

Review

Mine Site Restoration: The Phytoremediation of Arsenic-Contaminated Soils

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Abstract: Arsenic (As) is considered one of the most toxic chemicals to both human and environmental health. Mining activities represent one of the main anthropogenic sources of As; the concentration of As in mine soil can reach 9300 mg kg⁻¹. To overcome the major issue of soil As pollution, soil restoration is required. Biological restoration approaches are generally more cost-effective and environmentally sustainable than physical and chemical methods. In particular, phytoremediation, an environmentally friendly technique based on the use of plants to uptake contaminants from soil, has been successfully implemented to restore As-contaminated soils at mine sites. However, mine soils are generally depleted in essential plant nutrients, such as nitrogen (N). Recent research suggests that phytoremediation can be combined with other techniques (physical, chemical, and biological) to enhance the N content and plant biomass. The aim of this review is to assess the current state of knowledge in the field of the restoration of arsenic-impacted mine site soils, focusing on phytoremediation. We critically assess recent work examining the potential of the co-application of amendments with phytoremediation and identify promising technologies and key research gaps. More studies are required to test the effectiveness of using various soil additives to enhance the phytoremediation of As, not only in pot-scale experiments but also in the field, to enable an improved management strategy for mine site restoration in the future.

Keywords: arsenic; mine soils; biological restoration; phytoremediation; nitrogen limitation



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1. Introduction

Arsenic (As) is considered one of the most toxic chemicals to both human and environmental health; it was ranked at the top of the Agency for Toxic Substances and Disease Registry's (ATSDR) Substance Priority List in 2022 [1]. Arsenic is a chemical pollutant, classified as a metalloid, originating from natural and anthropogenic sources [2]. Mining activities—for example, gold mining—represent one of the main anthropogenic sources of As. Gold (Au) extraction methods, such as refractory ores and free-milling ores, produce As wastes in the form of gas, leachate, and calcinated soil, which pose significant environmental risks [3,4]. It has been reported that, globally, the cumulative As production as a result of mining activities increased from 0.06 to 3.29 million tonnes between 1900 and 2000 [5]; numerous studies indicate increasing As pollution as a result of mining in many countries [6,7]. In mining soils, the As concentrations may rise to 9300 mg kg⁻¹ [8], almost

400 times higher than the acceptable As levels of 24 mg kg^{-1} in soil regulated by the United States Environmental Protection Agency (USEPA) [9].

To overcome As pollution, soil restoration is required. Restoration is the process of recovering degraded, damaged, and destroyed soil using biological, chemical, and mechanical methods [10]. Biological restoration methods are more cost-effective and eco-friendly than physical and chemical methods [11]. Phytoremediation, a biological method that utilises plants, can be applied to remediate As-contaminated soils [12,13]. Phytoextraction, which mainly refers to the accumulation of heavy metal(loid)s in plant biomass, and phytostabilisation, which is the stabilisation of metals in the environment, are two phytoremediation mechanisms that are utilised for heavy metal(loid) uptake from contaminated soils [14]. However, to increase the productivity of plants, soil amendments are needed [15,16]. Nitrogen (N) deficiency in mine soils is reported as an important issue that can significantly reduce plant productivity [17]. Several studies suggest that phytoremediation can be combined with other techniques (physical, chemical, and biological) to enhance the plant biomass and soil N content [18–23].

Previous reviews have examined As pollution along with remediation strategies. For example, Sevak and Pushkar [24] evaluated sources of As contamination, its impacts on human health, and remediation methods. Another review by Kumar et al. [25] analysed several in situ techniques to remediate As. However, to the best of our knowledge, the description of the N limitation in mine soils and the approaches adopted to increase the soil N content alongside phytoremediation have not been reviewed. Here, we examine the nature of As, its distribution and impacts, and the methodologies to remediate As-contaminated soils. The focus of this review is on the potential of the co-application of amendments with phytoremediation to enhance the soil N content, identifying promising technologies and identifying key research gaps that may lead to improved phytoremediation approaches for As-contaminated mine soils.

2. Arsenic

2.1. Origin and History of Arsenic

Arsenic derives from the Latin word “arsenicum” and the Greek word “arsenikon”, which means *bold* or *potent*. It has been known since ancient times and was used in medieval times and the Middle Ages. The discovery that As was a metallic element was attributed to the German scientist and philosopher Albertus Magnus around 1250 BCE. Although As is well known as a poisonous agent, it has also been utilised for medicinal purposes [26,27]. Scheindlin [28] stated that several illnesses, such as malaria, chorea, and syphilis, were effectively treated with a 1% solution of potassium arsenite (Fowler’s solution), discovered in 1786.

2.2. Chemistry of Arsenic

Arsenic is a chemical element, classified as a metalloid, with an atomic number of 33 and atomic mass of 74.9 [29,30]. Arsenic belongs to Group VA of the Periodic Table, with specific gravities of 1.97 and 5.73. It is odourless and occurs in the single form or as the modification of two solid forms with different colours: yellow and grey. Arsenic is divided into inorganic and organic compounds. Arsenic-containing inorganic compounds are mostly identified as arsenate (As[V]) and arsenite (As[III]), detected in soil and groundwater. Dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenobetaine (AsB), and arsenocholine (AsC) represent organic As compounds, mostly found in seafood and considered less toxic than inorganic As [31].

2.3. Sources of Arsenic

Arsenic is the 20th most common element in the Earth’s crust. It is derived from natural and anthropogenic pathways. The most frequent source of As in nature is volcanic rocks, especially their ash and weathered products; rocks sedimented in marine environments;

the sedimentation of hydrothermal ores; fossil fuels; hydrothermal veins; hot springs; and forest fires [32].

The arsenic concentrations in the environment can be elevated by anthropogenic sources, which originate from human activities. Arsenic can be released during metal procurement and processing, including the production and processing of ores. In addition, the utilisation of high temperatures during oil and coal burning has also been found to be a source of As. Moreover, the use of various arsenical pesticides, algacides, and insecticides can also result in the release of As into the environment. Mining activities and other industrial sources, including the utilisation of decolouring agents and the production of ceramics, glassware, and cement, may also release As. Finally, pharmaceutical substances and household waste disposal are other examples of anthropogenic sources of As [33].

2.4. Distribution of Arsenic in Nature and Living Organisms

Arsenic is distributed in the atmosphere, soil, water, and geological formations. Biotic systems are exposed to As through transformations that occur during the biogeochemical cycle of As (Figure 1). In the atmosphere, As occurs as dust particles or gas, produced by natural and anthropogenic sources, including volcanic eruption, fossil fuels, volatilisation, and coal smelting [34]. The estimated global emission of atmospheric As is 7.8×10^7 kg year⁻¹, whereas natural sources contribute to 1.2×10^7 kg year⁻¹. Emission from volcanoes amounts to 8.9×10^6 kg year⁻¹, while 2.1×10^7 kg year⁻¹ of As is emitted by microbial volatilisation [35]. Arsenic in the atmosphere can pass into biological systems via inhalation. The particle size and solubility of As determine the extent of its absorption by biota [34]. According to the ATSDR [36], the exact amount of As absorbed by respiration is unknown, but it ranges from 60 to 90% of the As present in the air. The utilisation of pesticides for vegetables, fruits, and crops leads to soil contamination through soil leaching or washing, brought by heavy rains or floods. In soil, As is present in three major forms: arsenite (As³⁺), arsenate (As⁵⁺), and organic arsenic. Arsenic enters humans and animals via inhalation or ingestion when contaminated vegetables, fruits, crops, and water are consumed [2,34].

