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Chapter- 5

Bioremediation of Heavy Metals by Microbial Process

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Abstract:

Heavy metal toxicity, persistence, bioaccumulation, and biomagnification make it a serious environmental problem. There are several natural and human-caused factors that can lead to environmental heavy metal pollution. The main natural sources of heavy metals are rock weathering and volcanic eruptions. However, burning fossil fuels and petrol, mining, incinerators for trash, industrial and agricultural activities, metal-bearing rocks, and so on are human sources of heavy metals. The most common heavy metal pollutants that are extremely dangerous include lead, zinc, copper, mercury, arsenic, chromium, nickel, and arsenic. Oxidative stress development is the fundamental chemical process of metal poisoning. Stress weakens the immune system, ruins tissues and organs, leads to birth abnormalities, and reduces the ability to procreate. One innovative and promising technique that can be used to remove and reduce heavy metals from water and contaminated soil is bioremediation. An important component of heavy metal bioremediation is microorganisms. Genetically modified organisms can be created by genetic engineering, and these organisms have the potential to produce fewer polycyclic hydrocarbons (PAHs). There are numerous methods by which metals and microbes interact, including biosorption, bioaccumulation, and bioleaching. To preserve lives and carry out legislation relevant to heavy metal conservation in the environment, it is imperative to investigate the origins of these metals and the potentially harmful impact they have on human health.

Introduction:

As the world's population rises, so do human requirements and actions, which lead to an enormous buildup of toxic waste from many sources that contaminate our environment. Some of

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the negative effects of industrialization, like pollution, rising carbon emissions, and resource depletion, pose a threat to human health and the health of every region in the world. A few of the drawbacks of industrialization are that it forces technological advancements and alters human civilizations in ways that are both social and economic. High concentrations of non-essential heavy metals and metalloids (lead, cadmium and arsenic) in soils and irrigation water are harmful to the environment, food safety, and the health of people and animals (Samal et al., 2017; Chakraborty et al., 2019; Witkowska et al., 2021; Roy et al., 2022; Rangamani et al., 2023;). Flavobacterium, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Corynebacterium*, *Methosinus*, *Rhodococcus*, *Mycobacterium*, *Stereum hirsutum*, *Nocardia*, *Methanogens*, *Aspergilus niger*, *Pleurotus ostreatus*, *Rhizopus arrhizus*, *Azotobacter*, *Alcaligenes*, *Phormidium valderium*, and *Ganoderma applantus* are a few of the microbes that help in the bioremediation of heavy metals (Rajak, 2017; Thakur et al., 2021; Bala et al., 2022; Rajalakshmi and Paari, 2023). The most effective microbial resources for use in bioremediation or bacterial-assisted phytoremediation strategies that may help improve plant growth (Ghosh et al., 2023) and yield in contaminated soils have not yet been thoroughly analyzed, even though several bacterial strains have been reported to be capable of remediating heavy metal-affected soils (Wang et al., 2022).

Table 1: Heavy Metal-Induced Diseases: Unraveling the Pathway of Exposure.

Heavy Metal	Disease(s)	Pathway of Exposure
Lead	Lead poisoning	Ingestion of lead-contaminated food, water, or paint; Inhalation of lead dust or fumes (e.g., from lead-based paints, certain occupations)
Mercury	Minamata disease	Consumption of mercury-contaminated fish and seafood; Inhalation of mercury vapours (e.g., from industrial processes, dental amalgams)
Cadmium	Itai-Itai disease	Ingestion of cadmium-contaminated food, water, or tobacco; Inhalation of cadmium dust or fumes (e.g., from industrial processes)
Arsenic	Arsenicosis	Ingestion of arsenic-contaminated water, food, or air; Exposure through occupational activities (e.g., mining, smelting)
Chromium	Lung cancer (Hexavalent Cr)	Inhalation of hexavalent chromium compounds in certain occupational settings (e.g., welding, chrome plating)
Nickel	Lung and nasal cancers	Inhalation of nickel compounds, occupational exposure (e.g., nickel refining, alloy production)
Copper	Wilson's disease	Genetic disorder affecting copper metabolism; excessive intake from water or food
Aluminum	Alzheimer's disease	Although controversial, some studies suggest a link between aluminum exposure and Alzheimer's disease; exposure through food, water, and certain products

Heavy metal toxicity:

Globally water pollution is considered as one of the major threats that impact human health, ecosystem, and sustainable development. All over the world, massive industrialization and rapid urbanization adversely affect the ponds, lakes, and rivers water quality parameters (Bhattacharya, 2015; Mondal et al., 2022; Madhu et al., 2022). Nowadays the situation has become more adverse as the industries frequently release the wastes which contain metallic contaminants into the ecosystem, exceeding the permissible limit. Fishes are more susceptible to heavy metal pollution as they are continuously exposed to these pollutants and its potential for bioaccumulation. For this reason, fish are considered as an ideal biological indicator of heavy metal induced toxicity. A possible mechanism for heavy metal-induced toxicity in aquatic organisms is the production of reactive oxygen species (ROS) as they are exposed to the water-borne toxicants (Velma et al., 2009).

