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Bioremediation of Heavy Metals in Wastewaters: A Concise Review

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ABSTRACT

Wastewater is contaminated by heavy metals that come naturally and anthropogenically from the surrounding environments. In the latest years, the use of wastewater in agriculture has increased due to the ability of soil to the inherent remedy fundamental and micronutrient contents. Therefore, the need to eliminate heavy metals from wastewater has increased. Previous remediation methods have several limitations such as high cost and producing large quantities of hazardous wastes that cause secondary sources of pollution. Thus, it is recommended to use green technology with low economic cost and environmentally friendly to remediate heavy metals from wastewater before its use in agriculture. In this regard, the latest advances in microbial biotechnology revealed the effective role of fungi, which are considered an essential group of microorganisms that have several environmental properties including their ability to remediate these metals from wastewater via bioaccumulation and biosorption. The advantages of this method are green technology and environmental acceptance due to its non- interference in the ecosystem and economy.

INTRODUCTION

Rapid industrialization and urbanization to meet the growing call for crucial commodities boom increase environmental pollution. Environmental pollutants have been a primary difficulty during the last few years, influencing the high satisfaction of life **(Goutam *et al*., 2021)**. The overgrowing population and expansion of industries have produced massive quantities of waste/refuse water because of diverse anthropogenic activities e.g., households municipal corporations, a city nearby water bodies and industries producing a massive quantity of wastewater, reaching water bodies (streams/rivers) without a right remedy, which subsequently form water pollutants **(Bhatia & Sakhuja, 2020)**. Nowadays, waterbodies polluted with heavy metals have become a widespread crisis (**Bhafid *et al*., 2017)**. Heavy metals have several properties, which include persistent, non-degradable and accumulated, posing a health hazard and can be transported via bioaccumulation. Heavy metal accumulation is a public health concern since it may be transmitted via bioaccumulation but is also persistent and non-degradable in the environment **(Khan *et al*., 2015**; **Tufail *et al*., 2022)**.

Metals are crucial to living organisms including flora and animals for their biological functions, and the wide prevalence of those heavy metals results in negative health impacts, which include several organ failures and carcinogenicity. These impacts on residing organisms require searching for an alternative manner for metal removal **(Anahid *et al*., 2011)**. Environmental cleaning by traditional methods of remediation using physical and chemical approaches are rather degrading to the environment and can cause secondary pollution. Therefore, bioremediation is an alternative to these traditional approaches. Bioremediation would be applied for cleaning up the contaminated websites inclusive of water, soils, sludge and waste streams **(Kaur *et al*., 2021)**. Microbial biotechnology is an unexpectedly developing and rising discipline, with numerous packages for coping with environmental issues.

1. Heavy metals

Metals with an atomic weight ranging from 63.5 to 200.6 and a high density (5 g/cm^3) are recorded as heavy metals **(Shanab *et al*., 2012; Kumar *et al*. 2015)**. Few metals have vital roles in the biological function of living organisms under a certain limit **(Kumar *et al*., 2015; Joshi, 2018)**. Therefore, heavy metals can be classified according to their health importance into four major groups as follows: (1) Copper (Cu), zinc (Zn), cobalt (CO), chromium (Cr), manganese (Mn) and iron (Fe). These are all essential heavy metals. In addition, these metals are known as micronutrients **(Reeves & Baker, 2000)** and are hazardous when consumed in greater quantities than necessary **(Monni *et al*., 2000)**. (2) Heavy metals that are not required, such as barium (Ba), aluminium (Al), lithium (Li) and zirconium (Zr). (3) Tin (Sn) and aluminium are two less hazardous heavy metals (Al). (4) Toxic heavy metals such as mercury (Hg) and cadmium (Cd) **(Gajewska *et al*., 2022)**.

2. Sources of pollution with heavy metals in water bodies

Water is an important natural resource on our planet and is essential for survival. It is known that heavy metals are considered the principal source of water pollution. Thus, the concern about water bodies contaminated with heavy metals is turning global worldwide **(Bahafid *et al*., 2017)**.

Heavy metals obviously arise in the environment (water bodies) from two main sources, natural and anthropogenic sources. Natural sources include volcanic emissions, deep-sea vents, woodland fires and geysers. Anthropogenic sources include mine and smelting sites, painting and coating enterprises, metallic-production factories and tanneries as shown in Fig. (1) **(Fuller *et al*., 2003; Zhang *et al*., 2011)**.

