth of countries, particularly those lacking the necessary resources to combat soil environmental pollution.In light of this scenario, bioremediation offers a wide range of techniques, methods, and approaches as foundational tools provided by nature, which have proven to be a unique and successful treatment, with its effectiveness demonstrated in various ways across the globe(Cervantes et al.2023).

1. Methodology:

To gather the content of this article, electronic databases such as SID, PubMed, Elsevier, Springer, ScienceDirect, and Google scholar were utilized. In addition to the articles from these databases, the documentation from Ms. Tala Hayati's thesis (MS) was also referenced for discussion and conclusion.

3. Findings:

1. Soil and Effluent Pollution:

1.1 Soil Pollution:

Soil pollution refers to any alteration in the characteristics of soil components that renders its use impossible. Soil pollutants can be harmful solid or liquid substances that have mixed with natural soil. These pollutants generally adhere to soil particles through destructive physical, chemical, and even biological processes, or they may be lodged between soil particles if not adhered. These pollutants can be added to and transferred into the soil due to the disposal of contaminated materials into the soil, spillage of these materials onto the soil, or the leaching of contaminated soils (Hayati.2018). The types of soil pollutants include:

1. Various industrial pollutants and heavy metals (Hayati.2018).
2. Various types of waste and refuse (Hayati.2018).
3. Various agricultural pesticides (Hayati.2018).
4. Various petrochemical substances (Hayati.2018).
5. Various effluents from mining activities (Hayati.2018).

1.2 Effluent Pollution:

One of the most significant pollutants that currently poses numerous dangers to human, animal, and even plant communities is heavy metals. These metals are present in the effluents of several industrial factories, such as those producing pulp, petrochemicals, refineries, fertilizers, and agricultural pesticides, and they have considerable toxic effects on the receiving environment. Unlike other pollutants, the disposal of heavy metals from the environment is very challenging because these metals are not chemically or biologically degradable and are non-biodegradable; however, they can be oxidized, reduced, or complexed by organic materials. Therefore, reliable methods must be employed for the treatment of sites contaminated with these metals (Hayati.2018).

2. Metals and Their Types:

Metals are elements that naturally exist in relatively low concentrations. They possess beneficial properties and are important components of our daily lives. Based on their chemical and physical properties (chemical approach), metals are classified into the following categories:

1. Light (Hayati.2018).
2. Heavy metals (Hayati.2018).
3. Metalloids (Hayati.2018).

Metals and metalloids constitute about 75% of known elements. Only hydrogen (H), boron (B), carbon ©, nitrogen (N), phosphorus (P), oxygen (O), sulfur (S), halogens, and noble gases are excluded from this category (Hayati.2018).

2.1 Classification of Heavy Metals:

1. **Heavy metals are generally divided into three main categories:** Toxic Metals: Toxic metals encompass a wide range of elements that have harmful effects on biological systems and the environment. These metals exist in various forms, and exposure to them can lead to severe health issues. Types of toxic metals include:
2. **Highly toxic metallic pollutants**: Lead (Pb), Cadmium (Cd), Mercury (Hg), Chromium (Cr), Copper (Cu), Selenium (Se), Nickel (Ni), Silver (Ag), and Zinc (Zn) (Bathla & Jain.2016), as well as Barium (Ba) (Hayati.2018).
3. **Metals with lower toxicity:** Aluminum (Al), Cesium (Cs), Cobalt (Co), Manganese (Mn), Molybdenum (Mo), Strontium (Sr), and Uranium (U) (Bathla & Jain.2016), as well as Titanium (Ti) (Hayati.2018).
4. **Valuable Metals:** Precious metals are defined as rare metals with high economic value, including Palladium (Pd), Platinum (Pt), Silver (Ag), Gold (Au), and Rhodium (Ru) (Hayati.2018).
5. **Trace Metals:** Trace elements are inorganic micronutrient minerals that naturally occur in humans and are required in amounts less than 100 mg per day. These elements are essential components of biological structures and play significant roles in various processes vital for life, mediating crucial biochemical reactions. Excess levels of these elements, which exceed the amounts needed for biological functions, can be toxic to health. Therefore, it has been established that imbalances in optimal levels of trace elements can impair biological processes and are associated with many lethal diseases, including cancers. Recent efforts have focused on understanding the relationship between heavy metals, trace elements, and their roles in cancers. Many studies have shown that certain specific elements may be valuable and have predictive significance in the early diagnosis, prognosis, and treatment assessment of certain diseases, particularly various types of cancer. Trace elements include Zinc (Zn), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Nickel (Ni), Chromium (Cr), Cobalt (Co), Lead (Pb), Selenium (Se) (23), Iodine (I), and Fluorine (F) (Sousa et al.2019).

2.2 Classification of Metalloids:

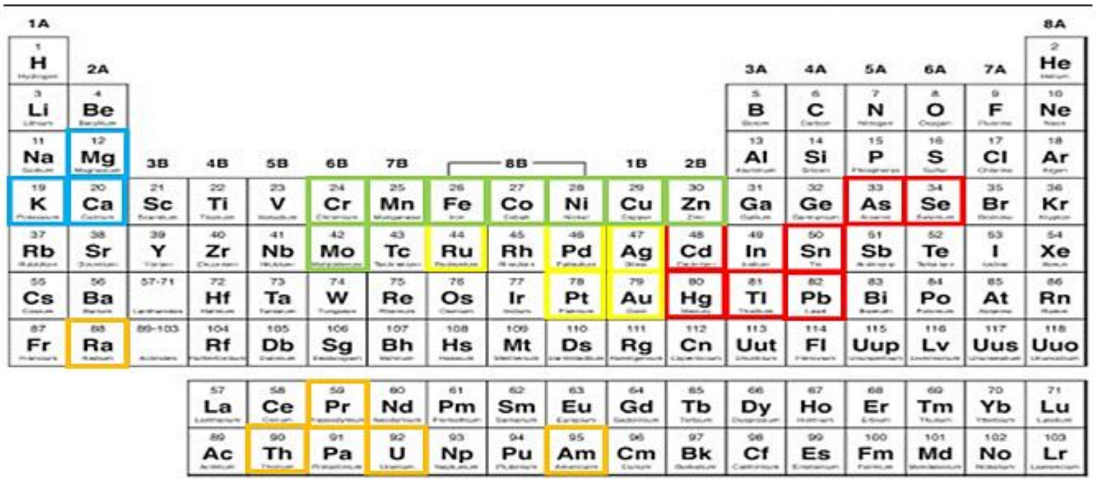
Metalloids encompass a group of chemical elements that are widely found in nature. Most of their physical and chemical properties are intermediate between metals and nonmetals, making their classification as either metal or nonmetal challenging. Physically, they are shiny, brittle, and exhibit moderate electrical conductivity similar to metals, while chemically, they behave like nonmetals. Commonly recognized metalloids include Arsenic (As), Antimony (Sb), Boron (B), Germanium (Ge), Silicon (Si), and Tellurium (Te) (Kesawat et al. 2020). Astatine (At) and Selenium (Se) are sometimes also considered metalloids as they exhibit metalloid characteristics under specific conditions (Lashani et al.2023).

