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Review

Arsenic Remediation through Sustainable Phytoremediation Approaches

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Abstract: Arsenic contamination of the environment is a serious problem threatening the health of millions of people exposed to arsenic (As) via drinking water and crops grown in contaminated areas. The remediation of As-contaminated soil and water bodies needs to be sustainable, low-cost and feasible to apply in the most affected low-to-middle income countries, like India and Bangladesh. Phytoremediation is an aesthetically appreciable and successful approach that can be used for As decontamination with use of the best approach(es) and the most promising plant(s). However, phytoremediation lacks the required speed and sometimes the stress caused by As could diminish plants' potential for remediation. To tackle these demerits, we need augment plants' potential with appropriate technological methods including microbial and nanoparticles applications and genetic modification of plants to alleviate the As stress and enhance As accumulation in phytoremediator plants. The present review discusses the As phytoremediation prospects of soil and water bodies and the usefulness of various plant systems in terms of high biomass, high As accumulation, bioenergy potential, and economic utility. The potential and prospects of assisted phytoremediation approaches are also presented.

Keywords: arsenic; hyperaccumulator; nanoparticles; microorganisms; phytoremediation; *Pteris vittata*

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1. Introduction

Arsenic (As) contamination of the soil and water is a serious problem in several parts of the world, especially in South and Southeast Asian countries. It is an issue of concern owing to the toxic impacts of As on plants and humans and due to the span of the affected areas being very large [1]. The contamination of As has been caused mainly by biogeochemical processes in countries in South and Southeast Asia and by industrial and agricultural processes in European and North American countries [2–4]. The severely affected countries of South and Southeast Asia are renowned for intensive rice cultivation along with the dense population [5]. Thus, if even a single well or hand pump is contaminated with As in an area, a large number of people are affected. Further, rice cultivation is performed for two seasons or even three seasons in a year with the use of groundwater plus rainwater for irrigation. Therefore, when the groundwater in the area has As contamination, its use for irrigation adds a huge amount of As to the soil every year [6,7]. Another important point to consider is the fact that rice is the best-known accumulator of As among crop plants [8].

The availability, solubility and toxicity of different forms of As depend on the pH, ionic conditions, phosphorous and other elemental contents in the environment, whereas differences in uptake rates contribute to the degree of cellular exposure to arsenic. A majority of As released into the environment is inorganic and is accumulated by binding to organic soil matter. In an aerobic environment, mostly the arsenate [As(V)] form predominates, whereas the arsenite [As(III)] form is predominant in anaerobic conditions. A higher As(III) contamination in paddy fields due to water logged conditions and the presence of a potential As(III) accumulator plant, rice, are both of serious concern [9,10].

The problem of As contamination is the need for use of sustainable and low-cost solutions for the remediation of groundwater and soil [5,11]. There are several physical and chemical methods for the treatment of contaminated water and soil [12]. The natural microbial or plant-based approaches are known as bioremediation and phytoremediation, respectively. These methods are dependent on natural resources (minerals, water and solar energy) and therefore cost less and do not add any xenobiotics [13]. However, both methods have merits and limitations. The treatment of huge amounts of water/soil under in situ conditions by physico-chemical methods would be extremely costly [14], while the use of plants for this purpose would make the process very slow. In this regard, any method should have feasibility for application at the site itself, low-cost and be sustainable. Therefore, future research endeavors will require an optimum integration of physico-chemical and biological methods for effective sustainable remediation of contaminated areas.

Plants enhance soil fertility and enrich microorganisms of the soil during the course of remediation. In addition, the application of economically useful plants in phytoremediation makes it feasible for farmers to adopt it [15]. Plants with a faster growth rate, high biomass, and high shoot As accumulation are desirable for phytoremediation [16]. However, it has been difficult to find all three qualities in one plant. The plants with high As accumulation in shoots and a short life cycle have been found to have low biomass, while there are other plants which have high biomass but accumulate As with low efficiency [17]. Further, some high biomass economically useful plants suffer from As toxicity and cannot grow at their full potential. To overcome such difficulties, microbial association as a sustainable strategy has been utilized to enhance the growth and biomass of plants and to enhance their As accumulation efficiency [18,19]. Currently, the application of nanoparticles has become an acceptable approach for the reclamation of polluted ecosystems [20–22]. The concept of nano-phytoremediation technology has been emerging for the removal of contaminants from soil/water, which involves the application of both nanotechnology and phytoremediation [23–26]. However, the main challenge in using nanoparticles for the remediation of pollutants is the lack of an adequate number of reports proving its efficacy.

2. Phytoremediation: A Sustainable Approach

There are various approaches of As phytoremediation that can be utilized judiciously for remediation of contaminated sites. Various approaches are summarized in Figure 1 and are discussed below. Recent studies demonstrating the potential of various approaches have been presented in Table 1.

