Research Progress on Bioremediation Technologies for Heavy Metal Contaminated Soils

Ruochu Liu *

International Curriculum Center of RDFZ, Beijing 100080, China

* Corresponding Author Email: lrc1104@outlook.com

Abstract. Soil heavy metal contamination is a growing concern due to industrial and economic development, making its remediation an urgent task. Phytoremediation, an eco-friendly and cost-effective technique, has become a research hotspot both domestically and internationally. Compared to the limitations of physical and chemical remediation methods, bioremediation of heavy metal-contaminated soil offers significant environmental benefits by reducing soil pollution and restoring ecological function, making it more suitable for contemporary soil treatment. This paper reviews the current status of heavy metal contamination and traditional remediation approaches. It then focuses on the mechanisms of phytoremediation for heavy metal contamination soil, particularly through gene-editing techniques. The advantages and disadvantages of traditional lead remediation are analyzed, alongside the benefits and underlying principles of hyperaccumulating plants for heavy metals. We address current challenges in applying phytoremediation, such as low lead extraction efficiency, and discuss gene-editing methods to enhance its effectiveness. The paper highlights key technical schemes and principles of plant genetic engineering, comparing the pros and cons of gene-edited plants for lead-contaminated soil remediation and their application methods. Finally, we explore the challenges and future research directions for genetic technology in heavy metal-contaminated soil remediation.

Keywords: soil heavy metal contamination; gene-editing techniques; phytoremediation.

1. Introduction

Soil heavy metal contamination has emerged as a significant environmental issue with industrial and economic growth. These contaminants can enter the human body through the food chain, posing serious health risks. Therefore, the remediation of heavy metal-contaminated soil is crucial. While various remediation technologies exist, such as soil replacement and electrochemical remediation, they often involve intensive labor and contribute to secondary environmental pollution [1].

Phytoremediation, an economical and environmentally friendly remediation technique, offers promising prospects and has gained widespread attention and recognition. For instance, a three-year phytoremediation effort using Celosia argentea Linn. on cadmium (Cd)-contaminated agricultural soil resulted in a 6.34% reduction in soil Cd content. Similarly, Sedum plumbizincicola has achieved Cd removal rates of up to 84.5% in contaminated soil. Despite its minimal secondary pollution, low operating costs, and good remediation efficacy, phytoremediation is currently limited by the scarcity of suitable plant resources, often restricted to a few hyperaccumulating species.

Recent advancements in genetic engineering offer a solution to this limitation. By modifying genes associated with heavy metal absorption and transport in plants, we can enhance their uptake capacity and tolerance to heavy metal stress. Additionally, inoculating plants with beneficial microorganisms or utilizing material chemistry techniques can improve plant growth and survival rates under heavy metal stress, thereby expanding the selection of remediation plants. This paper primarily discusses the research progress in combining genetic engineering with phytoremediation. It also reviews traditional heavy metal pollution remediation technologies, aiming to provide novel insights and references for the practical application of gene-editing techniques in conjunction with phytoremediation in the future.

2. Soil Heavy Metal Contamination

2.1. Sources of Soil Heavy Metal Contamination

Heavy metals are naturally occurring chemical elements characterized by high atomic mass and density. They become toxic to cells when their concentrations exceed certain thresholds. Toxic heavy metal ions include lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), thallium (Tl), and chromium (Cr). In recent years, with the rapid development of modern industries, high concentrations of heavy metals in soil and rivers have become a global issue, posing severe threats to various microorganisms, plants, and animals [2].

Most heavy metal contamination in the environment today originates from anthropogenic activities. The primary sources include mining, smelting, wastewater discharge, paints, irrigation with contaminated wastewater, fossil fuel combustion, incineration of municipal solid waste, and the use of fertilizers and pesticides. The majority of heavy metals in soil are non-biodegradable, leading to long-term and persistent negative impacts on ecosystem stability. The expansion of various industrial and mining operations generates large quantities of heavy metal-rich waste. In the past, driven by economic development, some enterprises pursued a "pollute first, clean up later" approach, leading to widespread heavy metal contamination across significant land areas in China, particularly in proximity to industrial and mining sites. This pollution severely affects the health and living environment of nearby residents.

