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Review

Phytoremediaton Strategies for Co-Contaminated Soils: Overcoming Challenges, Enhancing Efficiency, and Exploring Future Advancements and Innovations

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Abstract: Soils co-contaminated with petroleum hydrocarbons (PHs) and heavy metals pose significant challenges, such as reduced bioavailability of pollutants, toxic effects on soil microorganisms, and unpredictable chemical interactions. These complex interactions hinder effective remediation. Phytoremediation, which utilizes plant and microbial processes, offers a sustainable and eco-friendly approach. However, its effectiveness is often constrained by the intricate interplay among PHs, heavy metals, and soil components, which complicates pollutant degradation and microbial activity. This review explores the interactions between enhancement strategies, including soil amendments, plant growth-promoting bacteria (PGPB), and genetic engineering, which can synergistically enhance pollutant degradation and remediation efficiency. Key challenges include competition for soil adsorption sites among contaminants, microbial community disruptions, and environmental variability. Moreover, the limitations of these strategies, including their reliance on specific plant species, sensitivity to environmental variability, and the necessity for long-term monitoring, are discussed. The proposed solutions focus on integrating emerging technologies and interdisciplinary approaches to overcome these challenges and improve pollutant removal efficiency. Future advancements in interdisciplinary approaches, integrating biological techniques with technological innovations, are highlighted as key to addressing the complexities of co-contaminated environments and improving pollutant removal efficiency.

Keywords: phytoremediation; co-contaminated soil; petroleum hydrocarbons (PHs); heavy metals; phytoremediation enhancement

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1. Introduction

Soil is a vital component of the natural environment, providing essential services such as biomass production and water filtration as well as a habitat for various organisms [1,2]. However, rapid industrialization since the 20th century has led to significant soil degradation due to the release of harmful pollutants, particularly petroleum hydrocarbons (PHs) and heavy metals [3,4]. These contaminants pose serious threats to both ecosystems and human health [4,5].

PHs are persistent organic pollutants derived from petroleum-based activities, known for their stability and prolonged presence in soil [6,7]. Similarly, heavy metals are toxic and mutagenic, with long-lasting environmental impacts [8]. These pollutants often co-occur in contaminated sites due to overlapping anthropogenic sources, complicating

remediation [9,10]. Approximately 40% of contaminated sites in the United States are co-contaminated with PHs and heavy metals [9]. Similar trends have been observed globally, highlighting the pervasive nature of this issue [4]. The interactions of PHs and heavy metals with soil components can affect their bioavailability, reactivity, and toxicity, leading to unpredictable remediation outcomes [4,10,11]. Compared to single-pollutant remediation, restoring co-contaminated soils presents unique challenges. The competition between pollutants for binding sites on soil particles, their impact on soil biota, and complex interactions all contribute to the difficulty of remediating soils contaminated with PHs and heavy metals [4]. While conventional remediation methods are effective, they often come with high costs and may cause further soil disruption [10].

These limitations of conventional remediation methods highlight the need for alternative approaches that are both cost-effective and environmentally sustainable. Phytoremediation has emerged as a promising alternative for restoring co-contaminated soils due to its environmental benefits, cost-effectiveness, and ability to utilize plants' natural abilities to degrade, absorb, or stabilize pollutants [12,13]. This technology leverages the synergy between plants and soil microorganisms, offering a sustainable solution for contaminated soil restoration. While phytoremediation has been extensively studied for single pollutants, such as PHs or heavy metals, research on its application to co-contaminated soils remains limited [4]. Interest in phytoremediation has grown steadily over the past 20 years; however, much of the research has focused on soils contaminated with heavy metals (Figure 1), leaving multi-pollutant scenarios underexplored. As co-contaminated sites become increasingly prevalent, a deeper understanding of the interactions between PHs and heavy metals and their behavior in soil is essential for advancing phytoremediation techniques.

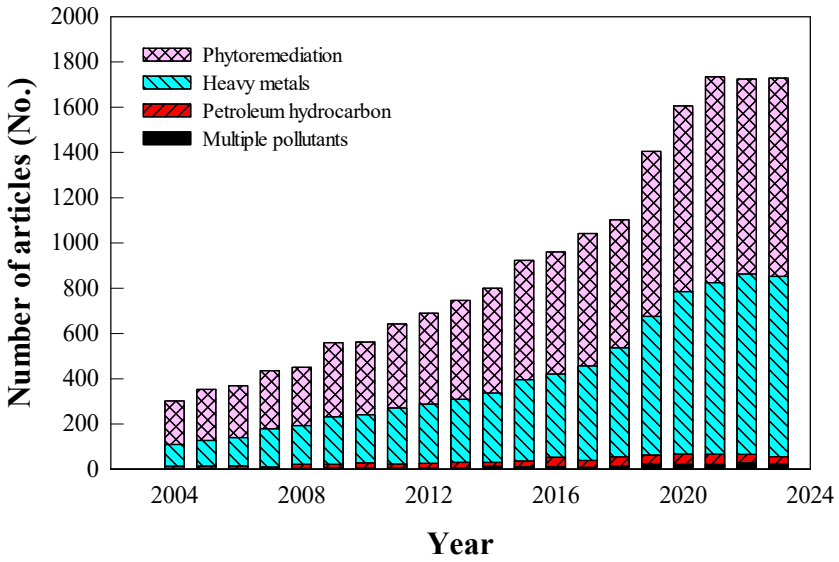


Figure 1. Number of articles on phytoremediation research related to heavy metals, petroleum hydrocarbons (PHs), and multiple pollutants over the past 20 years.

This review aims to address the critical challenges associated with remediating soils co-contaminated with PHs and heavy metals, emphasizing their complex interactions and impacts on pollutant bioavailability and microbial activity. Building on prior studies that predominantly focused on single pollutant remediation, this work provides a comprehensive analysis of the unique challenges and synergies involved in multi-pollutant phytoremediation. By bridging scientific insights with practical applications, this review not only expands the understanding of co-contaminated soil remediation but also seeks to guide future research and promote effective environmental management practices.

2. Methodology

This review was conducted through a comprehensive literature analysis of phytoremediation in co-contaminated soils. The research focused on four key areas: (1) the interactions and co-occurrence of PHs and heavy metals in contaminated soils, (2) key phytoremediation mechanisms, (3) challenges associated with remediating co-contaminated soils, and (4) emerging strategies to enhance phytoremediation efficiency.

Data were collected using the Web of Science, Scopus, and PubMed databases. The search terms included "phytoremediation", "petroleum hydrocarbons", "heavy metals", and "enhancement" combined with Boolean operators such as "AND", "OR", and "NOT" to refine the search results. The literature search primarily focused on studies published within the last 10 years (2014–2024) to capture recent advancements, while earlier studies (2004–2013) were reviewed selectively to provide historical context.

Studies were selected based on their relevance to co-contaminated soils, publication year, methodological rigor, and comprehensiveness. Relevance was determined by the study's focus on co-contaminated soils containing both PHs and heavy metals. Methodological rigor was assessed based on experimental design, data analysis, and reproducibility of findings, while comprehensiveness was evaluated by examining the depth of analysis in terms of phytoremediation strategies, mechanisms, and field applications.

Table 1 summarizes the key studies, categorizing them by the pollutants studied, plant species utilized, enhancement strategies employed, and major findings. This overview provides a concise representation of the current state of phytoremediation research and highlights areas for further exploration.

Table 1. Summary of key studies on phytoremediation strategies for contaminated soils.