The roles of metalloids in cellular processes are typically divided into four major categories (Kesawat et al. 2020):

1. Non-essential metalloids: Ge, Te(Kesawat et al. 2020).
2. Essential metalloids: B(Kesawat et al. 2020).
3. Beneficial metalloids: Si(Kesawat et al. 2020).
4. Highly toxic metalloids: As, Sb(Kesawat et al. 2020).

Figure 1 illustrates the descriptive position of heavy metals and metalloids in the periodic table.

Figure 1: The descriptive position of heavy metals and metalloids in the periodic table. (Jadaa. 2023)



The chemical properties and oxidation-reduction characteristics of metals have been beneficial for various cellular mechanisms, including transport, stabilization, categorization, and the binding of disrupted components such as DNA, proteins, and other molecules. Therefore, heavy metals and metalloids are defined as dense metals that occur naturally and can be toxic even at very low concentrations, possessing an atomic density greater than 4 grams per cubic centimeter, which is five times that of water (Das et al.2016).

3. Toxic Effects of Heavy Metals and Metalloids on Human Health:

These heavy metals and metalloids are also referred to as micronutrients due to their necessity at very low levels for the normal growth and maintenance of living organisms. The toxicity of heavy metals can persist in nature for extended periods; some can even transform lower toxic species into higher toxic forms in specific environments. For example, the biostorage and bioaccumulation of mercury in the food chain can disrupt natural physiological activities and ultimately harm human life. Compounds containing metals such as gold (Au), platinum (Pt), palladium (Pd), vanadium (V), rhodium (Rh), titanium (Ti), iridium (Ir), and other rare metals have recently been utilized in medicine for applications including imaging, radiotherapy, anti-arthritis treatments, and cancer therapies (Hayati T. 2018).

Heavy metals, whether in elemental form or as metal-organic compounds, can have significant impacts on the health of human, animal, and plant communities. Exposure to heavy metals can lead to neurological disorders, cellular aging, liver and kidney failures, gastrointestinal issues, and carcinogenic effects (Hayati T. 2018). Conversely, if the levels of heavy metals and metalloids drop below a critical threshold, it may disrupt essential plant processes such as photosynthesis, mitosis, and water absorption. Symptoms of toxicity in plants may include wilting leaves, short brown roots, and other indicators. High accumulation of metals from soil in plants can affect the food chain of humans and animals, leading to serious consequences. This issue impacts not only plant health but also the health of animals and humans that consume these plants, ultimately posing a threat to ecosystems (Das et al.2016). However, the increased concentrations of several metals in soil and water due to the industrial revolution have created a concerning situation for human life and aquatic organisms. This is evident from various reports highlighting the detrimental effects of heavy metals on human health. The toxic effects of certain heavy metals on human health are summarized in Table 1.

Table 1: Toxic Effects of Heavy Metals on Human Health

Row	Metal	Toxic Effect	Reference
1	Ag	Exposure to silver metal may cause a gray or bluish discoloration of the skin and other body tissues, respiratory issues, irritation of the lungs and throat, and stomach pain.	(Das et al.2016) (Jobby&desai.2017)
2	Ba	This metal can cause cardiac arrhythmias, respiratory failure, gastrointestinal dysfunction, muscle contractions, and increased blood pressure.	(Jobby&desai.2017)
3	Cd	Cadmium interferes with cellular proliferation, differentiation, apoptosis, and DNA repair mechanisms. Common clinical effects include demineralization of the skeleton, kidney and liver problems .as well as the induction of cancer and mutations. Additionally, it acts as an endocrine disruptor, causes lung damage, and affects calcium regulation in biological systems .	(Jobby&desai.2017) (Raffa et al.2021)

4	Cr	The main toxic effects of chromium on humans include hair loss, wounds, dermatitis, perforation of the nasal septum, and respiratory cancer. In soil, chromium alters the structure of microbial communities and reduces their growth .	(Jobby&desai.2017) (Raffa et al.2021) (Das et al.2016)
5	Cu	Excessive concentrations of copper lead to the formation of free radical species that damage cells and inactivate certain enzymes, posing a threat to the environment, microorganisms, and human health. Specifically, accidental ingestion of copper may cause nausea, vomiting, and abdominal pain, while long-term exposure can result in chronic effects, including damage to the brain and kidneys, increased levels leading to cirrhosis of the liver and chronic anemia, as well as irritation of the stomach and intestines.	(Jobby&desai.2017) (Raffa et al.2021)
6	Hg	Mercury tends to accumulate in various parts of the human body, causing damage to the brain, thyroid, lungs, myocardium, muscles, liver, kidneys, skin, and pancreas. Among these organs, the nervous system is most affected. Inorganic mercury can inhibit enzyme activity in the body and disrupt the normal metabolism of cells. The organic form has a significantly negative impact on brain function and can enter the body through the food chain. Additionally, it can lead to autoimmune diseases, depression, drowsiness, fatigue, hair loss, insomnia, memory loss, restlessness, visual disturbances, tremors, mood swings, brain damage, and respiratory and kidney failure.	(Jobby&desai.2017) (Raffa et al.2021) (Das et al.2016)
7	Ni	Allergic skin diseases such as itching, lung cancer, and conditions affecting the nose, sinuses, and throat can result from continuous inhalation. These conditions are immunotoxic, neurotoxic, and genotoxic, impacting fertility and causing hair loss.	(Jobby&desai.2017) (Das et al.2016)
8	Pb	Lead poisoning primarily occurs through the ingestion of contaminated food and water. This element is rapidly absorbed into the bloodstream and causes damage to various systems. Numerous studies have reported the hazardous effects of lead on the nervous system, including irritability, restlessness, headaches, confusion, ataxia, drowsiness, seizures, and coma, as well as impacts on kidney function, body development, and the lymphatic system. Additionally, excessive exposure in children leads to growth disturbances, decreased intelligence, short-term memory loss, learning disabilities, coordination problems, and an increased risk of cardiovascular diseases.	(Jobby&desai.2017) (Raffa et al.2021) (Das et al.2016)
9	Zn	This micronutrient is essential for all organisms and plays a crucial role in the metabolism of nucleic acids and proteins, as well as in cell growth, division, and function. Excessive concentrations of zinc in food or drinking water may lead to vomiting, muscle cramps, and kidney damage . It can also result in dizziness and fatigue in humans .	(Jobby&desai.2017) (Raffa et al.2021) (Das et al.2016)

Heavy metals and metalloids accumulate in the environment, where human activities increase their background levels. This effect becomes dangerous when natural concentrations reach levels that can cause harm to living organisms. Their toxicity is significant even at low concentrations, and the toxic effects of certain metalloids on human health are presented in Table 2.