Major toxicity effects of heavy metals in environment:

Chromium: Predominantly chromium is present in the workplace and in nature in two different valence state- 1st Cr^{6+} and 2nd Cr^{3+} Like many other metallic ions, chromium may be toxic and is non-biodegradable for this reason it remains in the ecosystem and only keep changing forms (Mallesh et al., 2015). In drinking water, the permissible limit of chromium is 50microgram/L (IS:10500:2012) (Lushchak, 2015) Consumption of high doses of chromium has been proven as lethal to animals and humans as Cr (VI) is highly carcinogenic (Benjamin and Kutty, 2019).

Lead (Pb): Generally, lead is present in a very low concentration although it induces a variety of toxic effects on fish which are exposed to it having toxic effects on its membrane structure (Eroglu et al.,2015) and causes hypocalcaemia (Rogers et al., 2003).

Cadmium: Cadmium is one of the known heavy metal toxicant which having a toxic effect on fish. It is a naturally occurring element but is also released into the environment through industrial activities, such as mining, smelting, and manufacturing. Biologically cadmium is a very reactive component and that is why it is capable of causing both acute and chronic poisoning (Liu et al., 2022).

Oxidative Stress and Reactive Oxygen Species:

In toxicology research, oxidative stress is an important area as it involves increased lipid peroxidation, alterations in antioxidant scavenger levels, changes in the antioxidant levels, which can be marked as the first step toward contamination (Alves et al., 2006). In fish tissues, heavy metal accumulation causes oxidative stress by the generation of reactive oxygen species (ROS) for example, hydrogen peroxide, hydroxyl particles and superoxide radicals. The generation of reactive oxygen species has been considered as a probable mechanism of heavy metal toxicity in aquatic organisms which are exposed to waterborne contaminants (Kim & Kang, 2017a). Activation of free radical occurs in the fish which are exposed to heavy metal compounds. These free radicals may alter many physiological and metabolic parameters. Farag had experimentally shown that due to chromium exposure lipid peroxidation in the tissues of Chinook salmon (*O. tshawytscha*) was activated and showed that higher concentration of chromium significantly affects fish health (Obasohan, 2007a). On the other hand, Ahmed has cited that Potassium dichromate causes oxidative stress in European eel's (*A. anguilla* L.) gills and kidneys (Ahmad et al., 2006). Hexavalent chromium induced genotoxicity and production of oxidative stress were confirmed in common carp (*C. carpio*) (Kumar et al., 2013a).

Metal-Microbes interaction:

Constant exposure to metals aids in the development of microorganisms' resistance to heavy metals, as previously discussed. It is also crucial to understand the several ways that microorganisms and heavy metals interact, including

Biosorption: The process of biosorption involves the interaction of metal ions with the cell surface's polysaccharides and proteins. microorganisms that are both gramme positive and gramme negative have peptidoglycan layers (Nanda et al., 2019a). Teichoic acid, alanine, glutamate, and meso-diaminopimelic acid are the main components of gram-positive bacteria, but gram-negative bacteria only have one peptidoglycan layer that contains phospholipids, glycoproteins, enzymes, and lipopolysaccharide. For the binding of metals, these molecules function as ligands. Teichoic acid-containing carboxyl groups and other acidic groups function as channels for the uptake of metals. Therefore, compared to gram negative bacteria, teichoic acid-containing bacteria absorb more metal ions. Nucleic acid, proteins, lipids, and carbohydrates combine to produce the complex cell wall of gram-positive bacteria, which also contains extra poly substances (EPS) (Ayangbenro & Babalola, 2017a).

Bioaccumulation: The transporter protein on the lipid bilayer drives this process, which allows the metal ions to pass through and reach intracellular regions. In bacteria, endocytosis, lipid permeation, carrier-mediated transport, and complex permeation are the ways in which heavy metals are collected. Studies using *Pseudomonas putida* 62BN for cadmium bioaccumulation by TEM have revealed cadmium accumulation in the periplasm and intracellular spaces (Pande et al., 2022a).