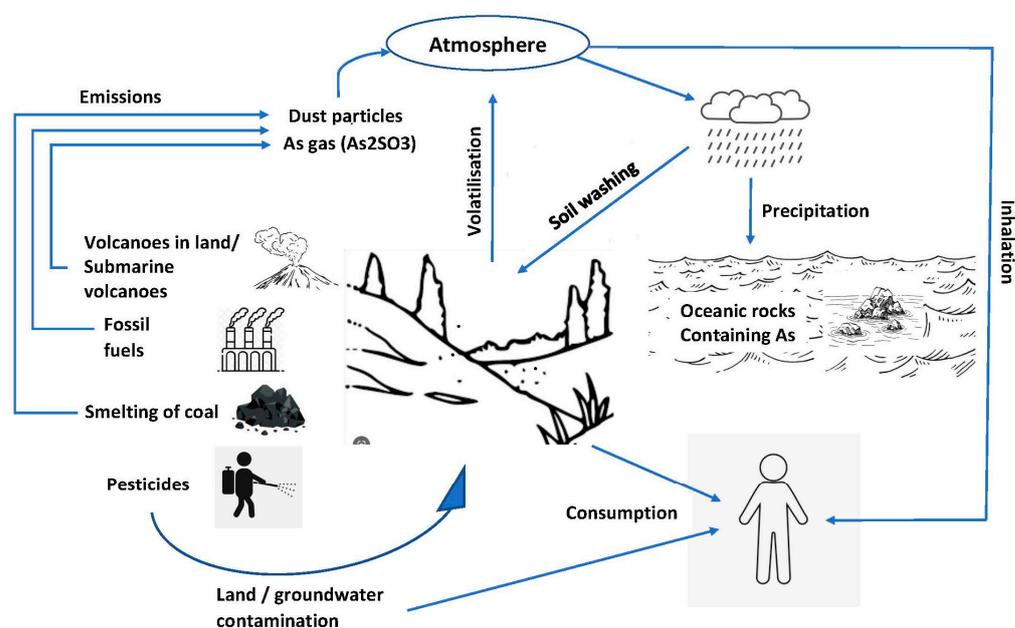


Figure 1. The arsenic cycle.

In water, As exists in the forms of arsenate and arsenite. Arsenic can be found in river water, lake water, seawater, estuaries, and groundwater, mainly due to precipitation. In addition, As is present in mine drainage, sediment porewaters, oilfields, and other brines [37]. Moreover, As occurs in igneous and sedimentary rocks at concentrations of

0.5–2.5 mg kg⁻¹ and 1–13 mg kg⁻¹, respectively. Several factors, including the rock type, pH, and organic and inorganic components, influence the concentrations of As in rocks [38].

2.5. Global Contamination of Arsenic

Murcott [39] stated that global As contamination is derived from anthropogenic, coal, geogenic, mining, petroleum, and volcanogenic sources that occur in soil and groundwater. Figure 2 shows a world map highlighting the 150 countries/territories that report As contamination in drinking water and the environment.

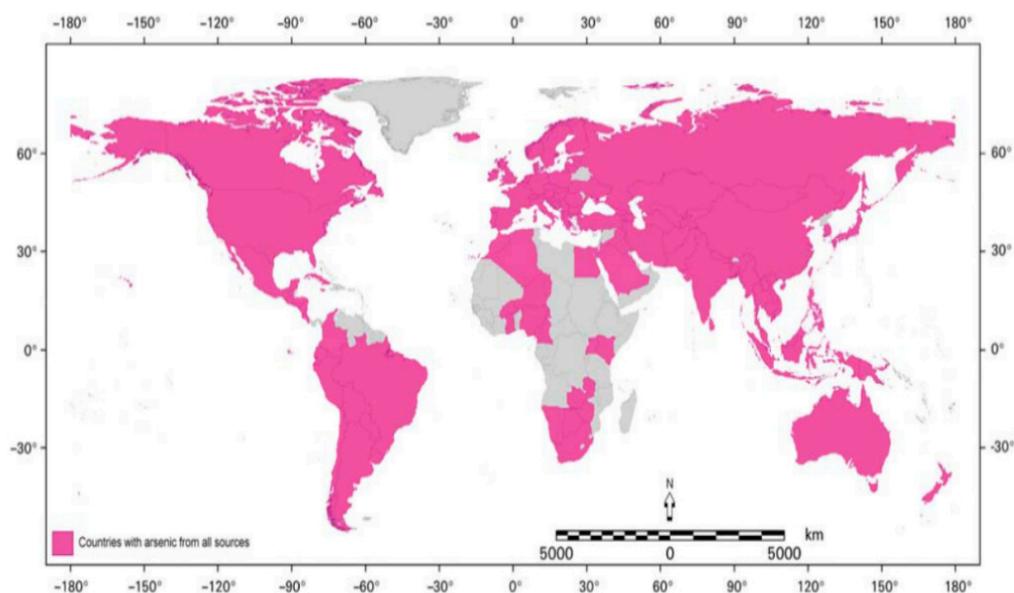


Figure 2. World map highlighting the 150 countries/territories that are contaminated by arsenic from various sources, namely anthropogenic, coal, geogenic, mining, petroleum, and volcanogenic; reproduced with permission from Susan Murcott, *Arsenic Contamination in the World: An International Sourcebook*; published by IWA Publishing, 2012 [39].

2.6. Arsenic Toxicity to Living Organisms and Human Health

Exposure to As has become a major global concern that affects living organisms and human health. Arsenic contamination in groundwater can reduce the quality of drinking water, which poses toxicity to human health. The As levels in groundwater should not exceed the acceptable level of 10 ppb that is regulated by the World Health Organization. It has been reported that more than 230 million people across the world are affected by As exposure in groundwater. The most affected countries are Bangladesh, India, and Southeast Asian countries [40]. Several studies have also reported that As can reduce the soil quality, leading to reduced crop productivity. A study by Muehe et al. [41] indicated that grain yields decreased by 39% due to As contamination in the soil. Irrigation water contaminated with As has also been reported, causing a decline in the shoot and fruit biomass of tomato [42]. In addition, As toxicity to aquatic organisms in freshwater has also been reported by Wang et al. [43]. They found that zooplankton *Daphnia magna* could not survive when exposed to As concentrations (6 mg L⁻¹) for 21 days. Another study by Cordero et al. [44] showed that 5 μM of As₂O₃ caused the necrosis of European sea bass cells after 10 days of As exposure.

