

## Review

# Types of Bioremediation and their Applications, in Heavy Metal Detoxification

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## ABSTRACT

Heavy metal contamination is the result of various human activities. Increased concentrations of heavy metals are responsible for causing lethal and chronic diseases in humans, affecting the metabolism of plants and causing metal toxicity in the environment. Available conventional techniques for metal detoxification are not found effective and economically feasible. Bioremediation is an ecologically and economically favorable technique that consists of natural biological processes to eliminate toxic heavy metal contaminants. Various microorganisms, fungi and plants can be used for heavy metal detoxification. This paper gives an idea about the concept, types, and principles of bioremediation.

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## Introduction

The survival quality of human on earth is completely linked to the good health and overall quality of environment. As a result of human activities every year, large amounts of various inorganic and organic compounds or elements are introduced into the environment. These compounds are either toxic or nontoxic but maximum of them are found toxic to human health. Heavy metals are the most hazardous form of toxic contaminants that can cause serious health issues and environmental concerns. The heavy metal elements have relatively high metal density *i.e.*, more than 5g/cm<sup>3.5</sup>. Heavy metals accumulated into the surface, soil, and groundwater more than the permissible limit are proven to be toxic, but in their balanced concentration, heavy metals play a crucial role in various metabolic activities of the body. Nickel, copper, iron, zinc and manganese are a few metals that are good for the body in small amounts but harmful (cytotoxic as well as mutagenic and carcinogenic in nature) in high concentrations<sup>30</sup>. These are also known as essential

metals. On the other hand, there are some high densities holding heavy metals that show toxic effects even at low concentration, these are known as non-essential heavy metals<sup>29</sup>. The exposure of toxic heavy metals to humans for a long period may affect human health and cause various diseases<sup>5</sup>.

The treatment of these metal contaminants becomes a necessity at this time to handle the alarming situation of metal toxicity. Membrane filtration, ion exchange, adsorption, and coagulation are some important methods that may be used to remove heavy metals from polluted soil and water<sup>23</sup>. Waste water can quickly dissolve heavy metal-containing salts, therefore over expensive physical segregation techniques cannot remove them. The available conventional methods of metal removal are very costly, non-efficient, and not a successful approach to remediate metal ions. Therefore, bioremediation may become an effective solution to handle the situation of metal toxicity in an efficient, successful, and cost-effective way. In this review paper, we elucidate the concept of bioremediation and describe

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the possible types of bioremediations to be applied for heavy metal detoxification in future.

### Concept of Bioremediation

Bioremediation involves the use of living organisms such as plants, bacteria, fungi, actinomycetes, etc. for the removal of toxic metal pollutants from the soil, water, or wastewater. Plants can detoxify the toxic metal contaminants through root absorption and leaf accumulation of metals. Diverse range of microorganisms are used to detoxify the toxic heavy metal pollutants available in the environment. Plants-based remediation is called phytoremediation and as usual microbes-based remediation is called microbial remediation. It is proven that bioremediation is a cost-effective, efficient, eco-friendly, and solar energy based technique as compared to other conventional methods.

### Types of Bioremediations

#### A. Phytoremediation

Greek word Phyto means plant and Latin word 'Remedium' indicates "to repair or eliminate an evil" are the two words that are combined to form the term "phytoremediation". Phytoremediation by plants is a promising approach for the clean-up of polluted environment. Green plants have remarkable capacity to absorb and detoxify toxic metal ions from their surroundings through a variety of ways<sup>1,22,31</sup>. The main goal of phytoremediation is to reduce pollutant concentrations or their harmful effects on the environment by using plants and the accompanying soil microbes. It can be used to remove organic contaminants such as insecticides, polychlorinated biphenyls, polynuclear aromatic hydrocarbons (PAH) and heavy metals. It is a novel, efficient, economical, effective, solar driven, adaptable, environmentally friendly, and *In-situ* clean-up strategy<sup>1</sup>. Additionally, some factors such as soil and plant type, pH, depth of contamination and climate can influence the success of phytoremediation projects. The binding of metal ions to plant cell walls, active ion transport inside cell vacuoles, and intracellular complex building with peptide ligands like phytochelatins (PCs) and metallothioneins (MTs) are some of the mechanisms involved in plant metal detoxification<sup>3</sup>. Metal-siderophore complexes are sequestered in the apoplasm or soil by the root. In addition, one of the primary mechanisms used by plants to handle high metal concentrations is exudate formation. The detoxification of As, Cd, and Pb use low molecular weight organic acids (LMWOAs) such as citric, oxalic, malic, and succinic acid<sup>3</sup>.