Table 2: Toxic Effects of Certain Metalloids on Human Health

Row	metalloids	Toxic Effect	Reference
1	As	Arsenic can cause health issues such as cancer (skin, lung, bladder, and liver . kidney) skin lesions including melanosis (hyperpigmentation), keratosis, and leukomelanosis (hypopigmentation). It can also disrupt the peripheral nervous system, lead to liver failure, leukopenia, circulatory diseases, anemia, and even death. Additionally, arsenic affects essential cellular processes such as oxidative phosphorylation and ATP synthesis (8). It can lead to ischemic heart diseases, impair cognitive abilities, motor functions, and hormonal regulation .	(Jobby&desai.2017) (Raffa et al.2021) (Das et al.2016) (Lashani et al.2023)
2	Se	Exposure to high levels of selenium can lead to disorders such as selenosis, hair and nail loss, disruption of redox regulation, mitochondrial dysfunction, and inhibition of cell growth . It can also affect endocrine function, disrupt the	(Jobby&desai.2017) (Lashani et al.2023) (Das et al.2016)

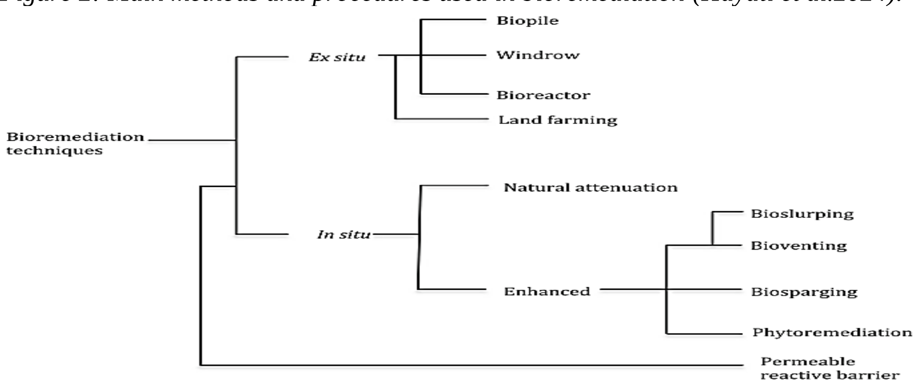
		activity of natural killer cells, cause hepatotoxicity, and result in gastrointestinal disturbances .	
3	B	At higher concentrations, this metal can be toxic and lead to numerous issues, including increased oxidative stress in cells, DNA damage, disruption of DNA repair systems, impairment of membrane function, inhibition of protein folding, and alterations in protein function and activities in living organisms.	(Lashani et al.2023)
4	Sb	Antimony is not found in living systems and, like arsenic, is highly toxic to humans and other living organisms. Long-term exposure to antimony can lead to issues such as eye, skin, lung, and mucous membrane irritation, oxidative damage to DNA, pneumoconiosis, and an increase in respiratory, cardiovascular, and gastrointestinal diseases. Additionally, antimony can impact the nitrogen cycle in soil and affect the activity of urease at a pH of 7.	(Lashani et al.2023) (Das et al.2016)
5	Te	Exposure to high levels of tellurium can lead to several health issues, including respiratory irritation, headaches, drowsiness, weakness, lethargy, fatigue, gastrointestinal symptoms, dizziness, and dermatitis.	(Lashani et al.2023)

4. Bioremediation:

Some microorganisms that inhabit soils and wastewater naturally utilize specific chemicals that are harmful to humans and the environment. These microorganisms can convert these chemicals into harmless water and gases, such as carbon dioxide. Many algae and bacteria produce exudates that absorb toxic metals at high levels. Metals are effectively removed from the food chain by binding to these exudates. The degradation of dyes is also carried out by certain bacteria and fungi, although this study will focus solely on the bacterial component (Girma.2015). It is important to note that when dry soils are contaminated with heavy metals, eradicating their effects is significantly more challenging than when metals and metalloids are present in moist soils or wastewater, making their restoration to their original state much more complex. In this context, there are very few alternatives, one of which is bioremediation—a diverse group of relatively modern processes, techniques, and methods that etymologically means “biological alleviation” (Cervantes et al.2023).

In recent decades, bioremediation techniques have advanced for the restoration of contaminated environments and their effective, economical, and environmentally friendly cleanup. Compared to both chemical and physical remediation methods, bioremediation is an environmentally compatible, more cost-effective, and sustainable approach. The restoration of contaminated environments can be achieved through various bioremediation techniques. The bioremediation methods covered so far are primarily driven by bacterial and archaeal floras, while other floras such as fungi, yeasts, and algae can also be part of selective systems; however, this study will focus solely on the bacterial aspect. As a reference point, the main branches of bioremediation can be summarized in Figure 2. Improving the efficiency of bioremediation is possible by enhancing the biodegradation capabilities of the employed microorganisms using the latest supporting technologies, which include genetically engineered microorganisms (GEM), nano and microbubbles, engineered biochar, mixed trophic microalgae, and nanotechnology (Hayati et al.2024).

Figure 2: Main methods and procedures used in bioremediation (Hayati et al.2024).



4. Microorganisms Involved in Bioremediation:
Microorganisms (bacteria, fungi, protozoa, and parasites) can be isolated from almost any environmental condition. They are capable of adapting and thriving in extreme and harsh conditions (extremophiles), such as lower and higher than normal temperatures near volcanic vents, desert conditions, low and abundant water, excessive oxygen or oxygen deficiency, the rumen of ruminants, the digestive tracts of insects, polar frost, and anaerobic conditions, among others. The primary requirements for microbial growth are a suitable energy source and a carbon source. Due to their adaptability to environmental conditions, microorganisms can be utilized for the degradation, decomposition, and absorption of pollutants to reduce environmental contaminants (Hayati.2018).

4.1. Aerobic Bacteria:
This group of bacteria is divided into two categories based on respiration:

1. Facultative Aerobes: These bacteria can use oxygen for respiration and are capable of generating energy through anaerobic processes in the absence of oxygen(Hayati.2018).
2. Obligate Aerobes: These bacteria can only grow in the presence of oxygen and require oxygen for energy production(Hayati.2018).

