A review of bioremediation techniques for heavy metals pollution in soil

Tuo Jina,b, Chenning Shic, Ping Wanga, Jinpeng Liuc*, Liuyang Zhand*

aCollege of Resources and Environment, Hunan Agricultural University, Changsha, Hunan, 410128, China
bRural Energy and Environment Agency, Ministry of Agriculture and Rural Affairs, Beijing 100125, China.
cNational & Local Joint Engineering Research Center on Biomass Resource Utilization, College of Environmental Science and Engineering, Nankai University, Tianjin, 300350, China
dBusiness School, Tianjin University of Finance and Economics, Tianjin, 300222, China
*Corresponding author’s e-mail: zhanly@nankai.edu.cn; nkliujp@126.com

Abstract. Heavy metals polluted soil caused by sewage irrigation, industrial pollution, pesticides and fertilizers, and atmospheric deposition have a harmful effect on the environment, which is difficult to completely eliminate and will threaten the ecological environment, food safety, and human health. The treatment of soil heavy metals and soil safe utilization have become the top priority of the treatment of industrial land and farmland soil. The existing main methods of soil heavy metals remediation include physical remediation, chemical remediation and biological remediation. With changes in governance scenarios and technological innovations, the joint application of interdisciplinary technologies has become an important development direction for soil heavy metals remediation. Especially in the treatment of heavy metals polluted farmland, the combined use of multiple technologies can further improve the safe utilization of farmland. In this paper, the main bioremediation techniques of heavy metals were summarized based on the domestic and foreign literatures in order to provide reference for further research in this field.

1. Introduction
Soil is an important natural resource, which is the foundation of agricultural development, provides raw materials such as clothing and food for human survival, and plays a huge role in the national economy. However, with the increasingly serious three waste pollution caused by the continuous development of modern industry, the large-scale use of chemical fertilizers, pesticides, and agricultural films, limited soil self-purification capacity cannot deal with more and more serious pollution, resulting in inevitable pollution. The population, the demand for food, as well as the role of soil in human life continue to increase, which leads to the great importance to repair the contaminated soil.

Among the existing soil pollution control technologies, bioremediation technology are receiving increasing attention and becoming a research hotspot with the characteristics of good effect, low cost, easy operation and management, and freedom from soil disturbance. In addition, some
environmentally friendly heavy metals passivation restoration technologies and modern agronomic technologies, could change the occurrence of heavy metals elements in the soil in order to stabilize their effective state, and effectively prevent the migration and transformation of biologically effective forms of heavy metals in the ecological environment. This has become an important development direction of soil in-situ restoration technology.

2. Microbial remediation

Microbial remediation technology refers to a remediation technology that uses indigenous microorganisms or artificially domesticated microorganisms with specific functions to reduce the activity of pollutants or degrade them into harmless substances through their own metabolism under adapted environmental conditions. Microbial remediation is mainly divided into bacterial remediation and fungal remediation, and its remediation principles mainly include biosorption, bioconcentration, and biotransformation. The use of microorganisms has advantages of small individuals and large specific surface area, fast reproduction and strong metabolism, multifarious types and wide distribution, strong adaptability and easy to cultivate, etc. The limitations of microbial remediation include the poor genetic stability so that easy to mutate, inability to completely remove pollutants, and need to compete with indigenous strains, which makes it easy to be affected by the environment [1].

2.1. Bacteria remediation

Using bacteria to remediate soil contaminated by heavy metals is the most common method in microbial remediation technology. Bacteria are widespread in the natural environment, especially in the soil, which provides a wide range of research materials for this technology. Generally, the research is to screen out bacteria resistant to high concentrations of heavy metals from the polluted soil, and then isolate and purify the bacteria. Finally, the obtained bacteria are used to repair the soil contaminated by heavy metals.

On the basis of optimizing cultivation condition, Xiaoming Li [2] et al. injected water into the oil field to isolate *Rhodobacter sphaeroides*. Under different pollution levels, the microorganisms were used to remediate simulated lead-contaminated soil, and the most optimal temperature, pH value and inoculum amount of the cultivation substrate was obtained. Valentina V. Umrania [3] et al. isolated microbial strains from metal-polluted soil, mud and water for bioremediation of toxic heavy metals. Through primary and secondary screening, 72 species of acidophilic thermophilic transformed microorganisms were isolated and screened for metal resistance and biosorption capacity. Kailasam Saranya [4] et al. studied the potential detoxification of heavy metals of phosphate-dissolving bacteria (PSB) isolated from coral, seaweed and mangrove. The biosorption of heavy metals is analyzed and confirmed by scanning electron microscope, fourier transform infrared spectroscopy and scanning electron microscope (SEM). Ramaraju Kalpana [5] et al. isolated a bacterium that produces extracellular polysaccharides (eps) and identified it as Bacillus cereus vk1 through 16S rRNA. The bacteria can effectively adsorb Hg\(^{+2}\), which provides a strategy for the bioremediation of Hg\(^{+2}\) polluted ecosystem. Chang-ho Kang [6] et al. studied the relationship between the resistance of urea bacteria to heavy metals and the resistance to antibiotics and found that the heavy metal resistance of these isolates is closely related to their resistance to antibiotics. One of the mechanisms of bacterial remediation is adsorption [7] (figure 1).
2.2. Fungal remediation
Fungal remediation refers to remediating contaminated soil using the ability of certain fungi in the soil ecosystem to degrade pollutants. Some fungi are super-enriched organisms, which can excessively absorb heavy metal ions in the environment and store them in the fruit body to achieve the purpose of repairing heavy metals in the soil.