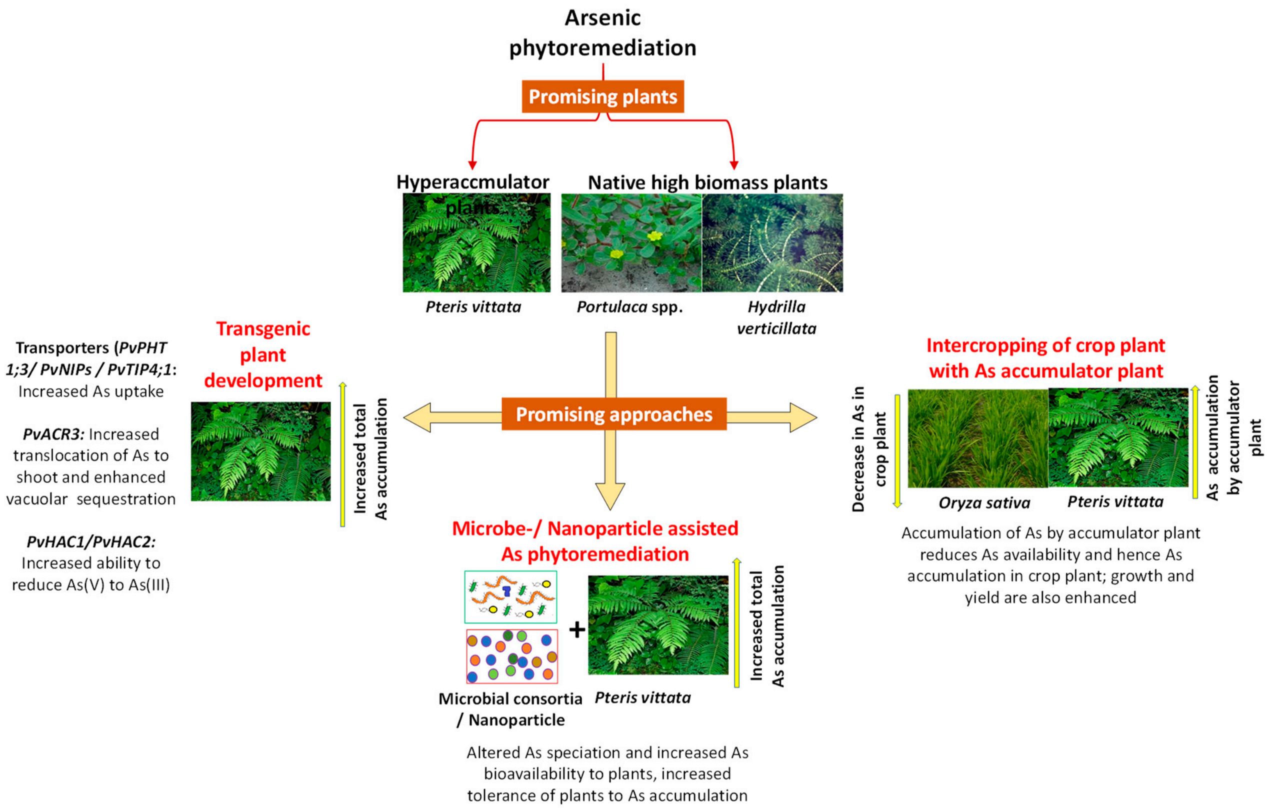

Figure 1. Various approaches to arsenic phytoremediation: use of hyperaccumulator plants or native high biomass and bioenergy plants; intercropping of arsenic accumulator plant with a crop plant for reduced arsenic toxicity to crop plant; microbe-or nanoparticle-assisted arsenic phytoremediation and the use of genetic engineering approaches to enhance phytoremediation potential of plants.

Table 1. A summary of recent studies on various phytoremediation approaches.

Plants	Arsenic Stress	Results	Ref.
	Arsenic Hyperaccumulator Plants		
Landolita punctata	As(V) (0.5–3.0 mg/L)	Plants showed As hyperaccumulation (>1000 mg/kg As) at or more than 1 mg/L As; however, higher than 1 mg/L As levels were toxic	[27]
Pteris vittata	As (average 8885 mg/kg) and thallium (3.91 to 178 mg/kg) contaminated mining area	*Pteris vittata* accumulated around 7215–11,110 mg/kg As, and 6.47–111 mg/kg of thallium	[28]
	High Biomass Producing Plants		
Calatropis prosera	Arsenic given in hydroponic and soil	*C. procera* reduced As concentration by 45% and 58% in hydroponics and by 30% and 36% in soil, after 15 and 30 days, respectively.	[29]
Portulaca oleracea	As (154 mg/kg and 193 mg/kg at site-I and site-II); other metals (Cd, Pb, Cu) were also present	At site I, As accumulation in stem was around 94.5 mg/kg, whereas at site II, it was 73.6 mg/kg	[30]
	Plants with Economic Utiliity		
Helianthus annus	Farmland soil containing As (84.85 mg/kg)	The mean As level 49.04 mg/kg in the above-ground parts. Average seed yield (45.90 kg/m^2) and oil production (34.65%)	[31]
Hydrilla verticillata	As(V) (15–375 µg/L)	Total As accumulation was 197.2 µg/g dry weight when As(V) was 375 µg/L	[32]

