

## **Improve Heavy Metals Bioremediation in Contaminated Soils by Using Microbial Biotechnology**

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### **Abstract**

Biotechnology offers new methods of solving environmental pollution, especially using microbial bioremediation applications, genetic engineering and biosensor technologies. This paper explores how microbial biotechnology and genome editing technology can be used to remove heavy metal in soils. The treatment of lead (Pb), cadmium (Cd), and zinc (Zn)-contaminated soil samples was done with the help of the bacterial cultures *Pseudomonas*, *Bacillus*, and *Streptomyces*. The experimental data showed that the heavy metal concentrations were greatly decreased and the efficiencies of the removal were between 55.0 and 73.3 %. In particular, the removal efficiency of Pb was 66.7 %, Cd 73.3 %, and Zn 55.0 %. Results of this study highlight the usefulness of microbial bioremediation as a potential tool in cleaning up the environment. Moreover, there are also opportunities to improve the functioning of microorganisms through the introduction of the latest genetic engineering techniques. Including CRISPR-Cas9, which can potentially significantly improve their pollutant-degrading activity. This study justifies the possibility of using modern biotechnology as a sustainable, environmentally friendly, and cost-effective method of environmental pollution reduction, and as a promising factor in ecological health and the control of pollution.

**Keywords:** Biotechnology, Environmental pollution, and Microbial biotechnology.

### **1. Introduction**

One of the most actively developing scientific directions is biotechnology that is significant in agriculture, medicine, environmental protection, and industrial

development [1, 2]. Contemporary biotechnology is the combination of molecular biology, microbiology, and genetic engineering into the creation of sustainable solutions to environmental problems on the global scale [3, 4].

The heavy metals, pesticides, and industrial chemicals that cause environmental pollution have become a grave menace to the ecosystem and human health [5, 6]. Heavy metals, in particular, lead, cadmium, mercury and chromium are very harmful as they can stay in the environment, and they cannot be easily broken down by natural processes [7]. Among the conventional remediation processes, which include chemical precipitation, filtration process, and excavation of soil, are costly and can lead to the occurrence of secondary pollution [8]. As such, green technologies must ensure that the issue of environmental contamination is reduced [9].

The application of microorganisms or plants to remove pollutants has also been receiving a lot of attention as a sustainable remedial method of restoring the environment [10, 11]. Bacteria, fungi and algae are microorganisms that have metabolic pathways that can convert toxic compounds into less toxic or harmless compounds. The microbial processes that may be used in the remediation of heavy metals are bio-sorption, bioaccumulation, bio-precipitation, and enzymatic detoxification [13].

It is the ability of microorganisms to live in polluted environments and at the same

time eliminate contaminants through these biological processes [14]. There are a few bacterial species that have been extensively identified to have the capabilities of eliminating heavy metals in contaminated soils and wastewater systems and they are *Pseudomonas*, *Bacillus* and *Streptomyces* [15]. Microbial bioremediation may effectively decrease the level of pollutants considerably and reclaim the environment in most situations [16].

The recent developments in biotechnology have also improved the capacity of bioremediation. There are genome editing tools like CRISPR-Cas9, which provide the ability of scientists to edit the genomes of the microorganisms in a very precise way. This gives a chance to create engineered microorganisms that could have a better capacity for degrading pollutants [17]. The biotechnology of CRISPR has also found use in environmental biosensors capable of detecting pollutants in high levels of sensitivity and specificity [18]. Also, in the new technologies of synthetic biology, bioinformatics, and nano-biotechnology, environmental biotechnology can open new opportunities.

Such technologies allow scientists to program microbial systems that can be used to target certain pollutants and enhance the

efficiency of remediation [19, 20]. Consequently, this research attends to assess the current biotechnological methods of environmental clean-up with emphasis on genetic engineering and microbial bioremediation methods [20].

The recent development of microbial bioremediation has been achieved with great success, but the combination of genetic engineering methods like CRISPR based microbial enhancement to remove heavy metals is still underdeveloped [19, 20].

## **2. Materials and Methods**

### **2.1 Sample Collection**

Soil samples were obtained in the regions of industries known to be contaminated with heavy metals. Elevations of 10-20 cm were used to collect samples, and they were preserved in sterile containers in the laboratory. All experiments were run three times to be statistically viable. ANOVA was used to analyze data, and the level of significance was  $p < 0.05$ . Soil samples were specifically sampled in industrial areas in Wasit Governorate, where industrial activities like chemical production and construction materials are known to cause heavy metal pollution.