Furthermore, with improved living standards, car ownership has significantly increased. In some Chinese cities, automobile exhaust emissions are a major source of heavy metal contamination in soil (Table 1). For instance, 1 kg of gasoline contains approximately 0.65 g of Pb, with about 35 μg of Pb being emitted through exhaust during vehicle operation. Data indicate that China's vehicle fleet has reached 395 million vehicles, with nearly 80 cities having over a million cars. Over time, the cumulative amount of emitted heavy metals has become substantial. Beyond exhaust, the wear and tear of automotive components also contribute significantly to heavy metal pollution. As vehicles operate, these parts abrade, releasing numerous heavy metal-containing particulates that are carried by wind into the soil, thus causing contamination.

Improper disposal of discarded electronic products in daily life is another major cause of soil heavy metal contamination. Used batteries, old mobile phones, and other electronic devices must undergo specialized treatment; otherwise, the heavy metals they contain can leach into the soil, leading to severe pollution. For example, a 20 g smartphone battery can contaminate 1 km^2 of soil, with the pollution persisting for over 50 years without natural elimination. Additionally, heavy metals present in general household waste also contribute to contamination. Studies have shown that heavy metal content in household waste is generally higher in summer than in winter, and higher in commercial areas than in residential areas [3].

Table 1. Table of Heavy Metal Contents in Soils of China

	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Sample Size (Number)	1853	2669	2711	3520	2141	1862	3269
Minimum Value ($mg\cdot kg^{-1}$)	0.75	0.12	13.2	3.62	1.03	5.2	5.48
Maximum Value ($mg\cdot kg^{-1}$)	39.8	2.62	121	111.3	0.93	63.12	257
Average Value ($mg\cdot kg^{-1}$)	13.39	0.68	63.04	38.17	0.31	26.18	47.34
Coefficient of Variation (%)	63.93	95.59	42.08	53.42	64.52	46.94	93.01
Soil Background Value ($mg\cdot kg^{-1}$)	11.2	0.097	61	22.6	0.065	26.9	26
Soil Grade I Standard ($mg\cdot kg^{-1}$)	15	0.2	90	35	0.15	40	35
Soil Grade II Standard ($mg\cdot kg^{-1}$)	30	0.6	200	200	0.5	50	300

2.2. Hazards of Soil Heavy Metal Contamination

The bioavailability and chemical forms of heavy metal pollutants can vary significantly. Some heavy metal ions can be absorbed by plant roots through diffusion, endocytosis, or ion transporters, accumulating in various plant tissues. Once absorbed by plants, these heavy metals enter the ecosystem's material cycle through consumption by herbivores, continuously accumulating in biological organisms and significantly impacting human health.

Soil primarily contains two types of metals: essential micronutrients vital for normal plant growth (e.g., Fe, Mn, Zn, Cu, Mg, Mo, and Ni) and non-essential elements with unknown physiological functions (e.g., Cd, Sb, Cr, Pb, As, Co, Ag, Se, and Hg). While essential micronutrients play crucial roles in various biochemical and physiological processes in plants and animals, non-essential elements typically cause damage to various physiological functions within organisms [4].

Even essential heavy metals, when exceeding certain concentrations, can adversely affect plant growth and metabolism. The accumulation of heavy metals in plants leads to cytotoxicity and genotoxicity, inducing physiological and morphological changes in cells. Plants suffering from heavy metal toxicity exhibit clear symptoms, including stunted growth, leaf chlorosis (yellowing), root browning and death, and reduced seed germination. Furthermore, heavy metals can cause the denaturation and inactivation of enzymes and proteins within plants, interfere with the absorption of essential metal ions, disrupt cell membrane structures, and impair normal respiration and photosynthesis. Additionally, heavy metal stress can induce the production of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals in plants, leading to DNA damage [5].

In natural ecosystems, when animals and plants absorb heavy metal elements from contaminated soil or water, these elements are transferred to humans through the food chain. Over long-term accumulation, these heavy metals can severely harm human health, often inducing gene mutations and significantly impairing normal bodily functions. In daily life, heavy metal elements commonly enter the human body through consumption of vegetables, fruits, and meat products.

2.3. Traditional Treatment Methods

Traditional approaches to pollution control, particularly for soil heavy metal contamination, primarily encompass physico-chemical methods. These strategies aim to either block the spread of contaminants or reduce their pathways in the soil.

2.3.1 Physical Methods

Physical remediation techniques typically involve physically isolating contaminated soil or altering its distribution to mitigate further spread.

Soil Encapsulation: This method involves excavating contaminated soil and encapsulating it to prevent further contamination of surrounding areas. It is particularly suitable for recently contaminated sites.