Table 1. *Cont.*

Plants	Arsenic Stress	Results	Ref.
		Microbe-Assisted Arsenic Remediation	
Arundo donax + consortia of two strains of *Stenotrophomonas maltophilia* and one strains of *Agrobacterium* sp.	As(III) (2–20 mg/L)	In the presence of bacterial consortium, 11.37 mg/kg As was volatilized by transpiration	[33]
Alfalfa + *Ensifer* sp. M14	Soil As(III) (10 mg/kg)	As concentration in leaves of inoculated plants was 11% higher than those cultivated without microorganisms.	[34]
		Nano-Phytoremediation Approaches	
Eucalyptus leaf extract mediated synthesis iron oxide NPs	Arsenic	Arsenic adsorption capacity was found to be 39.84 mg/g	[35]
Isatis cappadocica + glutathione modified superparamagnetic iron oxide NPs {nFe_3O_4@GSH}	Soil As (1000 µM)	nFe_3O_4@GSH treatment increased growth of plants and As tolerance by reducing As accumulation in plants	[36]
		Genetic Engineering Approaches	
Arabidopsis thaliana transformed with bacterial As transporter (ArsB) targeted to vacuolar membrane	As(III) (5 µM)	Transgenic plants showed higher As accumulation in shoots compared to wild type plants	[37]
Nicotiana tabaccum transformed with *PvPht1;3* from *P. vittata*	As(V) (20 µM) Soil As (9.66 mg/kg)	Arsenic accumulation in shoot tissues of transgenic tobacco increased in both hydroponic and soil experiments	[38]

2.1. Selection of Plants for Arsenic Phytoremediation

2.1.1. Arsenic Hyperaccumulators

Hyperaccumulator plants can accumulate metal in their shoots beyond a certain threshold limit, which is 1000 mg/kg for As [39]. Further, the bioaccumulation factor (BF; indicative of soil to plant metal transfer) and translocation factor (TF; indicative of root to shoot metal transfer) are also considered while categorizing a plant as a hyperaccumulator [40]. Both BF and TF should be more than one (>1) for an As hyperaccumulator plant. Hyperaccumulation of As has been observed mostly in fern plants of the *Pteris* genus like *P. vittata* [40], *P. longifolia* [41], *P. quadriaurita*, *P. cretica*, *P. ryiunkensis* [42], etc. and *Pityrogramma calomelanos* [43]. One of the plants from the Brassicaceae family, *Isatis cappadocica,* shows As hyperaccumulation [44]. *P. vittata* has worldwide distribution from North America to Europe and Asia and can grow in a wide range of environmental conditions ranging from temperate to tropical [45].

Arsenic can make up to about 2% of the biomass of *P. vittata* [40]. *P. vittata* is a perennial plant and, therefore, plantation of a field does not need replantation, and harvesting and collection of fronds is needed at regular intervals. Several studies have focused on the use of *P. vittata* for the remediation of As-contaminated soil in laboratory, pot and field studies [46]. Liao et al. [47] found that from soil containing 64 mg/kg As, *P. vittata* removed 7.8% of the As in seven months. *P. vittata* plants showed higher As accumulation when grown in soil with added phosphate rock than in soil without phosphate rock amendment [48]. Phosphorus addition in the form of phosphate rock induces mobilization of As to some extent that, in turn, helps to induce As removal by *Pteris* plants [49,50].

In a pilot-scale study [51], *P. vittata* was used to minimize As concentration from drinking water through a continuous phytofiltration system. During the 3 month experimental period, up to 1900 L/day water with an initial As concentration of 10.2 µg/L was remediated and was found to contain As concentrations as low as 2 µg/L. The fronds of *P. vittata* accumulated 66–407 mg/kg As [51]. Groundwater remediation has also been demonstrated with the use of *P. vittata* [52]. The authors tested the efficiency of one to four *Pteris* plants per container of 30 L and with variable nitrogen and phosphorus supply to remediate groundwater containing 130 μg/L As. The As concentration was reduced to less than 10 μg/L in 3 weeks with 4 plants while in 4–6 weeks with 1–2 plants. When fully grown plants with a high root density were reused, one plant per container gave good results. In a recent study, *P. vittata* was used in a hydroponic system without any mechanical aeration. The method used was simple in that the plants were grown with rhizomes over the water surface and nutrients were given in a low amount for achieving root proliferation (500 mm root length in four months). From a variable initial water As concentration of 50 μg/L, 500 μg/L, and 1000 μg/L, *Pteris* plants could bring down the concentration to 10 to 0.1 μg/L in 1–5 days, 4–6 days and 8–10 days, respectively [53]. The results suggest the potential of *P. vittata* for phytoremediation purposes; however, the use of *P. vittata* has been mostly in hydroponics limited to pilot-scale studies. Extension of the approach to field conditions will necessarily require higher biomass development of large scale hydroponic systems, large amounts of water for treatment, and maintenance with optimum nutrient supply and regular cleaning.