Authors and Year	Pollutant	Plant Species	Enhancement Strategy	Key Findings	Ref.
Choi et al. (2007)	HMs	*Populus* species	Genetic engineering: Mercuric ion reductase (*mer*A) gene	Introduced *mer*A gene from *Staphylococcus aureus* into plants (*Populus* species). Enhanced the plants' tolerance to ionic mercury.	[14]
Gasic and Korban (2007)	HMs	*Brassica juncea*	Genetic engineering: Phytochelatins synthase (*AtPCS1*) gene	Transgenic *B. juncea* expressing *AtPCS1* exhibited higher tolerance to Cd and As.	[15]
Israr and Sahi (2008)	HMs	*Sesbania drummondii*	Soil amendment: phytohormones (IAA)	The combination of IAA and EDTA significantly enhanced Pb accumulation in plant shoots.	[16]
Siddiqui et al. (2008)	HMs	*Brassica* plants	Soil amendment: phytohormones (gibberellin)	The combination of nitrogen and gibberellin (GA) effectively improved plant biomass and enzymatic activities.	[17]
Zhao et al. (2010)	HMs	*Zea mays*	Soil amendment: EDTA	EDTA significantly improved the efficiency of phytoextraction from metal-contaminated soil.	[18]
Brunetti et al. (2011)	HMs	*Nicotiana tabacum*	Genetic engineering: Phytochelatins synthase (*AtPCS1*) gene	The overexpression of the phytochelatins synthase genes (AtPCS1) enhanced Cd tolerance in tobacco.	[19]
Panwar et al. (2011)	HMs	*B. juncea*	Soil amendment: Vermicompost, EDTA PGPB inoculation: *Azotobacter* sp., *Pseudomonas* sp.	Vermicompost combined with PGPB inoculants significantly enhanced Cd uptake. EDTA and PGPB inoculant treatment showed the highest Cd concentration in shoots.	[20]
Park et al. (2011)	Both	*Festuca arundinacea*, *Helianthus annus*, *Brassica campestris*	Soil amendments: Humic acid	Humic acid increased degradation of TPH in the soil.	[21]
Al-wabel et al. (2015)	HMs	*Z. mays*	Soil amendment: Biochar	Increased shoot dry biomass of plants and reduced heavy metal concentrations in plants.	[22]
Chigbo and Batty (2015)	Both	*Z. mays*	Soil amendment: EDTA, citric acid	EDTA with citric acid improved Cu uptake in co-contaminated soils.	[23]
Agnello et al. (2016)	Both	*Medicago sativa*	PGPB inoculation: *Pseudomonas aeruginosa*	Bioaugmentation-assisted phytoremediation with *P. aeruginosa* and *M. sativa* achieved the highest TPH removal and stabilized heavy metals in roots.	[24]
Franchi et al. (2016)	PHs	*B. juncea*	PGPB inoculation: PH-degrading bacterial consortium	The inoculation of bacterial consortium increased seed germination, improved heavy metal uptake, and resulted in a higher removal rate of hydrocarbons.	[25]

Table 1. *Cont.*

Authors and Year	Pollutant	Plant Species	Enhancement Strategy	Key Findings	Ref.
Chen et al. (2017)	Both	*Scirpus triqueter*	PGPB inoculation: *Klebsiella pneumoniae*	The addition of PGPB efficiently decreased bioaccessible fractions of pyrene and increased the bioavailability of Ni.	[26]
Marchand et al. (2018)	Both	*M. sativa*, *H. annuus*	Soil amendment: Compost	Compost-amended soils planted with *M. sativa* effectively stabilized heavy metals (Cu and Pb) and hydrocarbons.	[27]
Steliga and Kluk (2020)	Both	*F. arundinacea*	Soil amendment: Fertilizer	A decrease in soil toxicity was observed during phytoremediation assisted by the fertilization process.	[28]
Wang et al. (2020)	HMs	*Z. mays*	Soil amendment: Organic manure, Biochar, Hydroxyapatite	Hydroxyapatite was the most effective amendment for stabilizing heavy metals (Cd, Pb, and Zn) in contaminated soil.	[29]
Choden et al. (2021)	Both	*Ocimum gratissimum*	PGPB inoculation: Pseudomonas putida MU02	The combination of *O. gratissimum* and *P. putida* achieved the highest TPH reduction, while the highest metal uptake was observed in plants without PGPB inoculation.	[30]
Zhang et al. (2020)	Both	*S. triqueter*	PGPB inoculation: *K. pneumoniae*	PGPB inoculation increased dissipation rates of pyrene and removal rates of Ni.	[31]
Cao et al. (2022)	Both	*M. sativa*, *Solanum nigrum*	PGPB inoculation: *Bacillus subtilis*, *Sphingobacterium multivorum*	Improved soil enzyme activity and microbial diversity.	[32]
Rahid et al. (2022)	HMs	*Z. mays*	Soil amendment: Compost, Biochar	Amendments reduced exchangeable-soluble fraction of HMs by lowering their mobility.	[33]
Song et al. (2022)	Both	*Kochia scoparia*	Soil amendment: Biochar PGPB inoculation: *Bacillus* sp. KSB7	KSB7-loaded biochar accelerated the phytoremediaton of PAHs and heavy metals.	[34]
Lee et al. (2023a)	Both	*F. arundinacea*	Soil amendment: Compost	*F. arundinacea* achieved a 63% TPH removal over 571 days, with Cd showing the highest uptake efficiency.	[35]
Lee et al. (2023b)	Both	*Z. mays*	Soil amendment: Compost	Phytoextraction achieved 75% TPH removal in co-contaminated soils, with heavy metal removability ranging from 3 to 63%.	[36]
Lee et al. (2023c)	Both	*F. arundinacea*	PGPB inoculation: *Novosphingobium* sp. CuT1	CuT1 increased the root biomass of plants and stimulated microbial enzyme activities.	[37]

Table 1. *Cont.*

Authors and Year	Pollutant	Plant Species	Enhancement Strategy	Key Findings	Ref.
Shi et al. (2023)	Both	*Lolium perenne*	PGPB inoculation: Pseudomonas aeruginosa	*P. aeruginosa* application improved the phytoremediation efficiency of *L. perenne* on soil contaminated with nonylphenol and Cd, with a minimal risk of alien microbial invasion.	[38]
Reddy et al. (2024)	Both	*Avena sativa*, *Lolium perenne*, *F. arundinacea*, *H. annuus*	Soil amendment: Compost	Improved soil conditions and enhanced the immobilization of heavy metals.	[39]

3. Soil Contamination by Petroleum Hydrocarbons and Heavy Metals

Rapid industrial development has led to widespread contamination of soils with various pollutants, particularly PHs and heavy metals [4,11]. These pollutants are persistent and pose significant risks to ecosystems and human health. PHs, primarily generated through petroleum-based anthropogenic activities, are highly stable and can remain in the soil for extended periods [6]. The sources of PHs in the environment are diverse due to the wide variety of hydrocarbon components. They are primarily released through uncontrolled anthropogenic activities, including oil and gas production, transportation, storage, tank leakages, petrochemical industry discharges, accidental spills, and ruptured underground pipelines [6,40]. PHs significantly alter the physicochemical properties of soil by disrupting nutrient composition, organic matter balance, and microbial communities, ultimately impairing nutrient cycling and plant growth [41]. At concentrations exceeding 3% in soil, PHs pose a severe threat to soil biota [42]. Their hydrophobic nature binds them to soil particles, reducing bioavailability while prolonging environmental persistence [42]. In humans, PH toxicity varies by composition and exposure level, causing short-term effects, such as dizziness and skin irritation, as well as long-term issues, such as cancer, mutagenicity, and neurotoxicity [6,43].

Heavy metals, such as lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn), are significant environmental pollutants due to their toxicity and mutagenic effects [8]. Unlike organic pollutants, heavy metals do not degrade, making them a long-term challenge for soil remediation [4,11]. Anthropogenic sources of heavy metals include mining and smelting, industrial processes, atmospheric deposition, agriculture, and waste disposal. Among these, mining and smelting are the most significant contributors, generating large volumes of tailings and causing extensive environmental damage, particularly around abandoned sites [44]. Industrial activities, such as fossil fuel combustion, electroplating, and battery production, release heavy metals, such as Cu, Pb, and Cd, into the soil [45]. Similarly, the use of phosphoric fertilizers and pesticides in agriculture introduces toxic metals, including Cd and Pb, which gradually deplete soil fertility over time [46]. In ecosystems, heavy metals induce oxidative stress in plants by generating reactive oxygen species (ROS), leading to cellular dysfunction, DNA damage, and impaired photosynthesis, ultimately reducing plant growth and crop yield [47,48]. In humans, heavy metals are absorbed through ingestion or inhalation, accumulating in vital organs, such as the liver, kidneys, and brain. This bioaccumulation causes severe health issues, including dysfunction, cancer, and, in extreme cases, death [49,50].

When PHs and heavy metals co-occur in soil, their interactions create complex challenges for remediation efforts. For example, the interactions between PHs and heavy metals in soil can alter their speciation, reactivity, and bioavailability [4]. These interactions can affect the efficiency of remediation processes by changing pollutant mobility, adsorption–desorption dynamics, and the biological activity of soil organisms [11]. Moreover, PHs and heavy metals compete for sorption sites on soil particles, further influencing their environmental behavior [4]. Given the complexity of these interactions, soils co-contaminated with PHs and heavy metals require a comprehensive, tailored remediation approach specific to contaminants and soil conditions [4,10].