### **2.2 Isolation of Microorganisms**

Isolation of microorganisms was carried out to satisfy the necessity of the experiment. Isolation of microbial strains was done by serial dilution methods and grew in nutrient agar plates. Purification of colonies was done by streaking [14-16].

### **2.3 Identification of Bacteria**

Isolates of bacteria were determined through morphological characterization, biochemical tests, and 16S rRNA gene sequencing [14, 15].

### **2.4 Bioremediation Experiment**

The experiment was chosen with three bacteria that were selected. *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Streptomyces sp.* Samples of contaminated soil were inoculated with bacteria, and incubation time was 30 days with 30°C, pH 7, and soil moisture of 60% [15-17].

### **2.5 Heavy Metal Analysis**

Identification and isolation of microbes were done as per the normal microbiological procedures reported in another research [21]. Atomic Absorption Spectroscopy (AAS) was used to analyze

heavy metals; it is commonly used to detect heavy metals in the environment [22-25].

### 3. Results and Discussion

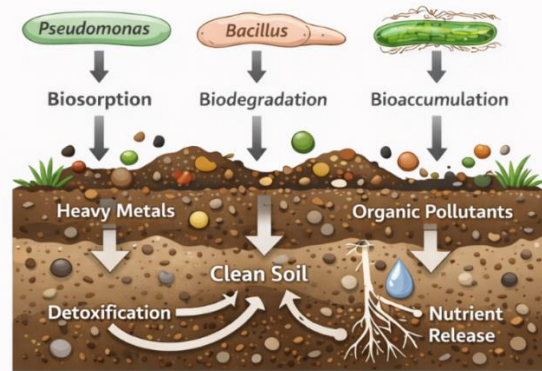
Experimental findings demonstrated that microbial treatment was very effective in decreasing levels of heavy metals in polluted soils. Similar results have been reported in earlier studies where bacterial species had good metal removal capabilities [23-25].

**Table 1:** Heavy metal concentrations before and after microbial treatment.

Metal	Initial concentration (mg/kg)	After treatment (mg/kg)	Removal efficiency (%)
Pb	120	40	66.7
Cd	45	12	73.3
Zn	200	90	55.0

These findings show that microbial biotechnology has the potential of reducing heavy metals contamination of the environment. Microbial bioremediation entails several biological processes which assist microorganisms to convert or fix toxic metals in the polluted soils.

The conceptual representation of the microbial bioremediation processes and their application in eliminating heavy metals in polluted environments are shown in figure 1 [21-24].



**Figure 1:** Conceptual framework of microbial bioremediation processes for heavy metal removal in contaminated soils.

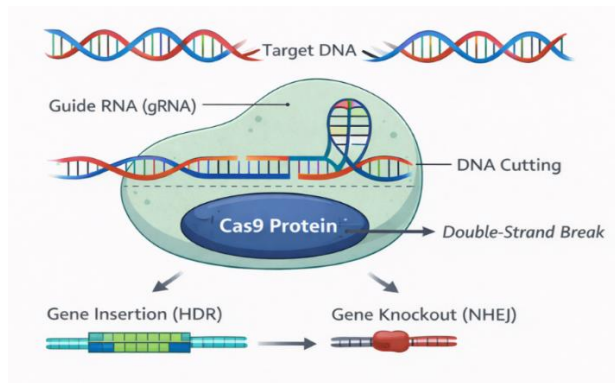
However, figure one reveals the main biological mechanisms of microbial removal of heavy metals, which include the biosorption, bioaccumulation, bioprecipitation, and enzymatic transformation. Biosorption is the binding of the heavy metal ions of the contaminated environment by the cell walls or extracellular materials of the microbial cells. While bioaccumulation, is the introduction of metals into microbial cells, where they are stored or converted to less toxic forms.

The Bio-precipitation represents the metabolic transformation of the metals into insoluble states and their extraction into the environment by the action of microorganisms. Also, enzymatic transformation related the toxic metals are transformed into non-toxic compounds by

microbial enzymes and this helps in their detoxification [20-24]. The findings of this paper affirm the high importance of microbial biotechnology in the environmental cleanup. Microorganisms can convert the toxic substances by using metabolic routes and enzymatic conversion reactions [23-25].

