PHYTOREMEDIATION FOR LEAD-CONTAMINATED SOIL

Luong Thi Thuy Van*, Hoang Thi Chanh, Cao Thi Phuong Thao

TNU - University of Education

ARTICLE INFO		ABSTRACT
Received:	08/4/2024	Lead-contaminated soil poses a serious concern for agricultural
Revised:	31/5/2024	production, food safety, and human health due to its detrimental effects and widespread accumulation in food chains. Phytoextraction, the
Published:	31/5/2024	extraction of pollutants using plants, is a technology that has been
KEYWORDS		researched and applied to extract or remove lead from polluted sites. This method is evaluated as an effective, environmentally friendly, and cost-effective technological solution. Based on a comprehensive review of
Phytoextraction		information and data spanning several years, this article aims to explore
Uptake		and analyze various strategies for overcoming the limitations of
Accumulation		conventional methods that rely on hyperaccumulating plants to uptake and
Lead		accumulate metals without intervening in the substrate or the plants themselves. These strategies include combining plant-assisted approaches
Genetic engineering		with chelates, integrating plant-assisted methods with microorganisms or mycorrhizal fungi, and merging plant-based technology with genetic engineering to shorten treatment time, increase absorption and accumulation of Pb in plant parts. Although many factors still need to be improved in terms of principles and treatment techniques, initial results have affirmed that this is a completely effective and feasible method for treating lead-contaminated areas, aligning with the trend of applying green technologies to environmental protection.

CÔNG NGHỆ XỬ LÝ ĐẤT Ô NHIỄM CHÌ BẰNG THỰC VẬT

Lương Thị Thúy Vân*, Hoàng Thị Chanh, Cao Thị Phương Thảo

Trường Đại học Sư phạm - ĐH Thái Nguyên

THÔNG TIN BÀI BÁO TÓM TẮT

Ngày nhận bài: 08/4/2024 Ngày hoàn thiện: 31/5/2024

Ngày đăng: 31/5/2024

TỪ KHÓA

Phytoextraction

Hấp thụ Tích tụ Chì

Công nghệ gene

Tách chiết chất ô nhiễm bằng thực vật (phytoextraction) là công nghệ đã và đang được quan tâm nghiên cứu, ứng dụng để chiết xuất hoặc loại bỏ Pb khỏi môi trường ô nhiễm. Phương pháp này được đánh giá là một giải pháp công nghệ hiệu quả, thân thiện với môi trường và chi phí thấp. Dựa trên các thông tin và số liệu đã được công bố trong nhiều năm, bài viết này nhằm tổng hợp và phân tích các cách tiếp cận khác nhau nhằm giảm thiểu những hạn chế của phương pháp tách chiết thực vật tự nhiên (không có sự can thiệp vào chất nền cũng như công cụ xử lý) như tách chiết thực vật kết hợp với chelate; tách chiết thực vật kết hợp với vi khuẩn hoặc nấm rễ; tách chiết thực vật kết hợp với chelate; tách chiết thực vật kết hợp với vi khuẩn hoặc nấm rễ; tách chiết thực vật kết hợp với công nghệ gen nhằm rút ngắn thời gian xử lý, tăng cường hấp thụ và tích lũy Pb trong các bộ phận của cây. Mặc dù còn nhiều yếu tố cần hoàn thiện về mặt nguyên lý và kĩ thuật xử lý nhưng những kết quả bước đầu đã khẳng định đây là phương pháp hoàn toàn hiệu quả, phù hợp và khả thi để xử lý những vùng đất ô nhiễm chì theo xu hướng ứng dụng công nghệ xanh bảo vệ môi trường.

229(06): 247 - 258

DOI: https://doi.org/10.34238/tnu-jst.10060

http://jst.tnu.edu.vn 247 Email: jst@tnu.edu.vn

^{*} Corresponding author. Email: luongvandhsptn@gmail.com

1. Introduction

The current state of heavy metal pollution in soil is regarded as a major ecological threat to the planet. Due to its great potential for toxicity, lead (Pb) is the second most dangerous heavy metal on the ATSDR's (The Agency for Toxic Substances and Disease Registry) list of priority toxic chemicals [1]. Small amounts of Pb are released into the environment through natural processes, including rock weathering, volcanic activity, and radioactive decay. Anthropogenic Pb emissions from mining, smelting, and metal processing activities, as well as Pb-containing product usage and recycling, have significantly increased the Pb content released into the environment. This poses a major risk to ecosystems and public health [2]. Therefore, it is essential to implement sustainable and effective management strategies for polluted areas.

Lead is harmful to animals, plants, and microbes in the environment, but its effects are mostly confined to places that are heavily contaminated [3]. Several ways lead can enter the body, such as through food, water, direct inhalation of Pb-containing dust in the air, etc. [4]. Numerous negative consequences on bodily functions, including the neurological system, blood, kidneys, and reproductive organs, have been linked to lead exposure. Many physiological dysfunctions are brought on by lead, including decreased IQ in youngsters, memory loss, peripheral neuropathy, anemia, decreased vitamin D metabolism, bone diseases, and renal dysfunction [5]. Globally, 600 mg.kg-1 is the suggested regulatory limit for lead in agricultural soil [6], whereas Vietnam's National Technical Regulation on Soil Quality (QCVN 03:2023/MONRE) sets a maximum Pb level of 200 mg/kg [7]. However, due to the non-biodegradable and non-thermal decomposition properties of Pb ions, they are extremely persistent in the environment. Even at low quantities in soil and water, Pb can upset the delicate balance of the ecosystem. Due to this feature, lead (Pb) builds up in organisms and is subsequently taken up directly via food chains, posing a major health risk to humans [8], [9].

Surface complexed, exchangeable, dissolved, and precipitated forms of lead can all be found in soil. Because lead is soluble and mobile, its chemical form determines how much lead is present in soil [10], [11]. Certain research indicates that plants' uptake of lead is more closely associated with free lead in soil porewater than with other types of lead [12], [13]. Most of the dissolved Pb species that reach the soil from different pollution sources are absorbed by colloidal particles in the soil, such as clay minerals, Fe and Al oxides/hydroxides, and organic materials [14], [15]. Among the routes by which lead is adsorbed include adsorption on the surface-active sites of mineral and organic colloids, precipitation processes, surface co-precipitation, and diffusion into particle pores [16]. Pb is frequently more highly adsorbed in soil components along with Cu than other heavy elements like Cd, Ni, and Zn, meaning that plants may only use a tiny amount of the absorbed Pb [17].