The behavior of pollutants and the efficiency of remediation in co-contaminated soils are influenced by various factors. Soil properties, such as pH, organic matter content, cation exchange capacity, and oxidation–reduction potential, play a critical role in determining the bioavailability and mobility of pollutants [12]. For example, acidic soils increase the solubility of heavy metals, enhancing their mobility, whereas alkaline conditions promote precipitation, reducing their availability [13]. Environmental factors, including temperature, moisture levels, and oxygen availability, significantly affect the degradation rates of PHs

and the activity of soil microorganisms [41]. Furthermore, microbial communities are essential for pollutant transformation, as microbial diversity, population density, and enzymatic activity influence the degradation and immobilization of pollutants [51].

Conventional soil remediation methods are classified into physical, chemical, and biological approaches, each offering distinct advantages and limitations [52]. Physical remediation separates pollutants from soil through mechanical or physical processes, such as electrokinetic treatment, vacuum extraction, thermal treatment, and soil washing [53]. Although effective, these methods demand specialized equipment, high energy consumption, and large-scale operations [53]. Chemical remediation utilizes agents like surfactants and oxidants to transform pollutants into less harmful forms [53]. While chemical methods are rapid and efficient, they are often associated with secondary pollution and high costs [52,53]. In contrast, biological remediation relies on the metabolic activities of living organisms, including plants, microorganisms, and algae, to degrade pollutants in the soil environment [3]. The techniques, such as biostimulation, bioaugmentation, and phytoremediation, are environmentally sustainable, cost-effective, and rely on natural biological processes [53,54]. However, biological methods require extended timeframes, ranging from months to years, to achieve effective pollutant removal but have fewer long-term environmental impacts compared to physical and chemical approaches [6].

4. Phytoremediation as a Sustainable Solution

Phytoremediation is an emerging, eco-friendly technology that utilizes the natural capabilities of plants to remove, stabilize, degrade, or sequester pollutants from contaminated soils [6,12,55]. Compared to traditional physical and chemical methods, phytoremediation is cost-effective, less environmentally disruptive, and sustainable [56]. Several mechanisms underlie phytoremediation, which vary depending on the plant species, type of contaminant, and soil conditions [12,13]. The main types of phytoremediation are described in detail below (Figure 2). While each mechanism has distinct advantages and limitations, their combined application can address the complexity of co-contaminated soils.

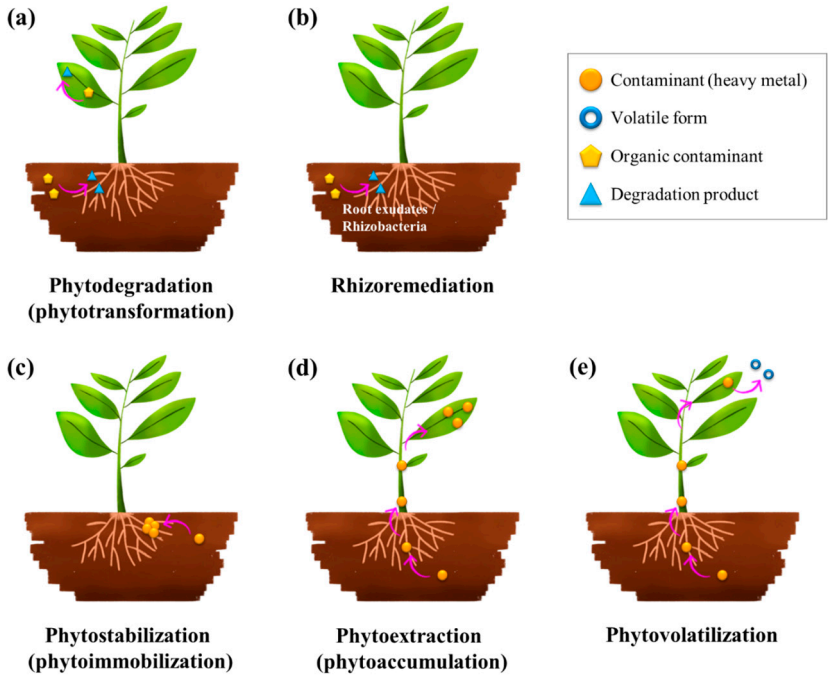


Figure 2. Overview of phytoremediation mechanisms. (**a**) Phytodegradation, (**b**) rhizoremediation, (**c**) phytostabilization, (**d**) phytoextraction, and (**e**) phytovolatilization.

4.1. Phytodegradation

Phytodegradation, also known as phytotransformation, involves the uptake and subsequent breakdown of organic pollutants by plants through enzymatic processes (Figure 2a) [57,58]. Plants secrete enzymes, such as dehalogenase, oxygenase, and peroxidase, which degrade complex organic pollutants, including PHs, into simpler and less toxic compounds [6,59]. These enzymes catalyze oxidation–reduction reactions that transform pollutants within plant tissues or the rhizosphere (the soil region near plant roots). Phytodegradation has demonstrated effectiveness in degrading polycyclic aromatic hydrocarbons (PAHs) and pesticides [6,59]. One advantage of phytodegradation is its ability to function effectively in soils with low microbial populations, allowing for plants to address higher pollutant concentrations that may be toxic to microorganisms. However, a notable limitation is the difficulty in tracking metabolic byproducts formed within plant tissues, making it challenging to confirm complete pollutant degradation [6]. Additionally, the efficiency of phytodegradation is influenced by site-specific factors, such as soil properties, pH, and temperature, which vary widely across contaminated sites [12,13]. Another challenge lies in selecting plant species with high enzymatic activity and pollutant tolerance, as only a limited number of species possess these capabilities [58]. Despite these challenges, phytodegradation has demonstrated promising results in real-world applications. For instance, a long-term study conducted on soil co-contaminated with diesel and heavy metals (Pb, Cu, Cd) evaluated the efficiency of *Zea mays* (maize) and *Festuca arundinacea* (tall fescue) over 571 days [35,36]. The study revealed total petroleum hydrocarbon (TPH) removal efficiencies of up to 94% with maize and 63% with tall fescue, highlighting the potential of phytodegradation to address complex contamination scenarios effectively.

4.2. Rhizoremediation

Rhizoremediation, also known as rhizodegradation, is a type of phytoremediation that harnesses synergistic interactions between plant roots and rhizosphere microorganisms to degrade pollutants (Figure 2b) [6,45]. Plant roots release exudates—such as organic acids, amino acids, and sugars—that stimulate microbial growth and activity in the rhizosphere [40,45]. These microbes are critical in degrading organic contaminants, including PHs [60]. Grasses, such as *Festuca arundinacea* (tall fescue), and legumes, such as *Glycine max* (soybean), have been shown to enhance the microbial degradation of PHs in the rhizosphere [40]. These plants support diverse microbial communities capable of breaking down complex hydrocarbons. Rhizoremediation is particularly advantageous for PH-contaminated soils, as it enhances the natural microbial breakdown of pollutants. However, the process can be slow and requires additional nutrient inputs to support plant and microbial growth [6]. The efficiency of rhizoremediation can be significantly improved by incorporating microbial inoculants that enhance microbial activity, pollutant bioavailability, and degradation rates in the rhizosphere [61,62]. For example, a study utilizing *Novosphingobium* sp. CuT1, isolated from the rhizosphere of tall fescue, demonstrated its potential in enhancing rhizoremediation in co-contaminated soil [37]. The inoculation of CuT1 increased tall fescue root biomass and stimulated microbial enzyme activity, resulting in improved pollutant treatment efficiency [37]. Similarly, *Bacillus subtilis* and *Sphingobacterium multivorum* have been shown to enhance the degradation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in association with alfalfa and black nightshade [32]. These bacterial strains improved soil enzyme activities (e.g., dehydrogenase, urease, and catalase) and increased microbial diversity, ultimately boosting the removal efficiency of PAHs and Cd [32].

4.3. Phytostabilization

Phytostabilization, also known as phytoimmobilization, is a remediation process in which plants immobilize soil contaminants, preventing their migration into groundwater or the atmosphere (Figure 2c) [59,63]. This technique is particularly effective for heavy metals and other inorganic pollutants. Rather than removing contaminants, plants restrict their mobility by adsorbing them onto root surfaces or precipitating them into less bioavailable forms within the rhizosphere [6]. For instance, plants such as *Vetiveria zizanioides* (vetiver grass) and *Populus* have been used to stabilize heavy metals (e.g., Pb and Cd) in contaminated soils, preventing these metals from leaching into groundwater or becoming airborne [63]. The key benefit of phytostabilization is its ability to mitigate contaminant migration without significantly altering the structure or composition of the soil. However, as this method does not eliminate pollutants, prolonged monitoring is essential to ensure that the contaminants remain immobilized and effective [45]. The effectiveness of phytostabilization can be enhanced by incorporating soil amendments, such as compost, which increase metal-binding capacity and organic matter content while promoting plant growth [27,39]. For instance, a study by Marchand et al. (2018) demonstrated that compost-amended soils planted with alfalfa effectively stabilized heavy metals (Cu and Pb) and hydrocarbons, reducing leachate concentrations and mitigating soil ecotoxicity [27]. Another study highlighted the potential of sunflower plants for phytostabilization in soils co-contaminated with PAHs and heavy metals [39]. This research emphasized the role of compost amendments in improving soil conditions, enhancing the immobilization of heavy metals, and supporting plant growth under toxic environmental conditions [39].