2.1.2. High Biomass Plants for Arsenic Cleanup

The remediation of a site in a short time warrants the need of high biomass plants with moderate to high As accumulation and a short life cycle enabling harvesting followed by the use of the field for subsequent cropping of the same or other appropriate plants. This would enable cultivation of phytoremediator crops in a contaminated field throughout the year in changing weather conditions. Some of the high biomass plants with good potential for As accumulation include *Jatropha curcas* [54], shrub willow (*Salix* spp.), sunflower (*Helianthus annuus*) [55] and Indian mustard (*Brassica juncea*) [56]. In a small field study, sunflower plants were exposed to different As levels in three soil types (sandy, loamy, and clayey) and As accumulation was found to vary from 270 mg/kg to 408 mg/kg in roots, 13 mg/kg to 28 mg/kg in stem and 35 mg/kg to 68 mg/kg in leaves in different soil [57]. The application of *Salix* in phytoremediation has been demonstrated [58]. Invasive plants like *Parthenium hysterophorus* can also be successfully used in remediation strategies as they can grow and cover an area at rapid rates in a wide range of environments and accumulate metals in high amounts [59]. Favas et al. [60] found *Callitriche lusitanica* to be a potential As accumulator with As concentrations reaching up to 2346 mg/kg DW. Other potential accumulators in higher plants have been identified in lab and field studies, e.g., *Isatis cappadocica* [44], *Sesuvium portulacastrum* [61], and *Eclipta alba* [62]. *Sesuvium* is a halophytic plant with a high tolerance not only to salt but also to a number of metals and showed As accumulation 155 μg/g dw upon exposure to 1000 μM As(V) in 30 d [61].

The contaminated water bodies may be remediated with the help of high biomass aquatic plants like *Ceratophyllum demersum* [63], *Hydrilla verticillata* [64], *Lemna gibba* [65], *Lemna minor* [66], *Azolla caroliniana* [67], *Pistia stratiotes* [68], *Salvinia natans* [69] and *Eichhornia crassipes* [70]. *Lemna gibba* has been demonstrated to accumulate As up to 1022 mg/kg dry biomass in 21 d from contaminated surface water containing 41.37–47 μg/L As. The biomass accumulation and As removal potential of *L. gibba* were found to be as high as 73.6 t/ha/y and 752 kg As/ha/y, respectively [65]. In another study, *E. crassipes* was found to accumulate about 498 mg As/kg dry weight from a solution of 0.5 mg/L As in 10 d with a reduction of initial As concentration by 83% [71]. *H. verticillata* plants were found to remove up to 72% of As from 8 L As (1500 ug/L) medium in 45 d with the maximum As concentration of 388 μg/g dry weight [72]. These plants show fast growth and high biomass accumulation, can be easily harvested and can reestablish themselves. Aquatic plants also need very little input for growth and have high tolerance to waste water. The use of water fern, *Mircanthemum umbrosum*, in As and Cd remediation was studied by Islam et al. [73]. The use of emergent aquatic plants like *Cyperus vaginatus* and *Vetiveria zizanioides* has also been demonstrated in phytoremediation studies [74]. With the use of a high biomass moderate As accumulator, the effective removal of As per year can be higher than that achieved with a low biomass hyperaccumulator. For example, the calculation of yearly As removal by *Sesuvium* was found to be as high as 1955 g As/ha/yr at 500 µM As, which was higher than the calculated As removal by *Pteris* (525–1470 g As/ha/yr) [61].

2.1.3. Plants with Bioenergy Potential and Economic Utility

Besides plant biomass, the economic value of the plant system such as high value metabolites, biofuel generation, compost formation, etc. is now considered as one of the prime criteria for selecting plants for phytoremediation. With such an approach, farmers can move from normal cropping patterns to phytoremediator plants [17,75]. Plant-based waste material can also be successfully reutilized in remediation projects. This approach not only handles the problem of plant waste utilization at one end but also remediates the contaminated site on the other. Rice husk, mustard husk, coconut coir waste, crop straw, etc. are some of the examples of materials derived from plant materials that can act as biosorbents and remediators of As and can sustain soil fertility and reduce As accumulation in crop plants [76]. The potential of aquatic plants can also be used with judicious controlled and proper management of generated biomass with biodiesel, biogas, biochar, or compost preparation [77,78]. Biochar has emerged as one of the most potential plant based materials that have a number of functional groups (hydroxyl, carboxyl, etc.), making it an excellent binder of metals and therefore its application in soil reduces As stress to crop plants. Further, the use of biochar has also been demonstrated in water filtration [79]. Zhu et al. [80] designed a biochar plus periphyton-based system for the removal of As from the wastewater. The first phase of the column contained biochar that removed up to 60% of As(III) from wastewater (containing 2 mg/L As(III); flow rate 1 mL/min) while subsequent a periphyton bioreactor enhanced As removal efficiency up to 90–95%.