Arsenic pollution results in human health issues ranging from acute to chronic poisoning. According to Schoolmeester and White [45], the lethal dose of As for acute poisoning ranges from 100 to 300 mg. However, based on the Risk Assessment Information System database, the lethal dose of acute inorganic As is around 0.6 mg kg⁻¹ per day [46]. The first clinical symptom refers to gastrointestinal issues, such as watery diarrhoea, nausea, vomiting, and extreme abdominal pain [47]. Other symptoms include prolonged salivation,

severe cardiomyopathy, a systemic rash of the skin, seizures, and acute psychosis. In addition, several studies show that pulmonary oedema and respiratory are features of acute As poisoning. In acute cases, people can die in between 24 h and 4 days depending on the amount of As. Chronic toxicity can occur when people are exposed to As for a long time, which leads to multi-range illnesses [48].

3. Arsenic Pollution in Mine Sites

Mining activities represent one of the main anthropogenic sources of As. Abandoned metal mine sites are notable sources of As contamination in the environment. Igarashi et al. [49] state that increasing levels of As (0.826 mg L^{-1}) have been detected in acid mine drainage (AMD) waste. Most of the As was found in the residual fraction, which comprises specific grains of As minerals derived from mine waste, which later merge with the soil mineral fraction [50]. Eisler [51] confirmed that mining residues, including those from soils and rock wastes, ore roasting, and tailings, were sources of As pollution at mining sites; tailings were noted as the main As source. Arsenic-containing tailings are commonly produced because of gold mining activities. The disintegration of sulphide minerals such as arsenopyrite (FeAsS) raises the concentrations of As in mine tailing dumps, especially in surface waters and groundwaters [52,53].

Several studies have reported high As concentrations in mine sites around the world. For example, in Ghana, As was detected at elevated concentrations ($1807\text{--}8400 \text{ mg kg}^{-1}$) in an abandoned gold mine site [54]. de Souza Neto et al. [3] investigated the total content of As in soils and tailings collected from gold mining sites in the Amazon. Elevated As concentrations were detected at all exploration sites, especially in tailings, which reached a maximum value of $10,000 \text{ mg kg}^{-1}$. This value is significantly higher than the health investigation value determined by the Brazilian National Council of the Environment (150 mg kg^{-1}). Arsenic ($83\text{--}2224 \text{ mg kg}^{-1}$) was also found in a realgar mine soil in China, with concentrations exceeding the Grade III values (40 mg kg^{-1}) listed by the China Environmental Quality Standard for Soils [55]. An excessive As concentration ($24.05 \text{ } \mu\text{g L}^{-1}$) was also found in groundwater in West Africa, a value significantly higher than the acceptable level given by the World Health Organization (WHO) ($10 \text{ } \mu\text{g L}^{-1}$) [56]. In Australia, As is known to be the contaminant found at the highest concentration at gold mining sites. Historically, gold mining occurred in many states in Australia but was greatest in Victoria. In Victoria, the As concentrations range from 280 to $15,000 \text{ mg kg}^{-1}$ [57].

4. Restoration of Arsenic-Contaminated Soil

An effective technology is required to manage As-contaminated soils. Wan et al. [58] reviewed the chemical, physical, and biological methods that have been used to remediate As-contaminated soils (Table 1). Soil washing and immobilisation represent two commonly used approaches to clean soil containing As. Soil washing is a remediation technique that utilises chemicals such as acids and bases as reagents to wash soil. For instance, oxalic acid has been used to wash As-contaminated soil at an abandoned mine site; the bioavailability of As was reduced, but still exceeded the acceptable limit of regulated As (5 mg L^{-1}) [59]. This method can be applied in situ or ex situ. However, soil washing is an expensive method and can cause environmental pollution due to leachate diffusion (Table 1). Moreover, the treatment can destroy soil structure and properties, making it unsuitable for rehabilitation [58]. Immobilisation is another method that uses chemical agents to immobilise As and decrease its mobility in soils (Table 1). Iron (Fe) oxides, solid waste, and biochar have also been used to facilitate soil restoration [60].

Recently, it has been reported that combining restoration treatments can significantly enhance As remediation; for example, Wu et al. [61] combined different types of Fe oxides and biochar to remediate As (Table 1). Among the Fe types, biochar-Fe-oxyhydroxy showed the highest percentage ($13.95\text{--}30.35\%$) of As remediation. Although this method is cheaper than soil washing, this approach requires the long-term observation of its efficacy, particularly regarding the potential for As to transform back into an active state.

Physical methods including soil replacement or soil cover, attenuation, and electrokinetic remediation have also been used to remove As from soil (Table 1). Contaminated soil can be replaced or covered with clean soil to reduce the As concentration. Soil replacement is usually applied in a highly contaminated sites, while soil cover is applied when the As concentration in a contaminated site is lower than in the surrounding site. The problems related to this approach include difficulties in obtaining clean soil and the possibility of environmental pollution during the transportation of contaminated soil (Table 1). Mixing contaminated soil with clean soil, termed attenuation or turnover, is another technique aimed at diluting the soil As concentration [58]. Wan et al. [62] conducted a field experiment for three years to remediate As-contaminated soils. The soil (0–60 cm depth) was raked and mixed and then planted with wheat and annually harvested. The result revealed that the As concentrations in the soils declined by 50% compared to the control treatment. Although the method successfully reduces As, it is a high-cost approach and requires regular maintenance and monitoring (Table 1). Electrokinetic remediation (EK) involves the migration of pollutants from the treatment area to the electrode area through ion exchange extraction. Hawal and Al-Sulttani [63] set up a chamber filled with contaminated soil. Tap water and cyclodextrin were used as an electrode solution and enhanced agent, respectively; it was demonstrated that EK could eliminate As from contaminated soil. However, this method is limited to laboratory experiments and, although promising, scaling up has not been attempted and the impact on the soil properties is unknown. Overall, despite the effectiveness of several physical methods, EK is expensive, and it has not yet been applied on a large scale (Table 1).

Bioremediation and phytoremediation are categorised as biological methods to remediate As. Compared to chemical and physical methods, biological methods are cheaper and represent environmentally friendly approaches. Bioremediation refers to the utilisation of microbes to remediate pollutants. Various microbes, including bacteria, archaea, and fungi, have been studied for their capabilities in remediating As. Yin et al. [65] reported that *Stenotrophomonas* spp., *Halorubrum* spp., and *Aspergillus* spp. are examples of As-remediating microbes that belong to bacteria, archaea, and fungi, respectively. Despite its low cost, bioremediation is sometimes only limited to laboratory experiments, as it represents a protocol requiring time and therefore significant preliminary research is needed to understand the potential efficacy of any field application (Table 1).

