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Review

Bioremediation of Heavy Metal-Contaminated Agricultural Soils: Mechanisms, Emerging Technologies, and Pathways to Field-Scale Application

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Abstract

Agricultural soils worldwide are facing escalating contamination by heavy metals, which present high risks for health due to their persistence, being non-biodegradable, accumulating across the soil profile, and being easily transferred into edible plant tissues, thus propagating through the food chain, with serious consequences for human health and ecosystem integrity. Conventional physical and chemical remediation approaches are costly, ecologically disruptive and operationally complex for the extent of contamination of agricultural land. Thus, there is an urgent need for sustainable and scalable alternatives. This review addresses the need by providing an integrated, mechanistically grounded synthesis of plant-based bioremediation strategies for heavy metal contamination removal, emphasizing the links between soil chemistry, plant physiology, and soil microbiology. First, the principal contamination pathways and controls on metal speciation and bioavailability are summarized, highlighting how parameters such as pH, organic matter, clay minerals, and redox conditions govern the metal fraction available for the plants. The molecular basis of plant heavy metal uptake, translocation and detoxification is examined in detail, including transporter-mediated root uptake, xylem loading and long-distance transport, and chelation by phytochelatins and metallothioneins. The performance and limitations of the main phytoremedation strategies are evaluated across representative hyperaccumulator species, then two major enhancement solutions are discussed: chemical enhancement using synthetic and biodegradable agents, and biological enhancement through plant growth-promoting rhizobacteria, arbuscular mycorrhizal fungi, and mycoremediation fungi. Integrating these perspectives, this review provides a critical assessment of when and how phytoremediation can offer a realistic and agronomically compatible route for managing heavy metal contamination in agricultural soils.

Keywords: heavy metals; soil contamination; bioremediation; phytoremediation; agricultural soils

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1. Introduction

Heavy metal contamination of agricultural soils has become the most widespread and persistent environmental crisis of the Anthropocene. A landmark study published in Science in April 2025, based on the analysis of almost 796,000 soil samples from 1493 regional

data sets, showed that 14 to 17% of global agricultural land is contaminated above the safety limits for at least one type of heavy metal, with more than one billion people living in high-risk areas [1]. The most widespread contaminant was cadmium, with a global spreading rate higher than 9%, followed by nickel (5.8%), chromium (3.2%), arsenic, cobalt, copper, and lead. Unlike organic pollutants, heavy metals are not biodegradable. Once they enter the soil matrix, they persist for decades or even centuries, sometimes accumulating in edible plant tissues and subsequently propagating through the food chain [1,2].

The origins of heavy metal soil contamination are both geogenic and anthropogenic. Natural sources include the weathering of rocks rich in metals, volcanic activity, and pedogenetic processes. On the other hand, human activities are the primary sources of pollution, especially when it comes to agricultural soils, through practices such as industrial smelting, mining, the overapplication of fertilizers and pesticides, sewage sludge application, and urban runoff [3–5]. In 2014, a survey conducted in China revealed that about 19% of farmland exceeded pollution limits [6,7]; similarly, a 2019 European Union survey showed that many of the analyzed soils surpassed the heavy metal safety thresholds [8]. South Asia is recognized as a global hotspot for heavy metal contamination of agricultural soils, particularly in countries such as India, Bangladesh, Pakistan, and Sri Lanka, due to wastewater irrigation, industrial emissions, and mining activities [1,9–11]. The issue extends to other regions worldwide, though the availability of data varies [12–14]. Agricultural soils are particularly vulnerable because they are actively managed for productivity. Practices such as tillage, irrigation, and organic matter inputs alter soil properties by modifying soil pH, redox potential, and microbial activity, which can mobilize previously stable metal fractions and increase their phytoavailability. The resulting transfer of toxic elements into cereal grains, vegetables, and root crops poses documented risks of kidney damage, neurological impairment, and carcinogenesis in the exposed human population [3–5,15].

Conventional remediation technologies, including excavation, landfilling, soil washing with chelating agents or electrokinetic treatments, offer rapid metal removal but have major limitations that restrict their feasibility for agricultural soils. Excavation and off-site disposal involve high costs; meanwhile, chemical washing agents not only exhibit variable removal rates depending on the amendment type and dose, but they also simultaneously strip macronutrients, disrupt microbial communities, and risk leaching heavy metals and chemical additives into the groundwater systems. Stabilization techniques based on using lime or phosphate amendments reduce the bioavailability of the metals but leave contaminants in situ, requiring long-term monitoring and leaving the area vulnerable to remobilization in case of acidification events [16–18]. In this framework, there is a huge interest in bioremediation and finding ecological and economically viable alternatives from both scientific and policy perspectives.

Bioremediation leverages the metabolic and structural capabilities of some living organisms, such as microorganisms and plants, to extract, immobilize, transform, or sequester heavy metals from contaminated soils through mechanisms such as biosorption, biotransformation, bioaccumulation, and biostabilization [15,17,19–21]. Phytoremediation exploits the accumulation capacities of some plant species; for example, *Thlaspi caerulescens*, *Arabidopsis helleri*, and *Salix* spp. can accumulate up to several grams of Cd and Zn per kilogram of aboveground biomass. Microbial remediation relies on the diverse enzymatic machinery of soil bacteria (e.g., *Pseudomonas*, *Bacillus*, *Streptomyces*) and fungi (e.g., *Aspergillus*, *Trichoderma*, *Glomus* spp.) to alter metal speciation and mobility. Compared to traditional physical and chemical methods, bioremediation is estimated to drastically reduce the costs and minimize secondary pollution, thereby restoring soil biological diversity and fertility over time [15,17,19–21].

This review synthesizes current, field-scalable plant and plant–microbe bioremediation strategies for heavy metal-contaminated agricultural soils. It explores the sources and impacts of heavy metals, the molecular mechanisms of plant uptake, and practical phytoremediation strategies. Furthermore, the review evaluates chemical enhancements—such as chelators and biochar—alongside biological enhancements like root-associated bacteria and fungi for improving soil health and remediation outcomes. These aspects are schematically represented in Figure 1. Overall, the ideas presented can be a starting point for moving promising case studies to field-scale applications for the bioremediation of heavy metal-contaminated agricultural soils, which is important for both food security and environmental health.

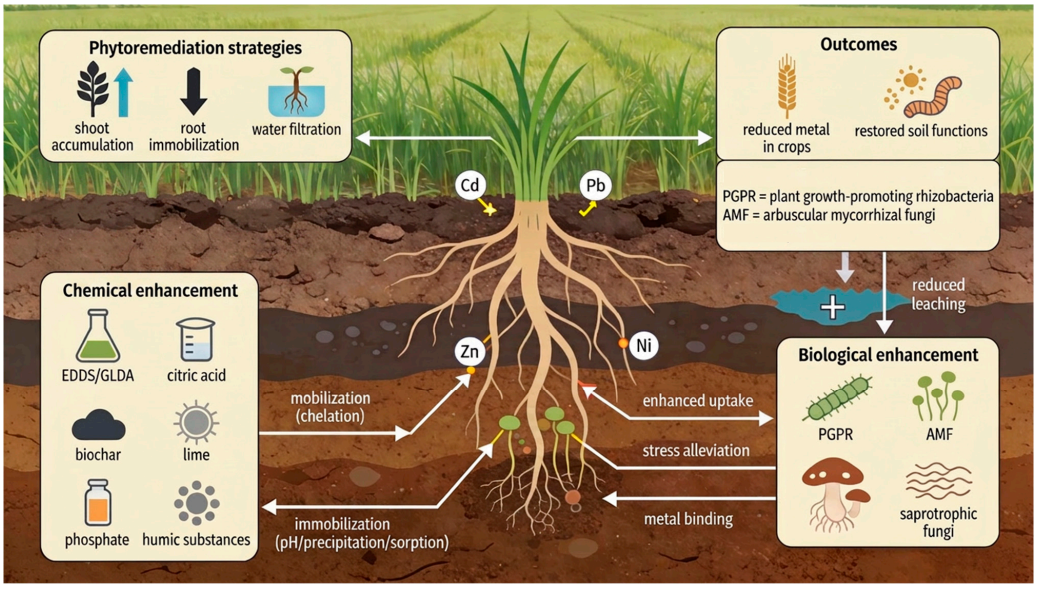

Figure 1. Schematic representation of heavy metal contamination of soils and remediation methods discussed in this article.

Considering all the aforementioned aspects, the present review provides a mechanistically integrated and agronomically focused synthesis of phytoremediation strategies for heavy metal-contaminated agricultural soils by bridging soil chemistry, plant physiological responses, and rhizosphere microbiology. Unlike previous reviews that address these components separately or focus predominantly on industrial contamination scenarios, this work critically evaluates the practical feasibility, enhancement strategies, and multiscale mechanisms governing phytoremediation efficiency in agricultural systems. This manuscript further advances the field through a comparative assessment of chemical and biological enhancement approaches and by linking metal bioavailability, molecular detoxification pathways, and field-level remediation performance within a unified conceptual framework.

2. Methodology

For this review, a systematic literature search was conducted within Web of Science, Scopus, and PubMed, complemented with ScienceDirect and Google Scholar for grey and emerging literature following PRISMA 2020 guidelines. Searches covered publications from 2000 to 2026 to capture both foundational mechanisms and emerging technologies for soil bioremediation. Search strategies were adapted slightly to the syntax of each database, and reference lists of key reviews on metal-contaminated soils and biological remediation were manually screened to identify additional studies. We combined keywords related to bioremediation as well as heavy metal contamination and agricultural soils: “bioremediation”, “phytoremediation”, “microbial remediation”, “heavy metal”, “Cd”,

"Pb", "Zn", "Cu", "Ni", "As", "Cr", "Hg", "agricultural soil", "cropland", "farmland". From the results, we included (i) peer reviews in English, (ii) studies on agricultural or arable soils contaminated with at least one heavy metal (Cd, Pb, Zn, Cu, Ni, As, Cr, Hg), (iii) bioremediation, phytoremediation, or green/biological technologies (e.g., microbes, plants, microbe–plant consortia, organic and nano-bio amendments), (iv) works that report at least one quantitative outcome (e.g., %removal, change in bioavailable fractions, plant uptake, yield, soil biological indicators). We excluded (i) purely chemical or physical remediation with no biological component, (ii) non-agricultural matrices, (iii) heavy metal contamination of water without soil, and (iv) editorials, conference abstracts without full data, and patents. Titles and abstracts were screened independently by two reviewers to remove clearly irrelevant records. Full texts of potentially eligible articles were then assessed against the inclusion criteria. Discrepancies were resolved by discussion or consultation with a third reviewer.

From each included study, we extracted soil type and setting (field, greenhouse, pot), contaminant metals and initial concentrations, biological agents (plant species, microbial taxa, consortia, amendments), mechanisms targeted (biosorption, bioaccumulation, biomineralization, redox transformation, phytostabilization, phytoextraction), experimental duration, outcomes, and any field-scale or pilot-scale implementation aspects.

The flow diagram of PRISMA analysis is presented in Figure 2. The database search yielded 1250 records, and an additional 50 records were identified through other sources. After removal of duplicates, 1000 records remained for screening. Titles and abstracts of these 1000 records were screened, and 820 were excluded as clearly irrelevant (e.g., wrong matrix, no bioremediation component, non-agricultural context). The full text of 180 articles was assessed for eligibility. Of these, 60 were excluded for the following reasons: not agricultural soils (n = 20), no biological or green remediation intervention (n = 15), no quantitative heavy metal outcomes (n = 15), or review-type papers used only for background and not for data synthesis (n = 10). In total, 120 studies met all inclusion criteria and were incorporated into the qualitative synthesis of mechanisms, emerging bioremediation technologies, and pathways to field-scale application.

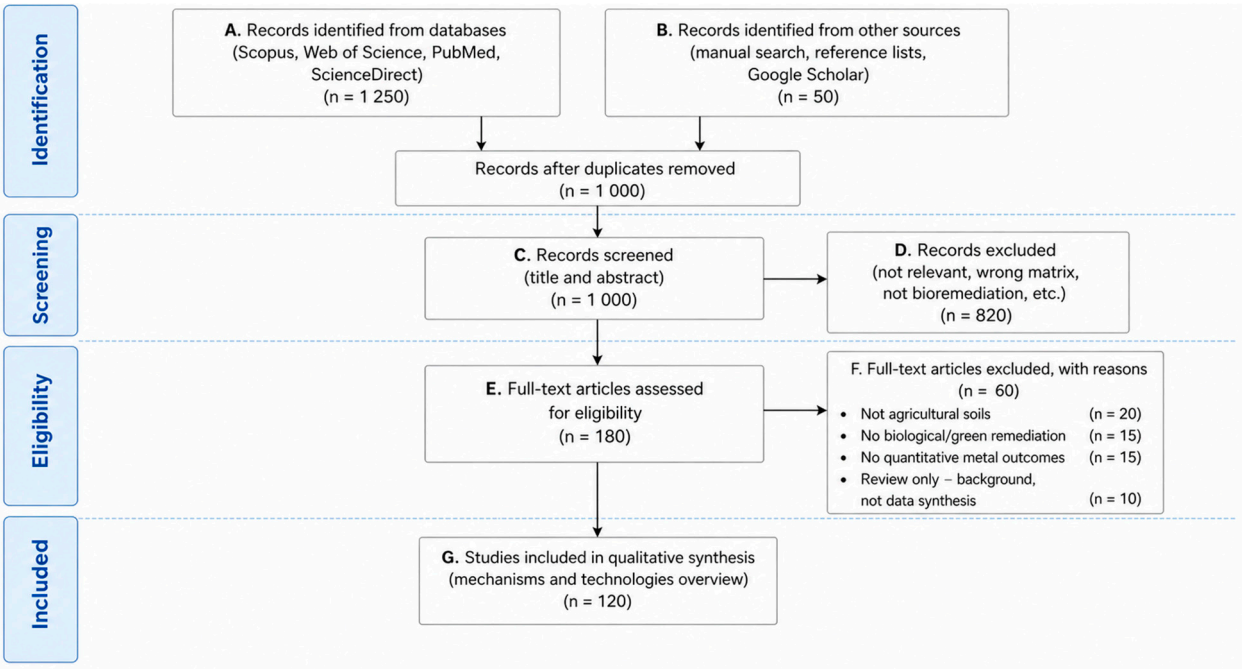

Figure 2. Prisma 2020 flow diagram for this study.

3. Heavy Metal Contamination of Agricultural Soils: Sources, Speciation, and Phytotoxicity

Before selecting or designing an appropriate remediation strategy, it is necessary to understand how the contamination of agricultural soils occurs, the chemical forms in which heavy metals are found, and how they interact with the ecosystem from microorganisms to plants, before entering the food chain. The term "heavy metal" is used throughout this review in its conventional sense, referring to metallic elements and metalloids with densities greater than 5 g cm^{-3} that exhibit toxic effects at elevated concentrations. We acknowledge that arsenic is formally a metalloid rather than a metal. For the purposes of risk assessment and remediation, our classification is therefore based on environmental persistence and toxicity rather than strictly on density-based or periodic-table criteria. In the context of agricultural soils, eight priority elements are considered to pose high risks due to their accumulation in crops and their potential impacts on human health: cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn).

The primary source of heavy metal contamination in agricultural soil is anthropogenic activity; industrial emissions, mining, and metallurgical operations emit metal-rich particulates and effluents that settle on the soil or infiltrate it via water systems [22]. Agricultural inputs, including phosphate fertilizers (Cd), pesticides (As), and sewage sludge, introduce these contaminants directly into the croplands. Furthermore, practices such as irrigation with contaminated water, as well as atmospheric deposition from traffic and industry, exacerbate the problem [23,24]. Because these elements, unlike organic pollutants, are not biodegradable, they persist in the environment and can only be transferred or sequestrated rather than destroyed.

Figure 3 illustrates the sources, behavior, and impacts of heavy metals in soil–plant systems. Once into the soil, heavy metal mobility and bioavailability are influenced by factors such as pH, organic matter content, and redox conditions. These parameters determine whether metals remain adsorbed, precipitate, or become available for plant uptake. The bioavailable fractions can then be absorbed by roots, leading to phytotoxic effects alongside broader ecological consequences, including reduced microbial activity, disrupted nutrient cycling, root damage, and inhibited plant growth. Ultimately, the accumulation of these metals in edible plant parts poses severe risks to food safety.