Soil disposal: For some sites (such as old factory sites), landfilling is a viable option for dealing with heavy metal pollution. This process can isolate pollutants.

Soil replacement: Replacing contaminated topsoil with clean subsoil, using natural processes for remediation. Although this method is very effective, it is labor-intensive and costly, so it is only suitable for small-scale pollution.

Electrodynamic remediation (electroreduction): This process uses the electrical migration characteristics of heavy metal ions under the action of electric current. However, its application is limited due to the high conductivity requirements of the soil and high costs.

Thermal treatment: Based on the characteristics of heavy metal pollutants that are easily volatile when heated, this method volatilizes heavy metals by heating the contaminated soil. Then, a special gas separator is used to collect the released pollutants for centralized treatment.

2.3.2 Chemical Methods

Chemical methods focus on altering the chemical state of heavy metals to reduce their mobility and toxicity.

Chemical Precipitation: This involves adding specific chemicals to the soil to react with lead ions, forming insoluble compounds. This process reduces the solubility and mobility of lead, thereby stabilizing the pollutant. For instance, adding phosphates can lead to the formation of insoluble lead phosphate. Similarly, lime and red mud can decrease the bioavailability of lead to plants.

Chemical Extraction: In contrast, chemical extraction uses chemicals to dissolve lead from the soil, followed by its removal through processes like leaching [6].

2.3.3 Advantages and Disadvantages of Traditional Methods

These conventional methods offer the advantage of directly treating contaminated soil, which can, to some extent, reduce the bioavailability and environmental risk of lead. For example, chemical precipitation is relatively straightforward, easy to implement, and potentially less costly than complete soil removal. Chemical extraction can effectively remove lead from heavily contaminated areas.

However, traditional contamination management methods have significant drawbacks. Firstly, they are often costly and can lead to secondary environmental pollution [7]. For instance, improper handling during chemical extraction can contaminate groundwater. Secondly, these methods are frequently labor-intensive, demanding substantial human and material resources. Furthermore, stabilization methods only immobilize lead in the soil; they do not remove the contaminant, thus posing a long-term environmental risk. Chemical extraction, on the other hand, can alter the soil's physical and chemical properties, negatively impacting its fertility. Consequently, developing cost-effective, environmentally friendly, and sustainable technologies for soil lead pollution remediation is critically important.

3. Transgenic Plants and Advanced Gene-editing Techniques

3.1. Research Landscape of Plant-Based Pollution Control

Phytoremediation stands as a green, sustainable, and cost-effective plant-based technology gaining significant attention for remediating heavy metals in contaminated soil and water. Its effectiveness is influenced by several key factors, including the type and concentration of heavy metals, the specific phytoremediation method employed, climatic conditions, plant species, biomass production potential, plant sensitivity, and the efficiency of signaling pathways.

Notably, ornamental, medicinal, and aromatic plants demonstrate considerable potential for heavy metal remediation in soil and water [8]. Their rapid growth and high biomass, coupled with their existing medicinal, pharmaceutical, cosmetic, and decorative applications, make them attractive candidates. Moreover, the emergence of novel gene-editing technologies, such as CRISPR/Cas9, offers a new tool for sustainable phytoremediation. Precise editing of target genes can improve the heavy metal remediation capacity of certain ornamental plants, thus solving a common problem with genetically modified organisms (GMOs).

Current research in this field not only summarizes the protective mechanisms and molecular mechanisms of these plant species against heavy metals, but also increasingly focuses on using the latest advances in CRISPR/Cas9 genome editing to enhance their phytoremediation capacity.

In addition to mechanistic studies, phytoremediation research has expanded to integrated risk assessment and regulatory approaches. These studies consider the impacts of genetically modified plants released into the environment on environmental protection, livestock, and human health. Many studies have evaluated the remediation potential of specific plants (e.g., different genotypes of broad beans) for heavy metals such as lead (Pb) and cadmium (Cd). The ability of aquatic plants to remove Cd from water bodies has also been studied.

Crucially, the utility of modifying plant genes through genetic engineering can significantly enhance plant tolerance to pollutants, thereby increasing their overall phytoremediation potential.