Phytoremediation, which utilises plants as a remediation tool, is relatively simple to undertake as it does not require heavy equipment. It is also relatively cost-effective because it involves few workers (Table 1). Moreover, it is categorised as an eco-friendly technique as it reduces the contaminant risks and protects the ecosystems surrounding the contaminated area [58,66,67]. Phytoremediation has been applied as a method for risk management in polluted sites. Moreover, this approach leads to increased soil quality for the cultivation of crops through phytoextraction, which can improve the market value, and it can be commercially applied. Generally, plants with high biomass and high As accumulation are beneficial for phytoremediation [68,69]. Table 1 confirms that, compared to other methods, phytoremediation does not pose a negative impact on the environment. However, Srivastava et al. [70] noted that several factors, including the selection of plant types applied for phytoremediation and the environmental conditions, should be considered before phytoremediation application.

Table 1. Advantages and disadvantages of approaches used for restoration of arsenic-contaminated soils, adapted from [58,64].

Approaches	Advantages	Disadvantages
Chemical technologies		
Soil washing	- Applicable both in situ and ex situ.	- Destroys soil quality. - Requires significant expenditure. - Promotes secondary pollution during soil transportation for ex situ. - Causes As diffusion. - High cost.
Soil immobilisation	- Low cost. - Convenient to apply. - Applicable for field experiments. - Can use multiple agents.	- Requires long-term monitoring. - Can return As to an active state.
Physical technologies		
Soil replacement/soil cover	- Not reported.	- Requires significant expenditure. - Energy demanding. - Difficulties in obtaining adequate clean soil. - Promotes secondary pollution during soil transportation. - Decreases soil nutrients.
Turnover and attenuation	- Reduces As quickly and efficiently.	- Expensive - Decreases soil nutrients. - Needs high maintenance and expert labour. - Difficulties in obtaining sufficient clean soil.
Electrokinetic remediation	- Decreases As rapidly. - Reduces high As in topsoil.	- Can only remove mobile fractions of As. - Reduces As at lower rate in deeper soil layers.
Biological technologies		
Phytoremediation	- Low cost. - Simple to operate. - Environmentally friendly.	- Needs a longer time for plant reproduction. - Requires pollution control during plant harvest.
Bioremediation	- Low cost. - Environmentally friendly.	- Requires a combination of microorganisms or other remediation methods to achieve high removal of As. - Limited to laboratory experiments. - Requires high adaptation of microorganisms to the environment.
Combined technologies		
Phytoextraction and soil washing	- Improves As removal.	- Needs safe eluents that do not have negative effects on hyperaccumulator growth.
Electrokinetic remediation and soil immobilisation	- Controls acidification. - Improves As removal.	- Requires the use of expensive chemicals.

5. The Concept of Phytoremediation

The word phytoremediation originates from the Greek word *phyto*, which means “plant”, and a Latin word, *remedium*, which means “to delete an evil”. It is an environmentally friendly method that utilises plants and related microorganisms to reduce the toxic impacts of contaminants within the environment. Phytoremediation can be differentiated into several mechanisms (Figure 3): phytoextraction, phytofiltration, phytostabilisation, phytovolatilisation, phytodegradation, rhizodegradation, and phytodesalination [71].

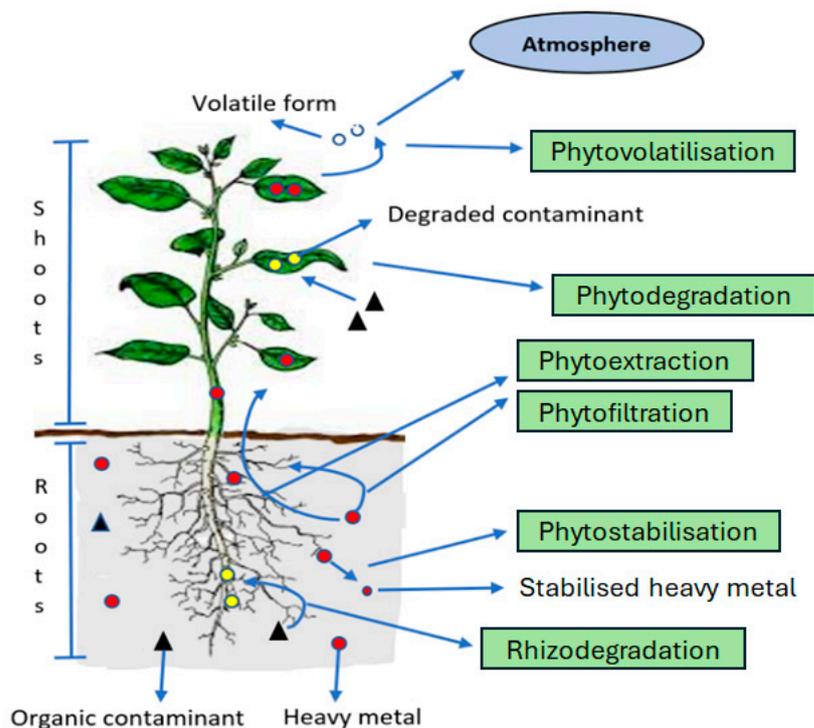


Figure 3. Different mechanisms of phytoremediation for the removal of heavy metals and organic contaminants from contaminated soils.

Phytoextraction, also termed phytoaccumulation, phytoabsorption, or phytosequestration, is a process in which the plant roots uptake the contaminants from the soil or water, which are then translocated and accumulated within the plant biomass. For effective phytoextraction, the translocation of metals to the shoots is desirable because the shoots are easy to harvest compared with the root biomass [72]. Susarla et al. [73] reported that plants can be effectively used for phytoextraction if they can accumulate more than 0.1% dry weight of pollutants in their shoots. Phytodegradation is a technique that involves degrading organic pollutants assisted by enzymes within plant tissues. Heavy metal(loid)s cannot be degraded by this method because they are non-biodegradable. In contrast, organic pollutants such as PAHs can also be broken down in the rhizosphere by microorganisms inhabiting the rhizosphere, a process defined as rhizodegradation. Another technique for phytoremediation is phytodesalination, which uses halophytic plants to remove salts from the soil to support plant growth [75].

Among these techniques, phytoextraction and phytostabilisation are recognised as the most widely used phytoremediation techniques for heavy metal(loid)s [76], representing promising techniques for the long-term removal of As from soil [77]. Phytoextraction is more efficient; however, several factors, including the plant species, the soil bioavailability, the soil properties, and the speciation of the heavy metal(loid)s, affect its efficacy [77]. The plant species used for phytoextraction are required to have some key characteristics; these include their ability to accumulate more than 1000 mg kg^{-1} (i.e., a hyperaccumulating plant) of the heavy metal, some 50–500 times higher than average plants [78,79]. In addition, they

must produce high quantities of biomass, tolerate high concentrations of heavy metals, and adapt to biotic and abiotic stresses to be easily cultivated [77]. The successful application of phytoextraction in contaminated soils has been reported. For example, Ranieri et al. [80] showed that Cr-contaminated soil was remediated with removal rates ranging from 49.2% to 61.7% through phytoextraction using *Phyllostachys pubescens*. Another study by Yu et al. [81] showed that *Bidens Pilosa* L. and *Xanthium strumarium* L. presented the highest phytoextraction capacity for various metal(loid)s (Pb, Cd, Cn) in contaminated soils.