2.2. Promising Approaches for Augmenting Arsenic Remediation by Plants

2.2.1. Microbe-Assisted Arsenic Phytoremediation

Even with the selection of an appropriate hyperaccumulator plant or a high biomass economically useful plant depending on the features of the site for As phytoremediation, it is desirable to further augment plants' remediation potential and growth so as to make remediation more lucrative and feasible. Plant associated microbiota and their synergetic interaction can be an effective strategy and is referred to as phytobial remediation [81]. There are successful examples of microbe-assisted enhanced phytoremediation efficiency for As [82,83]. There are certain crucial considerations like root colonization, survival, growth and competition with other pathogenic microbes and stimulation of plant growth. Microbial communities through their mutualistic association, either as free living, root symbiont or endophyte [84,85], produce certain metabolites which augment plant growth, alleviate stress and participate in As remediation [82,86]. Plant growth is promoted through the production of plant growth hormones and nutrient absorption is improved by siderophores [87–90]. In a study on isolation of As-resistant plant growth promoting microbes (PGPMs), *Microbacterium* sp. strain SZ1 from As-bearing gold ores was shown to be useful for phytobial remediation as the bacterial genome had the necessary genes responsible for siderophore production [91]. From a contaminated site in Spain, Moens et al. [92] reported reduced As toxicity on plant growth with concomitant lower As accumulation in rice plants by inoculating *Ochrobactrum tritici* As5 to the plant's rhizosphere. The As-resistant bacteria (*Pseudomonas gessardii* and *Brevundimonas intermedia*) and As-resistant fungi (*Fimetariella rabenhortii* and *Hormonem aviticola*) isolated from the Puchuncaví valley in Chile exhibited higher plant growth-promoting properties and good As remediation properties in soil cultivated with wheat [93].

In an interesting three year field study, Yang et al. [94] demonstrated that rhizobacteria (*Pseudomonas vancouverensis* strain m318) mediated As(III) to As(V) conversion, and efficient As phytoextraction. Treatment with rhizobacteria enhanced fern biomass, As accumulation, and As removal (<10 mg kg^{-1}) in the soil, suggesting that in a span of three cycles of fern growth, a clean field could be achieved. Awasthi et al. [86] studied the prospects of using a consortium of rhizobacteria (*Pseudomonas putida*) and alga (*Chlorella vulgaris*) for ameliorating As toxicity through measurements of growth and As uptake. An estimated 79–82% drop in As accumulation in rice was shown, suggesting the usefulness of the approach. Using isolates from the mangrove rhizosphere of Sundarban, Mallick et al. [95] applied two As-resistant halophilic bacteria, *Kocuria flava* AB402 and *Bacillus vietnamensis* AB403, for growth promotion and As remediation. Both bacteria showed up to a 52% reduction in As accumulation in the roots and shoots of rice seedlings.

Arbuscular mycorrhiza (AM), belonging to symbiotic fungi, have the remarkable feature of positively influencing plant growth and stress tolerance [96]. Plant-AM association has been studied and several reports have demonstrated AM application for alleviating heavy metal contamination [97,98] via mechanisms, which include converting inorganic As to less toxic forms and enhancing plant biomass [99,100], increased uptake of metals through metal transporters and activation of genes related to signaling and detoxification pathways [101,102]. AM has been shown to have good potential for reclamation of abandoned fly-ash containing heavy metals such as As, lead, cadmium and mercury [103]. In a study with the application of *Glomus mosseae* BEG167, Xu et al. [104] found higher phosphorous (P) accumulation and reduced As in *Medicago truncatula* grown in soils supplemented with As (10–200 mg/kg). AM-mediated As toxicity alleviation has also been demonstrated in tomato [105], ryegrass and clover [106].