4.4. Phytoextraction

Phytoextraction, also known as phytoaccumulation, involves the uptake of contaminants from the soil by plant roots, followed by their translocation to the aboveground parts of the plant (shoots and leaves) (Figure 2d) [45,52]. Once the contaminants, typically heavy metals, accumulate in plant tissues, the plants are harvested and removed from the site, thereby reducing the overall contamination level in the soil. Hyperaccumulator plants, such as *Brassica juncea* (Indian mustard) and *Helianthus annuus* (sunflower), are commonly used for phytoextraction due to their ability to accumulate high concentrations of metals [45]. Over time, repeated planting and harvesting of these plants can significantly reduce metal concentrations in contaminated soils [55]. Phytoextraction is particularly effective for sites contaminated with heavy metals as it offers a permanent solution by removing pollutants from the soil. However, the process is slow, and the plants used in phytoextraction may require multiple growing seasons to achieve meaningful pollutant removal [57]. Additionally, proper disposal or treatment of harvested plant biomass is essential to prevent secondary contamination [57]. Chelators can enhance the efficiency of phytoextraction by increasing the bioavailability of heavy metals, making them more accessible for plant uptake [12,20]. For example, the combined application of EDTA and citric acid significantly improved Cu uptake in co-contaminated soils using maize while simultaneously reducing pyrene dissipation in the soil [23]. This combination not only facilitated metal uptake but also minimized hydrocarbon loss, showcasing the potential for integrated remediation strategies.

4.5. Phytovolatilization

Phytovolatilization involves the uptake of contaminants from the soil or water, their conversion into volatile forms within the plant, and their subsequent release into the atmosphere through transpiration (Figure 2e) [55,57]. This mechanism is effective for specific volatile organic compounds and some heavy metals that can be transformed into gaseous

forms, such as mercury, selenium (Se), and arsenic (As). Specific species of poplar trees (*Populus*) have been shown to take up Se and release it as a volatile compound (dimethyl selenide) through their leaves [45]. One of the key advantages of phytovolatilization is its ability to remove pollutants from the soil while reducing their immediate environmental impact by converting them into less harmful forms [6]. However, this method has significant limitations. The volatilized pollutants may re-deposit in the environment, potentially causing secondary contamination or unforeseen ecological effects [6]. As a result, careful monitoring and risk assessment are essential to ensure the safe application of phytovolatilization.

By advancing research in phytoremediation mechanisms and addressing their limitations through integration with other technologies, this approach holds great potential for achieving sustainable soil remediation solutions. Combining phytoremediation with complementary strategies, such as microbial inoculants or soil amendments, could further enhance its efficiency, enabling practical applications in diverse environmental settings.

5. Challenges in Phytoremediation of Co-Contaminated Soils

Rapid industrial development has led to the widespread release of pollutants, resulting in the coexistence of multiple contaminants, such as PHs and heavy metals, in soil [4,11]. The presence of these pollutants with diverse physicochemical properties creates complex physical, chemical, and biological interactions, making their behavior unpredictable [4,11]. Unlike single-contaminated soils, co-contaminated soils involve various unpredictable physicochemical and biological processes, which complicate remediation efforts [4,11]. PHs and heavy metals can directly or indirectly interact, altering their reactivity, speciation, toxicity, mobility, and bioavailability [4]. Addressing such contamination necessitates a multifaceted remediation approach founded on a comprehensive understanding of these interactions and behaviors [4]. Below are some of the key challenges associated with phytoremediation in co-contaminated soils.

5.1. Chemical Interactions Between Pollutants

In co-contaminated soils, the complex interactions between PHs and heavy metals significantly impact their bioavailability, reactivity, and toxicity, complicating remediation efforts [4]. Figure 3 illustrates the key mechanisms of these chemical interactions, including cation-π interactions, complexation, redox reactions, and coprecipitation. These interactions not only increase the persistence of pollutants but also reduce the efficiency of phytoremediation methods, such as phytoextraction and phytodegradation, by altering pollutant mobility and bioavailability.

Cation-π interactions are particularly common in soils contaminated with both heavy metals and PAHs. Cationic heavy metals (or PAHs) adsorbed on soil particles create new sorption sites for PAHs (or heavy metals), thereby increasing pollutant binding to soil particles [4]. Studies have shown that the addition of heavy metals (such as Cu, Ni, Pb, and Zn) increases PAH sorption onto soil minerals [64,65]. For example, a study found that Cu and Zn enhanced the sorption of PAHs in soils through cation-π interactions, ultimately reducing PAH mobility and persistence [64,65]. Complexation, a crucial mechanism, occurs when PHs and heavy metals bind together, forming stable complexes that influence their environmental behavior [4]. One pollutant adsorbed on soil particles can act as a cross-linking agent, providing additional binding sites for other pollutants, thus enhancing their sorption and reducing mobility and bioavailability [4,66]. For instance, Jiang et al. (2013) observed the formation of a Cd–As(V)–goethite complex in co-contaminated soil, significantly reducing their bioavailability and environmental risk [66]. Complexation reactions are more common in cationic heavy metal–PH co-contaminated soil, particularly when

PHs contain O-, S-, and N-functional groups [4,66]. Redox reactions also play a crucial role in soils contaminated with heavy metals and PHs. Organic pollutants can alter the redox state of metals, influencing their mobility [4]. For example, organic matter facilitated the reduction of Cr(VI) to Cr(III), a less harmful and more stable form, improving remediation outcomes in co-contaminated soils [4]. Finally, coprecipitation occurs when cationic and anionic metals precipitate together, further immobilizing contaminants. A study by Jiang et al. (2013) found that the coprecipitation of Cd–As(V) in soil significantly reduced their mobility [66]. This process often occurs in iron-rich soils, where soil components such as hydroxides and sulfides contribute to co-precipitation [4].

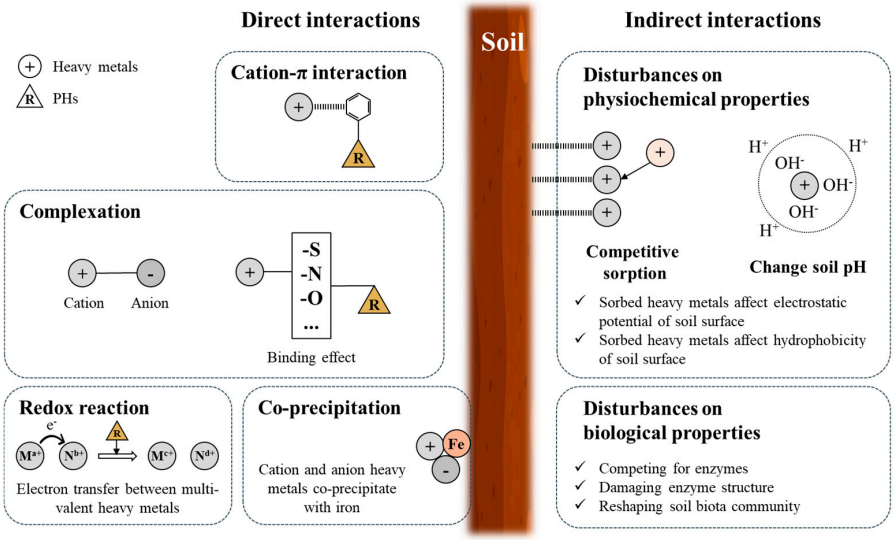


Figure 3. Physical and chemical interactions between PHs and heavy metals in soil, modified from [4].

The coexistence of PHs and heavy metals also alters the physicochemical properties of soil, including pH, cation exchange capacity (CEC), and electrostatic potential, indirectly affecting the behavior of other pollutants [4,67]. For example, Zhou et al. (2011) found that, in soils co-contaminated with PAHs and heavy metals (Cd and Zn), the formation of a complex between Cd^{2+} and Zn^{2+} decreased soil pH, which increased PAH mobility [67].

While chemical interactions present a significant obstacle, they also offer potential opportunities for targeted remediation strategies. Effective remediation efforts must address these complex interactions and be tailored to site-specific conditions to optimize outcomes. Furthermore, understanding these interactions is crucial for enhancing phytoremediation methods and integrating them with other technologies to overcome the challenges posed by co-contaminated soils.