3.2. Gene Editing Technology

Gene editing represents a powerful new approach to the site-specific modification of a genome. It is achieved by modifying gene sequences to cause different effects, such as gene knock-in, knock-out, up or down regulation. The basic mode of action is by provoking double-strand breaks (DSBs) in DNA at specific genomic sites and by utilizing the cell's own DNA repair mechanisms to trigger DSB repair. During this repair process, new base insertions or deletions can occur at the DSB sites, modulating target gene expression and facilitating gene-specific editing.

Early advancements in effective gene-editing technologies were notably achieved with zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs).

ZFNs are engineered DNA cleavage reagents comprising zinc finger proteins fused with FokI restriction endonucleases. They facilitate genome editing by creating DSBs in DNA, which are subsequently repaired by the cell's intrinsic repair mechanisms. Despite the considerable time and effort required for designing and constructing highly specific ZFNs, they were pivotal as early gene-editing tools. For example, ZFNs successfully induced targeted mutagenesis and gene targeting in Arabidopsis thaliana, demonstrating high frequencies of both processes.

TALENs emerged to overcome some technical limitations of ZFNs. Their editing effect is mediated by TALE proteins that specifically bind to a DNA sequence, coupled with the FokI nuclease, which acts as a molecular scissor to cut DNA. Naturally, TALEs are proteins used by plant bacteria to regulate plant genes during infection. By understanding the "code" by which TALEs bind to plant DNA and activate genes, researchers can customize TALE protein fusions to target any desired DNA sequence.

The discovery of these tools significantly broadened the scope of gene editing, making it applicable to both lower and higher organisms. However, ZFNs faced limitations in targeting diverse gene sequences due to the restricted availability of zinc finger proteins. While TALENs offered easier design and improved specificity, they could exhibit cytotoxicity. CRISPR/Cas9 gene editing technology represents a new generation, surpassing ZFNs and TALENs in its advancements. The CRISPR/Cas9 system is structurally simpler, primarily composed of a CRISPR RNA (crRNA) that guides the system to the target and a Cas9 protein that performs the cleavage, enabling accurate and precise editing of targeted sites.

3.3. CRISPR/Cas9 System

The CRISPR/Cas9 technology originates from the adaptive immune system of bacteria, where its primary function is to recognize and degrade invading exogenous gene fragments. In plant gene-editing applications, the Cas9 nuclease is precisely guided to a specific genomic locus by a guide RNA (gRNA), which then triggers DSBs at that target location. The cell subsequently activates its own DNA repair mechanisms to mend these breaks, facilitating modifications such as gene knockouts, insertions, or replacements. It's noteworthy that innovative modifications of Cas proteins have led to the development of base editing and prime editing technologies, which do not rely on DSBs, allowing for more precise single-nucleotide substitutions.

As a cutting-edge gene-editing technology, CRISPR/Cas9 offers broad application prospects in the plant field, primarily in the following areas:

First, this technology has been employed for editing specific crops to improve their agronomic traits. For example, CRISPR/Cas9 has successfully decreased cadmium accumulation in rice grains by mutagenic disruption of the OsNramp5 gene, lying at the root of the development of low-cadmium rice cultivars. Furthermore, the Xa13 promoter has been manipulated resulting in Xa13 transgene - free rice that is resistant to rice leaf blight. These lines can then be screened to select out exogenous gene-free plants in subsequent generations, which offers a hopeful way for disease resistance breeding of crop plants [9].

In addition, CRISPR is essential for the improvement of phytoremediation, and for adaptation to the environment. It is conducive to finding genes that are related with the phytoremediation mechanism and that help plants obtain tolerance to heavy metals and other pollutants in GM environmental contamination. This strategy can increase the root system and immobilize large amounts of contaminants or directly improve the plant's phytoremediation ability. Furthermore, CRISPR technology can introduce tailor-made instruction sequences into the plant genome and help the plants to sense and react to abiotic stress signals in polluted soil, paving the way for environmental pollution control and plant upgrading.

Furthermore, researchers have ingeniously created BEs and PEs by reprogramming CRISPR systems to marry Cas9 nickase or deactivated Cas9 with functional domains including deaminase and reverse transcriptase. These new tools overcome the shortcomings associated with traditional CRISPR technology that is based on DSBs. They enable ultra-precise modifications, such as targeted single-nucleotide substitutions, and the insertion or deletion of small fragments, all while maintaining genomic integrity. In crop genetic improvement, base editing systems have demonstrated significant application value: for example, base substitutions with extended editing windows have been achieved in rice, wheat, and other crops using Cas9-adenosine deaminase fusion proteins; and precise base conversions have been accomplished in rice, wheat, and maize using Cas9-cytosine deaminase fusion systems. These technological innovations provide DSB-free and efficient solutions for fine manipulation of plant genomes, pushing gene editing towards more precise regulation.