2.2.2. Intercropping and Co-Cultivation Methods

Intercropping is a common agricultural practice in which two different crops are grown together to improve soil conditions for plant growth, improved nutrient availability and soil enzyme activity [107]. The intercropping of As hyperaccumulator *P. vittata* with As sensitive and non-accumulator plants has been tested in order to reduce As contamination of the field and to mitigate As stress on the other plants. The intercropping of *P. vittata* and *Panax notoginseng*, two economically useful plants, was studied by Lin et al. [108]. It was observed that As concentrations in the rhizosphere of *Panax* plants were reduced. The intercropping of *P. vittata* with *Morus alba* was also found to reduce As levels in *Morus alba* plants due to significant As removal by *Pteris* plants [109]. The intercropping of *P. vittata* with maize (*Zea mays*) plants has also been studied [110] and the two plants were grown in both coordinate and malposed intercropping. It was found that level of Fe-hydroxides-associated As were lower in soil layers (10–20 cm and 20–30 cm) while As accumulation in *P. vittata* was higher in malposed intercropping than in coordinate intercropping. The rate of As removal was 2.4-fold higher in malposed than in coordinate intercropping. Maize grains showed lower As concentration in grains, within the suggested maximum contaminant limit, during malposed intercropping [110].

The roots of different intercropped plants may concentrate in different zones from the top layer to a few centimeters' deep. Correspondingly, As distribution also varies sharply in different layers of soil by a few centimeters (0–40 cm) [111]. Therefore, intercropping of *Pteris* with other cash crops/economically important crops can give interesting results. However, it has been considered as the best approach to remediate and use the field for economic gain at the same time [109,112,113]. If the harvesting of *P. vittata* can be managed in a timely manner along with management of fallen leaves and shoot tissues (not to be used) of intercropped cash crops, this strategy can effectively remediate the As-contaminated sites along with economic gains to the landowner [110]. Ye et al. [114] studied co-cultivation of *P. vittata* with rice and found that As removal by *Pteris* reduced the As level in rice with a significant decline in DMA content.

The combination or sequential use of aquatic plants has been found to enhance As removal from a medium in a given time frame as compared to that of a single plant. The successive application of three aquatic plants, *Lemna, Hydrilla, and Ceratophyllum*, for As removal was tested. The medium used contained 2500 µg/L As, and plants were used in succession for a total of 21 days with 7 days allocated for each plant. The study found reported the maximum As removal (27% in 21 d) when *Hydrilla-Cerotophyllum-Lemna* succession was used [115]. In a combination approach used by Srivastava et al. [116], the combination of *Ceratophyllum demersum* and *Lemna minor* achieved the maximum As removal (4365 μg) in 30 d from an As supplemented medium (2500 μg/L).

2.2.3. Nanotechnological Approaches to Enhance Phytoremediation

Nano-phytoremediation is an emerging strategy that has shown the potential to enhance plants' ability to grow in a polluted stressful environment and accumulate As in plant tissues. Fabrication of effective and eco-friendly nanoparticles for successful application in managing widespread contamination of hazardous metalloids has received much attention [117]. Nanoparticles (NPs) may increase the plant's stress tolerance to increase phytoremediation as well as help in the alleviation of toxicity [118,119]. Nano-phytoremediation can effectively remediate the polluted soils/water using those plants that possess high efficiency for NPs/metal uptake [26,120,121], and can be used as an alternative solution for As phytoremediation (Figure 2).