5.2. Disruption of Soil Biota

Soil microorganisms are essential for the success of phytoremediation, particularly in breaking down organic pollutants. However, the presence of heavy metals can inhibit microbial activity, disrupt microbial community structures, and interfere with enzymatic pathways essential for pollutant degradation [67]. For instance, heavy metals, such as Cd and Pb, hinder microbial enzymes, making it challenging for microbes to degrade hydrocarbons effectively [10]. In co-contaminated soils, the coexistence of PHs and heavy metals can further disrupt biological processes. Microorganisms responsible for degrading organic pollutants may experience reduced efficiency due to heavy metal toxicity. Additionally, some metals can directly damage microbial cells or inhibit the production of essential enzymes needed for bioremediation [4].

The coexistence of PHs and heavy metals also alters microbial community structure, leading to declines in microbial diversity and abundance and reducing the overall resilience of soil ecosystems [4]. For example, in a long-term study of soils contaminated with diesel and heavy metals, bacterial community structures underwent significant alterations due to co-contamination [35,36]. Over time, microorganisms with higher resistance to pollutants became dominant, resulting in a shift in microbial diversity. This shift initially inhibited the production of essential microbial enzymes, such as indole-3-acetic acid (IAA) and siderophores, which are critical for pollutant degradation [35]. Partial recovery of enzyme production was observed as the microbial communities adapted to the co-contaminated conditions, but the overall efficiency of the phytoremediation process remained reduced [35]. Choden et al. (2021) further illustrated the impact of heavy metal toxicity on microbial populations in co-contaminated soils with crude oil and zinc [30]. Initially, bacterial populations increased due to the abundance of carbon sources, but Zn toxicity eventually inhibited biomass synthesis, leading to reduced microbial growth. The bioaugmentation of *Pseudomonas putida* MU02 effectively enhanced microbial resilience in the co-contaminated soils. However, Zn toxicity still posed challenges, limiting biomass synthesis despite the presence of the inoculated strain [30]. This reduction in microbial activity impairs the overall phytoremediation process, making it slower and less effective [4].

The reduction in microbial activity caused by heavy metal toxicity poses a significant challenge to phytoremediation, undermining one of its key components. Future remediation strategies must focus on enhancing microbial resilience through bioaugmentation techniques and the use of adaptive soil amendments, such as organic matter or microbial inoculants, to support microbial recovery. These approaches could restore microbial communities, improve enzyme activity, and support more effective remediation of co-contaminated soils.

5.3. Unpredictable Pollutant Behavior

Compared to single pollution sources, the remediation of multiple pollutants, such as PHs and heavy metals, is highly unpredictable and complex [9,10]. Chemical reactions between PHs and heavy metals form complexes that alter their physicochemical properties, impacting their bioavailability [11]. Furthermore, the adsorption and desorption kinetics of pollutants are influenced by their competition for binding sites on soil particles, altering their transport within the soil [11]. These interactions not only impact the movement of pollutants but also affect the metabolites—such as enzymes, biosurfactants, and organic acids—secreted by plants and soil microorganisms, disrupting biological processes [11]. Such disruptions in biological processes can further influence the remediation efficiency of other pollutants.

Due to the significantly different physicochemical properties of PHs and heavy metals, remediating soil contaminated with both pollutants is highly unpredictable [4,10]. Remediation may require external chemicals and additional costs or result in alterations to the physicochemical properties of the soil [4,10]. For example, remediation methods that target PHs may inadvertently mobilize heavy metals, creating secondary contamination risks.

The unpredictable behavior of pollutants in co-contaminated soils highlights the complexity of remediating efforts. These challenges demand adaptive remediation strategies, such as flexible treatment combinations, tailored soil amendments, and site-specific monitoring systems, to respond to the dynamic interactions of pollutants effectively.

5.4. Environmental and Economic Considerations

Phytoremediation is widely recognized as an eco-friendly and cost-effective technology because it does not produce secondary pollutants, and its direct costs are significantly

lower compared to conventional methods [45]. Additionally, phytoremediation offers potential economic benefits through bioenergy production and phytomining, which can generate revenue and partially offset operational costs [13]. However, applying phytoremediation to co-contaminated soils introduces unique economic challenges. The complex interactions between multiple pollutants often necessitate additional inputs, such as fertilizers or soil amendments, to enhance pollutant bioavailability and support plant growth. These inputs can increase operational expenses and overall project costs [68]. Indirect costs, including the time value of extended remediation periods and opportunity costs from restricted land use, are significant considerations, particularly for industrial sites requiring rapid remediation [45]. Furthermore, the uncertainty associated with these indirect costs complicates the economic evaluation of phytoremediation, especially for large-scale applications.

Despite these challenges, phytoremediation remains a viable solution for specific co-contaminated environments. It is particularly suitable for remote or low-value lands, such as mining tailings, unused pipeline sections, and landfills, where opportunity costs are minimal and alternative land uses are limited [13]. Additionally, phytoremediation provides an environmentally sustainable option for restoring natural ecosystems, such as forests, grasslands, and wetlands, offering long-term ecological benefits beyond pollutant removal.

To balance economic feasibility with environmental benefits, integrating phytoremediation with complementary technologies and long-term site management strategies will be essential. These approaches can optimize resource utilization, enhance pollutant removal efficiency, and ensure the sustainability of remediation efforts in co-contaminated soils.

6. Strategies for Enhancing Phytoremediation

To enhance the efficiency of phytoremediation in co-contaminated soils, innovative strategies are needed to improve the bioavailability of pollutants, strengthen plant–microbe interactions, and boost overall plant resilience. Several approaches, such as soil amendments, phytohormones, plant growth-promoting bacteria (PGPB), and genetic engineering (Figure 4), have been developed to address the challenges associated with co-contaminated environments. These strategies aim to optimize the remediation process, achieving faster and more sustainable results.

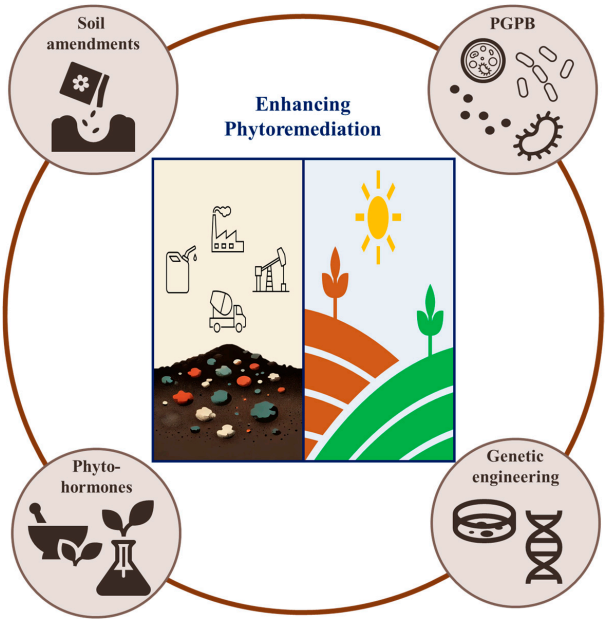


Figure 4. Strategies for enhancing phytoremediation efficiency.

6.1. Soil Amendments

Soil amendments are essential for enhancing phytoremediation efficiency by modifying the physical, chemical, and biological properties of contaminated soils. These modifications promote plant growth, boost microbial activity, and increase pollutant bioavailability, ultimately making phytoremediation more effective [12,13]. Soil amendments are generally classified into two main categories—environmental functional materials (EFMs) and chemical reagents.

6.1.1. Environmental Functional Materials (EFMs)

EFMs, such as biochar, woodchips, sawdust, and agricultural byproducts, show significant potential for enhancing phytoremediation [13,22,69]. Among these, biochar—a product derived from the pyrolysis of organic residues—has been extensively studied for its ability to improve soil structure, water retention, and nutrient availability [22,29,32]. Its porous structure supports plant growth and provides a habitat for beneficial microorganisms. Its large surface area and high CEC can enhance the bioavailability of heavy metals and organic pollutants [12,13]. Research has demonstrated that biochar amendments improve the removal efficiency of heavy metals and PHs. For instance, the application of biochar in soils contaminated with Zn, PB, and Cd increased the uptake of these metals by *Z. mays* (maize) and *M. sativa* (alfalfa) [22,29,33]. Additionally, biochar improves microbial diversity and boosts soil enzyme activity, both of which are crucial for the degradation of organic pollutants.