Industrial development in both the developing and non-developing countries has resulted in an uncontrolled release level of heavy metals into waterbodies (local water)

(Clark, 2001) as electroplating, manufacturing, fertilizer, pesticide, etc... that produce huge quantities of heavy metals in nearly water bodies. This caused severe problems for all different living organisms **(Ayele *et al*., 2021)**. The most recorded major water heavy metal pollutants categories are Hg, Cr, Pb, Zn, Ni, Cd and Cu **(Abdolali *et al*., 2014)**.

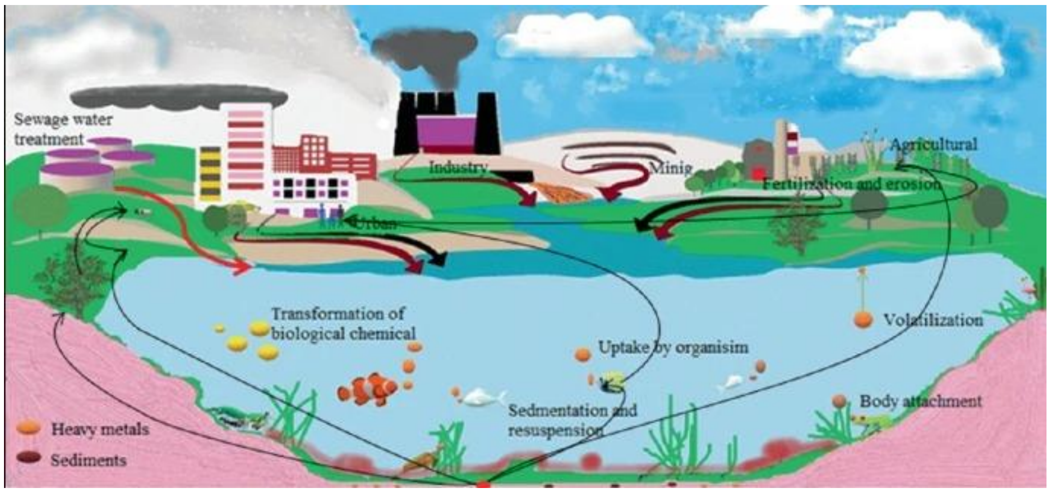

Fig. 1. Sources of heavy metals in water bodies **(Mishra *et al*., 2022).**

3. Toxicity of heavy metals on living organisms

Heavy metals are persistent in the environment, non-biodegradable and difficult for biological creatures to absorb (**Yahaya & Don, 2014)**. Heavy metals are unable to decompose in living species and remain in their body parts due to their long half-lives causing health risks **(Nabulo *et al*., 2011)**. Alleviating heavy metal concentrations is considered a determining factor for the quality of living aquatic organisms **(Poli *et al*., 2009)**. Even heavy metals that are found in traces in water bodies can be very toxic causing severe health problems to the ecosystem via transferring from polluted water into other aquatic organisms to humans through the food chain via bioaccumulation **(Stasinos & Zabetakis, 2013; Khan *et al.*, 2015)** as shown in Fig. (2).

Based on several factors, including the type of living species, dose and duration of exposure to these metals, the level of heavy metal toxicity is determined **(Ayangbenro & Babalola, 2017)**.