These types of bacteria are often reported to play a significant role in the reduction of pesticides and hydrocarbons. Many of these bacteria utilize pollution as their sole carbon and energy source (Hayati.2018).

4.2. Anaerobic Bacteria:
This group of bacteria is also divided into two categories based on respiration: (Hayati.2018).

1. Facultative Anaerobes: Bacteria that can grow in the presence or absence of oxygen but perform well in the absence of oxygen(Hayati.2018).
2. Obligate Anaerobes: Bacteria that can only survive in the absence of oxygen(Hayati.2018).

Consequently, there is increasing interest among researchers in cultivating anaerobic bacteria for bioremediation of polychlorinated biphenyls in river sediments, as well as for the dechlorination of trichloroethylene and chloroform (Hayati.2018).

4.3. Microaerophilic Bacteria:
These bacteria require low levels of oxygen and cannot survive in high concentrations of oxygen; in fact, high oxygen concentrations are toxic to them.

4.4. Microaerotolerant Bacteria:
These bacteria can grow in low-oxygen conditions but are also capable of tolerating high-oxygen environments. They can thrive in various oxygen concentrations and can even survive in anaerobic conditions.

Table 3 presents heavy metals and their associated microorganisms for bioremediation (genetically modified microorganisms), while Table 4 lists metalloids and related microorganisms classified by their respiration type.

Table 3: Heavy Metals and Associated Microorganisms for Bioremediation (Genetically Modified Microorganisms)

Row	Metal	Microorganisms	type of respiration	Reference
1	Hg	*Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas stutzeri, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Aeromonas hydrophila*	Facultative Aerobic	(Verm&Kuila.2019) (Purkan et al.2016) (Massoud et al.2019) (Figueiredo et al2016) (Kumari et al.2019)
		Acidithiobacillus ferrooxidans, Deinococcus geothermalis, Deinococcus radiodurans	Obligate Aerobic	
		Serratia marcescens, Cupriavidus metallidurans	Facultative Anaerobic	
		Achromobacter sp., Aeromonas hydrophila, Bacillus subtilis, Cupriavidus metallidurans, Deinococcus geothermalis, Deinococcus radiodurans, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas sp., Pseudomonas stutzeri, Saccharomyces cerevisiae, Staphylococcus aureus, Escherichia coli, Serratia marcescens	Microaerotolerant	
		Vibrio parahaemolyticus	Microaerophilic	

2	Pb	*Bacillus carotarum ،Bacillus barbaricus ،Bacillus lentus ، Geobacillus thermodenitrificans ،Pseudomonas vesicularis ، Pseudomonas aeruginosa ،Enterobacter cloacae ، Stenotrophomonas maltophilia*	Facultative Aerobic	(Verm&Kuila.2019) (Kumar et al.2016) (Sen et al.2014) (Massoud et al.2019) (Gupta et al.2014) (Coelho et al.2015)
		Aeromonas veronii ،Bacillus cereus ،Bacillus licheniformis ، Bacillus subtilis ،Pseudomonas fluorescens ،Staphylococcus aureus ،Escherichia coli	Microaerotolerant	
3	Cr	*Deinococcus radiodurans ،Methylococcus capsulatus*	Obligate Aerobic	(Hayati.2018) (Verm&Kuila.2019) (Purkan et al.2016) (Kumar et al.2016)
		Pseudomonas aeruginosa ،Staphylococcus epidermidis ، Escherichia coli ،Serratia marcescens ،Enterobacter cloacae ، Achromobacter eurydice ،Alcaligenes eutrophus ،Pantoea agglomerans ،Bacillus subtilis ،Bacillus megaterium ، Pseudomonas putida ،Pseudomonas maltophilia ،Pseudomonas synxantha ،Geobacillus thermodenitrificans ،Micrococcus roseus ،Pseudomonas ambigua ،Pseudomonas chromatophilia ، Staphylococcus capitis	Facultative Aerobic	
		Pseudomonas dechromaticans ،Shewanella algae ،Shewanella putrefaciens ،Geobacter metallireducens	Facultative Anaerobic	
		Desulfomicrobium norvegicum ،Desulfovibrio desulfuricans ، Desulfovibrio vulgaris ،Pyrobaculum islandicum ، Thermoanaerobacter ethanolicus	Obligate Anaerobic	
		Bacillus cereus ،Pseudomonas fluorescens ،Aeromonas dechromatica	Microaerotolerant	
4	Zn	*Pseudomonas aeruginosa ،Cupriavidus metallidurans ، Geobacillus thermodenitrificans*	Facultative Aerobic	(Das et al.2016) (Kumar et al.2016) (Gupta et al.2014) (Peng et al.2018)
		Acidithiobacillus ferrooxidans ،Acidithiobacillus thiooxidans ، Leptospirillum ferrooxidans	Obligate Aerobic	
		Desulfotomaculum nigrificans	Obligate Anaerobic	
		Bacillus cereus ،Bacillus licheniformis ،Bacillus lentus ،Bacillus carotarum ،Pseudomonas putida ،E. coli	Microaerotolerant	
		Rhodobacter sphaeroides	Microaerophilic	
5	Cd	*Pseudomonas aeruginosa ،Acinetobacter sp. ،Citrobacter sp. ، Geobacillus thermodenitrificans ،Pseudomonas putida ، Pseudomonas monteilii ،Stenotrophomonas maltophilia ، Salmonella typhimurium ،Micrococcus roseus*	Facultative Aerobic	(Kumar et al.2016) (Massoud et al.2019) (Priyalaxmi et al.2014) (Wu et al.2019) (Sen et al.2014) (Shen et al.2018) (Ma et al.2020) (Shi et al.2020)
		Treponema denticola	Facultative Anaerobic	
		Aeromonas veronii ،Bacillus safensis ،Bacillus mycoides ، Pseudomonas fluorescens ،Cupriavidus taiwanensis ، Saccharomyces cerevisiae	Microaerotolerant	
		Rhodobacter sphaeroides	Microaerophilic	
6	Ni	*Bacillus thuringiensis ،Proteus vulgaris ،Staphylococcus sp.*	Facultative Aerobic	(Das et l.2016) (Kumar et al.2016) (Das et al.2014)
		Pseudomonas fluorescens	Microaerotolerant	
7	Ag	*Citrobacter intermedius ،Pseudomonas diminuta ،Pseudomonas maltophilia ،Staphylococcus aureus ،Geobacillus thermodenitrificans*	Facultative Aerobic	(Das et l.2016) (Kumar et al.2016)
		Pseudomonas maltophilia ،Staphylococcus aureus	Microaerotolerant	