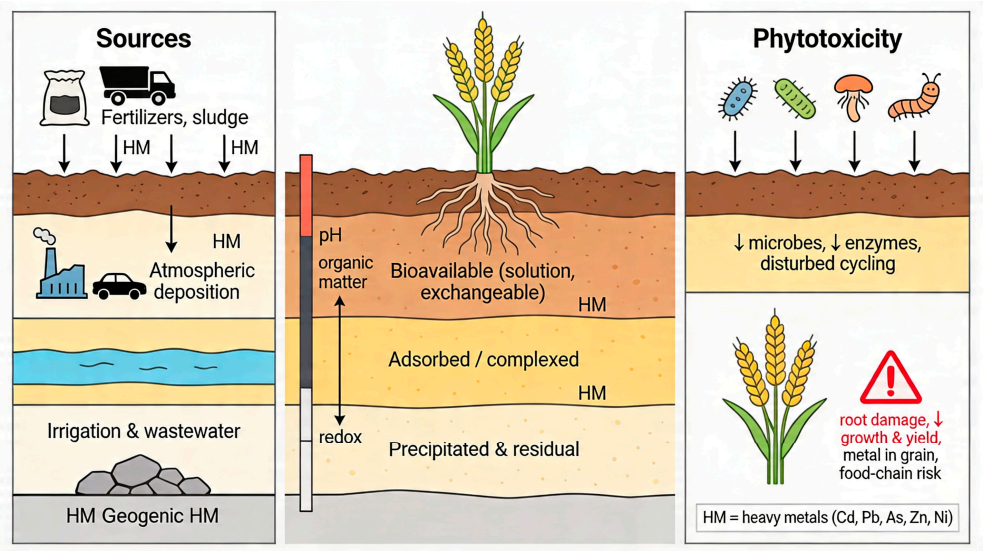

Figure 3. Schematic representation of sources, speciation, and phytotoxicity of heavy metals in agricultural soils.

3.1. Sources of Contamination

Heavy metals occur naturally in soil; their background concentrations vary by location due to local geology. These geogenic concentrations set the "baseline" against which the anthropogenic contamination should be assessed [24]. Geogenic metals are widely reported to occur in soluble crystalline mineral form, such as silicates, oxides, and sulfides, exhibiting low solubility and limited bioavailability under normal soil conditions [25]. This low solubility presents a significant complication for remediation. In contrast, metals from anthropogenic sources often occur in more soluble and labile fractions. A study of the soils in the area of a former mine found high contamination factors doe Cu, Pb, Zn, and Cd, with high bioavailable fractions for these contaminants, the content exceeding locally regulated threshold values [26]. This distinction between the total metal concentration and bioavailable metal concentration is a critical conceptual tool in soil remediation, which will be detailed in Section 3.2.

In some areas globally, elevated heavy metal concentrations exceed anthropogenic inputs, a fact with direct regulatory implications: the geogenic contamination is generally not amenable to source-control measures and may set permanent background concentrations that cannot be reduced below regulatory thresholds using any remediation technique [1]. Nevertheless, the primary source of agricultural soil contamination remains anthropogenic inputs, which operate through several distinct pathways [23].

Extraction and metal processing activities are the most spatially concentrated and well-documented sources of contamination for agricultural soils. Smelter emissions deposit lead, cadmium, zinc, copper, and arsenic in a declining gradient downwind of the processing site, sometimes extending several kilometers away into agricultural lands. The analysis published in *Science* used structural equation modeling to verify that mining and smelting processes are among the principal anthropogenic contributions to high levels of Cd, Pb, and As in the vicinity of industrial regions [1,24].

Phosphate fertilizers are a widely distributed anthropogenic source of cadmium contamination. Cadmium is a natural co-contaminant in phosphate rocks, the raw material used to manufacture synthetic phosphate fertilizers, with concentrations ranging from 1 to 90 mg Cd per kg P_2O_5 depending on the geographic extraction region [27]. Since phosphate fertilizers have been applied to nearly all intensively cultivated soils worldwide over the past century, accumulated cadmium additions are significant, even when the annual input rates are low. The EU addressed this through Regulation (EU) 2019/1009, which limits Cd content in EU-marketed phosphate fertilizers to 60 mg per kg P_2O_5. However, this threshold is contested by some of the member states led by France, which recommends a limit of 20 mg Cd per kg of fertilizer. Modeling of European soil cadmium balances suggests that, at the current average fertilizer concentration, leaching of Cd from the topsoil likely exceeds fertilizer inputs in most EU soils.

The application of sewage sludge is considered an economically attractive disposal pathway that recycles plant nutrients such as N, P, K, and organic matter back to agricultural soils, but it simultaneously imports heavy metals. EU Directive 86/278/EEC sets regulatory limits for seven heavy metals (Cd, Cr, Pb, Ni, Cu, Hg, and Zn) in both sludge and receiving soil, while the United States Environmental Protection Agency (UESPA) regulations cap nine metals in biosolids used for agricultural application. Other organizations, such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), have also defined strict regulatory thresholds and international standards. These guidelines set maximum allowable heavy metal concentrations for both agricultural soils and edible plant tissues, providing a critical framework for characterizing human health risks and ecosystems impacts. Despite these regulations, long-term application has shown that Zn, Cr, Pb, and Cd can accumulate beyond maximum permissible limits in treated topsoils,

with Cd and Pb detected at elevated concentrations down to 40–50 cm depth in some sites [28–30]. The toxicity of the metals in sewage sludge is partially mitigated by their strong association with the organic matter; however, as the organic fraction decomposes over time, sequestered metals can be released into more bioavailable fractions [28,29].

Arsenic-based pesticides, such as Paris green and lead arsenate, have left persistent As and Pb contamination primarily in orchards and market garden soils, residues that remain long after the use of these products was banned [31]. Similarly, copper-based fungicides (such as Bordeaux mixture) have enriched vineyard soils with Cu throughout Europe and South America, reaching concentrations several times higher than natural background values; studies in European vineyards consistently documented Cu levels at 50–500 mg kg^{-1} in the topsoil, far exceeding the EU soil regulatory thresholds of 100–200 mg Cu kg^{-1}, depending on the land use [32–35].

Industrial emissions of Pb, Cd, Hg, As, and Zn from coal combustion, cement production, and metal smelting deposit metals onto the agricultural land via atmospheric transport. This pathway is particularly significant for mercury, which undergoes global atmospheric transport, and for lead, whose concentrations in agricultural soils near major roads were significantly elevated during the era of leaded gasoline [23]. A 10-year comparative study of suburban agricultural soils in Chengdu, China, found that the dominant source signature for soil Pb shifted from industrial emissions to transportation between 2008 and 2017, reflecting the region's transition from heavy industry to urbanization and traffic as the primary drivers of Pb in peri-urban zones [36].

Finally, in water-scarce agricultural regions within South and Southeast Asia, parts of Sub-Saharan Africa, and Latin America, untreated or inadequately treated municipal wastewater is usually used for irrigation. This practice delivers a continuous stream of Cr, Pb, Cd, Zn, Cu, and Ni directly into crop-growing soils. Consequently, vegetables grown in sewage-irrigated peri-urban fields in countries like India and China consistently exhibit elevated levels of Cd, Cr, Pb, and Zn in their edible tissues [9,11,37].

The relative importance of heavy metal contamination sources in agricultural systems varies substantially with land use, management intensity, and proximity to anthropogenic emission sources. A critical distinction must be made between diffuse chronic contamination, which gradually affects large agricultural areas, and point-source contamination, which produces severe but spatially localized impacts.

In intensively cultivated arable systems, phosphate fertilizers represent one of the most significant long-term diffuse sources of cadmium contamination because of their universal and repeated application. Unlike industrial contamination, which is geographically restricted, phosphate-derived Cd affects nearly all fertilized cropland. Compared with sewage sludge, phosphate fertilizers introduce a narrower contamination profile, predominantly Cd, with less contribution from Pb, Cr, or Ni. Their significance, therefore, lies less in acute toxicity and more in chronic, widespread accumulation across conventional agriculture.

By contrast, sewage sludge presents a more complex but less uniformly distributed risk. Its agronomic appeal is clear, as it recycles nutrients and organic matter, improving soil fertility. Yet this benefit creates a contamination paradox: sludge introduces a broad spectrum of metals, including Zn, Pb, Cr, Cu, Ni, and Cd, often simultaneously. Compared with phosphate fertilizers, sewage sludge contamination is more heterogeneous and less predictable because composition depends strongly on wastewater origin and treatment efficiency. In nutrient-recycling agricultural systems, sludge may represent the greater long-term hazard per application, even if fertilizer-derived Cd dominates at continental scale.

In orchards and vineyards, legacy agrochemical contamination often exceeds modern fertilizer contributions. Historic arsenical pesticides created persistent As and Pb hotspots in orchard soils, while repeated copper fungicide application has made Cu accumulation a

defining contamination issue in viticulture. Unlike fertilizer contamination, these sources are crop-specific and management-driven, but concentrations can be substantially higher due to repeated direct application. In peri-urban agricultural systems, atmospheric deposition and wastewater irrigation frequently dominate. Industrial emissions, traffic-related Pb, and contaminated irrigation water introduce metals continuously, often with higher bioavailability than geogenic sources. Mining and smelting remain the most severe contamination sources in terms of concentration, but their relevance is primarily local rather than systemic [9,37].

3.2. Behavior and Speciation in Soil

The most commonly reported measurement of soil contamination is the total metal concentration, which represents the amount of a given metal present in a kilogram of dry soil, measured after complete acid digestion [8,33]. This is the basis of determining the degree of pollution and whether remediation is required in most regulatory frameworks. However, total concentration is a poor predictor of both ecological risk and needed remediation, because it does not give any information regarding the heavy metal fraction accessible to plants [30,38].

Bioavailability describes the percentage of total soil metal that is in a chemical form accessible to plants, microbial assimilation, or transfer to groundwater under given conditions [38]. A soil with 10 mg Cd kg^{-1} in crystalline sulphide minerals, which are highly stable and essentially unavailable, presents very different risks from a soil with 2 mg Cd kg^{-1} in the exchangeable fraction, which is easily dissolved and immediately available to plants. Thus, bioavailability-based risk assessment is starting to be incorporated into the remediation decision-making.

Bioavailability is characterized by sequential extraction, a stepwise procedure that dissolves different soil phases and quantifies the metal concentration present in each of them [26,30]. These operationally defined fractions broadly correspond to those used in the standard Tessier and BCR sequential extraction schemes, which facilitate comparison and reproducibility across studies. The extraction steps deal, in order, with the following fractions:

1. The most mobile fraction—the exchangeable and carbonate-bound fraction—which is immediately available to plants, holds metals by weak electrostatic forces on the surface of soil particles and through carbonate minerals;
2. The reducible fraction, which contains metals associated with iron and manganese oxides, released when the soil becomes anaerobic and redox conditions change.
3. The oxidizable fraction, which contains metals bound to organic matter and sulphides, released when organic matter oxidizes.
4. The residual fraction, which represents metals locked in primary and secondary mineral lattices and its essentially permanently immobile under natural conditions.

The practical implication of this fractionation is that the mobility of a metal and, therefore, its bioavailability and leaching risk, depend on the soil conditions. A study of organic matter-amended soils found that adding organic matter decreased the mobile fraction of Pb on spiked soils, by promoting its association with the more stable organic fraction, but increased the mobile fraction of Cd, demonstrating that the same amendment can simultaneously stabilize one metal while mobilizing another [39,40].

There are three key factors controlling the metal bioavailability: soil pH, soil organic matter (SOM), and the clay content and mineralogy [23,38,41]. Soil pH is the most important determinant of metal solubility in agricultural soils. As pH decreases, the soil becomes more acidic, and the exchangeable fraction of most metals increases dramatically. For example, in the case of Cd, a decrease from pH 7 to 5 can increase the availability of Cd to the plants

by 10 to 100-fold, depending on the total available Cd and organic matter content [41–43]. This is why soil acidification, caused by acid rain, excessive nitrogen fertilization, or natural leaching, is an aggravating factor that increases the heavy metal risks in contaminated soils. Liming, to raise the soil pH, is one of the most effective and immediately applicable tools used to reduce the bioavailability of Cd and Pb, while Cr and As have an atypical behavior in this case, becoming more available at higher pH, which complicates the management of soils contaminated with both Cd and Pb or Cd and As [38,41,44].

Soil organic matter (SOM) forms stable complexes with metal cations, generally reducing their exchangeable fraction and, therefore, their bioavailability. The effectiveness of SOM as a metal sorbent depends on the nature of the organic material: humic acids are stronger and more stable sorbents than fulvic acids, while freshly added plant residues have weaker sorption than stabilized humus. As organic matter decomposes, the metals can be released, an effect that has been documented in soils treated with sewage sludge, where metal availability increased decades after the sludge application as the organic matter slowly mineralized [11,29,30,45].

Clay particles carry a negative surface charge that adsorbs cationic metals (Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}). Thus, fine-texture soils exhibit a lower metal bioavailability than sandy soils at the same total metal concentration. The type of clay minerals is also important: 2:1 expanding clay minerals (smectite, vermiculite) have much higher cation exchange capacity (CEC) than 1:1 clays (kaolinite), and therefore, greater metal-buffering capacity [46].

Metal speciation is dramatically altered by waterlogged or anaerobic conditions, which are characteristic of paddy rice soils, constructed wetlands, and floodplain agricultural soils. Under reducing conditions, sulphate is reduced to sulphide, which precipitates many metals as highly insoluble sulphide minerals. When the soil is reoxygenated, those sulphide minerals oxidize, releasing previously immobilized metals back into the soil solution. This redox cycling is one of the principal reasons why Cd contamination in paddy soils is challenging to manage: drainage and re-flooding cycles repeatedly mobilize and remobilize cadmium [38,47].

While sequential extraction provides valuable mechanistic insight into the distribution of metals among operationally defined soil fractions, it does not directly quantify the pool immediately accessible to plants. For agricultural decision-making, complementary indicators are often required. Chemical extractions using chelating agents such as DTPA are widely applied to estimate phytoavailable fractions of Cd, Zn, Cu, and Pb under field-relevant conditions, although their predictive value varies with soil properties. Soil porewater measurements offer a more direct assessment of the dissolved metal fraction controlling immediate root uptake and leaching risk. Plant-based indices, including bioconcentration and transfer factors, further integrate soil bioavailability with actual biological uptake responses [30].

3.3. Phytotoxicity and Crop Impacts

Soil microorganisms are among the most sensitive indicators of heavy metal pollution and among the most important mediators of soil health and fertility. Heavy metals disrupt microbial life through multiple parallel mechanisms such as protein denaturation, disruption of cell membrane, and DNA damage [48,49]. Heavy metals, especially Hg, Pb, Cd, and Cu, bind thiol and carboxyl groups in enzyme active sites, inactivating them. Urease, invertase, catalase, dehydrogenase, and alkaline phosphatase are enzymes essential for nitrogen cycling, carbon decomposition, and phosphorus release, and consistently inhibited by heavy metal contamination [50,51]. Divalent metal cations displace calcium and magnesium from cell membrane phospholipids, altering membrane permeability and ion

transport. Some heavy metals, such as Cr and As, are directly mutagenic and genotoxic; others, such as Cd and Hg, generate reactive species that indirectly damage DNA [52].

The practical consequences for agricultural soils are significant. A meta-analysis confirmed that heavy metal contamination consistently reduces microbial biomass carbon, microbial diversity, and soil enzyme activity, all which are a direct measure of soil's capacity to mineralize organic matter, cycle nitrogen and phosphorus, and support plant growth [50,51,53]. Urease and dehydrogenase are the most frequently reported enzymes to be inhibited by Hg and Cd; cellulase is specifically inhibited by Pb through blockage of thiol and carboxyl groups. These inhibitions translate directly into impaired nitrogen mineralization, reduced phosphorus release, and slower decomposition of plant residues, which reduces the natural fertility of contaminated soils even before any reduction in yield from direct phytotoxicity [50,51]. Some microbial communities can adapt through metal-tolerance mechanisms; their resistance can be exploited in bioremediation, as we shall discuss in Sections 6 and 9. Their dominance in contaminated agricultural soils signals a deep ecological shift from the high-diversity communities that affect the stable soil fertility [49,53].