There are several mechanisms by which plants can remediate polluted environment. Phytoextraction is

a typical technique in which plants absorb pollutants from the soil or water and deposit or accumulate them in their tissues. Due to the fact that plants can store heavy metals like lead, arsenic, and manganese in their roots or leaves, this method is effective for removing them<sup>1</sup>. *Cerastium glomeratum*, *Medicago minima*, and *Persicaria glabra* were found effective in Phyto stabilization of metals and *Artemisia vulgaris*, *Nonea edgeworthii*, *Arabidopsis thaliana* were found feasible for the metal bioremediation by phytoextraction<sup>26</sup> (Table-1).

Rhizodegradation is another important method where plants release certain compounds through their roots that enhance the degradation of organic pollutants and heavy metals by microbes in the soil. These compounds can stimulate the growth of beneficial microorganisms that break down the contaminants into less harmful substances. Plants such as *Vigna unguiculata*, *Helianthus annuus* and *Zea mays* are examples of some plants that have ability to promote root-associated microbes involved in rhizodegradation<sup>29</sup>. Another method is phytovolatilization, in which plants take up pollutants from the soil or water and release them as volatile molecules into the atmosphere. This is frequently employed for some volatile inorganic compounds (VICs), such as Se and Hg, which can be dangerous when present at high concentrations, as well as volatile organic compounds (VOCs), such as TCE. *Medicago sativa*, *Populus* sp. are examples of some plants feasible in phytovolatilization<sup>17</sup>. Phyto stabilization is a method that involves using plants for immobilization of contaminants in the soil, preventing their movement and reducing their bioavailability. This is often used for sites contaminated with radioactive materials or lead that can be removed by using *Agrostis tenuis*<sup>32</sup>.

#### B. Microbial Bioremediation

Bioremediation attained through the use of microorganisms are called microbial bioremediation. It is based on the metabolic capacity of microorganisms to degrade environmental pollutants and transform them into harmless forms through redox processes<sup>34</sup>. In microbial bioremediation, poisonous heavy metals are used as a source of food by the microbial community. The basic principles of bioremediation include reducing the solubility of these environmental contaminants through pH changes, redox reaction<sup>33</sup> and adsorption of contaminants from polluted environments. The ability of microorganisms to degrade contaminants depends on the suitability of environmental conditions for microbial growth and metabolism, including appropriate temperature, pH and humidity. Under stressful conditions, microorganisms employ a variety of defence

TABLE-1: Name of some most potent species of plant for bioremediation<sup>18,26,35</sup>

Type of metal contamination	Plant Species
Arsenic	<i>Azolla caroliniana</i> , <i>Azolla filiculoides</i> , <i>Pteris vittata</i> , <i>Populus nigra</i> , <i>Populus alba</i> , <i>Populus tremula</i> , <i>Salix alba</i>
Lead	<i>Brassica campestris</i> , <i>Brassica carinata</i> , <i>Helianthus annuus</i> , <i>Amaranthus hybridus</i> , <i>Amaranthus paniculata</i> .
Manganese	<i>Artemisia vulgaris</i> , <i>Nonea edgeworthii</i> , <i>Arabidopsis thaliana</i> , <i>Rosularia adenotricha</i> and <i>Salvia moorcroftiana</i> .
Mercury	<i>Brassica juncea</i> , <i>Eichhornia crassipes</i> , <i>Pistia stratiotes</i> .
Zinc	<i>Stipa hohenackeriana</i> , <i>Brassica juncea</i> , <i>Scariola orientalis</i> , <i>Astragalus effusus</i> .
Cadmium	<i>Scariola orientalis</i> , <i>Stachys lavandulifolia</i> , and <i>Tragopogon collinus</i> .