In an effort to purge contaminated ecosystems, a variety of biological, physical, and chemical techniques have been used to detoxify heavy metals [18]. However, because of their high cost, potential to alter soil qualities, and harm to soil microbes, chemical and physical procedures are frequently less common than biological methods. It is important that products derived from such methods may lead to secondary contamination. Because of their high removal efficiency of heavy metals and minimal adverse effects on the environment, biological methods of treating heavy metal contamination are therefore regarded as a green, economical, and environmentally friendly alternative. Among these methods is phytoremediation. This method relies on plants' capacity to reduce or neutralise the negative impacts of pollutants [19], [20]. By directly employing plants and related soil microbes in an on-site treatment process, phytoremediation technology essentially refers to green cleaning technology that eliminates, detoxifies, and immobilises both organic and inorganic substances in the environment (including soil, sediment, mud, surface water and groundwater, etc.). This technique encompasses fundamental procedures including the extraction of pollutants by plants (phytoextraction or phytoaccumulation), the immobilization of pollutants by plants (phytostabilization), and the volatilization of pollutants by plants (phytostabilization), and the

In recent years, the trend of choosing phytoremediation technology has been increasing, in which the application of phytoextraction technology to soil contaminated with heavy metals has had demonstrated compelling evidence about the effectiveness and sustainability of the treatment procedures [23] - [26]. Since there is a dearth of general information regarding treatment strategies that target particular metal objects, this study focuses on reviewing the literature, synthesising, and evaluating Pb-contaminated soil treatment strategies using "hyperaccumulator" plants. These strategies range from conventional approaches to integrating genetic technology to boost the effectiveness of treatment. By offering this source of information, the researchers intend to help readers conduct more research in this area.

2. Research methods

The researchers have gathered, synthesised, arranged, and categorised the material on the subject of phytoremediation, examined the material on the phytoextraction, and chosen facts and figures that provide validity on the procedures and approaches for treating lead-contaminated soil before analysing and assessing how to proceed as well as the benefits and drawbacks of each processing method.

3. Results and discussion

3.1. Natural phytoextraction method

Phytoextraction technology is the most widely used biotechnology option for treating heavy metal-contaminated soil nowadays. Through their roots, "hyperaccumulator" plants draw metals from the soil, water, or sediment, which is subsequently transferred and collected in the above-ground parts of the tree. With this method, heavy metals are eliminated by plants in their natural habitat - that is, without causing harm to the substrate. The following processes allow for the phytoextraction of metals from the substrate: (1) absorption of the metal fraction at the root surface; (2) transfer of the bioavailable metal fraction across the cell membrane into root cells; (3) immobilisation of the absorbed metal in the vacuole; (4) intracellular mobile metal that penetrates the cell membrane into the root vascular tissue; and (5) metal transfer from roots to aboveground tissues (leaves and stems) [23], [27]. By means of composting, drying, thermal breakdown, compression, and other processes, post-harvest biomass containing metals is processed to lower its weight and volume [23]. When it is economically advantageous to do so, the metal-containing materials - such as trace elements - are repurposed or handled as hazardous wastes [24].

Selected plant species need to have some necessary features, such as high levels of the bioaccumulation factor (BF1) and transport factor (TF2); strong resistance to heavy metals; large biomass and rapid growth; robust root system; good assimilation rate; and ease of harvesting [28]. Scientists have found species of 45 plant groups that "hyperaccumulate" heavy metals to date. The families with the greatest number of "super-accumulator" species are the Brassicaceae family, Fabaceae family, Euphorbiaceae family, Asteraceae family, and Lamiaceae family [29]. For most plant species with high Pb accumulation capacity, the majority of Pb is absorbed (about 95% or more) and accumulates in the roots, with only a small portion being translocated to aboveground parts. Some representative species have been recognized to have the ability to accumulate Pb according to this rule such as Avicennia marina, Phaseolus vulgaris, Pisum sativum, Vicia faba, etc. Black beans (Vigna unguiculata), Lathyrus sativus, Tobacco (Nicotiana tabacum) and Maize (Zea mays); while Chinese Cabbage (Brassica pekinensis) and some species of Geranium (Pelargonium sp.) have a higher ability to absorb and accumulate Pb in aerial parts of plant than in the roots without affecting the exchange functions of the tree [30]. Many effective studies on "hyperaccumulator" plants have been conducted based on a thorough understanding of plant physiological systems linked to Pb absorption, transport, accumulation, and detoxification (Table 1).

¹BF= Heavy metal concentration in plants/Heavy metal concentration in soil

²TF = Heavy metal concentration in aerial parts of plant/Heavy metal concentration in roots

Table 1. Phytoremediating plant species for hyperaccumulation of lead under soil conditions

No	Plants	Experimental conditions	Locations	Results (mgPb.kg ⁻¹ DM)	Refer -ences
1.	Fragrant Eupatorium Herb (Chromolaena odorata)	Contaminated soil at the scene: 118,967 mgPb.kg ⁻¹ and 99,545 mgPb.kg ⁻¹	Bo Ngam Pb mine, Thailand	Accumulate 3,520 in shoots, 9,870 in roots; 3,730 and 6,698 in shoots and roots	[31]
2.	Davidii Bushclover Arabis paniculata Franch	Contaminated soil at the scene: 28,000 mgPb.kg ⁻¹	Lanping Pb/Zn mine, China	Accumulate 2,300 in shoots	[32]
3.	Sedum alfredii Hance	Contaminated soil at the scene: 3,525 mgPb.kg ⁻¹	Pb/Zn mine, China	Accumulate 1,182 in shoots	[33]
4.	Iris lactea var. chinensis	Plant in sandy soil with concentration from $0-10$ mmol.L ⁻¹	Nanjing, China	Accumulate 2,163 in shoots	[34]
5.	Pelargonium capitatum cultivar Atomic Snowflake	Contaminated soil at the scene: 39,250 mgPb.kg ⁻¹	SouthWestern, France	Accumulate 6,880 in shoots	[35]
6.	Polygala umbonata, Spermacoce mauritiana, Microstegium ciliatum, Dactyloctenium aegyptium, Pennisetum polystachyon	Contaminated soil at the scene: 175,500 mgPb.kg ⁻¹ ; 112,000 mgPb.kg ⁻¹ ; 104,860 mgPb.kg ⁻¹	Bo Ngam Pb mine, Thailand	Accumulate 21,670 in shoots, 14,580 in roots; 28,370 in shoots, 78,330 in roots; 12,200 in shoots, 128,830 in roots; 8,100 in shoots, 5,930 in roots; 6,205 in shoots, 24,705 in roots	[36]
7.	Brassicaceae <i>Thlaspi</i> praecox Wulf.	Contaminated soil at the scene: 67,940 mgPb.kg ⁻¹	Slovenia	Accumulate 3,500 in shoots	[37]