Table 4: Metalloids and Associated Microorganisms for Bioremediation (Genetically Modified Microorganisms)

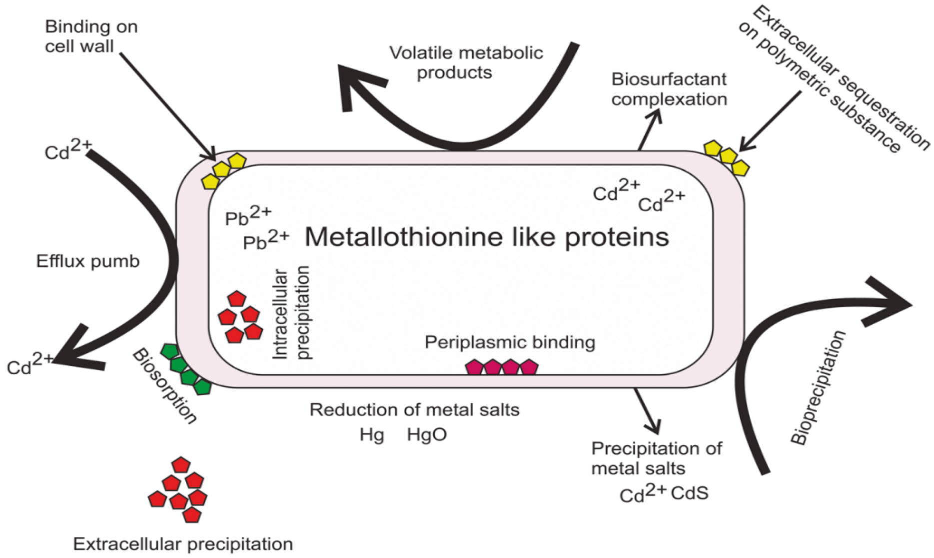
Row	Metalloids	Microorganisms	type of respiration	Reference
1	As	*Alcaligenes faecalis ،Bacillus arsenicus ،Bacillus idriensis ،*	Facultative Aerobic	(Verm&Kuila.

		Kocuria sp. ‹Rhodococcus sp. ‹Sporosarcina ginsengisoli ‹ Stenotrophomonas sp. ‹Escherichia coli (E. coli)		2019) (Purkan et al.2016) (Massoud et al.2019) (Figueiredo et al.2016) (Kumari et al.2019)
		Alcalilimnicola ehrlichei ‹Shewanella sp. ‹Chrysiogenes arsenatis ‹Sphingomonas desiccabilis	Obligate Aerobic	
		Geospirillum amenophis ‹Geospirillum barnesii ‹Bacillus selenitireducens ‹Herminiimonas arsenicoxydans	Facultative Anaerobic	
		Corynebacterium glutamicum ‹Escherichia coli (E. coli)	Microaerotolerant	
2	Sb	*Acinetobacter sp. ‹Chryseobacterium koreense ‹Comamonas sp. ‹ Ensifer sp. ‹Flavobacterium sp. ‹Fluviicola sp. ‹ Janthinobacterium sp. ‹Methylotenera sp. ‹Paracoccus versutus ‹ Pseudomonas sp. ‹Stenotrophomonas nitritireducens ‹ Stenotrophomonas sp. ‹Variovorax sp. ‹Shewanella sp.*	Facultative Aerobic	(Verm&Kuila. 2019) (Kumar et al.2016) (Sen et al.2014) (33) (Gupta et al.2014) (Coelho et al.2015)
		Gallionella sp. ‹Halomonas sp. ‹Mycobacterium sp. ‹Thiomonas sp.	Obligate Aerobic	
		Ferrovum sp. ‹Ferruginibacter sp. ‹Geobacter sp. ‹ Ignavibacterium sp. ‹Shinella sp. ‹Sulfuricurvum sp.	Facultative Anaerobic	
		Clostridium sp. ‹Desulfobulbus sp. ‹Leptospirillum sp. ‹ Phormidium sp. ‹Scytonema sp.	Obligate Anaerobic	
		Lactobacillus sp. ‹Pseudomonas sp.	Microaerotolerant	
3	Se	*Enterobacter cloacae ‹Pseudomonas syringae ‹Halomonas pacifica*	Facultative Aerobic	(Hayati.2018) (Verm&Kuila. 2019) (Purkan et al.2016) (Kumar et al.2016)
		Thauera selenatis	Obligate Anaerobic	
		Bacillus sp. ‹Staphylococcus warneri	Microaerotolerant	

5.Mechanisms of Heavy Metal and Metalloid Resistance by Bacteria:

Metals, metalloids, and bacteria can interact directly or indirectly with each other, depending on the microorganisms, metal ions, and external environment. Temperature, pH, nutrient sources, and metal ions are just some of the factors that influence how and how quickly heavy metals are absorbed by microbes. Due to their small size and rapid growth rate, bacteria can thrive in diverse environments. For this reason, they have been used to remove hazardous metals from the environment. The functional groups present in the bacterial cell wall (such as carboxyl, amino, phosphate, and sulfate) are typically where heavy metals bind. The efficiency of bio-remediation dependent on biofilms is significantly influenced by the adhesion of bacteria to the substrate and the three-dimensional organization of cells within biofilms, which varies greatly among microorganisms. Bacterial tolerance to metals may be affected by several factors, such as the transport of metal ions into the cell, efflux, extracellular and intracellular sequestration, and redox reactions. The extracellular barrier may act as a shield against the entry of metal ions. However, by utilizing ionizable groups in the cell wall or capsule, bacteria can adsorb metal ions. For the export of metal ions from cells, bacteria utilize active transport and efflux mechanisms. Metals are then complexed with various compounds (metallothioneins, phytochelatins) in the cytoplasm of the cell or accumulate through cellular components (periplasmic proteins, outer membrane proteins) in the periplasm or outer membrane. Some bacteria can generate energy using metals and metalloids as electron acceptors or donors. Metals in their oxidized state may serve as final electron acceptors in the anaerobic respiration of bacteria. Enzymatic reduction also leads to the formation of less toxic forms of heavy metals (Figure 3) (Das et l.2016).