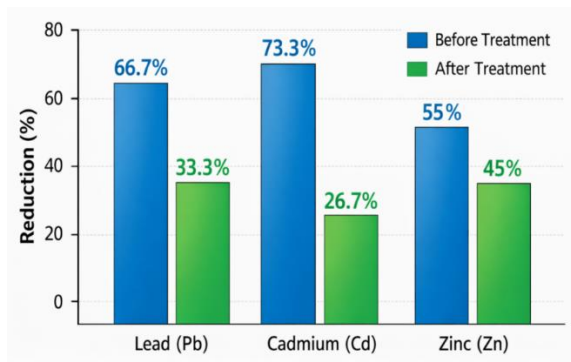
A high number of researchers have proven that bacteria and fungi can be used to effectively cleanse the environment with heavy metallic substances. Carboxyl, hydroxyl and phosphate groups in the microbial cell walls bind the metal ions and mediate the biosorption and detoxification processes [10-14].

Recent innovations in biotechnology present technologies of genome editing like CRISPR-Cas9 that allow one to carefully edit the genomes components of microbes to optimize their ability to degrade pollutants. The CRISPR-Cas9 mechanism of genome editing in microbial biotechnology is illustrated in figure 2 [18-22].



**Figure 2:** Schematic representation of the CRISPR-Cas9 genome editing mechanism in microbial biotechnology.

As demonstrated in figure 3, heavy metals were greatly reduced by microbial treatment with a removal efficiency of between 55 and 73 %. It has been shown to have similar efficiencies in removal of microbial bioremediation of contaminated soils in other studies [19-23]. Bioremediation has been regarded as an environmentally friendly and cheaper alternative to conventional remediation. In comparison to chemical treatment, microbial remediation does not generate secondary pollutants and may also be used to reestablish ecological balance in polluted environments [10-14].

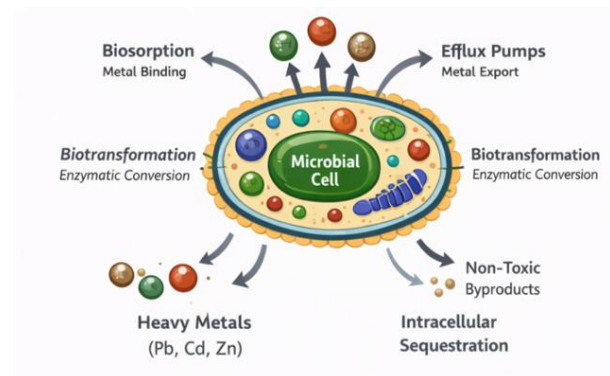


**Figure 3:** Removal efficiency of heavy metals (Pb, Cd, and Zn) after microbial bioremediation treatment

Moreover, synthetic biology approaches can be used to design engineered microorganisms that can degrade complex pollutants, and this has potential future uses in the next generation of environmental biotechnology applications. This method is consistent with the past literature, which has demonstrated the success of bioengineering in improving the pollutant degradation abilities of microbes [15-20].

There are several biological mechanisms that are used by microbes in the detoxification of heavy metals that have been widely reported in the literature in the past including biosorption, bioaccumulation, bioprecipitation and enzymatic transformation. These are the processes as shown in figure 4 that have played a central role in formulating microbial bioremediation strategies and their findings have been consistent with other studies that point out its

application in the reduction of environmental pollution [20-25].



**Figure 4:** Proposed microbial pathways for heavy metal detoxification including biosorption, bioaccumulation, bioprecipitation, and enzymatic transformation.

#### 4. Conclusion

This paper shows that biotechnology is significant in the management of the environment and control of pollution. Biotechnology, and / or Microbial biotechnology offers good methods of eliminating heavy metals and other pollutants in the contaminated environment. The outcome of the experiment indicated that levels of heavy metals decreased by a significant percentage once bioremediated by the microorganisms, which proves the effectiveness of different bioremediation methods.

Also, the recent developments in genetic engineering that have included CRISPR-

Cas9 suggest that enhanced opportunities to enhance microbial performance and create more effective environmental cleaning tools are possible. Future studies are recommended to incorporate biotechnology into synthetic biology, nanotechnology and bioinformatics to produce more advanced environmental remediation systems.

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