The phytotoxic effects of heavy metals on crops are similar to those discussed for microorganisms: inhibition of enzymes, disruption of the cell membrane, and generation of reactive species. However, they also include some specific interactions with photosynthesis, water relations, and nutrient transport [22,44]. Cadmium produces chlorosis in leaves by inhibiting chlorophyll synthesis and impairing iron and manganese uptake. It also reduces root elongation, diminishes stomatal conductance, and interferes with water and nutrient transport. At sublethal concentrations, Cd accumulates in grain and edible leaf tissues at levels that may exceed food safety limits, while the plant itself does not show any sign of stress. The "silent" contamination is particularly insidious in cereal crops such as rice, wheat, and sunflower, which are very well known as Cd-accumulating species. In Cd-contaminated soils in China and Japan, grain Cd concentrations sometimes exceed the EU food safety limit, while in France, there has been a recent warning about high concentrations detected in cereals that were subsequently transmitted to humans [3,44].

Lead is less mobile than cadmium, and most of what is absorbed by the roots is retained in the root cell walls rather than being translocated in the shoots. However, lead can accumulate in leafy vegetables and root crops when soils are heavy contaminated, while airborne lead deposits directly on the leaves, inhibiting the activity of delta-aminolaevulinic acid dehydrase (ALAD), a key enzyme in the tetrapyrrole biosynthesis pathways of chlorophyll and heme production [3,44].

In its inorganic forms (arsenate and arsenite) arsenic is a potent phytotoxin that disrupts the phosphate metabolism, inhibits the production of ATP, and generates reactive species through the reaction of arsenite with sulfhydryl groups. The crop most critically affected by arsenic contamination at a global level is rice: paddy soils create anaerobic conditions that reduce arsenate to the more mobile arsenite form, which is then taken up via aquaporin channels, resulting in arsenic concentrations in rice grain, being the main diet As exposure pathway for many people in Asia [3,44,54].

Chromium toxicity is mostly associated with the hexavalent form, Cr(VI), which is much more mobile and phytotoxic than the trivalent form, Cr(III). It enters plant cells via sulfate transporters, generating reactive oxygen species (ROS) in the cytoplasm and oxidizing the DNA. By contrast, the trivalent form is largely immobile and has low bioavailability under near neutral pH conditions [36,44].

The transfer of heavy metals from contaminated soil through the plants into human food is the ultimate driver of the public health concern surrounding the contamination of agricultural soil. This phenomenon is not uniform, varying between metal species (e.g., Cd

and As transfer easier to grains and vegetable tissues than Pb and Cr), between crop species (e.g., leafy vegetables and roots accumulate more than grain crops), and between soil conditions (e.g., low pH and high bioavailability increase the transfer dramatically) [3,24,55]. Cadmium has a biological half-life in the human body of about 20 years and accumulates mostly in the kidneys and liver; the European Food Safety Authority (EFSA) has identified dietary cadmium as a cause of kidney tubular dysfunction at current background exposure levels across EU population [56–58]. Inorganic arsenic is classified as a Group 1 human carcinogen; chronic dietary exposure is associated with bladder, lung, and skin cancers, as well as cardiovascular and neurological effects [59]. Lead causes irreversible neurodevelopment damage in children at blood concentrations with no established threshold [60]. These human health endpoints ultimately justify the investment in phytoremediation at an agricultural scale. Restoring the soil below safety thresholds for heavy metals is not merely an environmental act but a direct public health intervention.

Understanding the sources and speciation of heavy metals in agricultural soils shows why the plant-based remediation is so challenging. Plants interact with different metals simultaneously, in multiple chemical forms, at varying depths, in the presence of competing ions, and under different soil conditions that are also influenced by climate. The following section examines how plants have evolved to navigate these challenges and how they can be exploited as remediation tools.

4. Plant Mechanisms of Heavy Metal Tolerance and Accumulation

Heavy metal contamination poses a serious threat to plant growth and productivity, primarily through the disruption of cellular processes and induction of oxidative stress. Plants have evolved sophisticated detoxification mechanisms to cope with toxic metal accumulation, among which vacuolar compartmentalization plays a central role. By sequestering heavy metals into vacuoles, plants effectively isolate toxic ions from the cytosol, thereby reducing their interference with metabolic activities and preventing cellular damage. This process is mediated by specialized membrane transporters, including ATP-binding cassette (ABC) transporters and metal/proton antiport systems, which facilitate the movement of metal–ligand complexes into vacuoles [20]. In addition to compartmentalization, plants employ a range of biochemical strategies such as chelation by phytochelatins and metallothioneins. These molecules bind heavy metals, forming stable complexes that can be safely transported and stored within cellular compartments. Antioxidant defense systems also play a critical role, as heavy metal stress leads to the generation of reactive oxygen species (ROS). Enzymatic antioxidants such as superoxide dismutase and catalase, along with non-enzymatic compounds like glutathione, help mitigate oxidative damage and maintain redox homeostasis [61]. Together, these processes provide the molecular and physiological foundation for all subsequent responses discussed in this review. Hyperaccumulator plants exhibit enhanced versions of these mechanisms, allowing them to tolerate and accumulate exceptionally high concentrations of metals. These species possess efficient uptake systems, rapid translocation pathways, and highly effective detoxification processes, making them valuable for phytoremediation and phytomining applications. Understanding these integrated mechanisms provides important insights into improving plant resilience and developing sustainable strategies for environmental cleanup [62].

4.1. Metal Uptake at the Root–Soil Interface

Metal uptake at the root–soil interface represents a critical step in plant interaction with heavy metals, strongly influenced by physicochemical and biological processes occurring in the rhizosphere. Plants absorb metals primarily through transporters that also mediate the uptake of essential nutrients, which can inadvertently facilitate the entry of toxic elements

such as cadmium and lead [61]. The efficiency of this process depends on soil properties, including pH, redox potential, and metal speciation, which determine metal bioavailability.

4.1.1. Rhizosphere Mobilization

Rhizosphere mobilization plays a key role in enhancing or limiting metal uptake. Root cells actively acidify the rhizosphere by releasing protons via plasma membrane H^+-ATPases, which is particularly pronounced in hyperaccumulators such as *Thlaspi caerulescens* and *Arabidopsis halleri*. This drop in pH shifts metals from the carbonate-bound and Fe/Mn oxide-bound soil fractions into the exchangeable (plant-available) fraction. Root exudates—including organic acids (malic, citric, oxalic), amino acids, and phytosiderophores—further chelate soil-bound metals, maintaining them in soluble form at the root surface [63]. Additionally, interactions with rhizosphere microorganisms, including plant growth-promoting bacteria and mycorrhizal fungi, can further influence metal mobility and uptake efficiency. These microbes contribute to metal solubilization through siderophore production and enzymatic activity, thereby enhancing phytoremediation potential [63].

4.1.2. Transporter Families at the Root Plasma Membrane

Once mobilized in the rhizosphere, metal ions are imported into root-epidermal and cortical cells by a suite of membrane-spanning transporter proteins responsible for maintaining metal homeostasis and detoxification [20,22,64]. The constitutive overexpression of key transporter families in hyperaccumulators underpins their high phytoextraction efficiency and distinguishes them from non-accumulator species [62].

ZIP (Zrt/Irt proteins) transporters (Zrt—Zinc-regulated transporter; Irt—Iron-regulated transporter) represent a major entry pathway for divalent metal cations such as Zn^{2+}, Fe^{2+}, and Cd^{2+}. Their activity is tightly regulated, but in hyperaccumulator species, they are often constitutively expressed at higher levels, facilitating enhanced metal uptake and translocation [62,64,65]. Natural Resistance-Associated Macrophage Proteins (NRAMP transporters) mediate the uptake and intracellular transport of Fe^{2+}, Mn^{2+}, and Cd^{2+}. They are involved in both plasma membrane transport and internal remobilization processes, including vacuolar metal release under nutrient deficiency [64,66].

Iron-Regulated Transporter 1 (IRT1) is a high-affinity Fe^{2+} transporter that also mediates Cd^{2+} uptake due to low substrate specificity. Its expression is upregulated under iron deficiency, which can inadvertently increase cadmium accumulation in plants [62,64].

The multidrug resistance-associated protein (MRP) subfamily—ABC transporters (ATP-binding cassette), particularly ABCC-type proteins (ATP-binding cassette subfamily C)—are involved in detoxification by transporting phytochelatin–metal complexes into vacuoles, thereby reducing cytosolic toxicity [20].

Cation Diffusion Facilitators/MTP proteins are transporters that mediate the efflux and sequestration of metals such as Zn^{2+} and Cd^{2+}, contributing to intracellular metal homeostasis and tolerance [64].

Multi-antimicrobial extrusion (MATE) proteins contribute to detoxification through the transport of organic acids, which chelate metals and facilitate their efflux or redistribution [67].

4.2. Translocation from Root to Shoot

Long-distance translocation of heavy metals from roots to shoots represents a fundamental process in plant metal homeostasis and a key determinant of hyperaccumulation capacity. After uptake at the root–soil interface, metals must be efficiently transported through vascular tissues, primarily via the xylem, to reach aerial organs. This process is essential not only for plant survival under metal stress but also for phytoremediation appli-

cations, as efficient accumulation in aboveground biomass is required for metal removal from contaminated soils [62,64].

A critical step in this process is xylem loading, which is largely mediated by P1B-type ATPases belonging to the Heavy Metal ATPase (HMA) family. Among these, HMA2 and HMA4 are particularly important, as they facilitate the export of Zn^{2+} and Cd^{2+} from root cells into the xylem apoplast. Enhanced expression of these transporters has been strongly associated with increased root-to-shoot translocation, especially in hyperaccumulator species such as *Arabidopsis halleri* [64,68]. Conversely, transporters such as HMA3, localized on the tonoplast, sequester metals into root vacuoles and thereby limit their translocation, highlighting the balance between sequestration and transport in determining metal distribution within plants [69]. Heavy metals are not transported as free ions within the xylem due to their high reactivity and potential toxicity. Instead, they are complexed with low-molecular-weight ligands that ensure their solubility and stability during transport. Nicotianamine (NA) is one of the most important chelators involved in this process, forming stable complexes with metals such as Fe, Zn, and Ni, thereby facilitating long-distance transport in both xylem and phloem [67]. In hyperaccumulator species, elevated levels of NA contribute significantly to enhanced metal mobility and translocation efficiency. In addition to nicotianamine, other ligands such as histidine and organic acids (e.g., citrate and malate) also play crucial roles in metal transport. Histidine has been shown to facilitate nickel transport in hyperaccumulator species by forming stable complexes that enhance metal mobility in the xylem [64,70]. Organic acids contribute to the chelation and transport of various metals, particularly iron, and help maintain metal solubility under physiological conditions [67,71].

The efficiency of root-to-shoot metal translocation is commonly quantified using the translocation factor (TF), defined as the ratio of metal concentration in shoots to that in roots. A TF > 1 indicates efficient translocation of metals from roots to shoots, signaling high uptake potential, while a TF < 1 suggests the plant retains metals in the roots. This efficiency results from a coordinated interplay of enhanced transporter expression, increased chelator availability, and reduced vacuolar sequestration in roots [62,64]. A deeper understanding of these mechanisms provides valuable insights for improving phytoremediation strategies and developing crops with enhanced tolerance to metal stress.

4.3. Chelation and Detoxification in the Cytosol

Cytosolic chelation represents a central mechanism by which plants detoxify heavy metals immediately after their entry into the cell. Among the most important molecules involved in this process are phytochelatins (PCs) and metallothioneins (MTs), both of which are cysteine-rich ligands capable of binding toxic metal ions and reducing their reactivity. Their mechanisms of action and metal specificity are summarized in Table 1. Recent studies emphasize that these systems function as primary intracellular defense mechanisms, maintaining metal homeostasis and protecting cellular structures under stress conditions [22,72].

Phytochelatins are enzymatically synthesized peptides derived from glutathione and are rapidly induced in response to heavy metal exposure. Their structure, characterized by repeating γ-Glu-Cys units, allows efficient binding of metals such as Cd, Pb, and As. Once formed, PC–metal complexes are either retained in the cytosol or transported into vacuoles for long-term sequestration [73]. This rapid synthesis makes PCs particularly important during acute metal stress, where immediate detoxification is required to prevent cellular damage. Additionally, the biosynthesis of PCs is tightly linked to glutathione metabolism, highlighting their role within broader redox regulation pathways [74].

Table 1. PCs and MTs: mechanisms and metal specificity.

Feature	Phytochelatins (PCs)	Metallothioneins (MTs)
Molecular nature	Non-protein peptides	Gene-encoded proteins
Origin	Synthesized from glutathione	Produced via gene expression
Induction	Rapid, directly metal-induced	Transcriptionally regulated
Main function	Rapid metal chelation	Metal chelation + antioxidant protection
Target metals	Cd, Pb, As, Hg	Cd, Cu, Zn
Localization	Cytosol and vacuole	Cytosol, nucleus
Role in oxidative stress	Indirect	Direct (ROS scavenging)
Response dynamics	Immediate response	Long-term response

In contrast, metallothioneins are gene-encoded, low-molecular-weight proteins that also possess high cysteine content, enabling strong binding affinity for metal ions. Unlike PCs, which are synthesized enzymatically in response to stress, MTs are regulated at the transcriptional level and often provide longer-term protection against metal toxicity. MTs play a dual role by not only chelating metals but also contributing to reactive oxygen species (ROS) scavenging, thereby linking metal detoxification with oxidative stress responses [3].

The functional interplay between PCs and MTs is increasingly recognized as a coordinated detoxification system rather than independent pathways. PCs are typically synthesized rapidly upon metal exposure and act as immediate chelators, binding free metal ions in the cytosol. Subsequently, MTs may contribute to sustained detoxification and storage, particularly under prolonged stress conditions. This sequential and complementary action enhances the overall efficiency of metal detoxification [75,76].

Recent research also highlights that the relative contribution of PCs and MTs may vary depending on the type of metal and plant species. For instance, cadmium detoxification is predominantly mediated by phytochelatins, whereas metallothioneins may play a more prominent role in mitigating oxidative stress associated with metal toxicity [73]. Moreover, transcriptomic and proteomic studies suggest that the expression of MT genes is often correlated with stress intensity and duration, indicating a dynamic regulatory system that adapts to environmental conditions [3]. The integration of rapid peptide-based chelation (PCs) with gene-regulated protein systems (MTs) ensures both immediate and long-term protection against heavy metal toxicity. Understanding the balance and regulation of these systems is essential for advancing phytoremediation strategies and engineering plants with enhanced tolerance to metal stress.

4.4. Antioxidant Defence

Heavy metal stress is strongly associated with the excessive generation of reactive oxygen species (ROS), which represent one of the earliest and most damaging consequences of metal toxicity in plants. Metals such as cadmium, lead, and mercury interfere with electron transport chains in chloroplasts and mitochondria, leading to oxidative bursts characterized by elevated levels of superoxide radicals, hydrogen peroxide, and hydroxyl radicals [3,77,78]. These reactive molecules cause lipid peroxidation, protein oxidation, and DNA damage, ultimately impairing cellular integrity and plant growth.

To neutralize oxidative stress, plants have evolved a highly coordinated antioxidant defense system consisting of enzymatic and non-enzymatic components. The enzymatic antioxidant system includes key enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which function sequentially to detoxify ROS. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently converted into water and oxygen by CAT and APX [77]. This enzymatic cascade is essential

for maintaining cellular redox homeostasis and preventing oxidative damage under metal stress conditions.