Other EFMs, such as woodchips, husks, and sawdust, can also be used to enhance phytoremediation by improving soil porosity, field capacity, and organic matter content [13,70]. For example, sawdust significantly accelerates the biodegradation rate of PHs in contaminated soils [71]. These materials serve as bulking agents, promoting microbial growth and increasing pollutant bioavailability, thereby facilitating large-scale remediation efforts.

6.1.2. Chemical Reagents

Chemical reagents, including organic and inorganic fertilizers and chelating agents, are used to enhance phytoremediation [13]. Organic fertilizers derived from animal manure or plant residues provide essential nutrients that promote plant growth and increase biomass, thereby enhancing the overall efficiency of phytoremediation [33,72]. Phosphorus-based fertilizers are particularly effective in addressing nutrient deficiencies, stimulating root development, and enhancing plant tolerance to pollutant-induced stress [13].

Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), are particularly effective in enhancing plants' uptake of heavy metals. EDTA binds with heavy metals to form stable complexes, increasing their solubility and availability for plant absorption [18,20]. For example, the addition of EDTA to contaminated soils significantly improved the phytoextraction of heavy metals, such as Pb and Cd, by facilitating their uptake into plant tissues [18]. Similarly, Chigbo and Batty (2015) demonstrated that the combined application of EDTA and citric acid enhanced plant growth by 41% in co-contaminated soils and increased the phytoextraction of Cu while also promoting the dissipation of pyrene by up to 90.4% [23]. However, caution is necessary to avoid excessive metal mobility, which may contaminate groundwater.

While soil amendments offer significant advantages in enhancing phytoremediation, their cost and potential risks, such as secondary pollution from chelating agents, require careful consideration. These challenges are further discussed in Section 6.5.

6.2. Phytohormones

Phytohormones are crucial in regulating plant growth and enhancing resilience to abiotic stresses, such as soil contamination [73]. Low concentrations of phytohormones, such as 1-aminocyclopropane-1-carboxylate deaminase (ACCD), indole-3-acetic acid (IAA), gibberellins (GA), and abscisic acid (ABA), have been shown to significantly enhance plant tolerance, making them valuable tools in phytoremediation strategies [73].

ACCD hydrolyzes 1-aminocyclopropane-1-carboxylate (ACC), a precursor of stress ethylene, into α-ketobutyrate and ammonia, thereby mitigating ethylene-induced stress in plants [62]. IAA, an auxin hormone, promotes root elongation and shoot growth, aiding plants in managing the toxicity of heavy metals and organic pollutants [12]. GA helps reduce oxidative stress in plants by regulating antioxidant metabolism, improving their ability to survive and thrive in contaminated soils. ABA is crucial for regulating water use efficiency and stress responses, ensuring that plants continue to grow under adverse conditions [12]. Strategically applying these phytohormones to contaminated soils can accelerate plant establishment and improve overall phytoremediation efficiency. For example, a study on *Sesbania drummondii* demonstrated that the addition of IAA in combination with EDTA enhanced Pb accumulation in plant shoots by over 1200%, with Pb particles concentrated in vascular bundles [16]. This indicates a synergistic effect of phytohormones and chelating agents in improving pollutant uptake and translocation. Additionally, the combined application of nitrogen (N) and GA significantly alleviated salt stress in *Brassica* plants, improving attributes such as biomass, chlorophyll concentration, and enzymatic activity [17].

Phytohormones effectively enhance plant resilience and performance in contaminated soils. By regulating plant growth and stress tolerance, they significantly improve the outcomes of phytoremediation efforts.

6.3. Plant Growth-Promoting Bacteria (PGPB)

The utilization of rhizosphere bacteria's metabolic activity presents an alternative strategy to bolster phytoremediation efforts, emphasizing the synergistic interaction between plants and these bacteria [61,62]. PGPB are beneficial microorganisms that thrive in the rhizosphere, promoting plant growth and aiding pollutant degradation [61,62]. PGPB assist phytoremediation through various direct and indirect mechanisms. Directly, they improve plant nutrition by fixing nitrogen and solubilizing nutrients in the soil, ensuring the availability of essential elements for plant growth [61,62]. Additionally, PGPB produce enzymes, such as ACCD and IAA, which are critical in mitigating stress and promoting plant development [61,62]. Indirectly, PGPB enhance plant resilience by producing antibiotics, such as pyrrolnitrin and phenazines, which protect plants from pathogens and reduce environmental stress [61]. Their ability to increase the bioavailability of hydrophobic pollutants and heavy metals through biosurfactant production and chelation further supports phytoremediation efforts.

Certain PGPB species, such as *Bacillus*, *Pseudomonas*, and *Acinetobacter*, are particularly effective in degrading PHs by producing biosurfactants that enhance the solubility and bioavailability of hydrophobic pollutants [3,74]. These substances enhance the solubilization and dispersion of hydrophobic pollutants, increasing their availability for microbial degradation and plant uptake [3]. While PGPB do not directly degrade heavy metals, they impact the fate of these pollutants through various mechanisms [62]. For example, some PGPB produce chelating agents, such as siderophores, which bind to heavy metals, thereby reducing their mobility and enhancing plant uptake [62]. Additionally, PGPB promote phosphate solubilization and induce redox changes in the soil, further influencing the bioavailability and uptake of heavy metals by plants [62]. Therefore, inoculating soil with

PGPB holds significant promise for enhancing both plant biomass yield and pollutant bioavailability, offering a synergistic approach to phytoremediation [61]. By harnessing the capabilities of these beneficial bacteria, phytoremediation efforts can be optimized to address soil contamination challenges more effectively.

The genera *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Klebsiella* are prominent PGPB and have been widely studied for their beneficial traits [8]. However, research focusing specifically on enhancing phytoremediation in soils contaminated with both PHs and heavy metals through PGPB inoculation remains limited. Table 2 summarizes previous studies on this topic. Cao et al. (2022) demonstrated that inoculating a microbial consortium of *Bacillus* and *Sphingobacterium* into alfalfa and black nightshade (*S. nigrum*) grown in soil contaminated with PAHs and heavy metals (Zn, Pb, and Cd) improved both soil enzyme activities and bacterial diversity, leading to enhanced removal of PAHs and Cd [32]. Similarly, *Bacillus* sp. KSB7 was inoculated into Kochia (*Brassica scoparia*) in soil polluted with PAHs and heavy metals (Pb, Zn, Cu, and Cr), exhibiting plant growth promotion, metal tolerance, and PAH degradation capabilities [34]. Franci et al. (2016) showed that a bacterial consortium consisting of *Bacillus*, *Ralstonia*, *Microbacterium*, *Curtobacterium*, and *Pimelobacter* enhanced the phytoremediation of hydrocarbons, Cu, and Ni in mustard greens (*Brassica juncea*) [25]. This inoculation led to increased seed germination, improved heavy metal uptake, and a higher hydrocarbon removal rate. Chen et al. (2017) and Zhang (2020) found that *Klebsiella pneumoniae* significantly promoted the growth of a triangular club-rush (*Scirpus triqueter*) by producing ACCD and IAA, which increased pyrene degradation and Ni bioavailability [26,31]. In another study, *Novosphingobium* sp. CuT1 was inoculated into tall fescue (*F. arundinacea*) in soil contaminated with diesel, Cu, and Pb. This inoculation enhanced plant biomass and Cu bioavailability by producing ACCD, IAA, and siderophores [37]. *Pseudomonas* species are notable for their ability to promote plant growth and assist in the remediation of both PHs and heavy metals. They enhance phytoremediation by producing beneficial enzymes, such as ACCD, IAA, and urease, which support plant growth and pollutant degradation [24,30,38].

PGPB provide a synergistic approach to enhancing phytoremediation by promoting both plant growth and pollutant treatment. Their ability to degrade PHs and increase heavy metal bioavailability makes them essential in managing complex pollution situations. The integration of PGPB into phytoremediation strategies holds immense potential for addressing the complexities of co-contaminated soils, as their metabolic activities not only support plant growth but also enhance pollutant degradation, underscoring their critical role in achieving efficient and sustainable remediation outcomes.

6.4. Genetic Engineering

While PGPB have shown significant potential in enhancing plant growth and pollutant degradation, genetic engineering offers a more targeted approach to address the complexities of multi-polluted soils. Also known as genoremediation, genetic engineering has emerged as a powerful tool for enhancing phytoremediation efficiency by modifying plants and microorganisms to improve their tolerance and detoxification of pollutants [75]. This can involve the development of genetically engineered microorganisms (GEMs) to enhance phytoremediation processes [76] or the transfer of functional genes from microorganisms to plants [73]. By introducing specific functional genes, genetic engineering can improve the uptake, transformation, and removal of pollutants. The key mechanisms of genetic engineering in phytoremediation are discussed in Table 3.