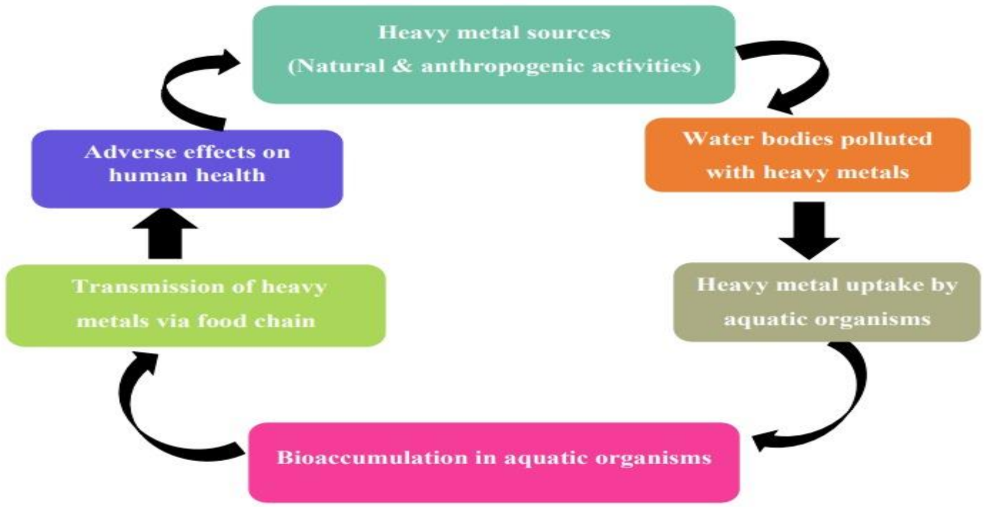

Fig. 2. The pathway of transporting heavy metals from contaminated water into the food chain to humans.

Fish is the most important aquatic organism in the aquatic ecosystem because it is receptive to heavy metal; they can be used as bioindicators of water polluted with heavy metals **(Cairns *et al*., 1984)**. Potentially, there are three ways for heavy metals to enter fish bodies, viz. the gills, surface body and digestive tract. The gills are considered the direct and immediate way for heavy metals uptake **(Roméo *et al*., 1999)**. Fish that inhabit contaminated waterbodies can store heavy metals in their tissues. Fish muscles are matched to other tissues that often have the highest quantities of these metals. Heavy metal accumulation in many organs of fish may result in skeletal diseases and operational problems **(Jezierska & Witeska, 2006)**.

Heavy metal concentrations in fish tissues cause several biochemical, physiological and histological changes in addition to some changes in the activities of different enzymes and metabolites in other freshwater fauna **(Nagaratnamma & Ramamurthi, 1982)**. It is worth mentioning that, the heavy metals accumulation in fish tissues is affected by several external factors such as 1) Concentration of metal. 2) Exposure period. 3) Metal uptake way. 4) Ecological parameters including water temperature or fundamental factors such as size and age **(Rajeshkumar, 2018)**.

In humans, different heavy metals cause several adverse effects and various diseases as shown in Fig. (3). Their toxicity has been related to birth defects, cancer, pores, skin lesions and liver and kidney lesions **(ATSDR, 2001)**.

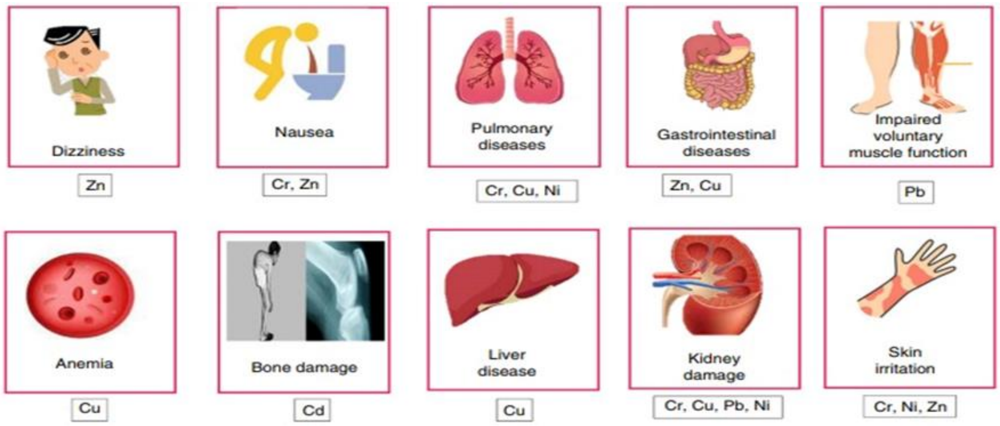

Fig. 3. The adverse effects of different heavy metals on the human body **(Cheng *et al*., 2019).**

4. Bioremediation

Precipitation, filtration, ion exchange, reverse osmosis, evaporation, membrane technology, carbon adsorption, electrowinning, pre-concentration, wastewater coagulation, chelation, redox, and electrochemical techniques are among the conventional remediation methods used to remove heavy metals from water bodies **(Jin *et al*., 2018; Ali *et al*., 2019)**. Nevertheless, these classic technologies have various disadvantages, including high cost for large-scale implementation, complexity, secondary contamination caused by inadequate removal of heavy metals from polluted waterbodies and interaction with environmental ecology **(Chatterjee, 2006)**. Moreover, these procedures are impractical and do not apply to metal-binding characteristics **(Ayangbenro & Babalola, 2017)**. Furthermore, it may result in eliminating the beneficial non-target microbial biotas, such as nitrogen-fixing bacteria and other fauna species **(Siddiquee *et al*., 2015)**.