Finally, the delivery methods for CRISPR/Cas9 components in plant gene editing have evolved into a robust technological system encompassing biological vectors, physical transformation, and DNA-free delivery. Agrobacterium-mediated transformation has become a commonly used method due to its ease of operation and wide applicability. Typical examples include floral dip transformation for mutation induction and gene targeting in Arabidopsis thaliana, and particle bombardment, direct DNA transformation, viral delivery systems, and Agrobacterium infiltration, all successfully applied in various plant species. Researchers now have a better understanding of the bacterial CRISPR defense system's mechanism against foreign gene invasion and have engineered it to simultaneously edit multiple genes. Furthermore, the application of CRISPR technology has been enhanced through structural modifications of the Cas9 protein [10].

The continuous development and refinement of gene-editing technology have demonstrated immense potential beyond the biomedical field, finding implementation in diverse areas including disease modeling, disease diagnosis, and gene therapy.

4. Application of Plant Gene Editing in Heavy Metal Contamination

4.1. Phytoremediation of Heavy Metal Contamination

The growth and development of plants cultivated on contaminated soils are often inhibited by the presence of heavy metals. However, certain heavy metal hyperaccumulating plants and transgenic plants expressing heavy metal resistance genes demonstrate the capacity to tolerate these stressors while simultaneously decontaminating the environment. This so-called phytoremediation involves the uptake of pollutants from soil or water by plants via their roots, which are then transported into above-ground tissues, including leaves and stems, where they become sequestered. The infected plant tissues may be harvested then disposed of in accordance with local regulations. This technique is especially useful for Pb remediation.

Phytoremediation types are of phytoimmobilization, phytoextraction, and phytovolatilization. In relation to heavy metal remediation:

• Phytoimmobilization refers to heavy-metal binding by plant root exudates, enzymes, and other materials that prevents the movement of these metals through the soil. In the end it can have a positive impact on the problem of polluted soil.

• Phytoextraction - uses hyperaccumulating plants to absorb and concentrate toxic elements, which are then removed from the soil in the latter stages by harvesting the plant biomass.

• Phytovolatilization which involves the plant transforming heavy metal in the soil to a volatile form and released into the atmosphere. Although this process can reduce the content of heavy metal in the soil, it may result in secondary air pollution that would require careful handling and control during operation.

A significant research area within heavy metal phytoremediation is the screening and application of heavy metal hyperaccumulating plants. The concept of plants accumulating heavy metals dates back to the 16th century, with continuous exploration by scientists globally. In 1983, American scientist Chaney and colleagues proposed the academic idea of using hyperaccumulating plants for heavy metal remediation, leading to the formalization of the phytoremediation concept. By the 1970s, the underlying academic theories gained widespread international recognition, prompting Chinese scientists to also embark on applied research tailored to national conditions. Currently, over 500 species of hyperaccumulating plants have been identified. Nickel hyperaccumulators are the most numerous, accounting for 80% of the total (Verbruggen et al., 2009). Common examples of hyperaccumulating plants include:

• Copper (Cu) hyperaccumulators: Elsholtzia splendens and Commelina communis.

• Arsenic (As) hyperaccumulators: Pteris vittata (Chinese brake fern) and Pteris cretica.

• Zinc (Zn) and Cadmium (Cd) hyperaccumulators: Sedum alfredii and Solanum nigrum (black nightshade).

• Lead (Pb) hyperaccumulators: Pteris vittata and Vetiveria zizanioides (vetiver grass) [11].

A key pathway in phytoremediation involves leveraging substances within plant root systems for effective pollution control. As mentioned, Chaney proposed the theory of using hyperaccumulating plants to clean heavy metals from soil in 1983. Currently, major remediation plants in use include maize, oilseed rape, and various weeds. While plant-based remediation technologies typically require a longer duration, often five years or more for a specific area, they offer strong remediation capabilities and the significant advantage of low cost.