mechanisms<sup>26</sup>. Resistant activity of microorganisms is determined by cell surface bio adsorption, cellular transport system by active efflux pump of metals from the cell, sequestration of metals in intracellular compartments, exclusion of metal chelates into the extracellular space, and enzymes that transform cells. It can also be explained by mechanisms such as oxidation-reduction reactions that convert metal ions to non-toxic to low-toxic ones<sup>3</sup>. Today, microbial-based bioremediation techniques are of great interest to scientists because of their outstanding advantages such as high efficiency, low cost and environmental friendliness, especially at low metal concentrations. Compared with plants, microorganisms can withstand environmental stress through rapid mutation and evolution.

## 1. Fungi

Fungi have the ability to break down a wide range of organic compounds, including petroleum hydrocarbons, pesticides, and polychlorinated biphenyls (PCBs). They produce enzymes that can degrade these contaminants and convert them into simpler compounds. Fungal bioremediation can be particularly effective in soil and water environments. Fungi can colonize contaminated soil and form extensive networks of hyphae, which enhance the degradation process by increasing the contact between the fungi and the pollutants. They can also tolerate harsh environmental conditions, such as high temperatures and low nutrient availability. Several fungal species belonging to the phyla of *Ascomycota* and *Basidiomycota* have also been

identified as metal oxidizing fungi<sup>2</sup>. Toxic substances in the environment can be detoxified by fungi using sophisticated enzyme systems, including CYP monooxygenase (cytochrome P450 monooxygenases) and GST (glutathione -S - transferase). Thus, fungi can effectively sequester the pollutants, minimizing the exposure of the living organism to refractory contaminants. For fungi to tolerate heavy metal compounds, they absorb metal ions into the cell and also chelate them. Because of their tremendous growth and exceptional binding abilities, fungi have become a superior option for bioremediating heavy metals. Due to their quick rate of multiplication and ease of morphological and genetic modification, they can be quickly and affordably cultured in large numbers<sup>2</sup>.

## 2. Algae

Algae, on the other hand, are photosynthetic organisms that can remove pollutants through a process called phytoremediation. They can absorb and accumulate heavy metals, such as lead and cadmium, from water bodies<sup>24</sup>. Algae can also help in the removal of excess nutrients, such as nitrogen and phosphorus, from waste water, preventing eutrophication<sup>10</sup>. Both fungi and algae have the potential to be used in combination with bacteria in bioremediation strategies<sup>25</sup>. This multi-species approach can enhance the overall efficiency of pollutant removal and provide a more comprehensive solution for contaminated sites<sup>25</sup>. Algae absorb metals by adsorption. Firstly, in a process known as physical adsorption the metal ions are adsorbed on the surface of the cell very quickly. Subsequently, in chemical

adsorption, these ions are slowly transported to the cytoplasm. These structures serve as a “detoxification mechanism” and a “storage pool” for metals in algae<sup>11</sup>. *Chlorella*, *A. inaequalis*, *W. prolifica*, *S. tenue*, and *Synechococcus* sp. are examples of algae that can withstand heavy metal exposure. However, numerous marine algae species, including *Chlorella* and *Anabaena*, have been utilized to remediate heavy metals<sup>11</sup>. A researcher<sup>8</sup> investigated the viability of biologically removing low-concentration aqueous lead from wastewater using live spirulina. Some researchers<sup>28</sup> grew *Scenedesmus* in various concentrations of Cu, Cd, Ni, Zn, and Pb. For *Scenedesmus* development, the Ni solution was less hazardous than Cu. High Pb concentrations were tolerated by the algae.