Source: Cited according to [38]

Plants used in these studies are mainly native, herbaceous plant species, capable of growing well in polluted, dry and nutrient-poor soil environments; have the ability to transport metals to aerial parts of plant and accumulate there in large concentrations. Even the accumulated Pb content in the shoots of two species *Polygala umbonata* and *Dactyloctenium aegyptium* is higher than in roots. However, grass species have a small biomass, so it requires a long treatment time and strict biomass management after each harvest to avoid metals returning to the environment to cause secondary pollution [38]. In Vietnam, on Pb and Zn mine (Hic Village, Tan Long Commune, Dong Hy district, Thai Nguyen), experimental models using Vetiver grass (Vetiveria zizanioides), Fern (Pteris vittata), and Betel grass (Eleusine indica L.) showed accumulation results in stems and roots for each species of 60 and 1600 mgPb.kg⁻¹, respectively; 760 and 5,000 mgPb.kg⁻¹; 450 and 3900 mgPb.kg⁻¹. Additionally, the process of using plants to treat Pb-contaminated soil is finished [39]. The use of these native plants is often of interest because they may thrive, multiply, and endure in contaminated areas more readily than plants brought in from other areas. Numerous studies have validated native plants' ability to mitigate contamination under field conditions [40], [41]. This method necessitates a lengthy treatment period because "hyperaccumulator" plants frequently have poor growth rates, shallow root systems, low biomass, and challenges with biomass management [25], so the solutions that promote metal accumulation in plants are one of the approaches that ensure the success of this environmentally friendly treatment technology.

3.2. Phytoextraction of Pb by plants combined with chelates

Although Pb is a common metal contaminant in soil, the mobility of Pb is poor due to the formation of insoluble precipitates. Consequently, the soil porewater contains a very small amount of the total Pb, which means that the concentrations of Pb transferred to plant shoots are relatively low. To increase the mobility of Pb in soil and the ability to absorb and transport Pb to the stems and leaves of "hyperaccumulator" plants, some artificial chelates have been added to the soil environment [26] such as EDTA (ethylenediaminetetraacetic acid), CDTA (trans-1,2-cyclohexylene-dinitrilotetra acetic acid), DTPA (diethylenetrinitrilo-penta acetic acid), EGTA (ethylebis[oxyethylenetrinitrilo]- tetraacetic acid), HEDTA (hydroxyethyl-ethylene-dinitrilo-tri acetic acid), citric acid and malic acid.

Among selected chelates, EDTA combined with several plant growth regulators was found to be the most effective in solubilizing bound Pb in soil. EDTA treatment enhanced the Pb accumulation and extraction ability of Indian Mustard (Brassica juncea), significantly reducing agricultural soil pollution caused by using sewage sludge as fertilizer [42], EDTA also significantly increased Pb concentrations in the shoots and roots of Chromolaena odora compared to the formula using only fertilizer. Many pot experiments have also demonstrated the effectiveness of EDTA in enhancing Pb accumulation and extraction by plant species. For example, Maize (Zea mays) increased 27 times, Beans (Phaseolus vulgaris) increased 70 times, Chinese carnation (Dianthus chinensis) increased 15 times, Sinapis alba increased 48 times, Bok choy (Brassica rapa) increased 60 times, Cauliflower (Brassica oleracea) increased 105 times, Lespedeza chinensis increased 32 times, and Lespedeza davidii increased 47 times [43]. A study by Lin, et al. (2009) [44] found that treatment of EDTA at low concentrations under medium nutritional conditions also improves sunflower's capacity to collect lead (Pb). Additionally, it can be concluded that applying EDTA twice would be more beneficial than applying it once in poor soils. Treatment with 5 mmol kg⁻¹ EDTA led Geranium (*Pelargonium zonale*) to accumulate 2,291 mgkg⁻¹ Pb (3.4 times more) in an artificially polluted environment with a Pb concentration of 7000 mg.kg⁻¹ [45]. Further research also shown that the addition of EDTA enhanced the concentration of lead (15-24 times) in the root and shoot tissues of Vetiver grass (Vetiveria zizanioides) [46] as compared to the control. In addition, an analysis of the treatment effectiveness of EDTA in comparison to various chelates reveals that EDTA is superior to chloride in terms of dissolving lead from the soil matrix [47], superior to propylenediaminetetraacetic acid (PDTA) in terms of accumulating lead in green onions (Allium fisulosum) [48], and superior to ammonium nitrate and ammonium sulphate in terms of encouraging lead accumulation in Sweet sorghum [49].

Although EDTA increases the mobility and accessibility of metals to plants, it also poses risks to the environment due to its high concentration of soluble metals, which are easily leached in the soil and are therefore easily enter food chains in the ecosystem. Freitas et do Nascimento, 2009; Wang et al., 2009 [50], [51] proposed using natural aminopolycarboxylic acids such as EDDS and nitrilotriacetic acid (NTA) to replace EDTA. The application of both NTA and EDTA is highly effective in solubilizing Pb from soil, redistributing Pb in soil into soluble and organic forms. The results of applying 5 mmol NTA.kg⁻¹ were successful with Maize [50] and Indian Mustard [52]. Accordingly, depending on plant cell toxicity and metal absorption kinetics, NTA is thought to be applied at the most suitable moment to enhance soil, combining the ability to biodegrade with the capacity to chelate very quickly [53].

Small quantities of organic substances known as "plant hormones" can control a plant's physiological functions. Thus, in order to treat Pb-contaminated soil, plant hormones are also a promising substitute for EDTA. A study by Israr and Sahi, 2008 [54] revealed that when auxins such 100 mM indole-3-acetic acid (IAA) and 1-naphthaleneacetic acid (NAA) were present, a flowering plant in the legume family called *Sesbania drummondii* deposited Pb in shoots 6.5 and

4.2 times higher, respectively. Specifically, the Pb concentration in shoots increased by 13.5 and 12.5 times when IAA or NAA was supplemented with EDTA.

However, some researchers believe that EDTA application does not enhance, or even reduces, the absorption of heavy metals by plants [55], [56]. Shen et al.'s 2002 study [57] revealed that while EDTA decreased Pb absorption in barley, Canola (*Brassica napus*), and Oats (*Avena sativa*), it was more efficient in increasing Pb absorption in Cabbage shoots. This discrepancy could be the result of variables including the features of the plant species, the experimental setup, the concentration, and the ratio of EDTA to Pb concentration. On the other hand, Pb translocation from roots to aerial parts of plant is greatly increased by EDTA [58].