In addition to enzymatic antioxidants, non-enzymatic molecules such as ascorbate, glutathione, carotenoids, and phenolic compounds play crucial roles in ROS scavenging. These molecules act as redox buffers and participate in the ascorbate–glutathione cycle, a central metabolic pathway responsible for detoxifying hydrogen peroxide and maintaining intracellular redox balance [77]. Glutathione, in particular, serves as a key molecule linking antioxidant defense with metal detoxification, as it is also a precursor for phytochelatin synthesis.

Recent studies highlight that antioxidant defense is not merely a protective system but also a regulatory network integrated with plant signaling pathways. ROS themselves function as signaling molecules that activate stress-responsive genes, transcription factors, and hormonal pathways, including salicylic acid and brassinosteroid signaling [77,79]. This dual role of ROS—as both damaging agents and signaling intermediates—requires a fine balance between production and scavenging to ensure optimal plant responses to metal stress. The efficiency of antioxidant defense varies among plant species and is particularly enhanced in metal-tolerant and hyperaccumulator plants. These species exhibit higher baseline and inducible levels of antioxidant enzymes, enabling them to maintain redox homeostasis even under severe stress conditions [20,78]. The upregulation of antioxidant systems is often coordinated with other detoxification mechanisms, including metal chelation and vacuolar sequestration, forming an integrated defense network [66].

Advances in omics technologies have provided new insights into the regulation of antioxidant defense under heavy metal stress. Transcriptomic and proteomic analyses reveal that genes encoding antioxidant enzymes are differentially expressed in response to specific metals and stress intensities [79]. These findings suggest that antioxidant responses are highly dynamic and tailored to environmental conditions. Through the coordinated action of enzymatic and non-enzymatic components, plants maintain redox balance, protect cellular structures, and regulate stress signaling pathways. A deeper understanding of these mechanisms is essential for improving plant tolerance and enhancing phytoremediation strategies.

Table 2 summarizes the key molecular mechanisms of plant heavy metal (HM) tolerance and highlights their coordinated roles in hyperaccumulation. At the root level, transporters such as ZIP, NRAMP, and IRT1 mediate metal uptake, often with enhanced activity in hyperaccumulator species, enabling increased influx of essential and non-essential metals. This is followed by efficient xylem loading, primarily driven by HMA2 and HMA4, which facilitate root-to-shoot translocation—a defining feature of hyperaccumulation. Within the cytosol, detoxification is achieved through chelation by phytochelatins and metallothioneins. While phytochelatins rapidly bind toxic metals and direct them toward vacuolar sequestration, metallothioneins provide sustained, gene-regulated metal binding and protection. Vacuolar sequestration, mediated by transporters such as MTP1 and HMA3, further reduces cytosolic toxicity by compartmentalizing metals. The antioxidant defense systems mitigate oxidative stress generated by metal exposure, ensuring cellular viability. Together, these interconnected mechanisms form a highly integrated network that enables plants not only to tolerate but also to accumulate heavy metals.

Table 2. Key molecular mechanisms in plant HM tolerance.

Mechanism	Key Proteins/Genes	Metals Targeted	Location	Role in Hyperaccumulation
Root uptake	ZIP, NRAMP, IRT1	Zn, Cd, Fe, Mn	Root plasma membrane	Enhanced in hyperaccumulators [66]
Xylem loading	HMA2, HMA4	Cd, Zn	Root/shoot vasculature	Upregulated in *A. halleri* [71]

Table 2. *Cont.*

Mechanism	Key Proteins/Genes	Metals Targeted	Location	Role in Hyperaccumulation
Cytosol chelation	Phytochelatins (PCs)	Cd, As, Hg	Cytosol	PC–Cd complexes for vacuolar storage [76]
Cytosol chelation	Metallothioneins (MTs)	Cu, Zn, Cd	Cytosol	Gene-encoded; stress-responsive [80]
Vacuolar sequestration	MTP1, HMA3, CAX2	Zn, Cd, Ca	Vacuole tonoplast	Prevents shoot toxicity [66]
Antioxidant defense	SOD, APX, CAT, GSH	All metals (ROS)	Chloroplast, cytosol	Enables survival under HM stress [61]

Enhanced transporter expression, particularly of ZIP, NRAMP, and HMA family proteins, significantly improve metal uptake and root-to-shoot translocation, but molecular efficiency alone does not guarantee successful remediation. For phytoextraction, total metal removal is determined not only by tissue metal concentration, but by the interaction between uptake efficiency, translocation capacity, and aboveground biomass production [62,64,81]. This explains why classical hyperaccumulators such as *Thlaspi caerulescens* demonstrate exceptional accumulation capacity but limited field-scale remediation efficiency due to their low biomass yield [82]. Conversely, high-biomass species such as *Brassica juncea* or *Miscanthus* may achieve greater cumulative metal removal despite lower tissue concentrations, because biomass productivity compensates for reduced accumulation efficiency [83,84]. Similarly, detoxification mechanisms influence remediation strategy selection rather than simply enhancing tolerance. Strong vacuolar sequestration in root tissues, mediated by transporters such as HMA3 and MTP proteins, reduces cytosolic toxicity and improves plant survival under contaminated conditions but simultaneously limits shoot translocation, making these species more suitable for phytostabilization than phytoextraction [66,69]. In contrast, species with reduced root sequestration and enhanced xylem loading, supported by HMA2/HMA4 overexpression and chelator-mediated transport, are more appropriate for active metal extraction [68,71].

Antioxidant defense and cytosolic detoxification also have practical agronomic implications. Although phytochelatins, metallothioneins, and ROS-scavenging systems do not directly increase metal export, they determine plant tolerance under prolonged stress, preserving photosynthetic capacity, growth, and biomass accumulation [20,78]. Since remediation efficiency in agricultural systems ultimately depends on harvestable biomass over multiple growing cycles, these protective mechanisms indirectly become critical determinants of field performance.

5. Phytoremediation Strategies: Mechanisms and Applications

Phytoremediation represents a sustainable and environmentally friendly strategy for mitigating pollution in soil, water, and air, particularly in response to the increasing presence of heavy metals and organic contaminants. This approach utilizes plants to absorb, detoxify, or immobilize pollutants, offering a cost-effective alternative to conventional remediation technologies. Due to the persistence and toxicity of contaminants such as cadmium, lead, and organic xenobiotics, phytoremediation has gained significant attention in environmental management [83].

The efficiency of phytoremediation depends on complex physiological and molecular mechanisms that regulate pollutant uptake, transport, and storage. These processes include mobilization in the rhizosphere, root absorption, translocation through the xylem, and sequestration in plant tissues. Metal transporters and chelating compounds such as

phytochelatins and metallothioneins play essential roles in detoxification and accumulation [85].

Hyperaccumulator species are particularly important, as they can tolerate and accumulate high concentrations of metals in their shoots. Their efficiency is linked to enhanced root uptake, increased translocation, and effective sequestration in leaf vacuoles, minimizing cellular damage [83]. Phytoremediation strategies include phytoextraction, phytostabilization, and rhizodegradation, each adapted to specific contaminants. Advances in biotechnology and plant–microbe interactions continue to improve their effectiveness, highlighting phytoremediation as a promising tool for sustainable environmental restoration [83,86].

5.1. Phytoextraction

Phytoextraction is the strategy most people envision when they think of "using plants to clean soil". The principle is straightforward: grow plants that absorb heavy metals from the soil through their roots, translocate those metals to their above-ground tissues (leaves, stems), and then harvest the plant biomass, removing the metals from the site along with it. Over repeated growing cycles, which may span several years, the total metal load in the soil is progressively reduced [87,88].

The effectiveness of phytoextraction is measured by two key indices [81,89]:

- The Bioconcentration Factor (BCF), also called the Bioaccumulation Factor (BAF), is defined as the concentration of a metal in the plant shoot divided by the concentration in the soil. A BCF greater than 1 means the plant is concentrating the metal relative to the surrounding soil; a BCF much greater than 1 (sometimes >10 or even >100 in exceptional hyperaccumulators) indicates a strong phytoextraction potential [81].
- The Translocation Factor (TF) is the ratio of metal concentration in the shoot to concentration in the root. A TF greater than 1 confirms that the plant is efficiently moving metals from root to shoot, which is essential for phytoextraction, since only the above-ground biomass is harvested. Plants with TF < 1 accumulate metals in their roots, which makes them useful for phytostabilization but not for phytoextraction [81].

A global synthesis of 547 data pairs from 82 studies published in 2025 found that cadmium hyperaccumulators achieve the highest average bioaccumulation factors of any metal group (BAF = 10.0 $\pm$ 1.3, with a maximum of 191), though their translocation factors are relatively modest (TF = 1.8 $\pm$ 0.1) because the small aboveground biomass of many hyperaccumulators limits total metal export. Nickel hyperaccumulators, by contrast, exhibit the highest TF values, with metals accumulating preferentially in leaves and stems rather than roots. This distinction has direct practical implications: for cadmium remediation, the challenge is not root uptake (which is very efficient) but scaling up above-ground biomass; for nickel, the challenge is finding ways to increase root uptake to match the plant's excellent translocation capacity [81].

Not all plants that tolerate heavy metals qualify as hyperaccumulators. The definition requires that concentrations in dry shoot tissue exceed the following thresholds, regardless of the metal concentration in the soil [81]:

- >100 mg Cd kg^{-1} dry weight in shoots,
- >1000 mg Pb, Ni, Cu, Co, or Cr kg^{-1} dry weight in shoots,
- >10,000 mg Zn or Mn kg^{-1} dry weight in shoots,
- >1000 mg As kg^{-1} dry weight in shoots (some sources use >1000 mg, others > 100 mg).

These thresholds are 10–500 times higher than metal concentrations found in the tissues of ordinary plants growing in the same contaminated soils. Approximately 700 plant species have been identified as hyperaccumulators for at least one metal, with the Brassicaceae family contributing more species than any other [87,90].

5.2. Key Hyperaccumulator Species

Hyperaccumulator species are a unique group of plants capable of tolerating and accumulating exceptionally high concentrations of heavy metals in their aerial tissues without showing toxic effects. These plants are typically adapted to metalliferous soils and can accumulate metals such as zinc, cadmium, or nickel at levels up to 100 times higher than non-accumulator species. Their efficiency is based on enhanced root uptake, effective translocation to shoots, and sequestration in vacuoles or cell walls, supporting detoxification and phytoremediation potential [85].

5.2.1. Brassicaceae Hyperaccumulators

Thlaspi caerulescens (now often reclassified as *Noccaea caerulescens*) is the model hyperaccumulator for cadmium and zinc research. It has been documented to accumulate up to 380 mg Cd kg^{-1} and 10,000 mg Zn kg^{-1} in its shoots. Such concentrations would be lethal to virtually every other plant species. The secret to its performance lies in the constitutive action of ZAP1, ZIP6, and HMA4 transporter genes (described in Section 3), which remain active at high levels even when the plant is growing in uncontaminated soil, meaning the plant is always primed for metal uptake [85,86].

Field experiments have demonstrated that *T. caerulescens* can export 130–540 g Cd ha^{-1} yr^{-1} depending on soil pH, with acidic soils yielding substantially higher removal rates because cadmium is more bioavailable at lower pH. Modeling studies estimate that reducing soil Cd concentration from 10 to 0.5 mg kg^{-1}—the Swiss regulatory threshold—would require 11 years at a biomass production of 5 t dry matter ha^{-1} yr^{-1}, or 42 years at 1 t ha^{-1} yr^{-1}, confirming that the principal limitation of *T. caerulescens* for practical remediation is its small size and correspondingly low biomass yield, not its metal accumulation capacity. The plant does not significantly decrease the $NaNO_3$-extractable (immediately bioavailable) soil fraction, suggesting it preferentially draws from more strongly bound soil pools—an observation with important implications for modeling remediation timescales [82]. Despite an extraordinary accumulation capacity, *Noccaea (Thlaspi) caerulescens* provides an example of a major limitation of hyperaccumulator-based phytoextraction, i.e., high metal concentrations in the tissues do not necessarily imply efficient remediation at the field scale. Soil metal depletion rates are slow due to the small stature and limited biomass yield, and in realistic agronomic conditions, remediation periods often span decades. *N. caerulescens* is therefore an important mechanistic model species, but its direct practical application is generally limited unless incorporated into assisted phytoextraction strategies or niche remediation scenarios.

Arabidopsis halleri is the other intensively studied Brassicaceae hyperaccumulator, primarily for and cadmium. As discussed in Section 3, HMA4 overexpression is the principal genetic driver of its hyperaccumulation phenotype. A particularly important finding from field studies is that phytoextraction efficiency in *A. halleri* is driven by plant genetics rather than soil metal concentration—meaning the plant performs across a range of soil contamination levels rather than only performing well at high concentrations. This makes it a more reliable tool for moderately contaminated agricultural soils than species that require high soil metal concentrations to activate their uptake machinery [71].

Brassica juncea (Indian mustard) occupies a different strategic niche. It is not a true hyperaccumulator—its natural Pb and Cd concentrations are modest—but it produces very large amounts of biomass (up to 10 t dry matter ha^{-1}), which compensates for its lower metal concentration per unit biomass. When combined with chelating agents such as EDTA (discussed in detail in Section 5), *B. juncea* becomes one of the most practically effective plants for lead phytoextraction. Adding EDTA at 2.5 mmol kg^{-1} increased Pb shoot concentrations 14–26 times compared to controls, though geogenic lead (naturally

occurring in parent rock) was found to be 59% less available than anthropogenic lead from industrial contamination, underscoring that soil contamination history significantly affects phytoextraction outcomes. A separate study using 2 mM kg^{-1} EDTA found maximum shoot biomass of 65 g and root biomass of 35 g in heavily contaminated soils, with no visual signs of chlorosis or root rot—confirming the plant's robust tolerance to combined lead and EDTA stress. When biochar and EDTA were combined, Pb uptake reached 60.2 mg g^{-1}, compared to only 10.0 mg g^{-1} in untreated controls [91–93]. EDTA-assisted phytoextraction can significantly enhance the mobilization and uptake of metals by plants such as *Brassica juncea*; however, its practical application in open-field agriculture conditions is still controversial. Heavy metals may be solubilized to a greater extent, which may significantly increase the risk of leaching, allowing contaminants to migrate into deeper soil layers and possibly groundwater systems. Additionally, EDTA is poorly biodegradable and may be persistent in the environment, which raises regulatory and environmental concerns for agricultural use. Therefore, EDTA-assisted phytoextraction is a useful proof-of-concept or controlled remediation method, but its large-scale field applicability is often restricted, and more biodegradable chelating alternatives might provide safer agronomic solutions.

Brassica napus (oilseed rape/canola) offers a dual-use advantage: moderate tolerance to chromium, selenium, lead, and zinc contamination, combined with valuable oilseed production. On moderately contaminated soils, it can generate both biomass for remediation and economically valuable seed oil, partially offsetting the cost of the remediation operation [94].

5.2.2. Crassulaceae

Sedum alfredii is a native Chinese plant recognized as an important hyperaccumulator for Cd and Zn-contaminated paddy soils in East Asia. Under controlled hydroponic conditions, it can tolerate concentrations of up to 4933 mg Cd kg^{-1} and 19,900 mg Zn kg^{-1}, representing levels approximately 500 times higher than those tolerated by non-accumulator species. In field conditions, its accumulation capacity is lower but remains comparatively high, making it one of the more effective options for phytoremediation of Cd/Zn co-contaminated paddy soils [95].

A key finding for practical application is that phosphorus fertilization significantly improves performance. The application of KH_2PO_4 at 352 mg P kg^{-1} soil increases both shoot zinc concentration and biomass yield. After one growing season, Cd extraction can reach 162.6 µg pot^{-1}. This shows that agronomic management, not only plant genetics, is important for field-scale efficiency. Repeated harvesting (continuous clipping) further enhances cumulative metal removal, as plants regrow from the root system. The related species *Sedum plumbizincicola* exhibits similar Cd hyperaccumulation and is often studied together with *S. alfredii* [87].