Table 2. Enhancement of phytoremediation through plant growth-promoting bacteria (PGPB).

PGPB	Plant	Pollutant	Enhancement of Phytoremediation	Ref.
Bacillus subtilis, *Sphingobacterium multivorum*	*Medicago sativa*, *Solanum nigrum*	PAHs, Cd, Pb, Zn	Increased soil enzyme activities (dehydrogenase, urease, and catalase), altered soil bacterial diversity, increased polycyclic aromatic hydrocarbon (PAHs) and Cd removal	[32]
Bacillus sp. KSB7	*Brassica scoparia*	PAHs, Cr, Zn, Cu, Pb	Capable of promoting plant growth, metal tolerance, and PAH degradations	[34]
Bacillus kochii, *Ralstonia pickettii*, *Microbacterium luticocti*, *Curtobacterium oceanosedimentum*, *Pimelobacter simplex*	*Brassica juncea*	Hydrocarbons, Cu, Ni	Produced indole-3-acetic acid (IAA), increased the germination rate of *B. juncea* seed and uptake efficiency of heavy metals (Cu and Ni), improved the removal rate of hydrocarbons	[25]
Klebsiella pneumoniae	*Scirpus triqueter*	PAHs, Ni	Produced 1-aminocyclopropane-1-carboxylate deaminase (ACCD) and IAA, promoted plant growth, and increased pyrene degradation and Ni bioavailability	[26]
K. pneumoniae CX-1	*S. triqueter*	Pyrene, Ni	Produced ACCD, promoted plant growth, increased dissipation rates of pyrene and removal rates of Ni	[31]
Novosphingobium sp. CuT1	*Festuca arundinacea*	Diesel, Cu, Pb	Produced ACCD, IAA, and siderophore, increased plant biomass and bioavailability of Cu	[37]
Pseudomonas aeruginosa	*M. sativa*	PHs, Cu, Pb, Zn	Promoted plant growth (roots and shoots), increased TPH degradability and metal uptake by plant roots	[24]
P. aeruginosa	*Lollium perenne*	PAHs, Cd	Enhanced enzymatic activities (urease, dehydrogenase, and polyphenol oxidase) and improved Cd adsorption and PAH degradation	[38]
Pseudomonas putida MU02	*Ocimum gratissimum*	Crude oil, Zn	Produced ACCD and IAA, increased Zn uptake and accumulation by plant, improved TPH deduction	[30]

Table 3. Key applications of genetic engineering in enhancing phytoremediation efficiency.

Genetic Engineering Mechanisms	Functional Genes	Gene Source	Modified Organism	Application	Ref.
Metal resistance/detoxification	*pbr* operon (Pb resistance)	*Ralstonia metallidurans*	*E. coli*	Conferred Pb resistance by reducing lead toxicity	[77]
	*cad*A (Cd resistance)	*Staphylococcus aureus*	*Bacillus subtilis*	Enhanced Cd resistance	[78]
	*cop*M (Cu resistance)	*Synechocystis* sp. PCC6803	*E. coli*	Enhanced Cu resistance	[79]
	*aox*B (As oxidase)	*Pseuomonas* sp. strain AK9	*E. coli*	Increased resistance to As toxicity	[80]
	*mer*A (Hg reductase)	*Klebsiella pneumoniae*	*E. coli*	Facilitated Hg resistance	[81]
	*mer*A	*Staphylococcus aureus*	*Populus* species	Enhanced tolerance to Hg^{2+}, converting it to Hg^{0}	[82]
Metal uptake/transport	MT (metallothionein)	*Pisum sativum*	*E. coli*	Coexpression with Ni transport system increased Ni uptake six-fold	[83]
	PtMT2b (metallothionein)	*Populus trichocarpa*	*Saccharomyces cerevisiae*	Increased Cd uptake five-fold	[84]
	MT	*P. sativum*	*Arabidopsis thaliana*	Increased Cu accumulation	[85]
	AtPCS1 (phytochelatin)	*Arabidopsis* sp.	*Nicotiana tabacum*	Enhanced As and Cd accumulation	[19]
	AtPCS1	*A. thaliana*	*Brassica juncea*	Enhanced As and Cd tolerance	[15]
Degradation of organic pollutants	*alk*B (alkane monooxygenase)	*Alcanivorax borkumensis*	*Pseudomonas oleovorans*	Enhanced alkane degradation	[86]
	*alk*B	*Rhodococcus* sp.	*Pseudomonas* sp.	Enhanced alkane hydroxylase systems	[87]
	*alk*B	*Pseudomonas* sp.	*E. coli*	Facilitated the key step of alkane biodegradation	[88]
	CYP153 (cytochrome P450)	*A. borkumensis*	*E. coli*	Enhanced bioconversion of *n*-octane	[89]
Plant growth	*acd*S (ACC deaminase)	*Pseudomonas* sp.	*Camelina sativa*	Reduced ethylene levels and enhanced plant growth	[90]

6.4.1. Enhancing Metal Resistance and Detoxification

The primary objective of genetic engineering in phytoremediation is to enhance the resistance and detoxification capacity of organisms exposed to heavy metals. Genetically modified organisms can be engineered with genes that confer metal resistance, allowing for them to tolerate high concentrations of toxic metals more effectively. For example, the *pbr* operon from *Ralstonia metallidurans* has been successfully cloned into *E. coli*, conferring Pb resistance. The *pbr* operon contains several key genes—*pbr*T, *pbr*A, *pbr*C, and *pbr*D—that work together to reduce Pb toxicity in the host microorganism [77]. Similarly, genes such as *cad*A and *cop*M, which confer resistance to Cd and Cu, respectively, have been introduced into various bacterial strains to enhance their detoxification capabilities [78,79]. Additionally, microorganisms can be genetically engineered to transform highly toxic heavy metals into less harmful forms [91]. For instance, Khoshniyat (2018) isolated the mercuric reductase gene (*mer*A) from *K. pneumoniae* and inserted it into *E. coli*, facilitating Hg resistance [81]. Satyapal et al. (2023) isolated the arsenite oxidase gene (*aox*B) from *Pseudomonas* sp. strain AK9, demonstrating that recombinant *E. coli* exhibited enhanced resistance to arsenic toxicity [80].

Building on these advancements, a significant application of genetic engineering in phytoremediation involves introducing the *mer*A gene from *Staphylococcus aureus* into plant species, such as *Populus* [14,82]. This modification enhances the plants' tolerance to ionic mercury (Hg^{2+}) and facilitates its conversion to elemental mercury (Hg^{0}), a less hazardous form [14,82]. This approach demonstrates the potential of genoremediation to expand the range of pollutants that can be effectively managed through enhanced phytoremediation techniques. By leveraging the unique capabilities of engineered genes, researchers can further improve the efficiency of plants in detoxifying heavy metals, thereby contributing to more sustainable environmental remediation strategies.

6.4.2. Enhancing Metal Uptake and Transport

Genetic engineering can also enhance the efficiency of heavy metal uptake and transport in plants. A common approach involves modifying plants to overexpress genes that produce metal-binding proteins or peptides, such as metallothioneins (MTs) and phytochelatins (PCs).

MTs are essential proteins for heavy metal transformation. They bind to heavy metals, significantly improving metal uptake. Deng et al. (2003) showed that coexpressing MTs with an Ni transport system in *E. coli* increased the Ni uptake capacity six-fold compared to the unmodified host [83]. De Oliveira et al. (2020) transformed *Saccharomyces cerevisiae* with the PtMT2b gene encoding MTs, achieving a more than five-fold increase in Cd uptake [84]. Genetic engineering can enhance the synthesis of metal chelators or transporters to improve metal uptake and transfer in plants. For instance, introducing the *Pisum sativum* MT gene into *Arabidopsis thaliana* has led to increased Cu accumulation [85]. Similarly, PCs are crucial in metal detoxification and accumulation [73]. PCs are synthesized in response to toxic metals and form coordination bonds with them, facilitating their detoxification and accumulation [73]. The expression of PC synthase genes, such as AtPCS1, in transgenic plants has been shown to enhance the detoxification and accumulation of As and Cd in species such as *Nicotiana tabacum* (tobacco) and *Brassica juncea* (mustard green) [15,19]. Furthermore, the overexpression of various PC synthesis enzymes in *B. juncea* (mustard green) has demonstrated increased extraction of metals, such as Cu, Pb, Cd, Cr, and Zn, compared to wild-type plants [92].