3.3. Phytoextraction of Pb by plants combined with rhizosphere microorganisms or mycorrhizal fungi

Plant-soil bacterial interactions could be crucial for plants to adapt to metal-contaminated environments and improve their capacity to extract metals from metal-contaminated soils [59] -[61]. By dissolving insoluble phosphate complexes in the soil with the help of organic acids released by bacteria, plants are able to absorb and utilise orthophosphate [30]. Bacterial species in the rhizosphere increase plant resistance to heavy metals [62] by generating substances that promote plant growth, like siderophores, IAA, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase [63]. Additionally, bacteria also have different defense strategies to fight heavy metal stress such as prevention, elimination and synthesis of metal binding proteins such as phytochelatins, metallothioneins, cysteines (gcgcpcgcg) (CP), and histidines (ghhphg)₂ [64]. Some endogenous microbes, such as Bacillus sp., Pseudomonas sp., and Achromobacter sp., can help plants phytoextract heavy metal pollutants from soil. Studies that compare the endogenous microorganism populations in hyperaccumulators like Sedum alfredii, Veterinarian (Pteris vittata), and Indian Mustard (Brassica juncea) reveal links between endogenous microorganisms in terms of their capacity to metabolise and tolerate metals [65]. Burkholderia sp., a heavy metal-resistant microorganism strain, dramatically raised the biomass of maize and tomato and raised the tissue's Pb concentrations from 38 to 192% [66]. In a different study, the inoculation of Maize with several atmospheric nitrogen-fixing microorganism resulted in higher plant growth, biomass, and Pb extraction than inoculation alone. Due to its capacity to produce IAA, siderophores, and 1-aminocyclopropane-1carboxylate deaminase, Bacillus edaphicus (NBT strain) isolated from the rhizosphere of Cotton (Gossypium hirsutum) incubated with Indian Mustard (B. Juncea) increased Pb absorption from 18% to 46% in treated soil Pb at concentrations of 400 and 800 mg.kg⁻¹ [67]. Using IAA, siderophore, ACC deaminase, or Pb solubilization in soil, P. fluorescens G10 and Microbacterium sp. G16 isolated from Canola plants cultivated in heavy metal polluted soils can infiltrate the rhizosphere soil and plant tissues of Canola plants to enhance plant growth and Pb uptake [68].

Because mycorrhizal fungi create non-toxic oxalates that dissolve or bioadsorb onto melaninlike polymers, they have a high ability to immobilise toxic metals and have the potential to improve the phytoremediation of heavy metal-contaminated soils [69], [70]. Many studies have used mycorrhizal fungi to enhance phytoremediation of Pb-contaminated soils as described in Table 2.

It is evident that using plant technology in conjunction with mycorrhizal fungi and bacteria to treat Pb-contaminated soil is successful. Nevertheless, the process's efficacy is dependent on a number of variables, including: 1-Heavy metal concentration and mobility; 2-Polluted soil environment characteristics (pH, oxygen content, redox potential, and nutrients); and 3-Soil environment temperature and humidity [77].

Table 2. Application of mycorrhizal fungi to improve the effectiveness of phytoremediation of lead-contaminated soil

No	Plants	Mycorrhizal fungi	Results	References
1.	Canola (Brassica napus)	Mucor sp. CBRF59	Raised Pb's mobility noticeably by up to 77%	[69]
2.	Clover plants (<i>Trifolium</i> repens L.)	Rhizophagus irregularis	Changed the distribution of Pb in the stem and roots.	[71]
3.		Acaulospora, Glomus, Rhizophagus	Increase the ability to transfer Pb from roots to aerial parts of plant.	[72]
4.	Wavyleaf sea lavender (Limonium sinuatum)	Glomus.mosseae, G. intraradices	Two to three times higher accumulation of Pb in the roots.	[73]
5.	Alpine rock-cress (Arabis alpine)	51 species	Significantly enhanced the growth of host plants under Pb stress and reduced the amount of Pb that accumulated in the shoots	
6.	Cucumber (Cucumis sativus)	Aureobasidium pullulans BSS6	Antioxidant activities (catalase, peroxidase, and reduced glutathione) were markedly increased and lipid peroxidation was inhibited	
7.	Suaeda salsa	Trichoderma asperellum	A 9–23% increase in plant height and a 5–13% increase in fresh weight; plant oxidative damage was mitigated, and the soil's Pb bioavailability dropped by 6–21%.	

3.4. Phytoextraction of Pb combined with genetic technology

Genetic engineering has the ability to improve plant phytoremediation efficiency in addition to chelation and rhizosphere engineering [78], [79]. This makes genetic engineering a potentially useful remediation technology [80]. Through the use of transgenic technology, genetic engineering applies the heavy metal accumulation ability of specific plant species and genotypes to absorb pollutants in proportions that frequently surpass naturally accumulated plant concentrations [81] - [83].

The capacity of plants to absorb, bind, and sequester metals in cells in a complex network is the basis for their ability to accumulate certain metal ions, which is controlled by several genes [84]. Plant cells have a variety of cation transporters on their membranes that can allow toxic metals to enter. Pb is an extremely poisonous metal that is not necessary for plant sustenance, hence plant cells lack particular Pb transporters. Arazi *et al.* [84] found a series of membrane protein channels (NtCBP4, *Nicotiana tabacum* - calmodulin binding protein) in Tobacco (*Nicotiana tabacum*) that can transport Pb ions across the plasma membrane into plant cells; this protein is similar to the mammalian nucleotide-nonselective cation channel protein. The study showed that NtCBP4 can improve the ability of Tobacco to accumulate Pb, and Pb can be transported across the membrane by calcium channels. Thanks to this, NtCBP4 is considered the first demonstration of a plant protein that can regulate Pb tolerance and accumulation.

