

# Treatment for heavy metal pollution: Remediating soil environment by harnessing genetic and biochemical capabilities of bacteria

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**Abstract.** Pollution is the introduction of harmful substances, also known as pollutants, into the environment. As all living things depend on clean air, water, and land for survival, pollution is a significant problem on a worldwide scale. In addition, heavy metals are the most essential and common contaminants in soil environments. These pollutants are widely diffused, harmful to human health, and persistent in soil environments. Owing to the circumstances locally and worldwide, pollution assessment and bacterial remediation techniques for polluted soil have gained substantial attention and become essential. Here, this review covers four bacterial processes involved in bacterial remediation technology, including heavy metal adsorption and adhesion, redox transformation of heavy metals, and the function of mycorrhizal fungi. Also, a case study of a detailed experiment on pollution treatment is presented. This research aims to eliminate heavy metal pollution by using bacterial remediation technology in order to save human beings and the environment since long-term exposure to heavy metals can cause lung cancer and bone fractures in humans, thus posing a significant security threat and hidden danger to human society.

**Keywords:** heavy metals, pollution, bioremediation technology, bacteria, mycorrhiza.

## 1. Introduction

Pollution is the introduction of harmful elements, defined as pollutants, into the environment [1]. Pollutions can occur with natural pollutants, for example, volcanic ash. Human activities like factory runoff or waste can also contribute to pollution. Pollution is a global concern, and it harms the quality of the air, water, and land, which all living organisms depend on for life-hood [2]. All living forms are put in danger when these resources are contaminated. Furthermore, soil and water contamination are one of the most crucial aspects of pollution that people cannot neglect. Healthy soil is necessary to grow crops, provide food, maintain populations, and for humans to stay healthy. Water contamination is thereby caused by soil contaminants that run off into rivers. At least 9 million fatalities occur annually due to air, water, and soil pollution. Moreover, heavy metals are the most significant and prevalent contaminants in the soil environment. Among them are nickel (Ni), cadmium (Cd), chromium (Cr), mercury (Hg), cadmium (As), copper (Cu), lead (Pb), and chromium (Cr). This contamination is persistent in the soil environment, physiologically harmful, and widely diffused. The ecology and public health are at risk from heavy metals-contaminated soil as society and the economy grow swiftly. The

availability of cropland in China's agricultural regions has decreased due to heavy metal contamination of the soil. Plus, 800,000 tons of lead and 30,000 tons of chromium have been emitted into the environment globally for 50 years [3]. In the soil, the majority of these heavy metals have already accumulated [4]. Yet, long-term exposure to heavy metals can cause lung cancer and bone fractures in people [5]. Due to the situation, locally and globally, remediation methods for contaminated soil and pollution assessment have become vital and have received significant attention.

Many research has been done on ex-situ (encapsulation, electrokinetic extraction, surface capping, soil flushing, phytoremediation, chemical immobilization, and bioremediation) and in-situ (chemical immobilization) remediation methods for heavy metal-polluted soil (landfilling, solidification, soil washing, vitrification) [6]. These remediation methods concentrate on lowering the soil's highest or most bioavailable heavy metal concentration. However, these conventional techniques for cleaning contaminated soil have several drawbacks and pose risks. Despite their significant effectiveness in resolving the issue of heavy metal pollution in the soil environment, most of these approaches are costly, wasteful of resources, time-consuming, require high energy consumption, and easy to produce secondary pollution. Thus, it is crucial to use cutting-edge remediation methods that can efficiently and safely clean up soil contaminated with heavy metals. Since the 1980s, bioremediation technology, especially bacterial remediation technology (also known as microbial remediation technology) [4], has been discovered, and it is a new method to treat heavy metal pollution. Bioremediation is the fundamental guiding concept of bacterial remediation technology, which refers to methods for environmental restoration that use microorganisms to break down or detoxify the pollutants in a contaminated matrix. Microbe-assisted phytoremediation and anammox enzymatic remediation are examples of bacterial remediation techniques [7]. The United States Environmental Protection Agency (USEPA) has issued guidance on using radioactive materials in the environment. Later, bacterial remediation technology attracted widespread attention because it can remediate the soil environment effectively requires low cost and works simultaneously efficiently.