### 3. Bacteria

Bacteria are the ideal candidates for bioremediation because they are ubiquitous, abundant, diverse, and small, and they have a highly adapted ability to grow and propagate under controlled and uncontrolled circumstances with environmental resilience (Table-2). Adsorption on bacterial cell surfaces is the primary mechanism by which heavy metals in solution are remediated by bacteria<sup>35</sup>. *Micrococcus luteus* and *Pseudomonas pseudoalcaligenes* both absorbed a remarkable quantity of Pb and Cu<sup>16</sup>. Due to their prevalence, size, capacity for regulated growth, and resistance to unfavourable environments, bacteria are also used as biosorbents<sup>41</sup>. Many bacterial species like *Micrococcus*, *Pseudomonas*, *Bacillus*, and *Enterobacter* were studied to check their potential for heavy metal bioremediation. Due to their high surface area to volume ratios, and abundance of active chemical adsorption sites including teichoic acid on bacterial cell surfaces, they possess excellent absorption capacity<sup>19</sup>.

Rhizobacteria (PGPR) and bacteria endophytes are the most common types of bacterial species linked

to the bioremediation of heavy metals. PGPR consists of a group of free-living rhizobacteria or endophytic bacteria<sup>13</sup> such as *Bacillus*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Gluconacetobacter*, and *Pseudomonas*<sup>20</sup>, which can reduce the lethality of heavy metals, improve the growth of plants in soils contaminated with heavy metals, produce plant hormones including siderophores, and aid in the solubilization of phosphate<sup>33</sup>. Actinomycetes are types of bacteria. The pollutants can be metabolized by them for growth and reproduction<sup>7</sup>. Actinomycetes, such as *Amycolatopsis*, *Corynebacterium*, *Rhodococcus*, and *Streptomyces*, can use complex organic matter as a source of carbon and energy<sup>6</sup>. In addition to this, they can also resist and remove heavy metals, for example, mercury (II), cobalt (II), cadmium (II), chromium (VI), zinc (II), and nickel (II). Thermophilic bacteria that may be isolated from hot springs<sup>4,15,36-38</sup> are also having the potential for the bioremediation of heavy metals.

The microorganisms are ideal for this research since they are simpler to handle, cultivate and use. Microbial biomass has different biosorption capacities, which also vary widely among microorganisms. However, the ability of each microbial cell depends on its pre-treatment and experimental conditions. Microbial cells must adapt to changes in physical, chemical, and bioreactor composition to improve biosorption. The ability of microorganism, particularly bacteria to adsorb, solubilize, and precipitate heavy metals through bio adsorption (biosorption), bioaccumulation, bioleaching, and bioprecipitation have gained recognition in recent years. Bacteria are the ideal choice for bioremediation because of their omnipresence, diversity, abundance, ubiquity, small size, and special ability to thrive and spread under both controlled and uncontrolled circumstances with environmental resilience<sup>29</sup>. Bacteria are more stable and survive better when present in mixed culture. The culture consortium is therefore metabolically

**TABLE-2: Examples of some important bacterial species used in bioremediation of heavy metals<sup>9,12,14,21</sup>**

Potent bacterial species	Heavy metal
<i>Bacillus thuringiensis</i> , <i>Pleurotus cornucopiae</i> .	Cadmium
<i>Pseudomonas</i>	Lead, Copper, Zinc, Cadmium
<i>Stenotropho monasmaltophilia</i> ,	Copper
<i>Achromobacter</i> , <i>Pseudomonas</i> , <i>Enterobacter</i>	Lead, Chromium
<i>Mycobacterium</i> , <i>Pseudomonas</i>	Cadmium, Copper



superior for field applications. Some representative examples of metal oxidizing bacteria include *Leptothrix discophora*, *Pseudomonas putida*, and the *Bacillus* sp. strain SG-1<sup>2</sup>.

### Conclusion

Bioremediation is an effective solution for the metal treatment. The choice between phytoremediation and microbial remediation depends on various factors such as specific site conditions, and extent of contamination. Both methods have their advantages and limitations. Phytoremediation has the advantage of being aesthetically pleasing, providing habitat for wildlife, and promoting soil stabilization. However, it can be a slow

process and may not be suitable for sites with high levels of contamination or where rapid remediation is required. Microbial remediation, on the other hand, involves enhancing the activity of naturally occurring microorganisms to degrade or immobilize contaminants. It can be a more targeted and efficient method, particularly for sites with high levels of contamination or where rapid remediation is necessary. Although, a combination of both phytoremediation and microbial remediation has also been suggested to get better results.

### Conflict of interest

The authors declare that they have no conflict of interest.

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