According to Song *et al.* [85], overexpression of the yeast protein YCF1 in Arabidopsis was responsible for the first instance of effective vacuolar compartmentalization of Pb in shoots. Because YCF1 is functionally active, plants have increased resistance and accumulate significant levels of Pb²⁺ along with glutathione from the cytoplasm into the vacuole. Through genetic transformation mediated by Agrobacteria, the high biomass plant species YCF1 was introduced into Indian Mustard (*B. juncea*). Pb concentration in YCF1 transgenic plants could be 1.4 to 1.6 times higher than in wild-type plants, and Pb accumulation through the transgenic line was 1.7 to 2.2 times higher than in wild-type plants [86]. In another study, overexpression of the gene encoding phytochelatin synthase (TaPCS1) of Wheat (*Triticum aestivum*) in Bush Tobacco (*Nicotiana glauca* R. Graham) significantly increased the plant's tolerance to Pb; the seedling's

roots are 160% longer than those of the wild-type plant. Transgenic plant seedlings grown in mine soil containing 1572 mg.kg⁻¹ Pb accumulated twice the Pb content compared to wild-type plants [87]. These potential research results have confirmed genetic technology as an effective tool in plant treatment of Pb-contaminated soil. But unlike transgenic crops meant for human or animal consumption, the use of transgenic crops to treat soil contaminated with heavy metals requires stringent management to keep these dangerous materials under control. Potential hazards include the potential for cross-pollination with native plant species, the potential for invasive species development, and the potential for poisons to infiltrate the ecosystem's food chain [79].

4. Conclusion

Numerous phytoextraction methods have been employed to reduce lead contamination in soils. This approach has shown to be an excellent means to restore the ecology of contaminated land regions in a straightforward, cost-effective, and environmentally responsible manner. However, cleaning the metal with the naturally hyperaccumulating plant method takes months or even years. It is essential to use supporting solutions to reduce the length of treatment, such as chelates, endogenous microorganisms, and mycorrhizal fungi, to improve plant development and resistance, promote metal mobility in soil, and encourage the absorption and buildup of heavy metals in plant parts. To create transgenic plants that grow quickly and produce large amounts of biomass for the pollution treatment process, it is also necessary to continue researching the features of the processes of Pb absorption, translocation, sequestration, and detoxification in plants, as well as the identification and characterization of molecules, tolerance genes, and detoxification. Overall, although there are still some technical challenges to overcome, modern technologies will help make it possible on a large scale.

REFERENCES

- [1] ATSDR, CERCLA priority list of hazardous substances. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Sciences, Atlanta, 2019.
- [2] D. C. Adriano, "Lead," *in* Trace Elements in Terrestrial Environments, D. C. Adriano (Ed.), New York, NY: Springer, 2001, pp.349–410.
- [3] H. B. Bradl, Heavy Metals in the Environment, vol. 6, Elsevier Ltd., 2005, pp. 17-21.
- [4] I. Thornton, R. Rautiu, and S. Brush, LEAD the facts, London, UK: IC Consultants Ltd, 2001.
- [5] D. A. Gidlow, "Lead toxicity," Occupational Medicine, vol.54, pp. 76-81, 2004.
- [6] D. E. Salt, M. Blaylock, N. P. B. A. Kumar, V. Dushenkov, D. Ensley, I. Chet, and I. Raskin, "Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants," *Biotechnology*, vol. 13, pp. 468–474, 1995, doi: 10.1038/nbt0595-468.
- [7] Ministry of Natural Resources and Environment, QCVN 03:2023/BTNMT: National Technical Regulations on Soil Quality 3-6, December 12, 2023.
- [8] B. V. Tangahu, S. R. S. Abdullah, H. Basri, M. Idris, N. Anuar, and M. Mukhlisin, "A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation," *International Journal of Chemical Engineering*, vol. 31, pp.155–185, 2011, doi: 10.1155/2011/939161.
- [9] W. Sidhoum and Z. Fortas, "The beneficial role of indigenous arbuscular mycorrhizal fungi in phytoremediation of wetland plants and tolerance to metal stress," *Archives of Environmental Protection*, vol. 45, pp. 103–114, 2019, doi: 10.24425/aep.2019.125916.
- [10] M. Shahid, E. Pinelli, and C. Dumat, "Review of Pb availability and toxicity to plants in relation with metal speciation; role of synthetic and natural organic ligands," *Journal of Hazardous Materials*, vol. 219-220, pp. 1–12, 2012.
- [11] F. A. Vega, M. L. Andrade, and E. F. Covelo, "Influence of soil properties on the sorption and retention of cadmium, copper and lead, separately and together, by 20 soil horizons: Comparison of linear regression and tree regression analyses," *Journal of Hazardous Materials*, vol. 174, pp. 522–533, 2010.
- [12] E. V. Chapman, G. Dave, and J. D. Murimboh, "Bioavailability as a factor in risk assessment of metalcontaminated soil," *Water, Air and Soil Pollution*, vol. 223, pp. 2907–2922, 2012.
- [13] G. Daldoul, R. Souissi, H. Tlil, D. Elbahri, O. El Hamiani, N. Chebbi, *et al.*, "Assessment of heavy metal toxicity in soils contaminated by a former Pb–Zn mine and tailings management using flotation process, Jebel Ghozlane, Northern Tunisia," *Environmental Earth Sciences*, vol. 78, 2019, Art. no. 703.