5.2.3. Poaceae (High-Biomass Grasses—Energy Crops)

Poaceae spp. is a very important family in phytoremediation of agricultural crops, because many of them are already in the farming system. They have a fibrous system, high tillering capacity and tolerance to abiotic stress, frequently polyploidy, which enhances their resilience and sometimes, metal tolerance. The grasses can function as accumulators or excluders, depending on the species, metal, and soil conditions [96]. Representative species are *Miscanthus x giganteus* (which can accumulate Zn, Cd, and Pb) *Panicum maximum*, *Eleusine indica*, *Cynodon dactylon* (with reported accumulation of Zn, Ni, Cu and Pb), and *Perotis indica* and *Echinochola colona* (reported good accumulators for Cd, Cr). The members of this species are ideal for phytostabilization, low-input phytoextraction in rotations, or as temporary cover crops.

5.2.4. Other Hyperaccumulators

Pteris vittata (Chinese brake fern) is the primary arsenic hyperaccumulator. It can concentrate up to tens of thousands of mg As kg^{-1} dry weight while maintaining vigorous growth, with most arsenic stored as reduced arsenite complexed in vacuoles [97]. This species also tolerates the co-contamination with minimum effects on arsenic uptake. The antioxidant enzyme production increases under multi-metal stress, indicating a strong oxidative-stress management system. Most of the arsenic accumulates in the aerial tissues, which makes the phytoextraction easy. Other *Pteridaceae* members that are known for heavy metal accumulation are *Pityrogramma calomelanos*, which accumulates arsenic and lead, with some reported growth depression at high lead loads but overall high uptake capacity, and other *Pteris* spp. from mining areas that have shown high copper, chromium, and arsenic accumulation, with less tolerance than *P. vittata*. The uptake mechanism for this species is mainly via phosphate transporters, followed by reduction in the plant from As(V) to As(III) and sequestration, strongly dependent on soil P availability and pH. These plants prefer well-drained, often slightly acidic soils, and are less suited for dry continental cropland, but very relevant for subtropical and temperate regions, irrigation channels, and abandoned plots. Their value in agriculture is targeted remediation of arsenic hotspots.

Salicaceae, mainly *Salix* and *Populus* spp. are not classic hyperaccumulators but high biomass accumulators with fast growth and deep root systems, thus ideal for phytoextraction and phytostabilization at large scale. *Salix viminalis* and other short-rotation coppice (SRC) willows accumulate cadmium, zinc, and nickel effectively, with reported cadmium shoot concentrations of several hundred mg kg^{-1} and high bioconcentration factors in contaminated soils.

Populus spp. show strong uptake and translocation of zinc, cadmium, and occasionally lead, with deep rooting and high transpiration that enhances mass flow of metals towards the roots. They are useful in areas where the contamination extends deep into the soil profile, such as along the river levees or near the industrial discharge areas [84]. These accumulators support rich rhizosphere communities and arbuscular mycorrhizal colonization, which can be exploited and are well-suited for buffer strips and field edges, where they can have even more uses, such as erosion control, biodiversity, and bioenergy feedstock, in addition to remediation.

Many species have proven their ability as phytoaccumulators; however, there is still a place for many others. Considering each ecosystem, there might already be existing species with heavy metal accumulation capacity, so there is always a need for more studies. For example, *Urtica dioica* is a robust native accumulator and cheap biosorbent or industrial fiber, a spontaneous colonizer of polluted sites which can often reduce Zn, Pb and Cd contamination [98,99].

5.3. Phytostabilization

Phytostabilization involves the use of tolerant plant species to immobilize contaminants within the rhizosphere or root tissues, thereby limiting their dispersion through leaching, erosion, or uptake into the food chain. This process relies on processes such as adsorption, complexation with root exudates, precipitation, and binding to soil particles. In addition, dense root systems improve soil stability and reduce contaminant transport. As a low-cost and environmentally friendly approach, phytostabilization is particularly suitable for long-term management of contaminated sites [100,101].

Simultaneous mechanisms of phytostabilization:

✓ Root surface binding: metal ions adsorb onto the negatively charged surfaces of root cell walls (which are rich in pectin carboxyl groups), effectively trapping them in the apoplastic space before they even enter root cells;

- ✓ Rhizosphere chemistry modification: root exudates (phosphate, carbonate, sulfate) precipitate metals as insoluble minerals in the rhizosphere soil; for example, lead is immobilized as pyromorphite ($Pb_5(PO_4)_3Cl$) when phosphate is abundant;
- ✓ Root vacuolar storage: metals that do enter root cells are pumped into root vacuoles by HMA3 and MTP transporters (Section 3) and retained there indefinitely—a controlled biological sequestration that prevents translocation to shoots and therefore to the food chain;
- ✓ Reduced soil erosion: plant canopy cover and root networks physically stabilize contaminated surface soil, preventing wind and water erosion from dispersing metal-containing particles.

The perfect phytostabilizer has the opposite trait profile from the perfect phytoextractor: low TF (metals stay in roots), high shoot biomass (provides soil cover and erosion control), and strong metal tolerance (survives in highly contaminated conditions) [83]:

Festuca rubra and *Agrostis capillaris* (red fescue and bent grass): the pillars of European phytostabilization programs on former mining sites; metal-tolerant ecotypes of both species have been selected and commercialized specifically for revegetation of metalliferous soils, with documented tolerance to Pb, Zn, Cu, and Cd [83].

Anthyllis vulneraria: a nitrogen-fixing legume that establishes on calamine grasslands (natural Zn/Pb-rich soils) in central Europe; used in ecological restoration programs that combine phytostabilization with habitat creation [83].

Miscanthus giganteus: as described above, primarily a phytostabilizer for Zn and Pb due to preferential root/rhizome retention, while producing harvestable energy biomass above ground [49,83].

Phytostabilization is rarely applied in isolation—it is almost always combined with immobilizing soil amendments that chemically reduce the bioavailable fraction of metals [102]. Lime raises soil pH, precipitating metals as hydroxides and carbonates; it also reduces Cd, Pb, and Zn bioavailability by 70–90% in acidic agricultural soils. Biochar adsorbs metals onto its highly porous surface and simultaneously improves soil water retention and microbial activity; combined biochar and phytostabilization treatments show synergistic reductions in metal leachability. Phosphate fertilizers immobilize lead as pyromorphite, which is one of the least soluble and most stable Pb compounds in soils. This effect is particularly relevant for urban and periurban agricultural soils contaminated by legacy leaded gasoline or lead-based paint.

Phytostabilization and phytoextraction are not mutually exclusive across time. A logical sequential strategy for heavily contaminated agricultural soils is to apply phytostabilization first—to rapidly reduce acute risk and allow partial re-establishment of soil biology—followed by phytoextraction over subsequent years to progressively remove the total metal load.

5.4. Phytovolatilization

Phytovolatilization is a strategy in which contaminants are removed from soil through their conversion into volatile forms and subsequent release into the atmosphere via plant transpiration. In this process, plants absorb contaminants from soil, transform them biochemically into volatile compounds, and release them through stomatal diffusion [103,104]. Unlike other phytoremediation approaches, contaminants are not accumulated in biomass but dispersed into the atmosphere at low concentrations. In practice, phytovolatilization is applicable to two elements: selenium and mercury [102].

Selenium is taken up as selenate or selenite and converted into volatile organic forms such as dimethylselenide and dimethyldiselenide, which are released through leaves.

Species such as *Brassica juncea*, *Astragalus bisulcatus*, and *Allium* spp. are known for their capacity to volatilize selenium efficiently.

Mercury phytovolatilization involves the uptake of Hg^{2+} or methylmercury and its conversion to elemental mercury (Hg^0), which is then released into the atmosphere. This process is limited in wild-type plants but can be significantly enhanced in genetically modified species, which enable reduction and demethylation of mercury [104]. Additionally, selenium application can reduce mercury bioavailability in soils through the formation of stable HgSe complexes, influencing both uptake and translocation [102].

Phytovolatilization is treated with caution in many regulatory frameworks, especially in Europe, since it shifts contaminants from soil to the atmosphere rather than eliminating them from the environment. Consequently, it is not widely accepted as an independent remediation strategy for heavy metals in agricultural systems. Instead, it is typically regarded as a supplementary approach within integrated remediation schemes or as a tool for studying plant-based detoxification processes [103].

5.5. Rhizofiltration

Rhizofiltration is a phytoremediation technique that uses plant roots to absorb, adsorb, and concentrate heavy metals from contaminated water rather than soil. Plants are typically cultivated in hydroponic or semi-hydroponic systems, such as floating mats, constructed wetlands, or riparian buffer zones, where contaminated water passes through root systems. The accumulated metals are removed through periodic harvesting of biomass [63,101].

Rhizofiltration is particularly relevant in agricultural systems, where irrigation water is a major pathway for heavy metal contamination of soils. Treating contaminated water before field application can reduce metal input and prevent long-term soil degradation [103,105].

Helianthus annuus is one of the most studied species for rhizofiltration due to its rapid root growth and high adsorption capacity. It has been successfully used in large-scale applications, including removal of radionuclides from contaminated water systems [105,106].

Eichhornia crassipes is a fast-growing aquatic macrophyte with high biomass production and strong capacity for metal uptake, although its invasive nature requires careful management.

Vetiveria zizanioides is characterized by a deep and dense root system, allowing interception of contaminants in water and subsurface flows.

Phragmites australis and *Typha latifolia* are widely used in constructed wetlands due to their tolerance to polluted conditions and efficiency in removing metals from drainage water [81].

Rhizofiltration systems are increasingly integrated into agricultural water management infrastructure, such as irrigation canals and drainage systems, enhancing both water quality and landscape functionality.

5.6. Choosing the Right Strategy

The selection of an appropriate phytoremediation strategy depends on multiple factors, including the type of metal, its concentration, the depth of contamination, land use objectives, and economic constraints. No single approach is universally optimal, and the choice must be adapted to site-specific conditions [101,105]. Table 3 summarizes the selection of phytoremediation strategies based on contamination characteristics and site conditions.

It links specific contamination profiles, such as Cd, Pb, As, or multi-metal pollution, with the most appropriate remediation approaches, including phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization. For each strategy, suitable plant species, estimated timeframes, and key limitations are provided. The table highlights that phytoex-

traction is effective but slow, while phytostabilization offers rapid risk reduction without removing contaminants. Chelation-assisted approaches enhance efficiency but may increase leaching risks. Rhizofiltration is emphasized for water treatment, and integrated strategies are recommended for large or complex contaminated sites.

Table 3. Phytoremediation strategies.

Contamination Profile	Strategy	Best Species	Time Horizon	Key Limitations	References
High Cd, moderate Zn, arable soil	Phytoextraction	*Thlaspi caerulescens*, *Sedum alfredii*	5–15 years	Low biomass; slow Zn removal	[82,95]
High Pb, agricultural/peri-urban	Chelation-assisted phytoextraction	*Brassica juncea* + EDTA/EDDS	3–7 years	Leaching risk; geogenic Pb resistance	[91,92]
High As, paddy/irrigated land	Phytoextraction	*Pteris vittata*	4–10 years	Phosphate competition; biomass disposal	[103]
Multi-metal, moderate levels	Phytostabilization + amendments	*Festuca rubra*, *Miscanthus* spp.	1–5 years	Does not reduce total metal content	[101]
Hg or Se contamination	Phytovolatilization	Transgenic *Arabidopsis*, *Nicotiana*; *Brassica* spp.	Variable	Regulatory limits; atmospheric dispersion	[102,104]
Contaminated irrigation water	Rhizofiltration	*Helianthus annuus*, *Phragmites australis*, *Typha latifolia*	Continuous	Biomass disposal; water management	[81,105]
Large area, mixed heavy metals	Phytoextraction + bioenergy	*Salix* spp., *Panicum virgatum*	5–10 years	Limited translocation; harvest logistics	[81]

Phytoextraction is suitable for soils contaminated with relatively mobile metals such as cadmium and zinc, although its efficiency may be limited by low biomass production in hyperaccumulator species [82,95]. Chelation-assisted phytoextraction can enhance metal uptake, particularly for lead, but it carries risks of increased metal mobility and leaching [91,92].

Phytostabilization is preferred for sites with moderate contamination or where rapid risk reduction is required. This strategy reduces metal mobility and bioavailability without removing contaminants from the soil [101]. In contrast, phytovolatilization is applicable to specific elements such as mercury and selenium, where contaminants can be transformed into volatile forms [49,102].

Rhizofiltration is particularly effective for treating contaminated irrigation water and preventing the transfer of metals into agricultural soils [105]. In large-scale or multi-metal contaminated areas, combined approaches—such as phytoextraction integrated with bioenergy production—may provide additional economic benefits while contributing to remediation goals [81].

Recent genome-engineering tools, particularly CRISPR/Cas systems, allow precise modification of metal transporters, chelator biosynthesis, and stress-response pathways in plants, thereby enhancing metal uptake, sequestration, and tolerance without compromising biomass production. Synthetic-biology approaches extend this further by engineering plants and associated microbes with synthetic metal-binding systems, redesigned metabolic routes, and responsive genetic circuits, creating tailored plant–microbe consortia for more efficient and controllable phytoremediation [107–109].

Excavation and soil replacement represent fundamentally different remediation philosophies. Unlike biological systems, they provide immediate contaminant removal or physical isolation. These methods become preferable when contamination is severe, when human exposure risk is acute, or when land must be returned rapidly to productive use. The plant detoxification mechanisms described in Section 3—antioxidant defence, phytochelatin synthesis, vacuolar sequestration—allow survival under stress, but they do not overcome the practical limitation that extreme contamination reduces biomass estab-

lishment and plant productivity. In such cases, phytoremediation becomes biologically possible but agronomically idealistic. A practical decision framework emerges from this comparison. First, contamination intensity must be assessed. Low-to-moderate contamination favors phytoremediation; extreme contamination favors excavation or soil replacement. Second, metal behavior must be considered. Metals such as Cd and Zn are more suitable for phytoextraction, while strongly sorbed metals such as Pb are better managed through stabilization. Third, remediation objectives must be defined. If the goal is contaminant removal, phytoextraction is preferable; if immediate exposure reduction is the priority, phytostabilization is more appropriate. Fourth, land use constraints are decisive. Agricultural land requiring rapid reuse is poorly suited to slow phytoextraction, whereas buffer zones, degraded land, or industrial margins are ideal candidates. Finally, economic and operational feasibility must be evaluated, including biomass disposal, monitoring, amendment requirements, and timeline expectations.

Thus, phytoremediation should not be presented as a universal alternative to conventional remediation, but as a selective, context-dependent strategy. Its greatest strength lies in moderately contaminated sites where ecological restoration, agronomic compatibility, and long-term sustainability outweigh the demand for rapid decontamination.

6. Chemical Enhancement of Phytoremediation

Even the most efficient hyperaccumulator faces a fundamental constraint: it can only take up metals that are dissolved in the soil solution around its roots. As discussed in Section 3.2, the majority of heavy metals in contaminated agricultural soils are bound to mineral lattices, adsorbed onto clay surfaces, connected to organic matter, or precipitated as insoluble carbonates and phosphates. If the bioavailable fraction is low, the plant's molecular uptake will be low and phytoextraction efficiency will be low as well [110,111]. Chemical enhancement represents applying specific compounds to the soil, either to mobilize metals and increase their bioavailability for plant uptake, or to immobilize metals and reduce their bioavailability to prevent food chain transfer. Thus, it is possible to shift the distribution of metals between soil fractions [112]. The choice between them is an important decision in remediation design and we shall detail both directions in this section. This complex does not let the metal be re-adsorbed on soil particles, keeping it available for plant uptake. When the chelating agent is added to a contaminate soil, it keeps it in the soil solution long enough to be captured by the root transporters [112–114].

The principle was discovered in the 1990s when researchers found that adding ethylenediaminetetraacetic acid (EDTA) to lead-contaminated soils followed by growing *Brassica juncea* resulted in increased concentrations of lead in shoots by 10 to 100 times compared to plants grown without chelation, stimulating the lead phytoextraction and making it viable for the first time, since lead is otherwise among the least mobile and least translocatable metals in soil [115,116].