4.2. Application of Gene Editing Technology

Gene engineering for enhanced phytoremediation involves introducing exogenous target genes, which are effective in remediating heavy metal-contaminated soil, into hyperaccumulating plants. These genes are expressed within the plant and participate in processes such as heavy metal absorption, transport, transformation, sequestration, complexation, and volatilization [40]. The introduction of these exogenous genes can significantly increase the plant's capacity for heavy metal extraction, thereby improving phytoremediation efficiency. Examples of such genes include those encoding metal chelators, metallothioneins (MTs), phytochelatins (PCs), and heavy metal transporters.

For instance, Thomas et al. demonstrated that introducing the yeast MT gene CUP1 into tobacco plants grown on copper (Cu)-rich sandy soil resulted in Cu accumulation in tobacco seedling leaves that was 2 to 3 times higher than in control groups. Genetic modification of heavy metal transporters can markedly enhance plant tolerance and accumulation of heavy metals. Nagata et al. engineered transgenic tobacco plants by co-expressing a mercury (Hg) transporter (MerT) and a polyphosphate kinase gene (polyP) that promotes Hg hyperaccumulation. The resulting plants showed significantly increased Hg absorption and accumulation. Genetic engineering is thus considered an effective approach to improve the tolerance and enrichment capabilities of hyperaccumulating plants for heavy metals, opening vast application prospects for phytoremediation and becoming one of the most promising directions for enhancing phytoremediation.

With the aid of high-throughput sequencing technologies such as whole-genome association analysis, transcriptomics, and proteomics, numerous genes and proteins involved in heavy metal absorption and tolerance have been identified. The main expression products include transporter proteins, ligands, metalloreductases, and metallothioneins, all of which play roles in the regulatory pathways of heavy metal absorption, transport, and detoxification in plants.

Currently, research focused on using genetic engineering to improve the ability of plants to remediate heavy metal-contaminated soil primarily targets metal transporter proteins. These include:

• Iron-regulated transporter protein (ZIP) family: Involved in heavy metal uptake from roots and transport to stems.

• Natural resistance-associated macrophage protein (NRAMP) family: Facilitates heavy metal transport across cell plasma membranes.

• Heavy metal adenosine triphosphatase (ATP)ase protein family and Cation Diffusion Facilitator (CDF) family: Proven to function in heavy metal absorption, transport, and detoxification processes. For example, in Solanum nigrum (black nightshade) grown in Cd-contaminated soil, the expression of NRAMP genes increases. This elevated NRAMP gene expression enhances S. nigrum's absorption and transport of Cd, and the expression levels of NRAMP genes in different parts of S. nigrum correlate with Cd accumulation in those parts. DITUSA et al. also found that arsenate can induce the expression of PvPht1 and PuPht3 genes, which encode arsenate transporters (Pht protein), in Pteris vittata (Chinese brake fern), thereby improving its as absorption capacity.

Since a plant's genotype fundamentally determines its efficiency in phytoremediating heavy metal-contaminated soil, coupled with the complexity of plant genomes and long plant breeding cycles, the screening and cultivation of heavy metal hyperaccumulating plants have progressed slowly. However, the recently developed CRISPR/Cas9 gene-editing technology allows for simultaneous targeted editing of multiple genes, such as those within the ZIP, NRAMP, and CDF protein families. This capability enables modification of their expression patterns or levels, thereby enhancing the plant's heavy metal absorption or tolerance [12]. For example, WANG et al. [13] used CRISPR/Cas9 to knock out the OsARM1 gene, a metal transporter protein gene in rice (Oryza sativa), which improved rice's tolerance to arsenic. Despite the enormous potential of CRISPR/Cas9 gene-editing technology, it still requires further validation due to a lack of detailed genomic information and uncertainties regarding its current applicability.

Other studies indicate that introducing genes responsible for regulating phytochelatins and metal ligands into plants can significantly improve their ability to remediate heavy metal-contaminated soil. The introduction of the metallothionein gene MT26 can enhance the lead (Pb) tolerance and accumulation capacity of Hirschfeldia incana (shortpod mustard). In oilseed rape (Brassica napus L.), the introduction of APS and SMT genes, which encode ATP sulfurylase and seleno-cysteine methyltransferase respectively, significantly increased selenium (Se) accumulation. Furthermore, transferring the phytochelatin synthase gene IpPCS1t from Ipomoea pes-caprae (beach morning glory) into Arabidopsis thaliana markedly improved the latter's cadmium (Cd) tolerance [14]. Nevertheless, we have limited knowledge regarding the genetic loci, in vivo function and transport mechanisms of most transporter proteins in plants. Thus, more in-depth studies are required to understand the roles and action mechanisms of these proteins. Although there is great promise for genetic engineering in order phytoremediation of heavy metal contaminated soil, it is important to be aware of challenges and risks.