This review summarizes four mechanisms of the bacteria in charge of bacterial remediation technology (dissolution of heavy metals, redox transformation of heavy metals, adsorption and adhesion of heavy metals, and the role of mycorrhiza) by the author. Moreover, a detailed case study of "bacterial remediation of heavy metal polluted soil and effluent from paper mill industry" is illustrated. Besides, the prospects and the area for future investigations in bioremediation and remediation strategies of heavy metal pollution are discussed and elaborated. Finally, the author emphasizes the significance of the remediation of heavy metal pollution and enhances the importance of maintaining a clean and unpolluted environment locally and globally.

## **2. Mechanisms of the bacterial remediation technology**

### *2.1. Dissolution of heavy metals by bacteria*

Bacteria have the ability to remediate and dissolve heavy metals. Currently, the amount of sludge produced by various bacterial treatment methods of sewage is enormous, and many sludges contain heavy metals in different concentrations. Specifically, high amounts of organic matter and biogenic substances, particularly nitrogen (N) and phosphorus (P), which are essential for plant development, can be abundantly found in sewage sludge. Toxic heavy metals such as copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), cadmium (Cd), nickel (Ni), and zinc (Zn) are also present in them. As a result of its capacity to bioaccumulate in the food chain, a particular metal may provide threats to the environment and human health, depending on the quantity and duration of exposure. The most frequent sources of heavy metals in sewage sludge are surface runoff from metropolitan areas or highways, home and industrial wastewater, and sewer system degradation. Wastewater from the agro-industrial sector may also be sent to wastewater processing stations. In addition to personal care and cleaning items, medications are another source of heavy metals. Moreover, illicit wastewater releases are a significant source of heavy metal contamination in sewage sludge [8]. Based on these severe pollution situations discussed, many restrictions are forced to be present on the later utilization of sludge resources.

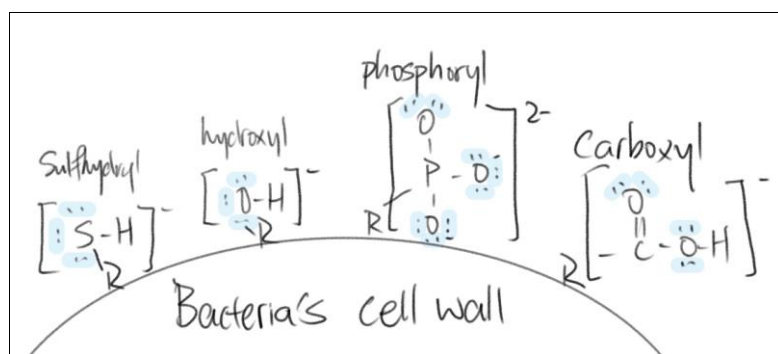
Sludge is crucial for the environment, and its re-usage can bring several benefits. When applied as dewatered sludge cake, the organic matter content in sludge can enhance the soil's physical, chemical, and physical qualities, assuring more excellent cultivation and aquifers capacity. Biosolids improve surface water retention by reducing runoff. Sludge's organic nitrogen is significantly less likely than artificial nitrogen fertilizer to pollute groundwater. The most outstanding technique to reuse the nutrients in sewage sludge is to apply it to agricultural land. When utilized as an organic fertilizer, it produces positive plant reactions regarding yield. Hence, sewage sludge is a crucial biological resource for environmentally friendly farming, and resolving the issue of heavy metal pollution in it is a crucial concern. To address this problem, the method of bacterial hydrometallurgy can be carried out. Bacterial hydrometallurgy is a process of chemical metallurgy that extracts and separates metals based on reactions that take place in an aqueous medium, and it is one of the processes of reducing metals from contaminated soil. Bacteria can alter the pH of the environment where heavy metals are located directly through their action or by using the small molecule, like organic acids, produced through metabolism. This will release heavy metal ions from their adsorbed and combined states. When the heavy metal ions are released, they will no longer be a part of the pollutants.

Recently, research on using bacterial hydrometallurgy to achieve this effect has progressed. In the investigations, it is discovered that there is a positive correlation between the decrease of pH value in the leaching solution and the removal rate of heavy metals. Still, some areas need further investigation. Firstly, more work needs to be done to understand the condition of the environment for the bacteria to work at the highest efficiency. Secondly, more investigations need to be carried out to make sure that the heavy metal-rich leachate is not causing secondary pollution.