6.1. Synthetic Chelating Agents: Mobilizing Metals for Phytoextraction

A chelating agent is a molecule with multiple functional groups, such as amine and carboxyl, that can simultaneously bind a metal ion, forming a stable, ring-shaped complex called chelate. It is a ligand with four carboxyl and two amine groups capable of binding almost any cationic heavy metal. Its effectiveness has been proven that 3–5 mmol diethylenetriamine (DETA) kg^{-1} soil increases diethylenetriaminepentaacetic acid (DTPA)-extractable metal concentrations and shoot metal contents of accumulator plants significantly compared to controls, with elevated antioxidant enzyme activities in EDTA-treated plants [113,117]. However, EDTA comes with a serious environmental liability that limits its regulatory acceptability for open-field application. EDTA is extremely

resistant to biodegradation, its half-life in soil being from months to years. The minimum effect half-life of EDTA on metal immobilization in soil was 36 days, with no measurable decrease in mobilization even 40 days after the application of high doses, meaning that the window of metal mobilization is long, and extends beyond the active plant uptake period [117].

Another problem with EDTA is the leaching risk. Because the EDTA-metal complexes are highly soluble and negatively charged, they are repelled by negatively charged soil particles that would normally retard metal movement through the soil profile. This means that EDTA-mobilized metals can rapidly leach to groundwater, especially in sandy soils or those with many macropores. Lead, which is almost immobile in unmodified soils, becomes highly leachable in the form of a Pb-EDTA complex [118,119]. EDTA disrupts calcium and magnesium nutrition in both plants and soil microorganisms, while at doses higher than 5 mmol kg^{-1} it partially reduces the chlorophyll content in the plants, having phytotoxicity [113,120]. The use of EDTA in agricultural soils is not approved under EU regulations for open-field remediation; its use is increasingly restricted to ex-situ conditions where leachate can be captured and treated [114,121]. Despite these limitations, EDTA remains the reference to which alternative chelating agents are evaluated, because its metal mobilization efficiency is among the highest achievable. Understanding its environmental impact helps in figuring out which are the important characteristics an ideal chelating agent should have.

The most promising alternative to EDTA is Ethylenediamine-N,N′-disuccinic acid (EDDS), specifically its S,S-isomer. It is the most scientifically validated biodegradable alternative to EDTA for the phytoextraction enhancement. Its structure is related to EDTA, but it is produced from naturally occurring S,S-amino acids. It was shown to be completely mineralized by soil microorganisms. EDDS performances are metal-specific [110,117,122]. For copper and zinc, EDDS performs similarly or better than EDTA in enhancing the phytoextraction, with EDDS-treated *Brassica juncea* and *Brassica rapa* exhibiting high shoot concentrations of the metals without the leaching risk [122]. EDDS is less effective than EDTA for lead in absolute mobilization terms, because Pb^{2+} forms a less stable complex in the case of EDDS, but due to its biodegradability, the window is shorter, with a half-life of one week or less, depending on the dose, compared to more than a month for EDTA, dramatically reducing the leaching risk [117]. For cadmium, EDDS is very effective and outperforms citric acid and tetrasodium glutamate diacetate (GLDA) in increasing the metal bioconcentration in plant tissues [122].

An important mechanistic discovery is that EDDS also stimulates the production of phytochelatin (PC) in the plants treated, through the increase in the activity of the glutathione pathway and the generation of increased concentrations of PC precursors. This dual action—on one side external soil metal solubilization, on the other side stimulation of internal plant detoxification—makes EDDS a very valuable tool to be integrated in phytoremediation strategies [110]. However, it also has some drawbacks. The shoot length of *B. juncea* and *B. rapa* is largely reduced in multi-metal contaminated soils, indicating a possible phytotoxicity effect at doses necessary for substantial metal mobilization. Thus, the optimization of the dose is necessary to balance metal mobilization against phytotoxicity [122].

Citric acid (CA) is the most widely used natural chelating agent to enhance phytoextraction. It is a component of the tricarboxylic acid cycle and degrades rapidly in soil in under a week. In comparative studies, CA was able to increase the extraction of Pb, Cu, Zn, and Cd, but it has an absolute mobilization efficiency lower than EDTA for most metals. Compared to EDTA, it is environmentally compatible, does not persist in soil, does not contaminate the groundwater and does not disrupt soil microbial communities, acting as a

biostimulant for some soil microorganisms, stimulating the enzyme activity, and potentially increasing the rate of decomposition of organic matter [114,123].

CA also has limitations; its relatively short soil half-life implies a narrow metal mobilization window, so its application must be precisely timed to peak plant root activity. CA chelating strength for Pb is much lower than that of EDTA and EDDS, making it less effective for lead removal. However, for other metals such as cadmium, zinc, and copper, CA is an effective, low-risk option, especially when combined with *Sedum alfredii* or *B. juncea* [114,124].

New biodegradable chelating agents have emerged as promising successors of EDTA as performance and regulatory perspective: tetrasodium N,N′-Bis(carboxymethyl)-L-glutamate (GLDA) and methylglycinediacetic acid, trisodium salt (MGDA). GLDA is derived from fermented glutamic acid, with a biodegradability higher than 60% in 28 days (OECD 301D certified). Its metal extraction performance closely mirrors that of EDTA for cadmium and nickel under optimal pH conditions, and it also has a better ecotoxicological profile. It does not produce a reduction in shoot length, nor chlorotic lesions, such as EDDS and CA. This phytotolerance is its single most important advantage over EDDS and CA for field-scale deployment, allowing higher doses to be applied without biomass loss [122]. MGDA is a phosphorus-free chelant with very high solubility (>500 g/L even in cold water) and stability across an unusually wide pH range (pH 2–13.5). Unlike EDTA, MGDA does not contribute to soil or water eutrophication, and it passed the OECD 301D biodegradation test. Its metal chelation spectrum makes it broadly applicable across contamination of multiple heavy metals. Its pH stability and selectivity over calcium make it suitable for calcareous soils where EDDS and CA perform poorly [125]. The EU regulation and sustainability chemistry frameworks are actively driving to replace EDTA with GLDA and MGDA in industrial applications, which will facilitate their use in soil remediation contexts.

Humic acid (HA) and fulvic acid (FA), the two major dissolved fractions of soil humic substances, are increasingly studied as multifunctional soil amendments that can simultaneously enhance the metal mobility and improve the soil biology and plant health. They are polydispersed molecules rich in carboxyl, hydroxyl and phenolic groups, which gives them strong capacity to form metal complexes, mostly with Cu^{2+}, Pb^{2+}, and Mn^{2+} [126–129].

Humic acid usually increases metal concentrations in both roots and shoots. In experiments using alfalfa, HA treatments increased the Mo concentrations in shoots and roots by almost two times compared to the control, promoting conversion from less mobile fractions to more mobile ones in the soil [128]. Optimal concentrations (500–2000 mg kg^{-1}) of HA improve phytoremediation performance in *Brassica rapa* under heavy metal stress, reducing the oxidative damage and increasing the biomass [126]. Fulvic acid has a smaller molecular size relative to HA, which allows it to form soluble metal complexes that remain in the soil solution, increasing the metal mobility through the soil profile but not necessarily into plants. Thus, FA is more relevant for applications that target metal leaching control than the promotion of phytoextraction [128]. The key practical advantage of humic substances over synthetic chelants is the promotion of soil microbial activity. The organic acids released during the decomposition of HA and FA stimulate the activity of microbial enzymes and favor the proliferation of plant growth-promoting bacteria (PGPB). This positions HA and FA amendments as natural bridges between chemical enhancement, as detailed in Section 6, and biological enhancement, as discussed in Section 7, making them ideal candidates for integrated treatment systems [123].

Therefore, synthetic chelators are effective in solubilizing metals, especially Pb and Cd, but they are not biodegradable, have a long soil persistence, and are prone to leaching, which leads to considerable environmental and regulatory concerns. In contrast, biodegrad-

able chelators such as EDDS, citric acid and low-molecular-weight organic acids generally have lower mobilizing efficiency but are more environmentally friendly because of their faster degradation and lower risk of groundwater contamination. Thus, chelator selection must be a compromise between extraction performance and persistence, ecological safety, and site-specific remediation objectives.

6.2. Soil Amendments for Metal Immobilization: Chemical Phytostabilization Support

While the chelating agents discussed in the previous section aim to increase the metal bioavailability for phytoextraction, a second class of chemical amendments aims to decrease the bioavailability and lock the metals in stable, insoluble forms that cannot be uptaken by plants, leach into the groundwater or be dispersed in the atmosphere. This approach is not remediation in the strict sense, but it is a necessary precursor to remediation or temporary protective measures for land that cannot be taken out of production long enough for extraction [112,130].

Agricultural lime, which is calcium carbonate, $CaCO_3$, and calcium oxide, CaO, increases the soil pH through the neutralization reaction between carbonate and soil acidity. As pH increases from 5 to 7, the bioavailable fractions of cadmium, lead, zinc, copper, and nickel decrease dramatically, because the metals precipitate as hydroxides, carbonates, and phosphates that are essentially insoluble near neutral pH. A study comparing different amendments derived from limestone found that different limestones had different performances, probably due to the purity, surface area and dissolution kinetic differences, which are very important practical factors when selecting the liming materials for field remediation programs in a specific geographic context [41,112,131]. The main limitation of pH-based immobilization is its reversibility: if the soil returns to acidic conditions, which could happen through acidifying fertilization, natural leaching, or cessation of lime application, the immobilized metals can be remobilized. Maintaining a soil pH above 6.5 requires periodic re-liming on most European agricultural soils, adding an ongoing management cost to the remediation program [41,132,133].

Biochar represents the carbonized residue produced by pyrolysis of organic materials such as crop residues, wood chips, sewage sludge, or animal manure, and has emerged as one of the most scientifically and agronomically attractive amendments for heavy metal immobilization in the past decade [134–136]. Biochar immobilizes heavy metals through several simultaneous mechanisms: surface adsorption, pH increase, enhancement of cation exchange capacity, and metal precipitation. Biochar has a very high specific surface area depending on the feedstock and pyrolysis temperature, with abundant functional groups on the surface that bind metal cations [134]. Most biochars are alkaline, and their application raises the soil pH, indirectly precipitating metals through the same mechanism as lime. At the high pH zones created around the biochar particles, metal phosphates and carbonate precipitate locally. Biochar application also increases the cation exchange capacity (CEC) of soil, providing more negatively charged surfaces for metal adsorption [134].

Beyond metal immobilization, biochar improves the water retention in soil, promotes soil microbial diversity, increases soil carbon storage, and improves aggregate stability [137,138]. It seems that combining biochar with phytostabilization plants produces a synergistic immobilization that exceeds what biochar or plant cover can achieve independently [139–141].

The combination of biochar and EDTA applied to *B. juncea* is particularly noteworthy since it achieved a Pb uptake of 60.2 mg g^{-1} compared to only 10 mg g^{-1} in the untreated control, suggesting that the biochar improved the conditions near the roots and enhanced the plant's capacity to exploit EDTA-mobilized lead. This apparent paradox was explained by the improvement of plant biomass and root architecture produced by the biochar,

which amplified the capacity of the plant to capture the metal even when it was externally mobilized by EDTA [44].

Phosphate fertilizers and hydroxyapatite are highly effective and selective for the immobilization of lead. Pb reacts with soil phosphate to form pyromorphite ($Pb_5(PO_4)_3X$, where X = Cl, OH, or F), which is one of the most stable lead compounds in soil. This reaction converts mobile bioavailable Pb^{2+} into an essentially permanent, insoluble mineral phase that does not respond to EDTA, rain or acidification [112]. The main risk of phosphate amendments is eutrophication; the excess phosphate that does not react with lead leaches to water bodies, potentially triggering algal booms. Application rates must be carefully calibrated to the content of lead in the soil to ensure stoichiometric phosphate-to-lead ratios that maximize pyromorphite formation while minimizing residual dissolved phosphate [112].

Farmyard manure (FYM), compost, and other organic amendments act as heavy metal immobilizers through mechanisms similar to those involved in the case of humic substances, such as metal complexation by carboxyl and phenolic groups, pH buffering, and promotion of the high-CEC organic fraction of the soil [130,142]. The limitations of organic amendments for metal immobilization mirror those of humic substances: as organic matter mineralizes, sequestered metals can be progressively released. However, if the organic amendments are applied recurrently as a normal agricultural practice, they will continuously regenerate the metal sorbing capacity of soil.

The efficacy of soil amendments for heavy metal remediation is highly site-specific and cannot be considered universally beneficial. Lime usually decreases the bioavailability of cationic metals such as Cd and Pb by increasing the pH but may increase the mobility of oxyanionic elements such as As and Cr in the alkaline conditions. Phosphate amendments can immobilize Pb by stable mineral precipitation; however, over-application may cause nutrient imbalance or eutrophication risks. Organic amendments, including compost, humic substances and biochar, generally decrease short-term metal mobility by sorption and complexation; however, decomposition dynamics, soil buffering capacity and varying pH conditions may subsequently remobilize certain metals [130].

6.3. Timing and Dose Optimization

The effectiveness of any strategy is strongly time-dependent, or it can cause more harm than benefits. For the phytoextraction based on the use of chelating agents, the optimal application window is the period of maximum root activity and transpiration, which is 4 to 6 weeks before harvest in annual species, during active elongation growth. Applying EDTA or EDDS too early, before the plant has built sufficient root biomass, wastes the chelant and extends the leaching risk window. If applying too late, after the plant has begun senescence, it will diminish the metal uptake [111,113].

For immobilizing amendments such as biochar, lime, and phosphate, the optimal timing is before planting, to allow the amendment to equilibrate with the soil matrix and establish stable metal-binding conditions before the roots of the plants begin developing in soil [112,130].

For all mobilizing chelants, there is also an optimal dose range beyond which increasing the concentration of the chelant will decrease the effects and increase phytotoxicity. The dose–response relationship is typically bell-shaped for shoot metal accumulation. Below the optimal dose, there is limited extraction due to insufficient metal mobilization. Above it, the oxidative stress induced in the plants by the chelant reduces the biomass and may produce the degradation and death of the roots, decreasing the total metal export per hectare despite higher shoot metal concentrations.

A comparative overview of the chemical enhancement approaches is presented in Table 4. These address the soil-side bottleneck of phytoremediation, which is the availability of the metals to plant roots. The plant-side bottleneck, which is maximizing the root uptake, translocation, and shoot accumulation, is addressed by biological enhancement through strategies described in Section 6.

Table 4. Comparative overview of chemical enhancement approaches. Information marked with N/A is not available.

Agent	Class	Target Metals	Biodegradability	Phytotoxicity Risk	Key Limitation	Best Combined with
EDTA	Synthetic chelant	Pb, Cd, Zn, Cu, Ni, Cr	Very low (months–years)	Moderate at >5 mmol kg^{-1} [113]	Groundwater leaching; EU restrictions	*B. juncea* (ex-situ only)
EDDS (*S*,*S*)	Biodegradable chelant	Cu, Zn, Cd, Pb	High (half-life 3.8–7.5 days) [117]	High (shoot length −38.5%) [122]	Less effective for Pb than EDTA; phytotoxic	*B. juncea*, *H. annuus*; dose-optimized
Citric acid	Natural organic acid	Cd, Zn, Cu	Very high (4–7 days) [114]	Low—improves plant [126]	Low efficacy for Pb; narrow mobilization window	*S. alfredii*, *B. juncea*
GLDA	Next-gen biodegradable	Cd, Ni, Zn, Cu	High (>60% in 28 days)	Very low (TI = 99–123%) [122]	Less studied than EDDS; performance varies by soil	*B. juncea*, energy crops
MGDA	Next-gen biodegradable	Zn, Pb, Cu, Ni	High (OECD 301D) [125]	Low	Limited field validation	Calcareous/alkaline soils
Humic acid	Humic substance	Cu, Pb, Mn, Mo	High (soil-derived)	Low—improves growth [126]	Dual-directional effects; dose-sensitive	PGPR consortia (Section 6)
Lime/$CaCO_3$	Inorganic amendment	Cd, Pb, Zn, Ni (via pH)	N/A (mineral)	None	Reversible; periodic re-application needed [41]	Phytostabilization grasses, biochar
Biochar	Organic amendment	Pb, Cd, Cr, As, Zn	N/A (stable C)	None—improves soil	Feedstock-dependent; not for phytoextraction alone	*Festuca*, *Miscanthus*, lime
Phosphate (KH_2PO_4)	Inorganic amendment	Pb (pyromorphite)	N/A (mineral)	None	Eutrophication risk; Pb-specific [112]	Phytostabilization in Pb hotspots
FYM/ compost	Organic amendment	Multiple HMs	High	None—improves fertility [130]	Temporary; mineralization re-releases metals	Phytostabilization; routine farm management

7. Biological Enhancement: Plant-Microbiome Interactions

The phytoremediation strategies discussed in Section 4 are ultimately limited by two interconnected constraints: the rate at which the plant can uptake metals from the soil, and the rate at which it can sustain the production of biomass needed to accumulate large quantities of metal per growing season. Chemical enhancement discussed in Section 6 addresses the first constraint, making the metals more available near the plant roots. Biological enhancement that addresses both constraints is based on the recruitment of microorganisms that colonize the rhizosphere and the root interior, and it will be detailed in this section. The logic of biological enhancement is that plants do not grow in isolation; in healthy soils, the plant roots are surrounded by complex and dense communities of microorganisms that have co-evolved with plants and exchange chemical signals, nutrients, and structural support with the host plants in ways that strongly influence the plant growth, stress tolerance, and resource acquisition. When the soils are contaminated with heavy metals, this natural relationship is disturbed, with metal stress suppressing the microbial diversity, reducing the microbial diversity near the roots, and weakening the ability of the plant to draw on its microbial allies [143–145]. This section examines the three principal categories of biological enhancement agents: plant growth-promoting rhizobacteria (PGPR),

arbuscular mycorrhiza fungi (AMF), and saprotrophic fungi, and ends with discussing the synergistic effects of PGPR-AMF, currently the most effective known enhancement strategy.