Plants selected for phytostabilisation require the ability to produce an extensive root system that immobilises the contaminants in the rhizosphere and reduces the translocation of As from root to shoot. The plants must also be easy to grow and quickly form a dense canopy and root system [82]. Fernández et al. [83] showed that three plants, *Atriplex atacamensis*, *Atriplex halimus*, and *Atriplex nummularia*, accumulated higher As concentrations in their roots than in their shoots, confirming the suitability of the plants for phytostabilisation.

6. Phytoremediation of Arsenic-Contaminated Mine Sites

Numerous studies have reported the application of various plant species for the phytoremediation of As-contaminated mine sites (Table 2).

Table 2. Studies showing the plant species used for the phytoremediation of arsenic-contaminated mine sites.

Plant Species	Group	Total As Concentration in Plants (mg kg ⁻¹)	Country	Reference
<i>Artemisia divarica</i>	Dicotyledons/flowering	50.88	China	[84]
<i>Acacia mangium</i>	Dicotyledons/flowering	1549	Malaysia	[85]
<i>Leucaena leucocephala</i>	Dicotyledons/flowering	6.83	Ghana	[86]
<i>Pityrogramma calomelanos</i>	Fern	161.82–280.18	Philippines	[87]
<i>Pteridium aquilinum</i>	Fern	722	Nigeria	[88]
<i>Pteris cretica</i>	Fern	4875	UK	[89]
<i>Pteris ensiformis</i> Burm.	Fern	2138	China	[90]
<i>Pteris vittata</i>	Fern	1911	Vietnam	[91]
<i>Pteris vittata</i> L.	Fern	7215–11,110	China	[92]
<i>Verbascum thapsus</i>	Dicotyledons/flowering	22,145	Turkey	[93]

Table 2 shows that a number of reported studies have used ferns and flowering plants [94], confirming their ability to uptake As. Fayiga and Saha [95] stated that ferns were mostly reported as hyperaccumulators of As. It can be seen from Table 2 that ferns such as *Pteris vittata* exhibit high As uptake. However, it also reveals that flowering plants can accumulate As. Phytoremediation can be used with a single plant but can also be combined with other plants. Srivastava, Shukla, Rajput, Kumar, Minkina, Mandzhieva, Shmaraeva, and Suprasanna [70] stated that the combination of hyperaccumulator plants and native high-biomass plants can be effectively used for As phytoremediation. Further, intercropping with crops such as *Oryza sativa* and As accumulator plants (e.g., *Pteris vittata*), and the use of transgenic plants, represent other approaches to improve As phytoremediation. However, the widespread application of transgenic plants in the environment remains a contentious issue.

Arsenic exists in different forms in soil and plants, including arsenate (As[V]), arsenite (As[III]), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) [96]. When As is predominantly in the inorganic form, it is taken up by plants through root absorption, along with other nutrients. Little information regarding the mobilisation of As in plants is known; however, it has been reported that As[V] and phosphate have the same transport pathway in higher plants. In addition to phosphate transporters, aquaporins are

also reported to be involved in As absorption. The reduction from As[V] to As[III] occurs inside the root cell, specifically in the vacuole. The reaction is $(AsO_4)^{3-}$ (arsenate) + 2 GSH (glutathione) \rightarrow $(AsO_3)^{3-}$ (arsenite) + GSSG (oxidised glutathione), catalysed by arsenate reductase (AR). Arsenic is mostly absorbed in the roots, which results in a reduced concentration within shoot material [97]. The uptake mechanisms of As by plants are illustrated in Figure 4.

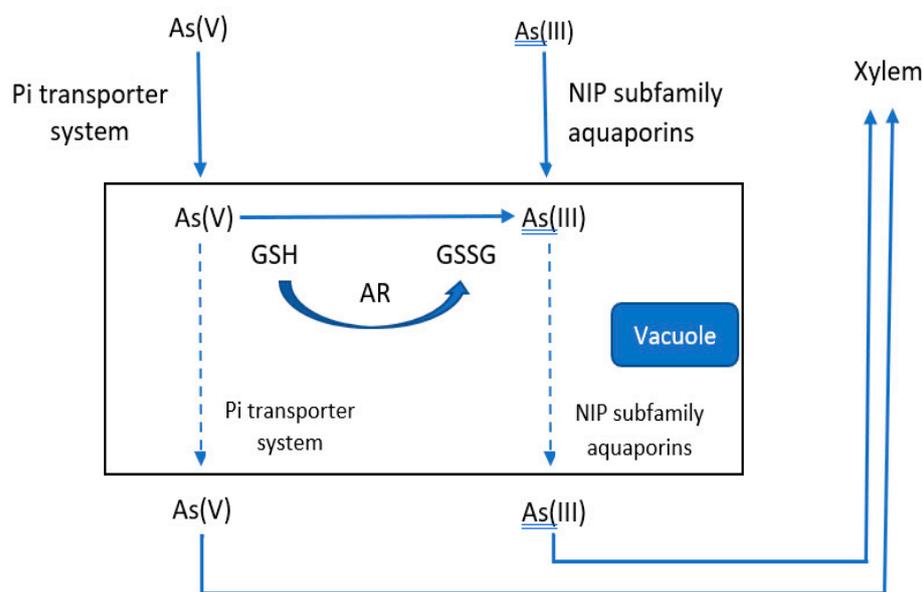


Figure 4. Mechanism of arsenic uptake by plant root cells.

7. Co-Application of Phytoremediation for Arsenic Removal from Contaminated Soils

Although phytoremediation represents a promising technology in terms of remediating As-contaminated soils, it has several limitations. Low plant biomass can promote the accumulation of high concentrations of As in the shoots, while plants with increased biomass may accumulate only low concentrations of As. Moreover, some plants are unable to grow because of As toxicity [98]. Fatima et al. [99] stated that the immunity of plants against toxicity, the fertility of the soil, and plant virulence represent several constraints affecting the phytoremediation performance. Recently, there has been research focused on improving plant growth, resulting in the increased efficacy of As phytoremediation. Reports show that phytoremediation can be co-applied with chemicals, as well as biological and physical methods, to enhance the effectiveness of As phytoremediation (Table 3).

Table 3. Co-treatments for arsenic phytoremediation.

Methods	Advantages	Disadvantages	References
Chemical - Chemicals (e.g., ethylenediamine tetraacetic acid (EDTA), diethylenetriamine pentaacetic acid, ethyleneglycol tetraacetic acid (EGTA), sodium dodecyl sulphate) - Chelating agents (e.g., acetic acid, oxalic acid (OA), malic acid) - Fertilisers, composts, biochar	Stimulate plant growth; increase the phytoextraction capacity of the plants.	Alter the soil microflora activity; may result in reduced ecosystem services.	[18–20,100]

Table 3. Cont.