Figure 3: Mechanisms of Bioremediation



Functional Type and Composition of Microbial Communities can also be evaluated using biological microplate methods, Restriction Fragment Length Polymorphism (RFLP) based on the 16S rRNA sequence of bacteria, as well as PCR-DGGE analysis. In a contaminated environment, the response of microbial communities depends on the type of metal, the nature of the substrate (such as pH, which affects the bioavailability of metals), and the species of microorganisms. In some cases, strains from contaminated and clean environments exhibit a high level of resistance to heavy metals, indicating the presence of inherent or structural resistance mechanisms. Microbial resistance mechanisms can be encoded by chromosomal genes or primarily by plasmid genes. Microorganism species with resistance mechanisms encoded on plasmids are of greater importance for ultimate applications in bioremediation, as these genes can be easily accessed by other microbial species through horizontal gene transfer. There are five main mechanisms of heavy metal resistance: (I) extracellular barrier (permeability), (ii) oxidation/reduction of heavy metal ions, (iii) intracellular sequestration, (iv) extracellular sequestration, and (v) efflux (active transport) of metal ions.

An Overview of How Microorganisms Respond to Some Heavy Metals and Metalloids:

Mercury-resistant strains of *Pseudomonas aeruginosa* can absorb mercury ions with a maximum uptake potential of about 180 mg/g. Since cysteine has a high affinity for mercury ions, cysteine-rich proteins with numerous sulfhydryl groups likely sequester mercury ions. Alternatively, mercury-resistant strains of *Pseudomonas putida* may absorb all Hg from the marine environment and then convert toxic mercury (Hg(II)) to Hg(0), leading to reduced mercury toxicity(Tarfeen et al.2022).

Both living and dead *Arthrobacter viscosus* can absorb Cr(VI) and convert it to Cr(III). Cr(VI) is effectively removed from the environment by biofilms of *Staphylococcus epidermidis*(Tarfeen et al.2022).

With a maximum absorption capacity of about 164 mg/g, *Rhodobacter capsulatus* may absorb Zn(II) and follows Langmuir and Redlich-Peterson isotherms(Tarfeen et al.2022).

Bacillus cereus strains can absorb Cd(II) with biosorption capacities of approximately 32 mg/g for dead cells and 24 mg/g for live cells. Extracellular polymeric substances (EPS) protect bacteria from any damage by preventing the entry of hazardous heavy metals into the cell (Tarfeen et al.2022).

Metal ions such as Hg, Cd, Co, and Cu can be sequestered by EPS. Heavy metals can change to another ionic state after absorption in bacterial cells to reduce their toxicity (Tarfeen et al.2022).

The common presence of arsenic in the environment has led microbes to develop defenses against arsenic. The presence of arsenic resistance operons is the most common defensive mechanism. The arsenic resistance operon typically (ars) includes (a) ArsR, a regulatory protein; (b) ArsB, an arsenite permease; and © ArsC, an enzyme necessary for arsenate reduction. Genome analysis of *Corynebacterium glutamicum* has shown that there are two complete arsenic resistance operons, ars1 and ars2, which contain the typical three-gene structure, arsRBC, along with an additional gene, arsc1(Saha et al.2017)(Ojuederie & Babalola.2017)

6 - Biochemical Reactions and Mechanisms of Bioremediation by Bacteria:

Bacterial signal transduction pathways assist in regulating or modulating cellular changes in response to external environmental stimuli. These signaling pathways can be divided into two categories: (Shweta et al.2021).

1. One-component pathways, which involve a single protein that combines both input and output domains, allowing for direct and simple signal transduction from stimulus recognition to cellular response (Shweta et al.2021).
2. Two-component pathways, which involve a membrane-bound histidine kinase that helps in recognizing environmental stimuli and a response regulator that mediates the cellular response. This second type of signaling pathway requires a phosphotransfer group between the two key proteins(Shweta et al.2021).

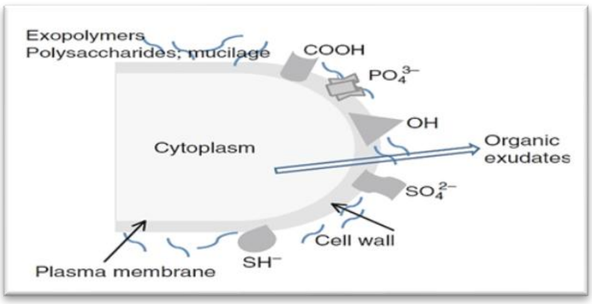
Among these pathways, the one-component system is the oldest and simplest system and is widely distributed in prokaryotes. However, findings suggest that the majority of prokaryotic signal transduction is carried out by the two-component system. Even bacterial chemotaxis pathways utilize this two-component system(Shweta et al.2021).

1-6 - Extracellular Materials:

1-1-6 - Chemical Functional Groups:

Metal and metalloid ions present in solution are adsorbed onto surfaces and react with chemical functional groups such as carboxyl, amine, imidazole, phosphate, thiol, hydroxyl, and other functional groups present in the biological polymers of the cell wall. The biosorption by these microorganisms is largely attributed to the ligands present in the biomolecules of their cell wall polymers. The bacterial cell wall is the first component that interacts with metal and metalloid ions. Therefore, the method of heavy metal absorption by non-living or inactive extracellular cells involves chemical functional groups present in the cell wall, such as carboxyl, phosphonate, amine, and hydroxyl groups, which play key roles in the absorption of heavy metals and metalloids. Since these groups carry a negative charge and are abundant on the cell surface, they actively participate in binding with metal cations. However, the efflux of metal cations and metalloids by microorganisms depends on surrounding environmental characteristics such as pH, temperature, metal concentration, and biomass (Hayati.2018).Sometimes, the ability of the biomass to absorb metal and metalloid ions can be enhanced by adding phosphate groups to the cell wall of photosynthetic microorganisms. This process can be achieved by the phosphorylation of hydroxyl groups in the polysaccharides of the cell wall using phosphoric acid in a urea-containing solution (Figure 4)(Gomes et al.2014).

Figure 4: Mechanism of Heavy Metal and Metalloid Uptake through Chemical Functional Groups



The binding of heavy metals and metalloids to extracellular materials stabilizes the metals and metalloids and prevents their entry into the cell. The anionic functional groups present on cell surfaces bind with a wide range of cationic metals such as cadmium, lead, zinc, and iron. For example, the stabilization of lead by extracellular polymers from various bacteria such as Staphylococcus aureus, Micrococcus luteus, and Azotobacter has been observed (Ariafar et al.2015).

2-1-6 - Siderophores:

These are compounds that form complexes with heavy metals and metalloids. Siderophores are low molecular weight extracellular materials that chelate iron and act as solubilizing agents for both inorganic and organic iron compounds under iron-limiting conditions. In addition to iron, siderophores can also form complexes with other metals such as aluminum, cadmium, copper, gallium, indium, lead, and zinc, as well as with radionuclides like uranium and neptunium. The complexes formed by siderophores with heavy metals and metalloids increase the concentration of soluble metals and reduce the toxicity of these metals to cells (Hayati.2018).