The major focus is on bioremediation as a green treatment (biological treatment) and commercially viable biotechnology **(Wang & Tam, 2019)**. It is highly recommended to combat those limitations since this treatment has the characteristics of low energy consumption, high efficiency and high environmental safety **(Joshi, 2018)**. To digest and eliminate environmental contaminants, this approach may use a variety of biological agents such as bacteria, fungi, higher plants and cellular free enzymes **(Coates & Anderson, 2008)**. Another benefit of a biological technique is that it can treat a high volume of wastewater while maintaining a low biomass content and a short operating period **(Akkar *et al*., 2016)**. The most known bioremediation techniques are shown in Fig. (4) **(Siddiquee *et al*., 2015)**.

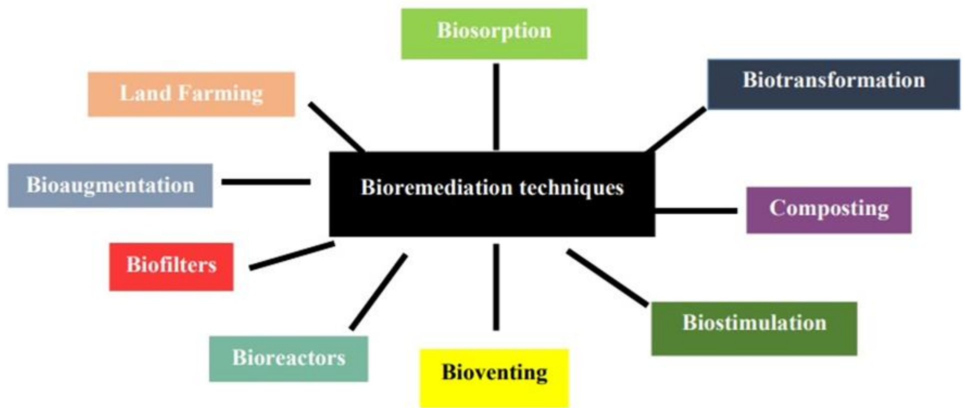

Fig. 4. Different types of bioremediation techniques.

4.1. Bioremediation strategies

There are two techniques for removing and transporting garbage for treatment.

4.1.1. In situ bioremediation

This approach method, which involves the removal of pollutants (hazardous substances) is widely employed in both saturated soils and groundwater **(Vidali, 2001; Girma, 2015)**. It is dependent on microbial activities, which include the destruction and detoxification of pollutants found in a location **(Ogbonna, 2018)**. This strategy is considered the most desirable choice due to its low cost and less disturbance owing to providing treatment in the same place, avoiding excavation and contaminants via transportation **(Vidali, 2001)**. This approach is used on-site and removes the need to transfer large amounts of garbage off-site, as well as the possible hazards to human health and the environment that might occur during transportation **(Harekrushna & Kumar**, **2012)**.

4.1.2. Ex situ bioremediation

This approach method entails extracting pollutants from contaminated areas and moving them to another location for treatment. When employing this bioremediation approach, various parameters must be considered, such as contamination depth, pollutant type, treatment cost and geographical location of the contaminated site **(Sharma, 2020; Dell'Anno *et al*., 2022)**.

4.2. Methods of bioremediation

Bioremediation is a form of biotechnology using biological sources, viz. plants, microbes, algae, etc… to clean the ecosystem from contaminants **(Garbisu &Alkorta, 2003)**. In addition, it uses relatively cheap, simple techniques (**Vidali, 2001)**. Bioremediation can be followed by these methods: phycoremediation, phytoremediation, bacterial bioremediation and mycoremediation **(EA, 2015)**.

4.2.1. Phycoremediation

Phycoremediation is a bioremediation method that uses algal species to adsorb and detoxify pollutants from wastewater to become appropriate to reuse, discharge and safer for organisms and environment **(Olguin, 2003)**. Algal species are considered a good natural source for remediation studies due to their growth, adaptation and manipulation within laboratory settings **(Dresback *et al*., 2001)**.

The algal cells adsorbe metal ions on their surfaces rapidly and then gradually transport them into their cytoplasm **(Dwivedi, 2012)**. Inside the cell, heavy metal may be detoxified through attaching to intracellular compounds and forming metal-binding peptides or proteins, such as metallothioneins, phytochelatins and gluthione **(Hirata *et al*., 2005; Hassinen *et al*., 2011**).