Methods	Advantages	Disadvantages	References
Physical			
Electrokinesis	Improves plant biomass production; increases metal uptake.	Exposure of heavy metals to the plants; may exacerbate plant stress.	[21]
Phytosuction partitioning	Time-efficient; greater tendency to remove As; enables nutrients to move towards the plants.	Suitable for only shallow contaminated soil layers.	[21,23]
Biological			
Genetic engineering (transgenic plants)	High specificity and sensitivity; improves phytoextraction capacity from root to shoot.	Time-consuming; high cost; regulatory issues; genetic pollution of natural environment.	[18,21,101]
Co-cultivation and intercropping	Economically profitable for contaminated land's owner; safe for environment.	Requires a commercial crop to be grown.	[21,22]
Bacteria, fungi, algae - Bacteria (e.g., <i>Pseudomonas</i> sp., <i>Delftia</i> sp., <i>Bacillus</i> sp., nitrogen-fixing bacteria) - Fungi (e.g., <i>Rhizoglyphus intraradices</i> , <i>Glomus etunicatum</i>) - Algae (e.g., <i>Chlorodesmis</i> sp., <i>Cladophora</i> sp.)	Cost-effective and environmentally friendly techniques; decrease the requirement for chemical fertilisers.	Microbes (bacteria) cannot always tolerate the high toxicity associated with As-contaminated soils.	[18,102–104]

7.1. Chemical and Physical Methods

Phytoremediation can be assisted by chemical and physical methods. Some chemicals, such as ethylenediamine tetraacetic acid (EDTA), diethylenetriamine pentaacetic acid, and sodium dodecyl sulphate, have been tested in combination with phytoremediation to remove As from contaminated soil [19,20]. Chelating agents and fertilisers have also been applied to improve phytoremediation (Table 3). All approaches resulted in enhanced As phytoremediation; however, they possess disadvantages—both the soil microflora and soil enzyme activity can be altered, and the environment can also be negatively impacted [105].

Biochar is a soil amendment that has recently gained attention in terms of its potential to improve phytoremediation. Biochar is a carbonaceous material produced by the pyrolysis of organic materials; biochar can also be produced by gasification, hydrothermal carbonisation (HTC), flash carbonisation, and torrefaction [106]. Biochar can be produced from various organic sources, such as pine wood, stinging nettles, olive pruning, rice husks, sewage sludge, and olive mill waste. Biochar has been proven to increase the soil quality, which promotes plant growth and protects plants from disease and other abiotic stresses [16]. Biochar has several potentially beneficial characteristics, including highly stable carbon, a high pH, and a high surface area [107]. Moreover, biochar can enhance carbon stability and microbial activity [16]. Many studies have reported that plant growth can be increased by biochar addition. For example, a pot experiment was conducted by Lebrun et al. [108] to investigate the effect of applying different rates (1%, 2%, 3%, 4%, 5%) of biochar on As phytoremediation in mine soil using *Linum usitatissimum* L. Increased As uptake was shown in plants grown with 3–5% (*v/v*) biochar, while soils with 1% and 2% biochar showed the lowest As uptake. Plant biomass was enhanced by the addition of biochar at all concentrations, but the highest total biomass was shown by plants grown in the presence of 5% biochar, in both the shoot and root.

Physical methods including electrokinesis and phytosuction partitioning have been applied to remediate As-contaminated soils. Electrokinetics (EK) is a physical method coupled with phytoremediation that was developed in the 2000s. This technique involves

the transmission between electrodes of a low-intensity electrical stream placed vertically in the soil [109] (Table 3). The application of EK-assisted phytoremediation in mine sites has been reported in several studies. For example, Couto et al. [110] utilised graphite rods as electrodes for As phytoremediation using Indian mustard and ryegrass, based on their cost-effectiveness and accessibility. The results confirmed an increase in As uptake in both plants following EK treatment. However, only Indian mustard showed a significant improvement in terms of As accumulation in the roots. During EK, the fate of the contaminated matrix is complex, dependent on the treatment time, soil properties, and types of electrolytic dissolution [111]. Phytosuction partitioning is a recently established physical technique in As phytoremediation [23]. This method has limitations, as it requires a deeper soil layer [21].

7.2. Biological Methods

Phytoremediation has also been combined with biological techniques. Genetic engineering has been employed to enhance As tolerance by modifying targeted genes in plants. Nahar et al. [112] found that tobacco plants were more resistant to As after the *arsenic reductase 2* gene, isolated from *Arabidopsis thaliana* was inserted. The use of transgenic plants is beneficial as they display high specificity and an improved translocation capacity in plants; however, the process is time-consuming, incurs high costs, and must overcome significant environmental regulations [18]. Combining As hyperaccumulator plants with other plants to reduce the As levels, termed intercropping, has been reported in several studies. Lin et al. [113] studied *Pteris vittata*, a hyperaccumulator plant that was intercropped with *Panax notoginseng*, a well-known medicinal plant. The results showed a decrease in the As concentration in *P. notoginseng*. In addition, *P. vittata* intercropped with *Morus alba* reduced the As concentration in *M. alba*, but increased As uptake in *P. vittata*. Without intercropping with *M. alba*, the As concentration in *P. vittata* leaf material was 404.9 mg kg^{-1} , which increased to 564.2 mg kg^{-1} after intercropping [22]. Intercropping can provide benefits for the owners of contaminated land as well as the ecosystem. Nevertheless, as this method is used to protect crops, it is unsuitable for applications to land without crops (Table 3).

The utilisation of bacteria, fungi, and algae is a promising way to improve phytoremediation, as reviewed by Gupta et al. [18]. These methods are low-cost, safe, and sustainable and can reduce the use of chemical fertilisers. Microbes play a significant role in phytoremediation through interactions with plants in the rhizosphere, which result in improved plant growth as they promote nutrient uptake (N, P, K) from the soil into the plant [114]. Additionally, they can assist in altering As speciation in the soil through methylation, which impacts As uptake in plants [115]. Many studies have been published that show the effectiveness of using microbes for As phytoremediation. Lampis et al. [103] conducted a study of the effects of five species of As-resistant bacteria (*Pseudomonas* sp. P1III2, *Delftia* sp. P2III5, *Bacillus* sp. MPV12, *Variovorax* sp. P4III4, *Pseudoxanthomonas* sp. P4V6) on As phytoremediation by *Pteris vittata*. The results suggested that a mixed inoculum of bacteria successfully increased As uptake by plant fronds from $21.1 \pm 1.9 \text{ mg}$ to $134.17 \pm 7.29 \text{ mg}$ and by the roots from $1.44 \pm 0.16 \text{ }\mu\text{g}$ to $12.79 \pm 0.37 \text{ }\mu\text{g}$. The presence of the bacteria also enhanced the plant biomass by up to 45%. Other studies indicated that the enhancement of As uptake by plants was correlated with an increase in plant biomass [116,117]. Nitrogen-fixing bacteria (NFB) have also been shown to increase plant growth [118–120]. NFB can uptake minerals such as N, P, K, and microelements, which stimulate plant growth. Further, they play an important role as biological control agents and can help plants to survive under abiotic stress [114].