- [14]I. Anastopoulos, I. Massas, E. Pogka, I. Chatzipavlidis and C. Ehaliotis, "Organic materials may greatly enhance Ni and Pb progressive immobilization into the oxidisable soil fraction, acting as providers of sorption sites and microbial substrates," *Geoderma*, vol. 353, pp. 482–492, 2019.
- [15] Z. Michálková, M. Komárek, H. Šillerová, L. Della Puppa, E. Joussein, F. Bordas, et al., "Evaluating the potential of three Fe- and Mn-(nano)oxides for the stabilization of Cd, Cu and Pb in contaminated soils," *Journal of Environmental Management*, vol. 146, pp. 226–234, 2014.
- [16] D. L. Sparks, Environmental soil chemistry, 2nd Ed., London, UK: Academic Press, 2003.
- [17] P. Punamiya, R. Datta, D. Sarkar, S. Barber, M. Patel, and P. Das "Symbiotic role of *Glomus mosseae* in phytoextraction of lead in Vetiver grass (*Chrysopogon zizanioides* L.)," *Journal of Hazardous Materials*, vol. 177, pp. 465–474, 2010.
- [18] K. T. Lim, M. Y. Shukor, and H. Wasoh, "Physical, chemical, and biological methods for the removal of arsenic compounds," *BioMed Research International*, vol. 2014, pp. 1-9, 2014, doi: 10.1155/2014/503784.
- [19] D. E. Salt, R. Smith, and I. Raskin, "Phytoremediation," Annual Review of Plant Biology, vol. 49, no. 1, pp. 643–668, 1998.
- [20] V. C. Pandey, D. N. Pandey, and N. Singh, "Sustainable phytoremediation based on naturally colonizing and economically valuable plants," *Journal of Cleaner Production*, vol. 86, pp. 37–39, 2015.
- [21] H. Sarma, N. Forid, R. Prasad, M. N. V. Prasad, L. Q. Ma, and J. Rinklebe, "Enhancing phytoremediation of hazardous metal(loid)s using genome engineering CRISPR—Cas9 technology," *Journal of Hazardous Materials*, vol. 414, pp. 1-12, 2021, doi: 10.1016/j.jhazmat.2021.125493.
- [22] S. Sonowal, A. R. Nava, S. J. Joshi, S. N. Borah, N. F. Islam, S. Pandit, R. Prasad, and H. Sarma, "Biosurfactants assisted heavy metals phytoremediation: green technology for the United Nations sustainable development goals," *Pedosphere*, vol. 2, no. 1, pp. 198–210, 2022, doi: 10.1016/S1002-0160 (21)60067-X.
- [23] J. Suman, O. Uhlik, J. Viktorova, and T. Macek, "Phytoextraction of heavy metals: A promising tool for clean-up of polluted environment?" *Frontiers in Plant Science*, vol. 9, p. 1476, 2018.
- [24] R. A. Wuana and F. E. Okieimen, "Heavy Metals in Contaminated Soils: A review of sources, chemistry, risks and best available strategies for remediation," *ISRN Ecology*, vol. 2011, pp. 1–20, 2011, doi: 10.5402/2011/402647.
- [25] A. J. M. Baker, S. P. McGrath, C. M. D. Sidoli, and R. D. Reeves, "The posibility of insitu heavy metal decontamination of polluted soils using crops of metal-accumulating plants," *Resources Conservation Recycling*, vol. 11, pp. 41-49, 1994.
- [26] S. Tandy, R. Schulin, and B. Nowack, "The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers," *Chemosphere*, vol. 62, pp. 1454–1463, 2006.
- [27] B. E. Pivetz, *Phytoremediation of contaminated soil and groundwater at hazardous waste sites*, Washington: US Environmental Protection Agency, 2001.
- [28] A. J. M. Baker and R. R. Brooks, "Terrestrial higher plants which hyperaccumulate metallic elements a review of their distribution, ecology and phytochemistry," *Biorecovery*, vol. 1, pp. 81-126, 1989.
- [29] H. Sarma, "Metal hyperaccumulation in plants: a review focusing on phytoremediation technology," *Journal of Environmental Science and Technology*, vol. 4, no. 2, pp. 118-138, 2011.
- [30] R. Prasad (Editor), Phytoremediation for Environmental Sustainability, Springer, 2021, doi: 10.1007/978-981-16-5621-7.
- [31] P. Tanhan, P. Pokethitiyook, M. Kruatrachue, R. Chaiyarat, and S. Upatham, "Effects of soil amendments and EDTA on lead uptake by *Chromolaena odorata*: Greenhouse and field trial experiments," *International Journal of Phytoremediation*, vol. 13, pp. 897–911, 2011.
- [32] Y. T Tang, R. L. Qiu, X. W. Zeng, R. R. Ying, F. M. Yu, and X. Y. Zhou, "Lead, zinc, cadmium hyperaccumulation and growth stimulation in *Arabis paniculata* Franch," *Environmental and Experimental Botany*, vol. 66, pp. 126–134, 2009.
- [33] S. K. Tian, L. L. Lu, X. E. Yang, S. M. Webb, Y. H. Du, and P. H. Brown, "Spatial imaging and speciation of lead in the accumulator plant *Sedum alfredii* by microscopically focused synchrotron X-ray investigation," *Environmental Science & Technology*, vol. 44, pp. 5920–5926, 2010.
- [34] Y. L. Han, S. Z. Huang, J. G. Gu, S. Qiu, and J. M. Chen, "Tolerance and accumulation of lead by species of *Iris* L.," *Ecotoxicology*, vol. 17, no. 8, pp. 853–859, 2008.
- [35] M. Arshad, J. Silvestre, E. Pinelli, J. Kallerhoff, M. Kaemmerer, A. Tarigo, A. Shahid, M. Guiresse, P. Pradere, and C. Dumat, "A field study of lead phytoextraction by various scented pelargonium cultivars," *Chemosphere*, vol. 71, pp. 2187–2192, 2008.
- [36] P. Rotkittikhun, M. Kruatrachue, R. Chaiyarat, C. Ngernsansaruay, P. Pokethitiyook, A. Paijitprapaporn, and A. J. M. Baker, "Uptake and accumulation of lead by plants from the Bo Ngam lead mine area in Thailand," *Environmental Pollution*, vol. 144, pp. 681–688, 2006.