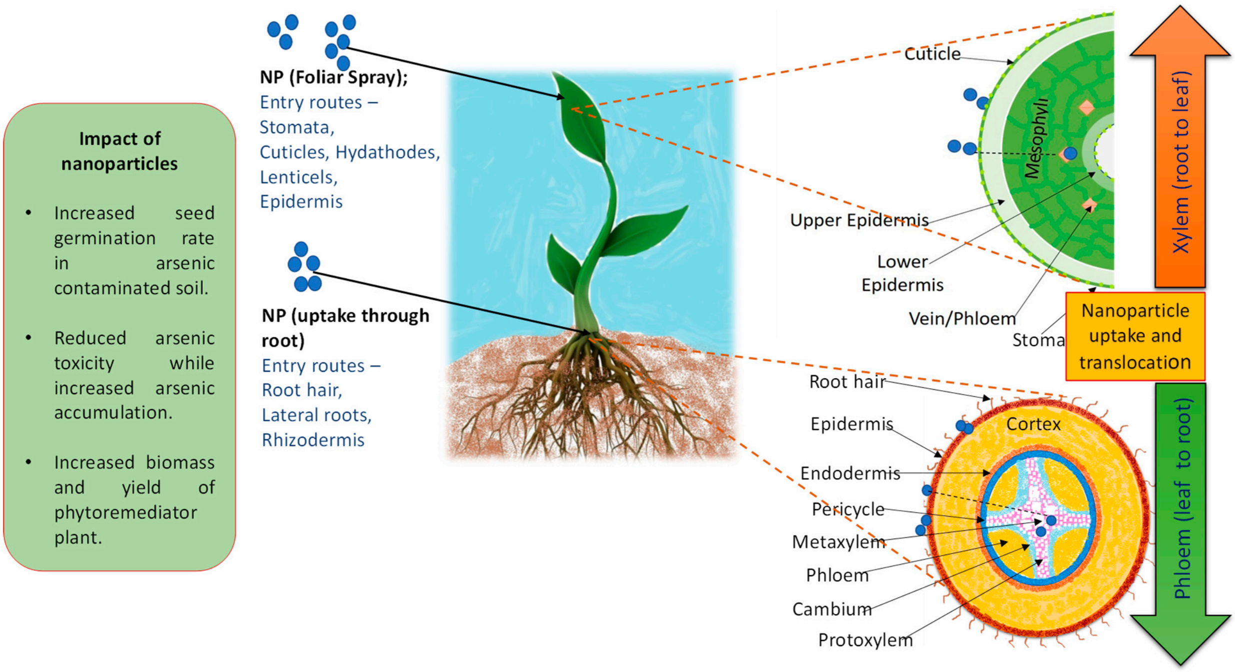

Figure 2. The use of nanoparticles through foliar spray and via roots can effectively enhance tolerance of plants to arsenic stress, improve their growth and biomass and also increase total arsenic accumulation.

Application of nanoparticles for the management of contaminated agricultural lands and improvement of plant growth and developments has shown significant prospects [26]. In this context, it was shown that nanostructured silicon dioxide can act as a potential agent that can improve the phytoremediation process to attain the desired outcomes [24,122]. Similarly, the NPs of aluminum oxide (nAl_2O_3) can be used in phytoremediation as they did not exert any toxicity consequences in *Arabidopsis thaliana* up to 4000 mg/L [123].

It was noted that the nanoscale zero-valent iron was widely used to facilitate the phytoremediation process [124]. It was found that the use of salicylic acid-based NPs enhances As remediation by *Isatis cappadocica* [125] while the use of nano-Zn improved As stabilization by *Helianthus annuus* [126]. A review summarized that the composites of nano titanium (Ti) such as Zr-TiO_2 and TiO_2-αFe_2O_3Ce-Ti oxide are frequently used to treat As-contaminated water [127]. The application of TiO_2, Si NPs and Au NPs has been found to counteract the toxic effects of different metals in *Zea mays* [128], *Glycine max* [129] and *Oryza sativa* [130], respectively. The application of fullerene nanoparticles could stimulate the phytoavailability of soil contaminants [124].

The application of NPs not only enhances the phytoremediation capability of As, but also reduces the bioaccumulation of As in crops. Recent research showed that the application of 1000 mg/L nano-TiO_2 reduced As accumulation in rice by 40–90% [131], and in *Vigna radiata* nano-TiO_2 reduced As phytotoxicity at the rate of 4000 mg/L [132]. The amendment of ZnO increased the growth of rice seedlings, reduced accumulation of As in roots and shoots, and saw a rise in phytochelatin level [133]. Noteworthy advances in nano-phytoremediation could form a basis for the development of non-toxic, cost-effective, and environmentally sustainable technologies for phytoremediation of As from various environmental matrices.

2.2.4. Genetic Engineering for Improving Arsenic Phytoremediation

The potential mitigation strategies for reducing the As burden involve As efflux and its sequestration in intracellular compartment [134]. Strategies for developing genetically engineered plants for As phytoremediation encompass increased uptake of As by roots, enhanced translocation of As from root to shoot including xylem loading, arsenate reduction, vacuolar sequestration and enhanced tolerance to As [135,136].

As(V) and As(III) uptake and transport are mediated by phosphate transporters (PHTs) and members of membrane intrinsic proteins (MIPs), respectively [137,138]. Thus, for designing a phytoremediation strategy, a high biomass crop can be genetically engineered by overexpression of the candidate MIP genes, particularly NIP3;1, NIP7;1, PIPs, Lsi2 and PvTIP4;1, which could increase As uptake and translocation and lead to enhanced As accumulation in genetically engineered plants. *P. vittata* showed increased As(V) uptake due to the increased expression of *PvPHT1;3* (a phosphate transporter) and higher affinity for As(V) over phosphate [139,140]. In *P. vittata*, As(III) is primarily sequestered into the vacuole by PvACR3 (Arsenic Compound Resistant 3), an arsenite effluxer localized the in plasma membrane of gametophyte, and its homolog is absent in angiosperm [141]. Interestingly, over-expression of *PvACR3* in Arabidopsis enhances As translocation in shoots [142], which could be a potential strategy for developing As-hyperaccumulating plants. *A. thaliana* was converted into an As hyperaccumulator by heterologous expression of *PvACR3* in *athac1* (arsenate reductase) mutant [143], and the same strategy could be tested in fast growing high biomass crop plants for efficient phytoextraction.

To mitigate As-induced stress to plants so as to enhance their As accumulation, redox transformation of As(V) and As(III), and further methylation of organic As species, can be targeted. The pioneering research on the development of a transgenic *Arabidopsis* plant for As phytoremediation involved stacking two bacterial genes by overexpression of arsenate reductase (*arsC*) in shoots and constitutive expression of γ-glutamylcysteinesynthetase (γ-*ECS*), which resulted in enhanced tolerance and higher As accumulation in the double transgenic plant [144]. Arsenate reductase (*AtACR2*) knock down lines of Arabidopsis resulted in enhanced translocation of As from roots to shoots [145].