8. Importance of Macronutrients and Micronutrients for Plant Growth

Nutrients are a crucial soil component, required for plant growth and development during phytoremediation. They are categorised into two types: macronutrients and micronutrients. Nitrogen (N), potassium (K), phosphorus (P), magnesium (Mg), sulphur (S), and calcium (Ca) represent key plant macronutrients, with N, P, and K being the most

critical [121]. Nitrogen plays a number of key roles integral to plant growth and development, including the synthesis of nucleic acids and proteins [122]. Nitrogen can also impact the development of the volume, diameter, and area of roots and leaves, improve nutrient uptake, and increase the nutrient balance and plant biomass. Nitrogen deficiency causes several symptoms in plants, such as narrow leaves and yellow leaf colouration and a reduction in plant growth [121,123]. Phosphorus has essential roles relating to plant energy metabolism and photosynthesis. Limited P also causes changes in the colour of older foliage, turning the leaves orange or red [124]. Potassium is the second most abundant macronutrient (after N) found in plant tissue. It is required for the completion of several physiological and metabolic processes in plants [125].

Numerous studies related to the impacts of N, P, and K on plant growth have been reported. For example, in rice production, the highest dry weight yield was most impacted by sufficient N availability; further, lowest yields were observed with N deficiency [122]. A study by Thalassinos et al. [126] revealed that the addition of N significantly increased the plant height of *Portulaca oleracea* during the phytoremediation of Cr(VI)-contaminated soils.

Micronutrients are also beneficial for plant growth, albeit at lower concentrations. Examples of micronutrients are boron (B), copper (Cu), iron (Fe), zinc (Zn), and nickel (Ni). They have various functions related to photosynthesis, plant production, and plant hormone regulation [127]. However, exceeding the optimum concentrations of micronutrients can be toxic for plants. Further, they might have synergic interactions with heavy metal(loid)s. For example, the increased uptake of Zn and Cu by plants can be caused by the presence of Pb, which results in plant growth inhibition [128].

9. Limitation of Nitrogen Availability in Mine Soil

Available N is required by plants in high concentrations ($1000 \mu\text{g kg}^{-1}$ dry matter) to enable them to grow, develop, and reproduce [17,129]. To improve plant productivity during phytoremediation, additional N may be required. The concentration of total N in most soils ranges from 500 to 20,000 mg kg^{-1} ; lower concentrations suggest that plants may suffer from N deficiency [17]. In mine soils, N has been reported as a major limiting nutrient. The impacts of mining activities, such as mixed soil horizons, the loss of soil organic matter, surface erosion, stockpiling, a coarse texture, nitrate leaching, the loss of vegetation, and heavy metal content, are the major causes of soil N loss. A deficiency in available soil N may occur due to the mixing of mine spoils and soils, which reduces the natural concentration of N [17]. A study conducted by Ahirwal et al. [130] showed differences in the N content at three different soil depths (0–20 cm; 20–40 cm; 40–60 cm). The N levels were higher in the top layer (0–20 cm) and gradually reduced in the lower layers. The low N levels at lower soil depths impacted plant growth [131]. Wang et al. [132] also reported that N was detected at very low concentrations (0.05 g kg^{-1}) compared to other soil nutrients in a gold mining site in China, which resulted in reduced plant growth. Domingo and David [133] reported that the N content in mine soil remained at 100 mg kg^{-1} in a reclaimed mine site in Philippines. This low N concentration may have been caused by high concentrations of heavy metals in the soil. In summary, N limitation plays a critical factor in restricting ecosystem development in mining-disturbed soils. Mining site recovery is highly reliant on N availability because of the soil properties [134–136]. Therefore, the addition of N in post-mine soils is often a prerequisite for mine soil rehabilitation [137].

10. Potential Approaches to Enhance Soil Nitrogen Content for the Phytoremediation of As and Other Pollutants

Several approaches have been applied to enhance the soil N content during the phytoremediation of As and other pollutants, as described below.

10.1. Nitrogen Fertilisers

Nitrogen fertilisers have been used to increase N uptake by plants. Liao et al. [138] used five different types of N fertiliser (urea, KNO_3 , NH_4HCO_3 , $(\text{NH}_4)_2\text{SO}_4$, and $\text{Ca}(\text{NO}_3)_2$).

for As phytoremediation. They found that the addition of the fertilisers increased N uptake by *Pteris vittata*, ranging from 12.4 to 27.1 g kg⁻¹, compared to the control treatment (7.8–8.7 g kg⁻¹); this resulted in enhanced plant biomass and As uptake. In contrast, another study reported that the addition of N fertiliser decreased the *Sedum alfredii* Hance biomass, N concentrations, and Cd and Zn uptake. Clearly, further research is required to understand the association between N fertiliser addition and phytoremediation, particularly with As. Although cost-effectiveness is reported as an advantage [139] of N fertiliser, significant negative environmental impacts result from its addition, including atmospheric pollution and leaching. It is expected that N pollution will increase by up to 156% by 2025 [140]. Further, Barłóg et al. [141] reported that the effectiveness of using N fertilisers has decreased from 68% to 47% over the last 10 years. In summary, despite some evidence for the effectiveness of N fertiliser, the efficacy of this amendment, together with the significant negative environmental impacts of its use, suggest that more sustainable means of soil N addition may be more effective.

10.2. Biochar

As shown in Table 3, biochar is one of the main approaches that has been applied with phytoremediation. In recent years, biochar has been widely used because of its effectiveness in restoring soil, including enhancing the soil N content [142]. Several studies report that biochar improves the N availability in soils together with plant N uptake. For example, Song et al. [143] found that maize straw biochar increased the N content in soil from 0.59 g kg⁻¹ to 0.82 g kg⁻¹. Enhancement in N uptake by plants (145.48 kg ha⁻¹ to 217.15 kg ha⁻¹) was also reported by Faria et al. [144], following sewage sludge-derived biochar application. Biochar produced from various sources contains N in both organic and inorganic forms that can be utilised by plants. The organic forms are hydrolysable N and water-soluble N; inorganic forms include nitrate (NO₃) and ammonium (NH₄). Biochar, with a high adsorption capacity, can also absorb NH₄ and NO₃ from soil to reduce the loss of N [145]. Hossain et al. [146] stated that biochar from various sources contained a wide range of N (0.24–6.8%). However, although the N content reached 6.8%, they stated that most of the biochar contained lower N (below 1.5%). They concluded that rehabilitation approaches that increase the N content of biochar should be considered. Lebrun et al. [108] noted that biochar produced from lightwood birch enhanced the soil N level and the growth of *Linum usitatissimum* on an As- and Pb-contaminated technosol. Biochar has been found to effectively assist the phytoremediation of As in polluted soils [16,147]. However, further studies are required to investigate the impact of biochar from different sources and with different concentrations on As phytoremediation.