3-1-6 - Biosurfactants:

Similar to siderophores, these are extracellular compounds capable of forming complexes with metals such as zinc, copper, and cadmium, thereby increasing the solubility of these metals and reducing their toxicity(Hayati.2018).

2-6 - Various Pathways for Heavy Metal Uptake into Cells:

These mechanisms vary depending on the type of metal and its compounds. Metals and metalloids can enter cells through natural transport processes or through competition to bind with the sites of transport proteins or ionic compounds, such as calcium channels. Bacteria that not only have the ability to absorb and sequester heavy metals and metalloids but also possess resistance and stability against these toxic metals can be utilized. These resistant and metal-accumulating microbes include groups of sulfate-reducing bacteria (SRB) such as Desulfovibrio desulfuricans. This bacterium utilizes various electron donors such as organic acids: lactate, acetate, pyruvate, fumarate, and hydrogen. It also has different electron acceptors such as sulfate, sulfite, thiosulfate, sulfur, nitrate, nitrite, and heavy metals. Thus, these bacteria can enzymatically reduce and render heavy metals insoluble(Kumar et al.2015).

Another mechanism is the active pumping of toxic metals out of the cell or enzymatic detoxification (generally redox chemistry), which involves converting a toxic ion to a less toxic ion or making that metal ion less bioavailable. Detoxification of metals is performed by forming complexes by many eukaryotes. Metallothioneins (MTs) are low molecular weight proteins (6–7 kDa) rich in cysteine found in animals, higher plants, eukaryotic microorganisms, and some prokaryotes. (No aromatic amino acids or histidine are present in MTs.) (Kumar et al.2015).

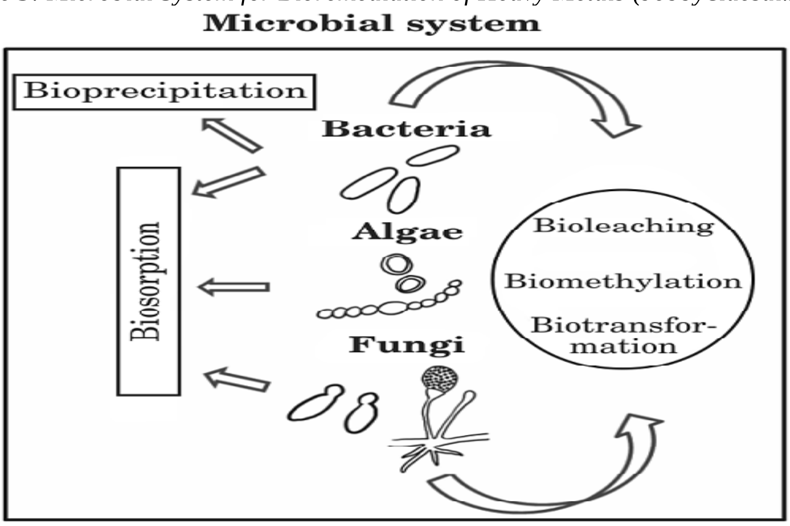
MTs are classified into three different categories based on cysteine content and structure: the predominant forms are Cys-Cys, Cys-X-X-Cys, and Cys-X-Cys (where X represents any amino acid). In several cyanobacterial species of the genus Synechococcus, a prokaryotic MT has been found. This MT, encoded by the smtA gene, contains fewer cysteine residues than mammalian MTs(Kumar et al.2015).

7 - Interactions of Metals, Metalloids, and Bacteria:

Microorganisms utilize heavy metals, metalloids, and trace elements as terminal electron acceptors or reduce them through detoxification mechanisms, which are employed to remove heavy metals and metalloids from contaminated environments. Microorganisms eliminate heavy metals and metalloids through mechanisms that extract energy from redox reactions of metals, using both enzymatic and non-enzymatic processes to counteract toxic metals. Continuous exposure to metals helps microbes become familiar with them and develop resistance. Therefore, understanding the nature of metal-microbe interactions becomes essential. These interactions can be categorized into the following types: (Amanso & Songachan.2021).

1. Biosorption(Amanso & Songachan.2021).
2. Bioaccumulation(Amanso & Songachan.2021).
3. Biomineralization(Amanso & Songachan.2021).
4. Bioleaching(Amanso & Songachan.2021).
5. Biotransformation (Figure 5) (Amanso & Songachan.2021).

Figure 5: Microbial System for Bioremediation of Heavy Metals (Jobby&desai.2017)



Biosorption:

The ability of biological materials to accumulate heavy metals and metalloids from wastewater through metabolic intermediates (using ATP) or spontaneous physicochemical adsorption pathways (not at the expense of ATP) is referred to as biosorption. This is an interactive process in which metal ions non-specifically bind to polysaccharides and proteins present on the cell surface. It is a characteristic in which both living cells and dead microbial biomass create binding sites, and these binding sites can sequester heavy metals and metalloids even from very dilute solutions(Amanso & Songachan.2021).

Several variables, including cellular physiology, physicochemical factors such as pH, temperature, contact time, ionic strength, and metal concentration, as well as the structure of microbial cell walls, all play a role in this complex process. Various groups have studied the biosorption of different heavy metals and metalloids such as cadmium, silver, lead, and nickel using microorganisms like fungi, algae, or bacteria. The advantages of biosorption include: (Namdeti.2023).

1. Lower cost biomass production (bacteria or fungi) (Namdeti.2023).
2. Utilization of biomass for heavy metal removal(Namdeti.2023).
3. Simultaneous uptake of multiple heavy metals(Namdeti.2023).

Bacteria in Biosorption:

Regarding microorganisms, bacteria are the most common, adaptable, and diverse species on Earth. According to various binding mechanisms, the cell wall generally controls the surface binding sites and the binding strength of different metal ions. Due to their small size and capacity to grow in various environmental conditions, several bacterial species, including *Bacillus, Pseudomonas,* and *Escherichia*, exhibit biosorption properties. The initial phase of biosorption occurs when a metal ion contacts the bacterial cell wall. The functional groups (amine, carboxyl, hydroxyl, phosphate, sulfate, and carboxyl) found in the cell wall serve as binding sites for metal ions. The overall process of internalizing metal ions in bacteria involves the binding of metal ions to active groups on the bacterial cell wall. Since glycoproteins are present, Gram-positive bacteria can absorb more metals. In contrast, Gram-negative bacteria, due to phospholipids and LPS, absorb fewer metals(Namdeti.2023).

In fact, biosorption is also a physicochemical process through which pollutants are passively attached to the cellular structure. In biotransformation, the chemical state of the pollutant is altered within the cell(Hayati.2018).