6.4.3. Degradation of Organic Pollutants

For the remediation of organic pollutants, genetically modifying microorganisms to include genes involved in hydrocarbon degradation is essential. Genes such as *alk*B, which encodes alkane monooxygenase, and *CYP153*, which encodes soluble cytochrome P450 enzymes, are often incorporated into GEMs to enhance the breakdown of PHs [56]. Research has shown improved hydrocarbon degradation by isolating *alk*B or *CYP153* genes from well-known hydrocarbon-degrading bacteria, such as *Alcanivorax* [86,89], *Rhodococcus* [87,93], and *Pseudomonas* [88]. In addition to modifying microorganisms, enhancing phytoremediation in plants has also proven effective. For instance, incorporating the CYP105A1 gene from *Streptomyces* into *N. tabacum* (tobacco) has significantly improved phytoremediation compared to wild-type plants [94].

6.4.4. Reducing Stress in Plants

Stress ethylene, produced in response to environmental stressors, such as heavy metal contamination, can inhibit plant growth and reduce phytoremediation efficiency. Genetic engineering has been used to reduce ethylene levels by introducing the *acdS* gene, which encodes ACCD, an enzyme that breaks down the ethylene precursor ACC [90]. By reducing ethylene production, plants can better tolerate stress, maintain higher growth rates, and improve root development in contaminated environments. This genetic modification has been applied to various plant species, enhancing phytoremediation performance in multi-polluted soils.

Genetic engineering offers targeted approaches to enhancing phytoremediation by focusing on specific mechanisms, such as metal resistance, pollutant degradation, and stress tolerance. These engineered organisms have significant potential to overcome the inherent limitations of traditional phytoremediation, particularly in complex, multi-polluted environments. Despite the transformative potential of genetic engineering in phytoremediation, ethical concerns, regulatory challenges, and environmental risks associated with GMO applications remain significant hurdles. These limitations and possible solutions are elaborated in Section 6.5.

6.5. Limitations and Challenges in Phytoremediation Enhancement

Despite significant advancement in strategies to enhance phytoremediation, several limitations and challenges remain that must be addressed for broader applicability and efficiency. First, the addition of external materials, such as fertilizers and chelating agents, though effective in improving pollutant uptake and plant growth, often increases the cost of remediation [12]. Moreover, these substances may pose risks of secondary pollution, highlighting the need for exploring cost-effective, biodegradable, and environmentally safe alternatives.

Second, while various strategies for phytoremediation enhancement have been explored, most studies have been conducted on a laboratory scale or under controlled greenhouse conditions [61,95]. This limitation raises concerns about the scalability and practicality of these techniques in diverse and uncontrolled real-world environments, where factors such as climate, soil variability, and pollutant heterogeneity can significantly influence outcomes [12,61]. One of the intrinsic limitations of phytoremediation in co-contaminated soils is the longer remediation period compared to conventional physical or chemical methods. However, recent advancements have demonstrated that this limitation can be mitigated through strategies such as soil amendments, PGPB inoculation, and the use of pollutant-resistant plant species [12,43]. These enhancements not only accelerate pollutant removal but also improve phytoremediation efficiency in co-contaminated conditions. Se-

lecting plant species with a high tolerance to PHs and heavy metals can further optimize remediation efforts and reduce the time required to achieve meaningful results [95].

Third, although phytoremediation has been widely studied, its application to co-contaminated soils remains limited by a lack of long-term field studies [35,36]. Several studies have explored the phytoremediation of soils co-contaminated with PHs and heavy metals, but many have been limited to short-term pot experiments under controlled conditions. For instance, most studies have examined the phytoremediation process over durations of 30–180 days and have been conducted on a laboratory scale using small pots [21,24,28]. Additionally, these experiments were often performed under greenhouse conditions with controlled temperatures and humidities [24,96]. While Lee et al. (2023) monitored phytoremediation over a relatively longer period of two years [35,36], these studies remain rare, and the controlled conditions fail to replicate the complexities of real-world environments. These limitations highlight the critical need for long-term in situ field studies to validate phytoremediation strategies for co-contaminated soils and optimize their application in diverse environmental settings.

Fourth, phytohormones have shown considerable potential in enhancing plant resilience and pollutant uptake. However, the precise mechanisms through which these hormones mitigate pollutant-induced stress remain poorly understood [73]. This lack of clarity hampers the development of targeted application methods and raises concerns about the potential risks associated with their overuse, including human exposure.

Finally, while genetic engineering presents targeted solutions to improve phytoremediation, it also introduces ethical concerns and ecological risks related to the release of genetically modified organisms (GMOs) into natural ecosystems [12]. Comprehensive risk assessments alongside regulatory and public acceptance are critical for advancing the safe application of genetic engineering in phytoremediation.

Addressing these challenges requires a multifaceted approach that includes long-term field studies, scalable technologies, and interdisciplinary collaborations to refine and validate phytoremediation strategies for large-scale and sustainable environmental restoration.

7. Future Prospects

Phytoremediation remains a promising and eco-friendly strategy for addressing soil contamination, particularly in co-contaminated environments containing both PHs and heavy metals. However, the inherent complexities of these soils demand further advancements to enhance remediation efficiency. Integrating biological products, such as beneficial microorganisms, with soil amendments has shown great potential for improving pollutant immobilization, microbial activity, and overall soil health [97]. For instance, biochar and compost have been demonstrated to reduce heavy metal mobility while enhancing plant growth, making them practical solutions for large-scale remediation projects [22,29,32]. While these amendments are effective, future efforts should focus on developing more sustainable and cost-effective alternatives to address concerns such as economic feasibility and environmental safety.

Advancements in genetic engineering and plant selection continue to offer exciting opportunities for improving phytoremediation. Developing plants with enhanced metal uptake mechanisms or pollutant degradation pathways and optimizing plant–microbe interactions could significantly expand the scope and efficiency of phytoremediation [98]. For example, reducing stress ethylene production in plants through biotechnological approaches could enhance tolerance to complex pollutants and accelerate remediation processes. Additionally, innovations such as combining phytoindicators with biomass accumulation and organic amendments have shown potential to improve soil restoration outcomes and

reduce toxicity levels [99]. These strategies not only address current limitations but also offer a pathway to achieving sustainable and scalable phytoremediation practices.

Expanding phytoremediation applications to diverse environments, such as wetlands and waterlogged soils, represents an exciting frontier. However, scalability and practicality remain challenges, particularly given the variability in soil properties, climate conditions, and pollutant types. Developing plant species that can tolerate a broader range of environmental conditions, including varying moisture levels and salinity, will be critical [100]. Long-term monitoring and tailored approaches will be essential to ensure the sustainability and effectiveness of remediation outcomes in these complex environments.

Future efforts should prioritize multidisciplinary research to overcome these limitations and challenges. Key areas of focus include the development of cost-effective and biodegradable soil amendments alongside adaptive remediation techniques suited for diverse environmental conditions. Advancing long-term field studies is vital for validating laboratory-scale successes and refining strategies for real-world applications. Additionally, transparent risk assessments and regulatory oversight will be necessary to address ethical and ecological concerns associated with genetic engineering. By addressing these research gaps and challenges, phytoremediation can evolve into a transformative tool for large-scale environmental restoration, bridging the gap between theoretical advancements and practical applications. Its success will not only mitigate soil contamination but also contribute to achieving broader environmental sustainability goals.

8. Conclusions

Phytoremediation provides a sustainable and cost-effective approach for addressing soil contamination, particularly in environments affected by PHs and heavy metals. This review has highlighted the key mechanisms involved in phytoremediation, the challenges associated with multi-polluted soils, and emerging strategies to enhance its efficiency. While significant progress has been made in understanding the interactions among pollutants, plants, and soil microorganisms, challenges, such as unpredictable pollutant behavior and disruptions to soil biota, persist. To overcome these challenges, future efforts should focus on enhancing plant resilience through genetic engineering, optimizing pollutant uptake, and improving plant–microbe interactions.

Integrating biological products with soil amendments and applying advanced biotechnological interventions hold significant promise for enhancing pollutant removal and accelerating soil restoration. Ultimately, the success of phytoremediation in practical applications relies on interdisciplinary collaboration, continued innovation, and robust field-based research. By adopting emerging technologies and optimizing existing strategies, phytoremediation can contribute significantly to addressing soil contamination challenges in the future.

These insights provide a framework for policymakers and practitioners to develop effective, sustainable soil remediation strategies. Integrating phytoremediation into environmental regulations can help address cost and sustainability challenges, particularly for large-scale contaminated sites. Future efforts should focus on site-specific approaches that incorporate suitable plant species, targeted soil amendments, and microbial inoculation to enhance pollutant removal efficiency. By aligning these strategies with evolving environmental policies, phytoremediation can support long-term soil management objectives and advance sustainable land restoration practices.

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