- [37] K. Vogel-Mikus, D. Drobne, and M. Regvar, "Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia," *Environmental Pollution*, vol. 133, pp. 233–242, 2005.
- [38] D. K. Gupta, H. G. Huang, and F. J. Corpas, "Lead tolerance in plants: strategies for phytoremediation," *Environmental Science Pollution Research*, vol. 20, pp. 2150–2161, 2013, doi: 10.1007/s11356-013-1485-4.
- [39] D. K. Dang, D. Le, V. T. Tran, T. K. A. Bui, and T. A. Dang, *Phytoremediation*, Agricultural Publisher Vietnam, 2011.
- [40] W. S. Shu, H. P. Xia, Z. Q. Zhang, C. Y. Lan, and M. H. Wong, "Use of Vetiver and three other grasses for revegetation of Pb/Zn mine tailings: Field experiment," *International Journal of Phytoremediation*, vol. 4, pp. 47–57, 2022.
- [41] O. P. Abioye, P. Agamuthu, and A. A. Abdul, "Phytotreatment of soil contaminated with used lubricating oil using Hibiscus cannabinus," *Biodegradation*, vol. 23, pp. 277–286, 2012.
- [42] H. Zaier, T. Ghnaya, A. Lakhdar, R. Baioui, R. Ghabriche, M. Mnasri, S. Sghair, S. Lutts, and C. Abdelly, "Comparative study of Pb phytoextraction potential in *Sesuvium portulacastrum* and *Brassica juncea*: Tolerance and accumulation," *Journal of Hazardous Materials*, vol. 183, pp. 609–615, 2010.
- [43] L. J. Zheng, X. M. Liu, U. Lutz-Meindl, and T. Peer, "Effects of lead and EDTA-assisted lead on biomass, lead uptake and mineral nutrients in *Lespedeza chinensis* and *Lespedeza davidii*," *Water, Air and Soil Pollution*, vol. 220, pp. 57–68, 2011.
- [44] C. C. Lin, J. Liu, L. Liu, T. C. Zhu, L. X. Sheng, and D. L. Wang, "Soil amendment application frequency contributes to phytoextraction of lead by sunflower at different nutrient levels," *Environmental and Experimental Botany*, vol. 65, pp. 410–416, 2009.
- [45] M. Hassan, M. Sighicelli, A. Lai, F. Colao, A. H. H. Ahmed, R. Fantoni, and M. A. Harith, "Studying the enhanced phytoremediation of lead contaminated soils via laser induced breakdown spectroscopy," *Spectrochimica Acta Part B*, vol. 63, pp. 1225–1229, 2008.
- [46] S. S. Andra, R. Datta, D. Sarkar, *et al.*, "Analysis of phytochelatin complexes in the lead tolerant vetiver grass *Vetiveria zizanioides* (L.) using liquid chromatography and mass pectrometry," *Environmental Pollution*, vol. 157, pp. 2173-2183, 2009.
- [47] M. Komárek, P. Tlustoã, J. Száková, *et al.*, "The use of poplar during a two-year induced phytoextraction of metals from contaminated agricultural soils," *Environmental Pollution*, vol. 151, pp. 27-38, 2008.
- [48] Y. Cho, J. A. Bolick, and D. J. Butcher, "Phytoremediation of lead with Green onions (*Allium fistulosum*) and uptake of arsenic compounds by moonlight Ferns (*Pteris cretica* cv. Mayii)," *Microchemical Journal*, vol. 91, pp. 6-8, 2009.
- [49] P. Zhuang, W. Shu, Z. Li, et al., "Removal of metals by sorghum plants from contaminated land," *Journal of Environmental Sciences*, vol. 21, pp. 1432-1437, 2009.
- [50] E. V. D. Freitas and C. W. A. do Nascimento, "The use of NTA for lead phytoextraction from soil from a battery recycling site," *Journal of Hazardous Materials*, vol. 171, pp. 833–837, 2009.
- [51] X. Wang, Y. Wang, Q. Mahmood, E. Islam, X. F. Jin, T. Q. Li, X. E. Yang, and D. Liu, "The effect of EDDS addition on the phytoextraction efficiency from Pb contaminated soil by *Sedum alfredii* Hance," *Journal of Hazardous Materials*, vol. 168, pp. 530–535, 2009.
- [52] S. K. Singh and S. A. Ramprakash, "Phytoextraction of lead from Pb contaminated soil by *Brassica juncea* as affected by chelating agents," *Environment and Ecology*, vol. 27, no. 2A, pp. 971-974, 2009.
- [53] E. Meers, F. M. G. Tack, S. Van Slycken, A. Ruttens, G. D. Laing, J. Vangronsveld, and M. G. Verloo, "Chemically assisted phytoextraction: a review of potential soil amendments for increasing plant uptake of heavy metals," *International Journal of Phytoremediation*, vol. 10, pp. 390–414, 2008.
- [54] M. Israr and S. V. Sahi, "Promising role of plant hormones in translocation of lead in *Sesbania drummondii* shoots," *Environmental Pollution*, vol. 153, pp. 29–36, 2008.
- [55] S. K. Tian, L. L. Lu, X. E. Yang, H. G. Huang, P. Brown, J. Labavitch, H. B. Liao, and Z. L. He, "The impact of EDTA on lead distribution and speciation in the accumulator *Sedum alfredii* by synchrotron Xray investigation," *Environmental Pollution*, vol. 159, pp. 782–788, 2011.
- [56] H. G. Huang, T. X. Li, S. K. Tian, D. K. Gupta, X. Z. Zhang, and X. E. Yang, "Role of EDTA in alleviating lead toxicity in accumulator species of *Sedum alfredii* H.," *Bioresource Technology*, vol. 99, pp. 6088–6096, 2008.
- [57] Z. G. Shen, X. D. Li, C. C. Wang, *et al.*, "Lead Phytoextraction from Contaminated Soil with High-Biomass Plant Species," *Journal of Environmental Quality*, vol. 3, pp.1893-1900, 2002.
- [58] G. D. L. Rosa, J. R. Peralta-Videa, G. Cruz-Jimenez, M. Duarte-Gardea, A. Martinez-Martinez, I. Cano-Aguilera, N. C. Sharma, S. V. Sahi, and J. L. Gardea-Torresdey, "Role of ethylenediaminetetraacetic acid on lead uptake and translocation by Tumbleweed (*Salsola kali L.*)," *Environmental Toxicology and Chemistry*, vol. 26, no. 5, pp. 1033–1039, 2009.