Bioaccumulation:

This is a method in which living microbial cells are used to purify heavy metals and metalloids. This process involves various biochemical interactions such as adsorption onto functional groups on the cell wall, intracellular accumulation, production of various complexes, oxidation, reduction, and precipitation. As a result, heavy metals and metalloids are actively absorbed. Bioaccumulation occurs when the rate of elimination of a toxic substance is generally less than the rate of absorption of that substance(Hayati.2018).

Bioleaching:

This is a simple and effective technology for extracting valuable heavy metals and metalloids from low-grade ores and mineral concentrates. (Dadban et al.2017)Bioleaching generally refers to the conversion of solid metals into a soluble form in water for microbial utilization(Hayati.2018).

Biotransformation:

In this method, the chemical state of the pollutant is altered within the cell(Eslami.2015).

Biomineralization:

This refers to the conversion of one mineral or organic substance into another mineral(Hayati.2018).

In addition to industrial applications for providing raw materials, microbial leaching is used for the remediation of certain mining sites, treatment of industrial mineral waste, detoxification of sewage sludge, and for the remediation of soils and sediments contaminated with heavy metals(Dixit et al.2015).

Table 2 summarizes the most important studies conducted in the field of bioremediation of heavy metals. As observed from these studies, the selection of the appropriate microorganism for the bioremediation of heavy metals should be based on the type of heavy metal and metalloids(Eslami.2015).

Table 2: Summary of the Most Important Studies Conducted in the Field of Bioremediation of Heavy Metals and Metalloids

Row	Metal	Microorganism Used	Microorganism Species	Findings	Reference
1	Hg(II)	*Limnothrix planctonica* *Synechococcus leopoldiensis* *Phormidium limnetica*	Cyanobacteria	Under aerobic conditions and a concentration of Hg(II) below 200 ppb, convert the solution to Hg(0) and insoluble HgS.	(Eslami.2015).

2	Cd(II) Cu(II) Co(II)	*Entrobacter cloacae*	Bacteria	This bacterium has an extracellular polysaccharide that chelates heavy metals. At a concentration of 100 mg per liter, it chelates 65%, 20%, and 8% of cadmium, copper, and cobalt, respectively	(Eslami.2015).
3	Cd(II) Cu(II) Zn(II)	*Rhodobium marinum 16NW* *Rhodobacter sphaeroides 24KMS*	Nonsulfur purple bacteria	The advantage of using these organisms is their ability to produce large amounts of extracellular polysaccharides, which facilitate the accumulation of metals and also provide protection against metal toxicity. The removal efficiency of heavy metals by the extracellular polysaccharides of these two species has been reported to be 29.97% for lead, 91.84% for zinc, 09.97% for cadmium, and 90.52% for copper.	(Eslami.2015).
4	Cd(II) Cu(II) Zn(II)	*Desulfovibrio desulfuricans*	Sulfate-reducing bacteria	While no effect on sulfate reduction was observed in the presence of Zn metal, Cu and Cd metals were found to reduce sulfate reduction efficiency by approximately 39% and 32%, respectively. The addition of Zn was reported to have no effect on the reduction of Cu toxicity, but it was effective in reducing Cd toxicity	(Eslami.2015).
5	As (V)		Bacteria	The ArsR gene and the arsenate reductase enzyme (ArsC) play a key role in the reduction of As(V) to As(III) and detoxify it by exporting it out of the cell through the ArsAB osmotic flow system.	(Pandey et al.2021)
6	As (V)	*Bacillus sp. XZM*	Bacteria	It can reduce the toxicity of As in Vallisneria denseserrulata.	(Irshad et al.2020)
7	As (V)	*Acidithiobacillus ferrooxidans BYQ-12*	Bacteria	The maximum bioleaching rate of As under optimal conditions was 97.73%, and the most effective factor for As leaching was the initial concentration of iron ions.	(Yan et al.2017)

4. Discussion and Conclusion:

Heavy metal pollution is a global environmental crisis that harms ecosystems and human health. Bioremediation, which utilizes microorganisms to detoxify heavy metals and metalloids, is a practical and environmentally friendly approach. Industrial, mining, and agricultural activities release heavy metals and metalloids, which subsequently accumulate in terrestrial and aquatic environments, posing significant environmental and health hazards. Several bioremediation techniques can remove heavy metals and metalloids, such as phytoremediation, mycoremediation, and microbial remediation (Safin et al.2024). Since microorganisms have various strategies for surviving in metal-contaminated environments, their detoxification mechanisms differ. Some of these strategies include biosorption, bioleaching, bioaccumulation, biotransformation, and biominization, which can be applied either in situ or ex situ for decontamination (Hayati.2018).

Zheng-Bo Yue and his colleagues conducted experiments in 2015 using sulfate-reducing bacteria (Desulfovibrio desulfuricans) and found that while no effect on sulfate reduction was observed in the presence of Zn metal, Cu and Cd metals were effective in reducing sulfate reduction efficiency by approximately 39% and 32%, respectively. They reported that the addition of Zn had no effect on reducing Cu toxicity but was effective in reducing Cd toxicity(Yue et l .2015).

Dadban Shahamat and colleagues published a paper in 2017 aimed at measuring heavy metals in the wastewater and sludge of the wastewater treatment plant in Gorgan city (Dadban et al.2017).

In 2018, Hayati conducted research on the isolation and identification of titanium dioxide-resistant bacteria and concluded that among 20 strains isolated from contaminated soils Staphylococcus and Bacillus genera, respectively. Both strains tolerated metal concentrations up to 1198 ppm, indicating that these bacteria are resistant to high metal concentrations and likely possess the ability to remove metals. Metal-resistant bacteria

are used in accelerating a biotechnological process for the recovery of precious metals (Bioleaching) and in the bioremediation of contaminated soils (Hayati.2018).
Safin Hassan Hussein and his colleagues studied the bioremediation of heavy metals in contaminated environments using Comamonas species in 2024 and reported that microbial remediation with Comamonas sp. is a promising option due to its potential in bioremediation of heavy metals. Comamonas thrive due to their adaptability to various environmental conditions in contaminated environments. Comamonas sp. can remove and detoxify heavy metals through various mechanisms, including biosorption, intracellular separation, and redox transformation. Therefore, evaluating the effectiveness of Comamonas sp.-based bioremediation for various heavy metals and identifying areas where further research is needed is essential (Safin et al.2024).
Based on the present study and other research, it is estimated that microorganisms, including bacteria, are suitable candidates for the bioremediation of heavy metal-contaminated sites (Hayati et al.2018).
and wastewater, only two strains, 1-2D cocci and 1-3D bacilli, based on their physiological and biochemical properties, belonged to the

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