While biological enhancement techniques like mycoremediation fungi, Arbuscular mycorrhizal fungi (AMF), and plant growth-promoting Rhizobacteria (PGPR) have significant potential to improve phytoremediation performance, their effects are still highly context-dependent and not always advantageous. Microbial partners have been shown to promote plant growth, nutrient uptake, stress tolerance, or metal mobilization in controlled or moderately contaminated environments. In other situations, however, the same biological agents may limit plant uptake and promote phytostabilization by overly immobilizing metals in the rhizosphere, hence decreasing phytoextraction efficiency. In addition to host plant genotype, pollutant speciation, and contamination intensity, other factors that affect their efficacy include soil physicochemical characteristics such as pH, organic matter content, texture, and native microbial competition. For instance, AMF may reduce translocation to harvestable biomass by improving metal sequestration in roots through glomalin synthesis and vacuolar retention. Similarly, PGPR-mediated siderophore synthesis may enhance metal solubility in certain soils while having minor or opposing effects in others. Therefore, biological improvement should be viewed as a site-specific technique that requires precise alignment between microbial function, remediation target (phytoextraction versus phytostabilization), and local agronomic conditions rather than as a solution that can be used regularly.

7.1. Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria are free-living soil bacteria that colonize the rhizosphere or the root surface and offer measurable benefits to the host plant through specific mechanisms. The roots are continuously releasing 5 to 21% of total photosynthetically fixed carbon as root exudates, such as sugars, amino acids, organic acids and secondary metabolites, creating a uniquely nutrient-rich microenvironment that selectively enriches PGPR populations relative to those in the soil bulk. In return, PGPR produce many compounds and enzymes that contribute to improving plant growth, nutrient status, and tolerance to abiotic stress factors, including heavy metals [144–146]. The most thoroughly documented PGPR genera in the context of heavy metal phytoremediation are *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, *Burkholderia*, *Arthrobacter*, *Klebsiella*, *Streptomyces*, and *Alcaligenes*, all of which have in common the ability to produce compounds useful for plant growth.

7.1.1. Phytohormone Production

The most universal mechanism by which PGPR stimulates plant growth is the production of indole-3-acetic acid (IAA), the most abundant form of the plant hormone auxin. IAA produced by PGPR in the rhizosphere is taken up by the root cells, stimulating root elongation, lateral branching, and root hair formation. In the context of phytoremediation, these changes are very important. More lateral roots and more root hairs imply a larger area in contact with the contaminated soil, increasing the uptake. It also means that more root exudates and more transporter proteins will be released at the soil–root interface, where the metal uptake occurs [144,145,147].

Under heavy metal stress, IAA partially compensates for the inhibition of endogenous auxin synthesis that occurs when plants are exposed to toxic metal concentrations. For example, cadmium disrupts tryptophan metabolism, a precursor pathway for IAA synthesis, reducing endogenous IAA in roots and slowing their elongation. PGPR—supplied exogenous IAA restores the root development even in soils contaminated with Cd. Several PGPR strains produce cytokinins, which are a second class of phytohormones that promote cell

division and shoot growth, further supporting the biomass accumulation and increasing the capacity of uptaking metals [144,147].

7.1.2. Phosphate Solubilization

Phosphorus is the most important nutrient for plant growth after nitrogen; however, most of the total soil phosphorus is found in soil in insoluble mineral forms that cannot be directly absorbed by plant roots. Phosphate-solubilizing bacteria (PSB) are a subgroup of PGPR and have the role of releasing soil phosphate into forms that are available for the plants, by secreting organic acids (e.g., gluconic, oxalic, citric, malic, fumaric), acid phosphatases, and phytases that hydrolyze insoluble phosphate complexes. PSB includes *Pseudomonas fluorescens*, *Bacillus mageterium*, and *Aspergillus awamori*, the latter of which are fungi [144–146].

In the context of phytoremediation, phosphate solubilization has two functions beyond the nutritional one: it improves the energy economy of the entire plant, supporting the high metabolic costs of phytochelatin synthesis and metal sequestration described in Section 3, and allows the reaction of released phosphate to form pyromorphite, as described in Section 5.3, reducing the phytotoxic fraction of lead in the immediate root zone while PGPR-supported plant growth takes place [144,145].

7.1.3. The Iron Competition Mechanism

Siderophores are small, high-affinity iron-chelating molecules secreted by PGPR when there is limited iron present. Their main biological function is to scavenge Fe^{3+} from the soil and deliver it to bacteria as a soluble complex that is taken up via specific membrane receptors. This mechanism is important for phytoremediation for several reasons. Firstly, PGPR siderophores can also chelate and mobilize cadmium, lead, zinc, and nickel, metals that share sufficient chemical similarity with Fe^{3+} to form siderophore complexes. In soils contaminated with cadmium, siderophore-producing *Pseudomonas* and *Bacillus* strains increase the Cd concentrations in the rhizosphere, making more cadmium available for the plant roots and increasing the efficiency of the phytoextraction [148,149]. On the other hand, siderophore-producing PGPR compete with plant pathogens for available soil iron, thus protecting the plants growing on contaminated land from fungal and bacterial root diseases. This biotechnological function is especially relevant for phytoremediation plantings on abandoned industrial or agricultural land, where pathogen pressure can be high, and the plants are already under abiotic metal stress [148,150]. The third implication is that siderophores improve plant iron nutrition directly by delivering the Fe-siderophore complexes to the roots, where plant ferric reductase can free the Fe^{2+} for root uptake. Keeping adequate iron nutrition in phytoremediation plants is critical because iron deficiency up-regulates the expression of transporters, which increases cadmium uptake through molecular cross-reactivity [148,151].

7.1.4. ACC Deaminase

1-aminocyclopropane-1-carboxylate deaminase (ACC) is an enzyme produced by PGPR that promotes plant growth and stress tolerance. When a plant detects an abiotic stress, it synthesizes the amino acid derivative ACC in its root cells. ACC is then transported upward into the shoot, where it is converted into ethylene by the enzyme ACC oxidase, ethylene being the primary stress signaling hormone in plants. At low concentrations, it promotes root development and triggers protective responses. At high levels, it becomes inhibitory, suppressing root elongation, promoting early leaf senescence, reducing photosynthetic capacity, and decreasing the total plant biomass [152,153]. In the context of phytoremediation, this stress-induced biomass reduction is counterproductive: the plant

is accumulating metal in its tissues, but it is simultaneously decreasing the contaminated biomass [154,155].

PGPR that produce ACC deaminase intercept it before it can be converted to ethylene, the enzyme metabolizing ACC into α-ketobutyrate and ammonia, compounds that the bacteria can use as carbon and nitrogen sources. Consuming ACC at the root surface, ACC deaminase-producing PGPR maintain ethylene at levels low enough to allow normal root growth even under heavy metal stress. Multiple studies have demonstrated the increased root and shoot biomass, and thus, a greater metal uptake per growing season compared to plants without ACC deaminase-producing inoculants [144,145,147,155].

7.1.5. Metal Biosorption and Biotransformation by PGPR

PGPR directly interacts with heavy metals in the rhizosphere through biosorption and biotransformation. Biosorption is a passive process in which the cell wall components of PGPR (e.g., peptidoglycan, teichoic acids, lipopolysaccharides) have negatively charged functional groups that bind cationic heavy metals electrostatically. This creates a metal buffer in the rhizosphere, temporarily sequestering metals at the root surface in concentrations that are manageable for plant transporter systems [146,156]. Biotransformation is a metabolically active process. Some PGPR genera, such as *Bacillus*, *Pseudomonas*, and *Arthrobacter*, can change the oxidation state or the chemical form of specific metals through enzymatic reactions. The most agriculturally relevant example is the reduction of toxic hexavalent chromium, a water-soluble carcinogen, to the far less soluble and less phytotoxic trivalent form, through the activity of chromate reductase [157]. Similarly, arsenate reduction to arsenite by PGPR changes arsenic speciation and disrupts its root uptake dynamics, with important implications for arsenic phytoremediation using *Pteris vittata* [158].

7.1.6. Key PGPR Strains and Their Performance

Among the many PGPR strains that have been tested for phytoremediation enhancement, several stand out for the consistency and magnitude of their documented effects [144,145,159]:

- *Pseudomonas putida* produces high-affinity siderophores, stimulating the accumulation of Pb and Cd in shoots of *Brassica* spp. [160].
- *Bacillus subtilis* and *B. thuringiensis* are exceptional producers of ACC deaminase, maintaining root biomass under Cd and Pb stress [144,161].
- *Azospirillum brasilense* is a primary nitrogen fixer that is also responsible for phytohormone production, improving shoot biomass of hyperaccumulator plants under Ni and Zn stress [162].
- *Burkholderia cepacia* produces phytohormones to counteract the metal stress and is effective for Cu and Ni immobilization combined with *Thlaspi caerulescens* in Ni-contaminated soils [163].
- *Streptomyces* spp. is unique among PGPR in producing ligninolytic enzymes and being relevant for soils with co-contamination with organic pollutants alongside heavy metals [159,164].
- *Rhizobium* spp. are primarily nitrogen fixers used with leguminous phytoremediation cover crops and have a secondary role as phosphate solubilizers and metal complexation by exopolysaccharides [144].

7.2. Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (AMF) are members of the phylum Glomeromycota and are in mutualistic symbiosis with almost 80% of terrestrial plant species, including most of those that are relevant for phytoremediation, as discussed in Section 4. The term

"arbuscular" refers to the characteristic tree-like branching structures called arbuscules, of which fungal hyphae form inside the root cortex cells. These structures are the sites of the central nutrient exchange that define the symbiosis: the plant delivers photosynthetically fixed sugars to the fungus through the periarbuscular membrane while the fungus delivers phosphorus, zinc, copper, manganese, and other low-mobility nutrients from the soil into the plant [165,166].

The critical structural feature that makes AFM so important in phytoremediation is the extraradical mycelium (ERM), the network of fungal hyphae that extends outwards from the root surface into the surrounding soil, sometimes reaching 10 to 15 cm beyond the root tip. Individual fungal hyphae are between 2 to 10 μm in diameter, far thinner than the finest root hair, allowing them to penetrate soil pores and aggregates that are inaccessible to roots. The total hyphal length associated with a single plant can reach 100 m per gram of root, creating an enormous metal-sensing and metal-intercepting surface that dramatically extends the effective root system [167,168].

7.2.1. The Heavy Metal Paradox in AMF

One of the most complex aspects of AMF-assisted remediation is the so-called "heavy metal paradox", whereby AMF can either protect the plant from metal toxicity or enhance the metal accumulation depending on environmental and biological conditions [165,169]. This dual behavior arises from the complex interactions among soil metal concentration, contaminant speciation, fungal strain, host plant genotype, and soil physicochemical properties such as pH and organic matter content. Under certain conditions, AMF improve root growth, nutrient acquisition, and metal mobilization, thereby increasing plant uptake and, in some cases, root-to-shoot translocation. Such responses are advantageous in phytoextraction systems, where species such as *Glomus mosseae*, *Rhizophagus clarus*, and *Diversispora spurca* have been reported to enhance metal transfer to harvestable biomass, particularly in association with hyperaccumulator plants [166,170]. Conversely, under other conditions, AMF contribute to metal immobilization through glomalin production, rhizosphere sorption, and sequestration within fungal structures or root tissues, thereby reducing shoot translocation and limiting food-chain transfer. This outcome is particularly desirable in phytostabilization, where the reduction in contaminant mobility represents the primary remediation objective. *Rhizophagus irregularis*, one of the most extensively studied AMF species, has demonstrated the capacity to reduce cadmium and arsenic accumulation in several crop species [165,171]. Consequently, AMF inoculation should not be considered a universally beneficial enhancement strategy, but rather a site-specific intervention whose effectiveness depends on alignment between microbial function and the intended remediation objective.

7.2.2. Glomalin

A very important recent discovery in AMF-heavy metal research is the identification of glomalin-related soil protein (GRSP), a glycoprotein secreted abundantly by AMF hyphae and spores both inside and outside the root, as a key mediator of heavy metal immobilization in the rhizosphere [172,173]. Its metal-binding properties were only studied recently, despite its discovery in the early 90s. GRSP contains numerous functional groups on its outer surface that bind heavy metal cations, especially Pb^{2+}, Cd^{2+}, Cu^{2+}, and Zn^{2+}, with high affinity. A recent study designed to separate the contributions of extraradical mycelium (ERM) and glomalin-related soil protein (GRSP) to cadmium dynamics in soil showed that when ERM was disrupted by physically severing the hyphal network, cadmium concentrations in maize shoots and roots increased by more than 50% compared to plants with intact ERM. The disruption also increased cadmium concentrations in the

soil solution inside and outside of the growth core, as well as cadmium leaching. Modeling confirmed that the ERM reduced cadmium uptake and leaching indirectly through GRSP secretion, which increased cadmium adsorption in the soil phase and reduced free Cd^{2+} in the soil solution. This has important implications for remediation management, showing that the mechanical disturbance of the soil that destroys AMF hyphal networks removes this glomalin-based metal buffering capacity, potentially mobilizing previously stabilized metals. Thus, in programs based on the use of AMF, no-tillage or minimum tillage management practices are preferable to conventional ones [167,172].

7.2.3. AFM Improvement of Plant Nutrition and Antioxidant Defense

AFM have an important role in plant phosphate acquisition; under heavy metal stress, when the plant capacity of phosphorus acquisition is compromised, AMF-supplied P maintains the energy budget required for phytochelatin synthesis and active metal vacuolar transport [165,168]. The mycelium behaves as an extended water conducting network, which is very important for maintaining the metal flow driven by the transpiration under drought co-stress, which is increasingly relevant as climate change intensifies the summer drought [174]. AMF colonization also increases root branching and root hair density, producing an increased surface area able to absorb the metals, which complements the PGPR-driven root growth promotion [143,168]. Moreover, several AMF species increase the activity in roots of enzymes such as SOD, CAT, POD, and APX, reducing the oxidative damage and maintaining photosynthetic capacity, similar to that described for PGPR ACC deaminase, but achieved through a different molecular pathway [15,166]. The AMF species with the strongest evidence base across multiple metals, partners, and soil types are presented in Table 5.

Table 5. AMF species, key mechanisms and phytoremediation modes.