However, transgenic lines generated with heterologous expression of Arabidopsis AtACR2 in tobacco were more tolerant to As, but accumulated reduced As level in shoots [146], which suggested that the identification of the *ACR2* gene from high biomass crop plants is a potential candidate, and its knock down/knock out by a gene editing approach can be a promising tool for developing genetically engineered plants for phytoremediation. Recently, two novel arsenate reductases (PvHAC1 and PvHAC2) from *P. vittata* were isolated, where *PvHAC1* was expressed in the rhizomes, while *PvHAC2* was expressed in the fronds and played a crucial role in As hyperaccumulation [147]. Therefore, heterologous expression of the phosphate transporter (*PvPHT1;3*) and arsenate reductase (*PvHAC1/2*) in a high biomass crop plant can be utilized as a potential strategy for efficient phytoextraction of As. In a recent report regarding As stress, RNA-seq analysis of *P. vittata* identified three upregulated genes viz. glyceradehyde 3-phosphate dehydrogenase (*PvGAPC1*), organic cation transporter 4 (*PvOCT4*), glutathione S-transferase (*PvGSTF1*) and RNAi demonstrated that the identified genes are essential for As tolerance. PvGAPC1 converts As(V) to 1-arseno-3-phosphoglyerate (1-As-3-PG), PvOCT4 transports 1-As-3-PG into the vesicle and PvGSTF1 acts as arsenate reductase, which sequestered (AsIII) into vesicles and moved it long distances for storage [148]. These genes can be utilized in genetically modified plants for phytoremediation after proper and thorough investigation of the pathways involved.

3. Conclusions and Future Directions

Arsenic contamination in the ecosystem has created serious environmental concerns due to the toxicity and carcinogenicity of this metalloid. In light of this, research and development efforts have been made for As remediation from soil and water sources through sustainable biostrategies which are environmentally friendly and easy to adopt in contaminated sites. The available options include 'phytoremediation' involving exploitation of plant species with high As-hyperaccumulating efficiency and a good biomass and bioprospecting potential. Based on the mechanistic view of As uptake, metabolism and transport and identification of novel candidate genes, biotechnological methods have been refined to genetically manipulate plants for enhancing the efficiency of phytoremediation and reducing the As load in crop plants. The application of plant growth promoting microorganisms and nanoparticles has immense potential for managing As contamination in plants and in the ecosystem. Extensive studies should be conducted to realize the prospects of microbe-/nano-assisted phytoremediation for the decontamination of As polluted soils/water. However, various approaches of phytoremediation have some merits and limitations (Table 2) and, therefore, future research must be focused on integration of different methods, suitably at a site so as to enhance the phytoremediation potential and speed up the process in addition to providing economic benefits to the landowner.

Table 2. Merits and limitations of various phytoremediation approaches.

Merits	Limitations
Arsenic Hyperaccumulator Plants	
Owing to As hyperaccumulation, large amount of As is concentrated in above-ground harvestable tissues	The biomass of hyperaccumulator plants is generally low and hence, total As removed in one cycle/harvest is low
Hyperaccumulator plants do not need much care and additional inputs for sustaining their growth	The habitat of hyperaccumulator plants may be limited and their application may not be practiced in all environment
High Biomass Producing Plants	
High biomass of plants allows large As removal in a single crop	For sustained growth of high biomass plants, additional nutrient (fertilizer) inputs and efforts may be required.
Native high biomass plants may be chosen to avoid habitat related issues	Native plants may be preferable feed for native wild/pet animals and may therefore pose risk
Plants with Economic Utiliity	
Plants with economic utility like oil-seed plants which restrict As accumulation in oil would allow farmers to dedicate fields for phytoremediation	For such plants also, animal consumption of leaves and shoot portion of plants must be avoided
Plants may find applications for bioenergy, biofuel and biochar preparation	The research on practical utility and problems is limited; volatile nature of some As species may be of concern
Microbe-Assisted Arsenic Remediation	
Arsenic tolerant and plant growth promoting microorganisms may enhace plants potential for As removal per crop cycle	Microbial supplementation might interfere with natural microbiome of plants and soil and thus, it still needs research
Nano-Phytoremediation Approaches	
NPs mediated plant growth improvement and increased As bioavilability would enhance As removal per crop cycle	The accumualtion of NPs may intself cause toxicity to plants
Genetic Engineering Approaches	
Genetic modification of plants as per the need would allow the generation of high biomass superhyperaccumulators of economic utilizability and would allow speedy phytoremediation	The issues related to approval and public acceptance of genetically modified plants are of concern

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