Genetic imprint that determines bacterial resistance to heavy metals:

When bacteria are exposed to heavy metals over time, the findings indicate that the bacteria develop resistance to the heavy metals, which include lead, zinc, copper, arsenic, chromium, nickel, cadmium, and mercury. Bacterial plasmids and chromosomes include metal resistance genes. Compared to plasmids, chromosomal genes are more complicated. The first metal resistance genes were found in bacterial plasmids. For instance, the bacterial plasmid PMOL28, which localizes the cnr operon, functions as a chromium, nickel, and cobalt genetic determinant. According to Nies and Cooksey, the cop operon is a copper-resistant gene found in Pseudomonas sp. Four proteins, including copA, copB, copC, and copD, are encoded by this cop operon. The cop protein gathers copper ions and creates spaces in the periplasm and outer membrane of the cell. Certain bacteria exhibit comparable functions for both chromosomal and plasmid genes. For instance, the ars operon in plasmids and the genetic determinant in *E. coli* and *Bacillus subtilis* are physically identical. The mechanism could be different, though, as chromosomes should have the genes for homeostasis, while plasmids should include the genes for resistance to heavy metals (Shahpiri & Mohammadzadeh, 2018a; Mergeay et al., 1985a).

Table 2: Heavy Metal-Microbe Interactions: Exploring Modes of Action.

Heavy Metal	Microbe Interaction and Mode of Action
Mercury	Methylating bacteria, such as Desulfovibrio spp., convert inorganic mercury to toxic methylmercury, which bioaccumulates in aquatic organisms.
Arsenic	Certain bacteria, e.g., Bacillus spp., can transform arsenate into less toxic arsenite or volatile arsine gas.
Cadmium	Pseudomonas species and other metal-resistant bacteria can accumulate and detoxify cadmium through metallothioneins and other cellular mechanisms.
Lead	Lead-resistant bacteria, such as Cupriavidus metallidurans, can sequester lead through intracellular and extracellular binding mechanisms.
Chromium	Some bacteria, including Pseudomonas and Bacillus species, can reduce toxic hexavalent chromium [Cr (VI)] to less toxic trivalent chromium [Cr (III)].
Copper	Copper-resistant bacteria like Cupriavidus necator may employ efflux pumps and metal-binding proteins to tolerate high copper concentrations.
Nickel	Nickel-resistant bacteria, for instance, Staphylococcus aureus, can tolerate high nickel levels through active efflux systems and metal-binding proteins.

Mechanism of bioremediation by microbes:

Bioremediation is a process that uses living organisms, typically microorganisms, to degrade or remove pollutants from contaminated environments. Microbes play a crucial role in bioremediation due to their ability to metabolize and break down various contaminants. The process involves various microbial activities and interactions that contribute to the degradation of different types of pollutants.

Table 3: Unveiling the Intricacies: Microbial Bioremediation Mechanisms and Pathways for Environmental Cleanup.

Process Step	Description/Pathway
Adhesion	Microbes adhere to the metal surface through biofilm formation. This involves the secretion of extracellular polymeric substances (EPS) by the microbes.
Electron Transfer	Microbes may transfer electrons to or from the metal surface, utilizing it as an electron acceptor or donor in metabolic processes. This can lead to corrosion or microbial metal reduction, depending on the specific microbe involved.
Biofilm Growth	The microbial biofilm on the metal surface continues to grow, providing protection and a conducive environment for microbial activity. This layer can consist of various microbial species, forming a complex community.
Metabolic Processes	Microbes engage in metabolic activities such as metal ion uptake, precipitation, or redox reactions. This can result in the alteration of metal speciation and its physical properties.

Metal Transformation	Microbes may induce transformations in the metal structure, such as the formation of metal oxides, sulfides, or other compounds. This alteration can influence the metal's stability and reactivity.
Corrosion or Protection	Depending on the microbial activities, corrosion of the metal may occur due to microbial metabolism or, conversely, the microbial biofilm may act as a protective barrier against environmental corrosion agents.
Nutrient Cycling	Microbes participate in nutrient cycling processes, utilizing and recycling nutrients present in the surrounding environment, which can indirectly affect the metal-microbe interaction.
Quorum Sensing	Microbial communication through quorum sensing may influence the behaviour and activities of the microbial community on the metal surface. This coordination can impact the overall metal-microbe interaction.
Biomineralization	Some microbes have the ability to induce the formation of mineral deposits on the metal surface, contributing to the overall stability or instability of the metal-microbe interface.
Detachment	Microbial detachment from the metal surface may occur, leading to the release of planktonic microbes into the surrounding environment. This can contribute to the spread of microbial colonization to new surfaces.

Conclusion:

This process of bioremediation for heavy metals by microorganisms is environment-friendly. As microbes grow fast, so this this process by microorganisms is also very fast. Microorganisms interact with heavy metals and develop resistance against heavy metals, and they help to find solutions for heavy metal pollution. More research for finding new strategies which can detoxify metal ions should be done by genetically engineered microorganisms (GEMs).

Authors’ contribution

The original concept and design of the book chapter has been done by AKP and SHM. SHM did the original article drafting, review and editing.PH did the review and editing.

Conflict of interest

The authors declare that they have no conflict of interest.

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