Many microalgae have already been used to remove pollutants, particularly heavy metals from diverse effluent types, either as single species such as *Chlorella*, *Spirulina* sp., *Cladophora*, *Oscillatoria*, *Anabaena*, *Arthrospira*, *Scenedesmus* and *Phaeodactylum tricornutum* **(Mulbry & Wikie, 2001; Bwapwa *et al*., 2017)** or as mixed cultures/consortia **(Mulbry, 2001; Tarlan, 2002)**.

4.2.2. Phytoremediation

The term "phytoremediation" is made up of the Latin suffix "remedium," which means "to correct," and the Greek prefix "phyton," which means "plant." Phytoremediation is an approach that removes pollutants from the environment using specific plants such as trees, aquatic plants, grasses and shrubs **(Favas *et al*., 2014)**.

Phytoremediation offers many benefits compared to other traditional remediation methods; it can minimize environmental damage and is affordable for large volumes of water with various contaminants **(Schwitzguebel, 2000; Mudgal *et al*., 2010)**. Substances that can undergo phytoremediation include heavy metals, explosives, metalloids, inorganic substances, radioactive elements, petroleum hydrocarbon pesticides and herbicides, organic manufacturing wastes and chlorinated solvents **(Ensley, 2000)**. Plant-based technologies for heavy metal decontamination are extraction, volatilization, stabilization, rhizo-filtration and degradation (**Raskin & Ensley, 2000**; **Hooda, 2007; Jeyakumar *et al*., 2022)** as shown in Fig. (5).

Aquatic plants are highly efficient in removing heavy metal contaminants. For wastewater treatment, plant-based adsorbents are preferred for they are inexpensive and eco-friendly. Heavy metals are removed by chemicals extracted from plants and numerous plant wastes. Some plants have excellent metal storage efficiencies such as duckweed (*Lemna minor*) (**Ali *et al*., 2020**). **Nazir *et al*. (2020)** reported that, the metal-removing capacity of the hyacinth plant for mercury, cadmium and arsenic is great using atomic absorption spectroscopy. Dry *Miscanthus* (silver grass) plants have the greatest potential to remove heavy metals from wastewater due to their chemical, physical, and leaching properties **(Osman *et al*., 2018)**.

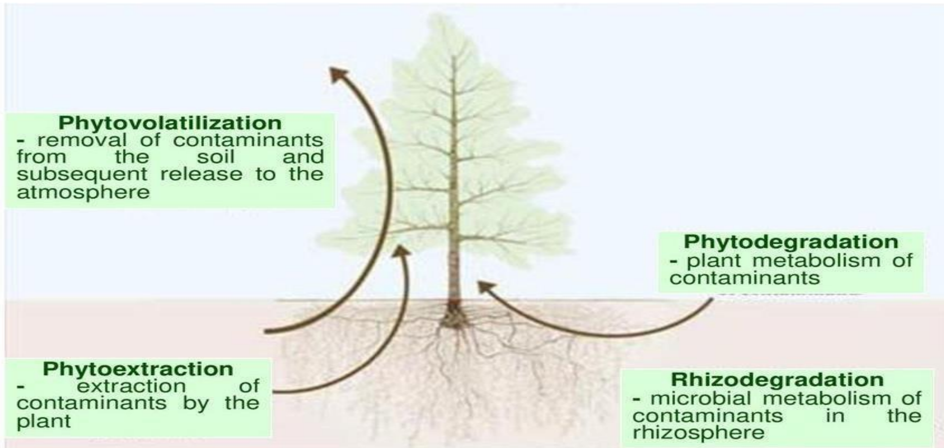

Fig. 5. Phytoremediation techniques used in wastewater remediation **(Zouboulis *et al*., 2019).**

4.2.3. Microbial bioremediation

Microorganisms are considered a major alternative key to overcome challenges. Metals have an important role in microbial differentiation, growth and metabolism **(Gadd, 1992)**. Although the increased contamination poses a greater risk to microbe toxicity, microbes have developed a number of techniques to adapt to the presence of heavy metals **(Gadd, 2009)**. Several mechanisms underlying the interactions between metals and microbes that remove metals from solutions are based on several mechanisms, such as biotransformation, bioaccumulation, biosorption, bioseparation, biodegradation, biobleaching and biodetoxification; they are all processes used in bioremediation **(Barrech *et al*., 2018)**.