5. Conclusion

Industrial activities pose a significant threat to our groundwater and soil resources, with lead contamination standing out as particularly detrimental to human health, especially concerning intellectual development. The pervasive nature and severe health impacts of excessive heavy metal concentrations, including lead, underscore the urgent need for heightened attention and proactive measures. Management strategies and technological solutions need to be in place to tackle this environmental menace. Currently, the treatment and remediation of pollution are mostly carried out using chemical, physical and biological methods. Both approaches have specific uses and interventions. Nevertheless, the conventional treatments have the advantage of employing direct possible treatment, but they tend to be expensive, a laborious process with a secondary pollution risk.

Therefore, a potential direction for green remediation in the future is to explore the mechanisms of absorption and transport of heavy metals in plants. Such knowledge will help to enable precise gene manipulation of plants by different engineering strategies. The goal is to design ideal plant varieties characterized by rapid growth, extensive root systems, enhanced heavy metal absorption capabilities, and superior heavy metal tolerance. Such "designer" plants, applied through advanced phytoremediation techniques, would offer an environmentally friendly, cost-effective, and sustainable solution to lead and other heavy metal contamination.

This integration of fundamental research into plant mechanisms with cutting-edge gene-editing technologies represents a significant step forward, providing a powerful new option for the future governance and remediation of heavy metal-contaminated soils.

References

[1] Tong Yali, Yue Tao, Gao Jiajia, Wang Kun, et al. Partitioning and emission characteristics of Hg, Cr, Pb, and as among air pollution control devices in chinese coal-fired industrial boilers. Energy & Fuels, 2020, 34(6): 7067-7075.

[2] Singh S, Parihar P, Singh R, et al. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Frontiers in plant science, 2016, 6: 1143.

[3] Wu, Budiman Zhu, Hao. Two-dimensional empirical mode decomposition of heavy metal spatial variation in agricultural soils, Southeast China. Environmental Science and Pollution Research, 2017, 24(9).

[4] Ghori N H, Ghori T, Hayat M Q, et al. Heavy metal stress and responses in plants. International journal of environmental science and technology, 2019, 16: 1807-1828.

[5] Gill S S, Khan N A, Tuteja N. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (Lepidium sativum L.). Plant Science, 2012, 182: 112-120.

[6] Jagetiya B, Kumar S. Phytoremediation of lead: a review. Lead in Plants and the Environment, 2020: 171-202.

[7] Rigoletto M, Calza P, Gaggero E, et al. Bioremediation methods for the recovery of lead-contaminated soils: a review. Applied Sciences, 2020, 10(10): 3528.

[8] Zhou W, Yang J, Zhang Y, et al. Current landscape of gene‐editing technology in biomedicine: Applications, advantages, challenges, and perspectives. MedComm, 2022, 3(3): e155.

[9] Nayeri S, Dehghanian Z, Lajayer B A, et al. CRISPR/Cas9-Mediated genetically edited ornamental and aromatic plants: A promising technology in phytoremediation of heavy metals. Journal of Cleaner Production, 2023, 428: 139512.

[10] Sandhya D, Jogam P, Allini V R, et al. The present and potential future methods for delivering CRISPR/Cas9 components in plants. Journal of Genetic Engineering and Biotechnology, 2020, 18(1): 25.

[11] Verbruggen N, Hermans C, Schat H. Molecular mechanisms of metal hyperaccumulation in plants. New phytologist, 2009, 181(4): 759-776.

[12] Schiml S, Fauser F, Puchta H. The CRISPR/C as system can be used as nuclease for in planta gene targeting and as paired nickases for directed mutagenesis in A rabidopsis resulting in heritable progeny. The Plant Journal, 2014, 80(6): 1139-1150.

[13] Rono J K, Zhang B Q, Liu X S, et al. Identification of novel rice (Oryza sativa) HPP and HIPP genes tolerant to heavy metal toxicity. Ecotoxicology and Environmental Safety, 2019, 175: 8-18.

[14] Su H, Zou T, Lin R, et al. Characterization of a phytochelatin synthase gene from Ipomoea pes-caprae involved in cadmium tolerance and accumulation in yeast and plants. Plant Physiology and Biochemistry, 2020, 155: 743-755.