## 2.2. Redox transformation, adsorption, and adhesion of heavy metals by bacteria

Different interactions and survival strategies are used by bacteria when inorganic metals are present. Biotransformation, extrusion, the usage of enzymes, and the creation of exopolysaccharides (EPS) are some of the techniques utilized by bacteria to resist metal toxicity. Bacteria have created inventive metal resistance and detoxifying systems in response to metals in the environment. The fixation of heavy metals by bacteria mainly relies on three common methods, extracellular complexation, extracellular precipitation, and intracellular accumulation. Furthermore, the mechanisms involved are electrostatic contact, ion exchange, precipitation, redox, and surface mechanism complexation.

As shown in Figure 1, the bacteria's cell wall surface contains functional groups like sulfhydryl, phosphoryl, hydroxyl, and carboxyl, and these groups contain atoms such as P, S, O. These atoms have lone pairs of electrons that can provide the coordinated complexation for heavy metals, allowing heavy metals to be adsorbed by the bacteria's cell walls.



**Figure 1.** The functional groups and lone pairs of electrons (highlighted in blue) on a bacteria's cell wall.

By adsorption, bacteria can alter the valence state of metals to change and reduce their toxicity, solubility, and mobility through their metabolic activities or through the organic molecules it generates. When the heavy metals' valency states become lower, their toxicity decrease, and thereby their hazards to the environment and living organisms around will be reduced, and they will not give rise to such

severe pollution. For example, acidophilus can convert highly toxic  $\text{Cr}^{6+}$  to  $\text{Cr}^{3+}$ , a less toxic and soluble state, through its metabolic activities, thereby reducing the hazards of chromium ions. Some other bacteria can reduce the insoluble  $\text{Pu}^{4+}$  to  $\text{Pu}^{3+}$  and reduce  $\text{Hg}^{2+}$  to elementary substance Hg. The adsorption strength of bacteria to heavy metals is affected by temperature, pH, and nutrition. Therefore, to increase the working efficiency and quality of bacteria, more investigations should be carried out to maximize the adsorption capacity of bacteria and further explore their adsorption principle.

### 2.3. *The role of mycorrhiza*

Mycorrhiza is a symbiotic phenomenon; it is the combination of soil fungi and higher plant roots within a mutualistic relationship where both organisms receive benefits. Mycorrhiza's primary function is to increase the host plant's intake of nutrients and water by using more soil than roots alone can. Many kinds of mycorrhizae exist, depending on the host plant and fungus taxonomy [9]. These forms are distributed in ecosystems concerning the distribution of the host plants and the climatic and soil conditions. The performance of the host plant can be changed by mycorrhizae's capacity to enhance host plant nutrition and water uptake, as well as support defense against root infections and grazing. Mycorrhizae can affect the makeup of the plant community, increase competition, and foster synergism by allowing various species to share resources. These effects can be achieved by variably influencing the performance of individual species within the community. Furthermore, in forestry, agriculture, and restoration, mycorrhizae are utilized to increase production and reduce pollutants from growing on disturbed land. Mycorrhizal fungi are potential candidates for restoring and cleaning up damaged habitats because of their capacity to gather radioactive materials and heavy metals. They can remediate and effectively resolve the issue of heavy metal pollution at the same time as they carry out the mutualistic relationship with the higher plant's roots.

The mutualistic interaction between mycorrhizal fungi and plant roots can be regarded in three ways: plant roots reduce heavy metal toxicity for mycorrhizal fungi, mycorrhizal fungi reduce heavy metal toxicity for plant roots, and mycorrhizal fungi improve the activity and growth of the plant. First, because heavy metal hyper-accumulation plants can absorb and transfer heavy metals, the toxicity of heavy metals to soil bacteria is reduced. Moreover, the plant root system can not only provide complexation with heavy metals through the secretion of organic acids and amino acids; but also can provide nutrients for mycorrhizal fungi living there to improve their activity. Moreover, the metabolic activities of the mycorrhizal fungi secrete organic acids, and extracellular precipitation, extracellular complexation, and redox of heavy metals are happening because of it. As a result, the toxicity of heavy metals is reduced, and the toxic effect of heavy metals on plant roots is also reduced. Finally, the existence of mycorrhizal fungi can significantly improve the activity and growth of higher plants. Especially in soils with poor nutritional conditions, mycorrhizal fungi can provide the plant roots with resources necessary for their growth, like water, nitrogen, phosphorus, and zinc, through their vast hyphae network.