AMF Species	Primary Target Metals	Best Plant Partners	Key Mechanism	Phytoremediation Mode
Rhizophagus irregularis	Cd, As, Pb, Zn	Maize, wheat, *B. juncea*	Glomalin, P nutrition, antioxidant activity	Phytostabilization
Glomus mosseae	Cd, Cu, Zn	*S. alfredii*, sunflower	Enhanced translocation, root architecture	Phytoextraction
Rhizophagus clarus	Pb, Ni, As	*T. caerulescens*, *P. vittata*	Hyphal P uptake, As speciation	Phytoextraction
Diversispora spurca	Cd, Pb	*S. alfredii*, *B. rapa*	Shoot translocation stimulation	Phytoextraction
Funneliformis mosseae	Cr, Ni, Cu	Legumes, *Festuca*	Oxidative stress reduction	Phytostabilization

7.3. Mycoremediation: Saprotrophic and White-Rot Fungi

While AMF are plant symbionts that require a host root to complete their life cycle, there is another category of fungi, saprotrophic fungi, particularly white-rot basidiomycetes, which can colonize contaminated soil or organic substrates and directly remove heavy metals through biosorption, bioaccumulation, and enzymatic biotransformation. These mechanisms are different from the AMF phytoremediation, being called mycoremediation.

White-rot fungi (WRF) have a white appearance when breaking down lignocellulosic organic matter, degrading the brown lignin fraction. This capacity is mediated by some extracellular oxidative enzymes which produce reactive oxygen species able to oxidize not only lignin but also metals [175]. The first mechanism involved in metal reduction is passive bisorption. The cell walls of WRF are rich in chitin, glucuronic acid, and galacturonic acid,

all highly negatively charged at physiological pH, and therefore very effective biosorbents for cationic heavy metals [175,176]. Another mechanism is the active intracellular accumulation: living WRF cells take up metals across the plasma membrane into the cytoplasm, where they are chelated by metallothioneins and organic acids and sequestered in fungal vacuoles. The oxidative stress response of WRF to heavy metals is similar to that of plants: at sub-lethal doses, the activity of some antioxidant enzymes increases, while at toxic doses, the activity declines as the antioxidant capacity is overwhelmed [130,175].

Among the most studied WRF for heavy metal remediation are *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, and *Trametes versicolor*. *P. chrysosporium* removes Cd, Pb, Zn, and Cr from contaminated media with a very high efficiency, also producing extracellular oxidative enzymes, and has high chitin content in its cell wall [177]. *Pleurotus ostreatus* is an edible species with practical applicability, tolerant of cobalt and copper at moderate concentrations, and can accumulate Cu and Ni in its mycelium from contaminated soil. Its inoculation in co-contaminated soil due to acid rain and heavy metals led to a significant reduction of soil heavy metal availability in a short time on field-scale application [178,179]. *T. versicolor* has a very high biosorption capacity due to its multi-layered fruiting body structure. Its mycelia embedded in carboxymethylcellulose beads were very effective biosorbents for lead, cadmium, copper and chromium in contaminated water column experiments [176].

Besides the laboratory-models WRF species, several studies have characterized indigenous fungal strains isolated directly from contaminated industrial soils. *Aspergillus fumigatus* and *A. flavus* isolated from Pakistani industrial soils showed very high lead efficiencies in standard laboratory media. *Aspergillus niger* and *A. terreus* from the same study achieved very high mercury removal efficiency, positioning *Aspergillus* strains as promising candidates for field bioaugmentation programs in lead and mercury-contaminated agricultural soils [74]. The use of indigenous strains rather than commercial laboratory isolates offers a significant advantage since they do not require any acclimatization and are less likely to be outcompeted by the resistant microbial community after inoculation, which is a persistent problem with non-adapted commercial inoculants [74].

7.4. PGPR-AMF Combined Strategy

The individually reported effects of PGPR and AMF as remediation technologies are meaningful, but the most advances in the field of phytoremediation came from studies combining the two in a single treatment. The idea behind it is to improve the plant performance under heavy metal stress through largely complementary mechanisms [47,143]. PGPR operate in the bulk rhizosphere, improving nutrient solubilization and reducing ethylene through ACC deaminase activity, directly modifying the speciation of metals through siderophores and biotransformations. AMF operate at the root–soil interface and in the root cortex, extending the effective root system through ERM, providing phosphorus via arbuscular transfer, and sequestering metals via glomalin in the perirhizosphere. When combined, their spatial domains of action add together, each one enhancing the conditions for the other [143,144,165,167].

The synergism seems to take place at the molecular level: some PGPR strains produce compounds that facilitate the AMF spore germination and hyphal growth under metal stress conditions, which means that the inoculation of PGPR improves not only the plant performance but also the functional efficiency of the AMF; overall, the effects are amplified [143]. However, the amplification of the outcome depends very much on the compatibility between PGPR and AMF strains, the plant species, soil pH, and metal type. Incompatible combinations can even produce worse results than the separate inocula-

tion, which underlines the importance of strain compatibility screening before using the combination in the field [143].

8. Knowledge Gaps and Future Perspectives

Despite the rapid progress in understanding the interactions between plants, microbes, and soil under heavy metal stress, there are still many gaps that limit the reliable large-scale use of bioremediation in agricultural lands [180–182]. Most studies show successful bioremediation in short-term pots or lysimeter experiments, where the conditions are controlled, but only a few field trials have been reported, spanning over one to three growing seasons, with rarely monitored post-harvest distribution of the heavy metals in soil profiles or drainage water. This makes it difficult to estimate the realistic remediation time frames, the extent of risk reduction and what happens when the treatments stop. Thus, there is a need for long-term and multi-year experiments on farms in different climate areas and cropping systems.

Another issue is the co-contamination of the same soil with different metals, and the presence of another stressor, such as drought, salinity, nutrient imbalance, or pesticide residues. Most studies focus on single metals under optimal conditions; synergistic and antagonistic effects between these factors are still poorly characterized. Future work should prioritize experimentally realistic multi-metal and multi-stress scenarios, including response surface rather than single-factor designs.

Most studies present sequential extraction schemes, free ion activity models, and bioavailability indices, but not so much quantitative links between specific spoil fractions, plant uptake dynamics, and human and ecological risks. For example, the thresholds at which the reductions are meaningful are not stated. Integrating geochemical speciation models with plant uptake models and validating them against field data in different soil types would allow establishing clear targets for remediation.

The number of studied species used for phytoremediation is limited. Yet, there are other wild relatives, orphan crops, well-adapted to marginal soils that may harbor useful potential for bioremediation. A systematic screening of regional germplasm collections and wild flora, especially historically contaminated or from metalliferous regions, could identify new candidate species and genotypes better suited to local climate conditions and farming systems.

Besides the already mentioned gaps, the mechanistic basis for the positive effects, such as those proven for PCPR or AMF, is still not very well known. Figuring the insights of these mechanisms would allow a predictive understanding of when a given combination will increase phytoextraction, favor phytostabilization, or just improve plant growth with little effect on metal fluxes (Table 6). The practical selection of remediation strategies requires evaluation beyond mechanistic effectiveness alone, as field implementation is constrained by economic, operational, and environmental factors. While excavation and soil replacement provide rapid contaminant removal, they are often associated with high financial costs, major site disturbance, and secondary waste management challenges. In contrast, phytoremediation approaches generally offer lower implementation costs and improved ecological compatibility, but require extended timeframes, careful biomass management, and may be limited by contaminant bioavailability and site-specific agronomic constraints. Chemical enhancement strategies may accelerate remediation efficiency but introduce additional risks related to metal leaching, amendment persistence, or environmental acceptability. Biological enhancement approaches similarly show variable scalability due to context-dependent performance. Therefore, practical remediation planning should integrate not only contaminant characteristics and removal mechanisms, but also cost,

treatment duration, biomass disposal, leaching risk, scalability, and compatibility with intended land use.

Table 6. Comparative practical assessment of major heavy metal remediation strategies for contaminated agricultural soils.

Remediation Strategy	Primary Mechanism	Target Metals	Typical Time Scale	Relative Cost	Secondary Contamination Risk	Biomass Management Issues	Scalability Applicability	Main Limitations
Excavation	Physical removal of contaminated soil	All metals	Immediate to weeks	Very high	Low (if managed properly)	Requires disposal of hazardous excavated soil	Low–moderate (site-specific)	Expensive, highly disruptive, unsuitable for large agricultural areas
Soil replacement	Removal and substitution with clean soil	All metals	Immediate	Very high	Low	Disposal of contaminated soil required	Low	Logistically difficult, costly, major ecosystem disturbance
Phytostabilization	Immobilization in rhizosphere/root zone	Pb, Cr, As, Cu	Months to years	Low	Low–moderate	Minimal biomass concerns	High	No contaminant removal; requires long-term monitoring
Phytoextraction	Plant uptake + harvest removal	Cd, Zn, Ni, Cu	Years to decades	Low–moderate	Moderate	Contaminated biomass requires controlled disposal	Moderate	Slow remediation; dependent on biomass productivity
Chelator-assisted phytoextraction	Increased metal solubilization and uptake	Pb, Cd, Zn	Months to years	Moderate	High	Contaminated biomass disposal required	Limited	Leaching risk, groundwater contamination, regulatory restrictions
Soil amendments (lime, phosphate, biochar, compost)	Immobilization/adsorption/precipitation	Pb, Cd, Cu, Zn (context-dependent)	Months to years	Low–moderate	Variable	Usually minimal	High	Effects strongly dependent on pH, amendment stability, metal speciation
PGPR-assisted phytoremediation	Growth stimulation/metal mobilization/stress tolerance	Cd, Zn, Cu, Ni	Years	Low–moderate	Variable	Biomass management depends on strategy	Moderate	Strong soil- and host-specific variability
AMF-assisted phytoremediation	Enhanced uptake OR immobilization (“heavy metal paradox”)	Cd, Zn, As, Pb	Years	Low–moderate	Low–moderate	Limited biomass concerns	Moderate	Outcomes highly context-dependent; may favor stabilization instead of extraction
Mycoremediation (fungal systems)	Biosorption/bioaccumulation/enzymatic transformation	Pb, Cd, Cr, Hg	Months to years	Moderate	Variable	Fungal biomass management required	Limited–moderate	Lower field standardization, environmental sensitivity

As field-scale phytoremediation transitions into active agricultural landscapes, future deployments must increasingly account for the disruptive impacts of climate change. Extreme weather events, such as prolonged droughts and recurrent flooding, dramatically

alter soil moisture and redox conditions, fundamentally shifting metal speciation and bioavailability. For instance, flooding-induced anaerobic conditions can rapidly mobilize highly toxic arsenite, while severe droughts tend to concentrate bioavailable metal cations in the root zone. Furthermore, elevated global temperatures alter rhizosphere microbiology and compound the abiotic stress on hyperaccumulator crops, potentially reducing their overall biomass yield and transpiration-driven heavy metal uptake. Consequently, optimizing bioremediation strategies for the future will require designing climate-resilient plant–microbe combinations capable of sustaining metal extraction and stabilization under increasingly volatile environmental extremes. Most assessments of phytoremediation emphasize removal efficiencies rather than costs, labor requirements, mechanical needs, or opportunity costs of taking the land partially or totally out of production. Developing simple but transparent decision tools that integrate agronomic performances, costs and benefits would help the farmers choose a context-appropriate strategy. Last, but not least, there is a need for regulatory frameworks that do not focus only on the total metal concentration thresholds but recognize bioremediation in soil protection and agricultural policy, with standardized protocols for certifying that a field has been safely remediated using this technology. Also, farmers and the community may be wary of using the bioremediation based on hyperaccumulator crops or microbial inoculants, especially when biomass handling and disposal routes are not clear. Future work should address these institutional and social dimensions.

In perspective, progress is likely to come from integrated approaches that combine moderately accumulating, high-biomass crops with carefully selected microbial consortia and tailored soil amendments, designing crop rotations where remediation species alternate with crops that can maintain the farm income, or embedding bioremediation trials within the farmland to accelerate learning in the respective climate conditions. Addressing these knowledge gaps and implementing integrated strategies is essential to move bioremediation of heavy metal-contaminated agricultural soils from promising case studies to reliable field-scale tools.

9. Conclusions

Heavy metal contamination of agricultural soils represents a persistent and escalating environmental issue that cannot be effectively remediated through natural attenuation processes alone, particularly in the context of the high and continuously increasing rates of anthropogenic inputs. The evidence reviewed here shows that bioremediation based on plants in combination with targeted chemical and biological enhancement offers a technically viable and agronomically compatible way to reduce the metal concentration in contaminated fields. The outcome depends on treating bioremediation as a flexible toolbox that can be tailored to site-specific contamination profiles, soil properties, and farming objectives.

Heavy metal remediation in agricultural soils is best understood as risk reduction and soil function recovery rather than absolute decontamination. Mechanistically, remediation outcomes hinge on (i) soil chemistry controls on speciation and bioavailability that govern root-accessible metal pools; (ii) plant uptake and detoxification pathways that set species-specific capacity and tissue partitioning; and (iii) microbially mediated processes that either mobilize or immobilize metals. At the soil and plant level, progress in understanding root uptake pathways and detoxification mechanisms has clarified why some species behave as hyperaccumulators while others are very sensitive to heavy metal stress. This analysis highlights important limitations and trade-offs. Taken together, the evidence suggests that heavy metal remediation is best framed as risk management and soil rehabilitation, not absolute decontamination, the most promising strategies being those that:

- ➢ Define realistic endpoints in terms of reduced plant uptake (for the agricultural crops), leaching, and exposure of humans and animals;
- ➢ Use site-specific combinations of plants, amendments and microbes to fit the metal type, contamination depth, soil properties, hydrology, and other constraints related to the use of the land;
- ➢ Integrate remediation crops into rotations, buffer zones, aligning them with existing agricultural operations and value chains;
- ➢ Ensure that the harvested contaminated biomass is handled safely.

Framed as a flexible toolbox that combines plants with targeted chemical and biological enhancement, tailored to metal type, contamination depth, soil properties, hydrology, and farm objectives, phytoremediation can deliver credible, agronomically compatible reductions in exposure risk when evaluated against standardized, regulatory-relevant endpoints and supported by mature biomass management pathways.

Future research should focus on expanding long-term field evidence, refining the decision tools that consider all factors, and aligning policy frameworks so that the plant and microbe-based approaches are explicitly recognized as valid remediation options. This can take the bioremediation from scattered case studies to a mainstream component of heavy metal risk management in agricultural landscapes.

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Abbreviations

The following abbreviations are used in this manuscript:

HM	Heavy metals
EU	European Union
UESPA	United States Environmental Protection Agency
SOM	Soil organic matter
DNA	Deoxyribonucleic acid
ALAD	delta-aminolaevulinic acid dehydrase
EFSA	European Food Safety Authority
ABC	ATP-binding cassette
ROS	reactive oxygen species
ATP	Adenosine triphosphate
ZIP	Zrt/Irt proteins
Zrt	Zinc-regulated transporter
Irt	Iron-regulated transporter
NRAMP	Natural Resistance-Associated Macrophage Proteins
MRP	multidrug resistance-associated protein
ABCC	ATP-binding cassette subfamily C
MTP	Metal tolerance proteins
MATE	Multi-antimicrobial extrusion
HMA	Heavy Metal ATPase

SOD	superoxide dismutase
CAT	catalase
APX	ascorbate peroxidase
BCF	Bioconcentration Factor
BAF	Bioaccumulation Factor
TF	Translocation Factor
EDTA	ethylenediaminetetraacetic acid
DTPA	Diethylenetriaminepentaacetic acid
DETA	diethylenetriamine
EDDS	Ethylenediamine-N,N′-disuccinic acid
GLDA	tetrasodium glutamate diacetate
MGDA	methylglycinediacetic acid, trisodium salt
PGPB	plant growth-promoting bacteria
CEC	cation exchange capacity
FYM	Farm yard manure
PGPR	plant growth-promoting rhizobacteria
AMF	arbuscular mycorrhiza fungi
IAA	indole-3-acetic acid
PSB	Phosphate-solubilizing bacteria
ACC	1-aminocyclopropane-1-carboxylate deaminase
GRSP	glomalin-related soil protein
ERM	Extraradical mycelium
WRF	White-rot fungi

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