Elham Mohammadian [8] et al. investigated the fungal populations in soil samples from lead and zinc contaminated areas in Zanian Province, Iran. The microflora’s effects on cadmium, lead, and zinc are determined by measuring the "minimum inhibitory concentration after exposure to increased concentrations of heavy metal chlorides", and their resistance to copper and copper was evaluated by measuring the total metal adsorption capacity after combustion. Amna Bano D [9] et al. studied the biosorption of heavy metals by obligate halophilic fungi. Fungi with the function of remediation of soil polluted by heavy metals are generally adaptive to the polluted environment. They may have evolved mechanisms to escape the damage that heavy metals do to them (figure 2) [10].

3. Phytoremediation
Phytoremediation refers to using plants to transfer, contain or transform pollutants to make them harmless to the environment, which purify the soil through the effects of plant absorption, volatilization, root filtration, degradation, and stabilization. It has the advantages of low cost, easy control of secondary pollution, and the effects of protecting topsoil, reducing erosion and soil erosion, and thus can be widely used in mine reclamation, preparation of heavy metal contaminated sites, and landscape restoration.

Xiaoming Li [2] et al. planted wheat in heavy metal contaminated farmland, and washed, separated, dried and weighed the leaves and roots of wheat seedlings after 30 days, measured the concentration
of lead in the soil, and studied the changes in the availability of lead in the soil. Although the efficiency of wheat bioremediation of lead is not high, it is still a promising alternative method for the remediation of lead in contaminated soil (figure 3). A.C. Agnello [11] et al. used alfalfa phytoremediation to remediate soil contaminated by heavy metals and petroleum hydrocarbons in pot experiments. Alfalfa plants have strong tolerance and growth ability in co-contaminated soil, which can promote plant growth and reduce plant stress. Alfalfa plants have limited ability to accumulate heavy metals, and heavy metals are mainly concentrated in plant roots. Hui Li, Xing Li [12] used pot experiment method to study the migration and transformation of cadmium in solanum nigrum and found that solanum nigrum treated with Fusarium moss or cyclodextrin has a good ability to enrich Cr (figure 4).

Figure 3. Pb speciation distribution with and without R. sphaeroides (Left: 0 d; Right:30 d).[2]

Figure 4. A potential strategy for remediation of Cd and BDE-209 co-contaminated soil.[12]

4. Multi-techniques joint remediation
Joint remediation refers to using two or more remediation methods to deal with the polluted soil. It can not only increase the rate and efficiency of contaminated soil remediation, but also overcome the limitations of individual remediation technologies.

4.1. Plant-microbe joint remediation
Dana Luminita Sobariu et al. [13] established a system composed of rhizosphere nitrogen-fixing bacteria and leprosy plants to grow in different concentrations of Gr(VI) and Cd(II) heavy metal solutions. Nitrogen-fixing bacteria can increase the germination rate, root length, stem length, and dry biomass of Jatropha, while the plant's tolerance to heavy metals in the bacteria-plant system is enhanced, indicating that the two have a good symbiotic ability and so that can form a reliable joint remediation system. AC Agnello [8] evaluated alfalfa phytoremediation, *pseudomonas* bioremediation and bioaugmentation assisted phytoremediation (planting alfalfa plants in the soil inoculated with *Pseudomonas aeruginosa* to jointly repair heavy metals and petroleum hydrocarbon contaminated soil), among which the best effect is the bioaugmentation assisted phytoremediation treatment.
4.2. Chemicals along with bacteria
Nalan Oya San Keskin [14] found that electrospun cyclodextrin fiber (CD-F) can encapsulate bacteria for bioremediation (figure 5). They encapsulated the bacteria into the CD-F matrix for wastewater treatment, and the results proved that the pollutant removal ability of bacteria/CD-F is higher than that of free bacteria. The reason is that the natural and non-toxic properties of CD-F are fiber biological composites. The material provides better bacteria viability, and bacteria can use CD as an additional carbon source to promote growth. According to the concept of bacterial cell encapsulation, new bio-composite materials with bioremediation capabilities can be developed for the treatment of heavy metal pollution.

![Figure 5. Images of bacteria before and after encapsulation. [14]](image)

4.3. Combined physical-chemical-biological remediation
The combined application of physical, chemical, and bioremediation technologies can combine the advantages of the three. The physical and chemical technologies can reduce the inhibitory effect of toxicity on microorganisms, and the restoration of microorganisms can reduce the burden of physical and chemical remediation. Zhi-yong Dong [15] and others used EDTA-assisted electrokinetic (EK) remediation combined with biodegradation technology to treat soil contaminated by crude oil and lead. The EDTA-enhanced EK treatment has greatly reduced toxicity, allowing microorganisms to continue to decompose pollutants. These results indicate that when the initial toxic metal content is high, the direct use of microbial treatment will lead to the restriction of microbial growth, while the direct use of physical and chemical techniques will lead to excessive costs and cumbersome operations, so the two can be combined to achieve the unity of economy and environment.

5. Conclusion
Soil heavy metal pollution is difficult to control due to its strong toxicity, wide distribution, and easy transformation. The remediation of heavy metal contaminated soil is a long-term and arduous process. Remediation technology is also constantly developing, from a single remediation with a single effect to a combination of advantages. The restoration is shifting from high-cost technologies with obvious side effects to low-cost, green and environmentally-friendly technologies, and new and more efficient restoration materials are continuously synthesized from ordinary materials. Among all remediation technologies, compared with physical and chemical remediation methods, microbial remediation can be carried out in situ, saves treatment costs, has little impact on the environment, is environmentally friendly and economical, is an enhancement of natural processes and thus generally does not produce secondary pollution, so it has a wide range of application prospects. But meanwhile, there are disadvantages such as long time consumption, specificity in most cases, and small remediation scope. Therefore, when selecting remediation technology, environmental, time, and economic factors should be considered comprehensively to choose the most suitable remediation plan.
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