Toxic contaminants may be transformed, altered and used by microorganisms to generate energy and biomass, repairing the ecosystem and averting additional pollution **(Goutam *et al*., 2021)**. Remarkably, microorganisms can absorb heavy metals via a passive process through the extracellular binding on both living and dead biomass, or by an active process through intracellular accumulation (bioaccumulation) in their living biomass. The application of single, consortium and immobilized metal-resistant strains for heavy metals remediation has produced successful results **(Kim *et al*., 2015)**.

The efficiency of remediation-mediated microorganisms over other bioremediation processes can be assessed by the following criteria: the increase of microorganism biomass, the tolerance to pollutants and the favorable regulation of environmental factors promoting microbial growth and degradation **(Taniguchi *et al*., 2000; Vidali, 2001)**.

4.2.3.1. Bacterial remediation

Due to bacterial abundance, size, capacity to grow under controlled conditions and resistance to environmental conditions, whole bacteria and/or their extracts have become widely used to combat pollutants **(Srivastava *et al*., 2015; Guerra *et al*., 2018)**. According to **Mosa *et al*. (2016)**, several bacterial species, including *Flavobacterium*, *Pseudomonas*, *Enterobacter*, *Bacillus* and *Micrococcus* sp., can survive in the heavy metal's presence. In mixed cultures, bacteria are more dependable, with a higher chance of surviving **(Sannasi *et al*., 2006)**.

For heavy metals removal, bacterial consortiums are more suitable for field applications, with better metabolic capabilities **(Kader *et al*., 2007)**. According to **De *et al*. (2008)**, chromium (Cr) was reduced by 78% using a mixture of bacteria of the species *Acinetobacter* and *Arthrobacter* at 16 mg/L of metal ion concentration. **Puyen *et al*. (2012)** reported that, a significant amount of lead was removed from a synthetic medium using *Micrococcus luteus.*

4.2.3.2. Mycoremediation

First, Paul Stamets created the word "mycoremediation," which means "removal of harmful chemicals by fungi". Molds, yeasts and mushrooms are diverse eukaryotic microorganisms known as fungi, which are of great practical value **(Stamets, 2005; Mohmand *et al*., 2011; Carris *et al*., 2012)**. The fungal cell membrane is a thin, double-layered sheet of lipids composed mostly of phospholipids, sterols (approximately 40% of the membrane composition) and protein molecules (about 60 percent) **(Ghaed *et al*., 2013; El-Bondkly & El-Gendy, 2022)**.

Fungi have become a superior option for remediating heavy metals due to their heavy biomass and adsorption abilities. Their production in large quantities is inexpensive, and genetic makeup is both easily modifiable. Moreover, they secrete several extracellular metabolites, including proteins, organic acids and enzymes, and their biomass (dead/live) may be used as an excellent natural source for treating industrial effluents **(Dhankhar & Hooda, 2011; Pantidos, 2014**; **Dell'Anno *et al*., 2022)**.

5. Mechanisms of mycoremediation

Fungi are everywhere, which has made it possible for them to adapt to most waste types. As illustrated in Fig. (6), fungi clean the heavy metal-filled environment via mechanisms that are both metabolically active (biomineralization, biotransformation, bioprecipitation and bioaccumulation) and metabolically passive (biosorptive) **(Goutam *et al*., 2021; Mohamadhasani & Rahimi, 2022)**. Bioaccumulation and biosorption processes have been proven to offer the most potential to replace existing metal removal technologies **(Dhankhar & Hooda, 2011)**.