10.3. Compost and Manure

An increase in soil N as a result of compost addition as an organic amendment during the phytoremediation of As using barley and wheat plants was reported by González et al. [148]. Further, Wan et al. [149] stated that the total soil N was significantly higher (0.81 g kg⁻¹) as a result of the addition of sewage sludge compost compared to the control treatment (0.5 g kg⁻¹) during the phytoremediation of As-contaminated mine soils by *Pteris vittata*. Several studies report that compost is beneficial for phytoremediation, but few focus on the investigation of its impacts on N content. Compost is a low-cost and sustainable approach to increase the N content, but it may develop additional pollution in soils if applied in large quantities, due to the presence of additional heavy metals in some composts [150].

Farmyard manure (FYM) has been applied with *Penistum americanum* and *Sorghum bicolor* for the phytoremediation of heavy metal(loid)-contaminated mine soils. Soil amended with FYM had higher N levels (6.81%) than the control treatment (0.42%) and promoted plant growth. However, FYM significantly reduced heavy metal(loid) uptake [151]. It has also been found that other types of manure, such as green manure and cattle manure greatly

increase soil N content. However, manure may reduce the total soil microbial community through increased soil acidity [152].

10.4. Nitrogen-Fixing Bacteria

Nitrogen-fixing bacteria (NFB) play an important role as an input of fixed N in most terrestrial habitats [153]. This N input improves the fertility of mine soil, confirming the potential of using NFB to enhance the N availability to plants during phytoremediation (Table 3). Nitrogen-fixing bacteria promote N availability through the production of organic acids and siderophores. Their presence is reported to improve the plant biomass and the uptake of heavy metal(loid)s and improve soil nutrient levels [154]. Jach et al. [155] stated that it is vital to identify and select symbiotic NFB and plants that are resistant to metals to tackle N input issues and enhance the organic matter in mine sites. Several studies have shown the impacts of NFB on plant growth, N concentrations, and pollutant uptake in mine sites; however, to the best of our knowledge, no studies have focused on As uptake.

Table 4 summarises several studies that have used various NFB inoculated in plants that were applied to mine soils. Some studies detected the pollutant types and their levels in mine sites and tested the efficacy of the bacterial inoculation of the plants on metal(loid) uptake, plant biomass, and N content. Most studies reported that NFB addition led to substantial increases in heavy metal(loid) uptake as well as increased plant growth and soil N content. Although N is known as a limited nutrient in mine sites, Padda et al. [156] showed that *Pinus contorta* var *latifolia* thrived in mine sites with low N content. However, the study found that a *Pseudomonas* strain isolated from the plant tissue of *Pinus contorta* var *latifolia* could improve the N content, resulting in enhanced plant growth. Oubohssaine et al. [157] reported that NFB are paramount for mine site restoration as they can enhance N availability in soil. The presence of free-living, symbiotic, or endophytic bacteria, which play important roles in nitrogen fixation, is firmly linked to N accumulation in soil.

Table 4. Studies assessing the impacts of NFB on plant biomass, nitrogen levels, and/or pollutant uptake in mine sites.

Nitrogen-Fixing Bacteria	Plant	Pollutants	Results	Reference
<i>Pseudarthrobacter oxydans</i> , <i>Rhodococcus qingshengii</i>	<i>Sulla spinosissima</i> (L.)	-	- <i>Pseudarthrobacter oxydans</i> LMR291 increased the root biomass by 120 mg plant ⁻¹ . - <i>Pseudarthrobacter phenanthrenivorans</i> LMR429 increased the shoot biomass by up to 70 mg plant ⁻¹ .	[157]
<i>Rhizobium</i> sp. G-14, <i>Pseudomonas</i> sp. Y-5	<i>Bidens pilosa</i>	-	- <i>Rhizobium</i> sp. G-14 treatment doubled the fresh weight of plant roots and shoots compared to the control and <i>Pseudomonas</i> treatment. - <i>Rhizobium</i> sp. G-14 and <i>Pseudomonas</i> sp. Y-5 increased the N content in plant shoots and roots.	[158]
<i>Mesorhizobium loti</i> HZ76	<i>Robinia pseudoacacia</i>	Cd, Zn, Pb, Cu	- <i>Mesorhizobium loti</i> HZ76 addition led to higher shoot biomass of plants (up to 0.10 ± 0.025 g plant ⁻¹) in Pb-contaminated soil. - <i>Mesorhizobium loti</i> HZ76 addition led to higher Zn uptake by plants (up to 200 mg kg ⁻¹ in shoots; up to 800 mg kg ⁻¹ in roots).	[159]
<i>Sinorhizobium saheli</i> YH1	<i>Leucaena leucocephala</i>	Cd, Mn	- <i>Sinorhizobium saheli</i> YH1 increased the plant biomass, plant height, and root length by 67.2, 39.5, and 27.2%, respectively. - Enhanced N in plants by 10.0%.	[160]
<i>Bacillus megaterium</i> , <i>Bacillus mojavensis</i> , <i>Bacillus subtilis</i>	<i>Medicago sativa</i>	HMs	- <i>Bacillus megaterium</i> improved the root length by 17.4 cm, compared to the control treatment (7.7 cm). - <i>Bacillus megaterium</i> improved the shoot length by 4.6 cm compared to the control (2.7 cm).	[161]
<i>Bradyrhizobium</i> , <i>Rhizobium</i> , <i>Ochrobactrum</i>	<i>Pongamia pinnata</i>	Ni, Cd, Mn, Cu	All rhizobia increased the plant biomass and N content, but <i>Rhizobium</i> addition resulted in the highest biomass and N content.	[162]

11. Conclusions

The detrimental effects of As pollution in mine sites on human and ecosystem health have attracted the attention of researchers to provide solutions for As removal. The restoration of As-contaminated mine sites has been conducted using physical, chemical, and biological methods. Physical methods, such as soil replacement and electrokinetic remediation, involve significant equipment and workers, resulting in elevated costs and increased ecosystem damage. Chemical methods also pose negative impacts to the environment. Phytoremediation, a biological approach, is a safe, low-cost method that can be applied to remediate As-contaminated mine sites. The use of a variety of plants as remediation tools is beneficial in terms of As uptake, but plant growth may be limited in mine soils due to a lack of available nutrients, particularly N. Recent studies have shown that the co-addition of soil additives, such as nitrogen fertilisers, nitrogen-fixing bacteria, and composts, can result in improvements in the efficacy of phytoremediation in terms of enhancing the N content and heavy metal(loid) uptake. However, not all approaches promote plant biomass and pollutant uptake or represent sustainable approaches. Further research is required to investigate the impacts of soil additives on soil N content for As phytoremediation. Moreover, in addition to pot-scale experiments, field experiments should be conducted to gain a broader perspective on the effectiveness of soil additives and achieve a better management strategy for mine site restoration in the future.

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