- [59] R. Prasad, S. C. Nayak, R. N. Kharwar, and N. K. Dubey, Mycoremediation and environmental sustainability, vol. 3, Springer, 2021.
- [60] V. Kumar, R. Prasad, and M. Kumar, Rhizobiont in bioremediation of hazardous waste, Springer, Singapore, 2021.
- [61] M. Jitchanok, N. S. Thomas, and P. Benjaphorn, "Bacterial-assisted phytoremediation of heavy metals: Concepts, current knowledge, and future directions," *Environmental Technology & Innovation*, vol. 33, pp. 1-15, 2024, doi: 10.1016/j.eti.2023.103488.
- [62] M. Miransari, "Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals," *Biotechnology Advances*, vol. 29, pp. 645–653, 2011.
- [63] P. Waranusantigul, H. Lee, M. Kruatrachue, P. Pokethitiyook, and C. Auesukaree, "Isolation and characterization of lead-tolerant *Ochrobactrum intermedium* and its role in enhancing lead accumulation by *Eucalyptus camaldulensis*," *Chemosphere*, vol. 85, pp. 584–590, 2011.
- [64] H. Sarma, N. Forid, R. Prasad, M. N. V. Prasad, L. Q. Ma, and J. Rinklebe, "Enhancing phytoremediation of hazardous metal(loid)s using genome engineering CRISPR—Cas9 technology," *Journal of Hazardous Materials*, vol. 414, pp. 1-12, 2021, doi: 10.1016/j.jhazmat.2021.125493.
- [65] W. He, M. Megharaj, C. Y. Wu, S. R. Subashchandrabose, and C. C. Dai, "Endophyteassisted phytoremediation: echanisms and current application strategies for soil mixed pollutants," *Critical Reviews in Biotechnology*, vol. 40, pp. 31–45, 2020, doi: 10.1080/07388551.2019.1675582.
- [66] C. Y. Jiang, X. F. Sheng, M. Qian, and Q. Y. Wang, "Isolation and characterization of a heavy metal-resistant Burkholderia sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil," *Chemosphere*, vol. 72, pp. 157–164, 2008.
- [67] X. F. Sheng, C. Y. Jiang, and L. Y. He, "Characterization of plant growth promoting *Bacillus edaphicus* NBT and its effect on lead uptake by Indian mustard in a lead-amended soil," *Canadian Journal of Microbiology*, vol. 54, no. 5, pp. 417–422, 2008, doi: 10.1139/w08-020.
- [68] X. F. Sheng, J. J. Xia, C. Y. Jiang, L. Y. He, and M. Qian, "Characterization of heavy metal-resistant endophytic bacteria from Rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of Rape," *Environmental Pollution*, vol. 156, pp. 1164–1170, 2008.
- [69] Z. Deng, L. Cao, H. Huang, X. Jiang, W. Wang, Y. Shi, and R. Zhang, "Characterization of Cd- and Pb-resistant fungal endophyte Mucor sp. CBRF59 isolated from Rapes (*Brassica chinensis*) in a metalcontaminated soil," *Journal of Hazardous Materials*, vol. 185, pp. 717–724, 2011.
- [70] Z. Deng, R. Zhang, Y. Shi, L. Hu, H. Tan, and L. Cao, "Enhancement of phytoremediation of Cd- and Pb-contaminated soils by self-fusion of protoplasts from endophytic fungus Mucor sp. CBRF59," *Chemosphere*, vol. 91, pp. 41–47, 2013.
- [71] E. Malekzadeh, N. Aliasgharzad, J. Majidi, J. Abdolalizadeh, and L. Aghebati-Maleki, "Contribution of glomalin to Pb sequestration by arbuscular mycorrhizal fungus in a sand culture system with clover plant," *European Journal of Soil Biology*, vol. 74, pp. 45–51, 2016, doi: 10.1016/j. ejsobi.2016.03.003.
- [72] J. Schneider, J. Bundschuh, and C. Nascimento, "Arbuscular mycorrhizal fungi-assisted phytoremediation of a lead-contaminated site," *Science of The Total Environment*, vol. 572, pp. 86–97, 2016, doi: 10.1016/j.scitotenv.2016.07.185.
- [73] M. Sheikh-Assadi, A. Khandam-Mirkohi, A. Alemardan, and E. Moreno-Jiménez, "Mycorrhizal *Limonium sinuatum* (L.) Mill. enhances accumulation of lead and cadmium," *International Journal of Phytoremediation*, vol. 17, pp. 556–562, 2015, doi: 10.1080/15226514.2014.922928.
- [74] V. K. Sharma, X. Y. Li, G. L. Wu, *et al.*, "Endophytic community of Pb-Zn hyperaccumulator Arabis alpina and its role in host plants metal tolerance," *Plant and Soil*, vol. 437, pp. 397–411, 2019, doi: 10.1007/s11104-019-03988-0.
- [75] A. Ali, S. Bilal, and A. L. Khan, "Endophytic *Aureobasidium pullulans* BSS6 assisted developments in phytoremediation potentials of *Cucumis sativus* under Cd and Pb stress," *Journal of Plant Interaction*, vol. 14, no. 1, pp. 303–313, 2019, doi: 10.1080/17429145.2019.1633428.
- [76] X. X. Li, X. Zhang, X. L. Wang, X. Y. Yang, and Z. J. Cui, "Bioaugmentation-assisted phytoremediation of lead and salinity co-contaminated soil by *Suaeda salsa* and *Trichoderma asperellum*," *Chemosphere*, vol. 224, pp. 716–725, 2019, doi: 10.1016/j.Chemosphere.2019.02.184.
- [77] R. Monica, C. Paola, G. Elisa, M. Mery, and F. Debora, "Bioremediation Methods for the Recovery of Lead-Contaminated Soils: A Review," *Appied Science*, vol.10, pp. 1-17, 2020, doi:10.3390/app10103528.
- [78] A. P. G. C. Marques, A. O. S. S. Rangel, and P. M. L. Castro, "Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology," *Critical Reviews in Environmental Science and Technology*, vol. 39, no. 8, pp. 622-654, 2009.
- [79] P. Kotrba, J. Najmanova, T. Macek, *et al.*, "Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution," *Biotechnology Advances*, vol. 27, pp. 799-810, 2009.

- [80] V. Hooda, "Phytoremediation of toxic metals from soil and wastewater," *Journal of Environmental Biology*, vol. 28, no. 2, pp. 367-376, 2007.
- [81] I. Raskin, R. D. Smith, and D. E. Salt, "Phytoremediation of metals: using plants to remove pollutants from the environment," *Current Opinion in Biotechnology*, vol. 8, pp. 221-226, 1997, doi: 10.1016/S0958-1669(97)80106-1.
- [82] R. B. Meagher, "Phytoremediation of toxic elemental and organic pollutants," *Current Opinion in Biotechnology*, vol. 3, pp. 153-162, 2000.
- [83] S. P. McGrath and F. J. Zhao, "Phytoextraction of metals and metalloids from contaminated soils," *Current Opinion in Biotechnology*, vol. 14, pp. 277-282, 2003.
- [84] T. Arazi, R. Sunkar, B. Kaplan, and H. Fromm, "A tobacco plasma membrane calmodulin-binding transporter confers Ni²⁺ tolerance and Pb²⁺ hypersensitivity in transgenic plants," *The Plant Journal*, vol. 20, pp. 171– 182, 1999.
- [85] W. Y. Song, E. J. Sohn, E. Martinoia, Y. J. Lee, Y. Y. Yang, M. Jasinski, C. Forestier, I. Hwang, and Y. Lee, "Engineering tolerance and accumulation of lead and cadmium in transgenic plants," *Nature Biotechnology*, vol. 21, pp. 914–919, 2003.
- [86] M. S. U. Bhuiyan, S. R. Min, W. J. Jeong, S. Sultana, K. S. Choi, W. Y. Song, Y. Lee, Y. P. Lim, and J. R. Liu, "Overexpression of a yeast cadmium factor 1 (YCF1) enhances heavy metal tolerance and accumulation in *Brassica juncea*," *Plant Cell, Tissue and Organ Culture*, vol. 105, pp. 85–91, 2011.
- [87] C. Gisbert, R. Ros, A. De Haro, D. J. Alker, M. P. Bernal, R. Serrano, and J. Navarro-Avino, "A plant genetically modified that accumulates Pb is especially promising for phytoremediation," *Biochemical and Biophysical Research Communications*, vol. 303, pp. 440–445, 2003.