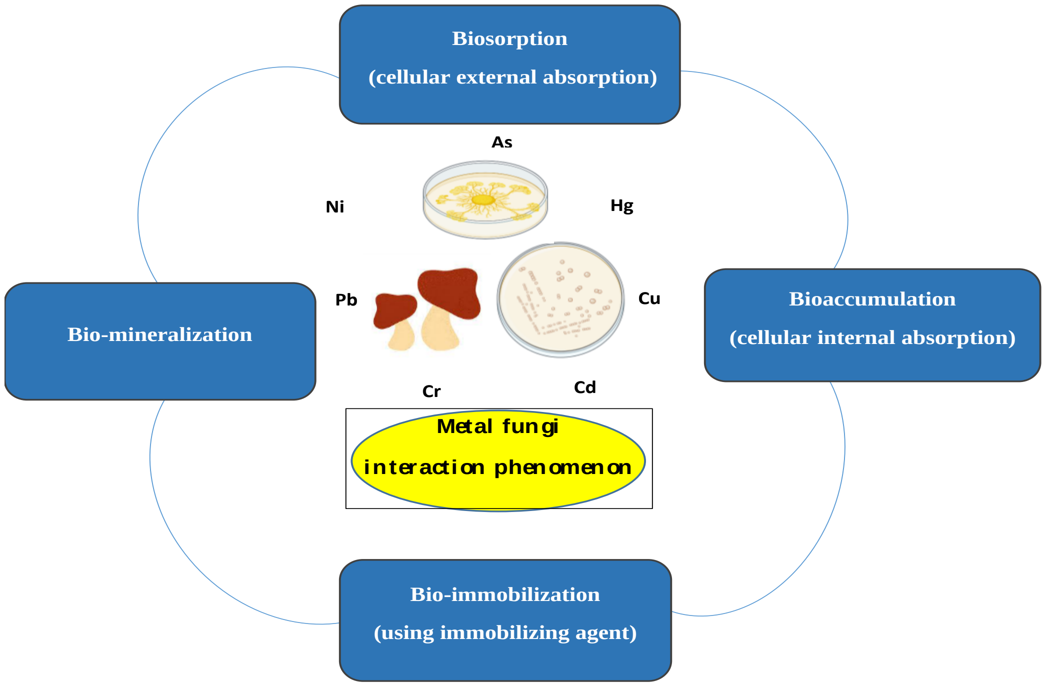

Fig. 6. Main fungal mechanisms facilitating heavy metal removal.

5.1. Biosorption and bioaccumulation

Biosorption is one of the important processes that depend on a greater affinity between a biosorbent (biological material) and sorbate (metal ions). It continued until the two components were in equilibrium. Fungi remove heavy metals by adsorbing metals on their surfaces and binding them with functional groups such as amine, carboxyl, phosphate, hydroxyl and sulfhydryl group, which is found on the cell's surface **(Das *et al*., 2008; Kisielowska *et al*., 2010; Jeyakumar *et al*., 2022)**.

The outer fungal cell shield contains anionic molecules that provide binding sites for cationic heavy metals. Cellular structures of fungi belonging to different phylogenetic units are characterized by having different chemical groups on their outer structures that are involved in metal binding **(Krgiel *et al*., 2008)**. There has been considerable confusion in the literature about the words "bioaccumulation" and "biosorption," which are dependent on the biomass state. When defining a process as "bioaccumulation," living cells are largely utilized, while dead biomass is used through "biosorption" processes **(Dhankhar & Hooda, 2011)**.

The process of bioaccumulation combines passive and active heavy metal bioremediation methods. When the rate of heavy metals absorption is higher than the rate of loss, they accumulate inside the organism **(Chojnacka, 2010)**. Through the use of importer complexes that are present in the fungal cell membranes, it can absorb heavy metals into their intracellular space. In the intracellular space, heavy metals can be detoxified by generating antioxidant compounds **(Bellion *et al*., 2006; Mishra & Malik, 2013)**.

According to **Annibale *et al*. (2007)**, some fungi including *Stachybotrys* sp., *Phlebia* sp., *Botryosphaeria rhodian*, *Pleurotus pulmonarius*, *Saccharomyces cerevisiae* act as a biosorbent for Zn and Cd removal **(Chen & Wang, 2007; Talos *et al*., 2009)**. **Tigini *et al*. (2010)** reported that, *Cunninghamella elegans* is considered an attractive biosorbent to heavy metals in textile wastewater. The filamentous fungi, *Monodictys pelagic* and *Aspergillus niger* can accumulate chromium and lead in intracellular spaces **(Sher & Rehman, 2019)**.

5.2. Bio-immobilization

The bio-immobilization technique uses microorganisms to reduce metal mobility and transform soluble metal ions into insoluble solids **(Yahaya *et al*., 2009)**. Many studies have shown that fungus may immobilize heavy metals and subsequently eliminate them. Table (1) shows several fungal species that have been immobilized to eliminate heavy metals.

Table 1. List of fungi utilized in the bio-immobilization process for remediation of various heavy metals from aqueous solutions.