Currently, the main difficulty in mycorrhizal restoration is obtaining bacteria with strong restoration abilities and suitable plants. Due to the importance of mycorrhiza fungi, humans started to use several techniques to maintain them and help them survive. Mycorrhizal Applications was established more than twenty years ago to address an issue that had plagued foresters and restoration specialists for years. The strong mycorrhizal network under the soil will be destroyed by disturbances like clear-cutting, fire, and erosion. Professional mycorrhizal inoculants are the best approach to rebuilding these symbiotic partners.

### 3. **Case study**

This case study explores bacterial remediation of heavy metal-polluted soil and effluent from a paper mill industry [10]. It is an experiment investigating whether proteobacteria or non-proteobacteria are better at resolving heavy metal pollution. The phylum of proteobacteria includes all Gram-negative bacteria, and only a small number of them have the ability to create energy through photosynthesis. Gram-positive and Gram-negative bacteria are both part of the non-proteobacteria group, and all of its

members can produce energy through photosynthesis. The color that gram-positive bacteria take on when stained is used to identify and categorize them. Hans Christian Gram created the staining method in 1884. The thick peptidoglycan cell wall present in gram-positive organisms retains the crystal violet dye used in the staining process.

On the other hand, the bulk of currently available antibiotics and other treatments are no longer effective against Gram-negative bacteria. These bacteria have the genetic capacity to transmit information that allows other bacteria to evolve drug resistance, and they also naturally find new ways to do so. Regardless of their division of phylum, they are bacteria, and all have genetic and biochemical capabilities to resolve the issue of heavy metal pollution to a certain extent and are all essential implements under molecular biology.

During the bioremediation investigation, researchers used soil and water samples from polluted (inside the paper mill environment) and unpolluted (a control location outside the paper mill polluted site). Three treatments comprise the experiment: Proteobacteria are used in Treatment 1, non-proteobacteria are used in Treatment 2, and no bacteria are used in Treatment 3. (control experiment). Readings were obtained every 30 days during the course of the six-month trial. Water was added constantly to keep the moisture content at 60-65%. For all treatments, the procedure was repeated three times.

This study shows that some bacteria can survive and convert toxic heavy metals into less damaging versions. This research has shown that, despite the soil and effluent discharge not having been prepared, bacteria isolated from the paper mill were capable of sorbing the very dangerous heavy metal accumulation in a time-dependent manner. The data tables in the research report for the study, which is shown on this slide, show that heavy metals, including Pb, Cr, As, Ni, and Zn were successfully mended in soil and effluent samples, with proteobacteria having better remediating capability than non-proteobacteria. The most effective waste management plan for paper mills maybe this one. Future studies will use this strategy continuously and focus on heavy metal bioaccumulation in bacteria and molecular characterization.

#### **4. Conclusion**

This study reviewed the mechanisms of bacterial remediation technology. Also, it presents a case study about an experiment on pollution and remediation strategy in detail. Furthermore, the properties of heavy metal elements in the soil, such as mobility, valency state, and toxicity, are affected by soil properties. Bacteria are essential components of the soil environment, and their metabolic activities directly impact soil's physical and chemical properties. Moreover, bacteria can also act on heavy metals through various activities. These mechanisms are the theoretical basis for bacterial remediation of soil heavy metal pollution. Researchers have extensively studied the interaction mechanism between bacteria and heavy metal ions, but there are few studies on the interaction between bacteria and rare-earth elements. Due to years of mining in irregular earth mining areas, severe ecological problems with heavy metal pollution have been raised to varying degrees, posing a threat to farming, animal agriculture, and domestic water in mining areas and causing huge environmental risks. The effect of bacteria on heavy metals shows that it's a feasible solution to control heavy metal pollution. Therefore, in future research, more investigations should focus on the effect of bacteria on rare-earth elements to find a possible way to control pollution in occasional earth-mining areas. Besides, if bacteria can be manipulated through gene engineering to increase their ability to remediate heavy metal pollution, their treatment process on this issue can be carried out with higher efficiency, thus resolving it at a higher degree. In short, if a better understanding of the condition that is most suitable for bacteria's work can be gained and if the remediation process can be manipulated to fit a wider variety of metal pollution, the working efficiency for the bacteria will be higher, and the technology of bacterial remediation (bioremediation) will be scaling new heights.

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