Fungal species	**Metal**	**Reference**
Rhizopus arrhizus	Copper, lead, zinc, manganese and cadmium	**Lewis (1998)**
Pycnoporus sanguineus	Copper	**Yahaya *et al*. (2009)**
Penicillium janthinellum	Copper, lead and cadmium	**Cai *et al*. (2016)**
Aspergillus fumigatus	Uranium	**Wang *et al*. (2010)**
Asperigillus terreus UFMG- F01	Chromium and nickel	**Dias *et al*. (2002)**

5.3. Biomineralization

Biomineralization is a biological approach for heavy metals remediation by mineralizing heavy metal ions through microorganisms that decrease the bioavailability and mobility of metals by immobilizing them and turning them into insoluble minerals. Biologically induced mineralization and biologically controlled mineralization are the two types of biomineralization **(Gadd, 2010)**.

Numerous studies have demonstrated that fungi can remove heavy metals through mineralization. The fungus *Neurospora crassa* can precipitate Cd as $CdCO_3$ **(Li *et al*., 2014)**. **Dhami *et al*. (2017)** reported that, *Fusarium oxysporum* and *Aspergillus* sp. are capable of precipitating vaterite, aragonite, calcite carbonates, lead and strontium hydroxides. Additionally, **Li *et al*. (2015)** reported that, *Pestalotiopsis* sp. and *Myrothecium gramineum* can mineralize $CaCO_3$ and $SrCO_3$.

5.4. Fungal nanoparticles–mediated heavy metal remediation

According to **Lövestam *et al*. (2010)**, a nanomaterial is a substance with a nanoscale external dimension, internal structure or surface structure. Compared to larger-sized materials, nanomaterials often have various physical or chemical characteristics **(Savage & Diallo, 2005; Shen *et al*., 2007)**. Recent research proved that nanomaterials have intentionally been used to remove pollutants from water, including heavy metals, hazardous organic dyes, oily waste and numerous agricultural and manufacturing industrial wastes **(Ray & Shipley, 2015; Anadão, 2017)**.

The development of nanotechnology has a unique function in detecting heavy metals using green synthesized fungal nanoparticles **(Taherzadeh *et al*., 2003)**. Fungi pay more attention to the biogenic production of metallic nanoparticles due to their tolerance and capacity for metal bioaccumulation **(Sastry *et al*., 2005)**.

Numerous silver and gold nanoparticles have been developed using various fungi **(Siddiqi & Husen, 2016)**. Moreover, fungi can produce other metal nanoparticles uptaking toxic heavy metals. These particles can also be used as biosensors that help the cleaning up the environment. Their detection technology may be employed in environmental detection (heavy metal-ion sensing) and bio-monitoring services **(Shan *et al*., 2009; Goutam *et al*., 2021)**. Table (2) presents a list of fungi as alternatives to various treatments for environmental restoration leading to nano-bioremediation that have been recommended as efficient, affordable and ecologically benign **(Dastjerdi & Montazer, 2010)**.

Table 2. Some fungal synthesized nanoparticles used in nanobioremediation.

Fungus	Nanoparticle	Reference
Rhodotonila mucilaginosa *Penicillium aurantiogriseum* *Penicillium citrinum* *Penicillium waksmanii*	Copper nanoparticles	**Salvadori *et al*. (2014) & Honary *et al.* (2012)**
Aspergillus aculeatus	Nickel oxide nanoparticles	**Salvadori *et al*. (2015)**
Phanerochaete chrysosporium	Cadmium sulfinde nanoparticles	**Chen *et al*. (2014)**

CONCLUSION AND FUTURE PERSPECTIVE

The uneven distribution of heavy metals in the environment is the outcome of human impact brought about by fast industrialization. The use of traditional methods has several downsides, including costly treatment, the formation of hazardous and difficult-to-handle sludge. As a result, biological therapies are effective, low-cost and ecologically friendly alternatives. Because of their biotechnological potential, microbes have removed a substantial amount of heavy metals in recent years. The role of fungus in heavy metal removal has been discussed in this review. Fungal cells have a particular shape that allows them to survive in a harsh environment including hazardous metals. Fungi employ biosorption, bioaccumulation, bioimmobilization and biomineralization techniques to survive in metal-polluted environments. Future research should look at new fungal species that have a high potential for heavy metal cleanup. Future bioremediation research may place a strong emphasis on the utilization of genetically modified fungal strains for specific industrial applications in order to create commercially appealing agents.

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