Phytoremediation: a sustainable environmental technology for heavy metals decontamination

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Abstract

Toxic metal contamination of soil is a major environmental hazard. Chemical methods for heavy metal's (HMs) decontamination such as heat treatment, electroremediation, soil replacement, precipitation and chemical leaching are generally very costly and not be applicable to agricultural lands. However, many strategies are being used to restore polluted environments. Among these, phytoremediation is a promising method based on the use of hyper-accumulator plant species that can tolerate high amounts of toxic HMs present in the environment/soil. Such a strategy uses green plants to remove, degrade, or detoxify toxic metals. Five types of phytoremediation technologies have often been employed for soil decontamination: *phytostabilization*, *phytodegradation*, *rhizofiltration*, *phytoextraction* and *phytovolatilization*. Traditional phytoremediation method presents some limitations regarding their applications at large scale, so the application of genetic engineering approaches such as transgenic transformation, nanoparticles addition and phytoremediation assisted with phytohormones, plant growth-promoting bacteria and AMF inoculation has been applied to ameliorate the efficacy of plants as candidates for HMs decontamination. In this review, aspects of HMs toxicity and their depollution procedures with focus on phytoremediation are discussed. Last, some recent innovative technologies for improving phytoremediation are highlighted.

Keywords Phytoremediation · PGPB · PGR · Hyper-accumulator plants · Heavy metals · Nanoparticles (NPs) · Transgenic plants

Abbreviations

ABC	ATP-binding cassette	Nramp	Macrophage protein
AMF	Arbuscular mycorrhizal fungi	nZVI	Nanoscale zero-valent iron
APX	Ascorbate peroxidase	PCs	Phytochelatins
AtACR2	Arsenic reductase 2	PGPR	Plant growth-promoting bacteria
CAT	Catalase	PGR	Plant growth regulators
GR	Glutathione reductase	ROS	Reactive oxygen species
GSH	Glutathione	SOD	Superoxide dismutase
HMs	Heavy metals	RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
MeP	Methyl parathion	TDZ	Thidiazuron
<i>merA</i>	Mercuric ion reductase		
<i>merB</i>	Organomercurial lyase		
MTs	Metallothioneins		
NPs	Nanoparticles		

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1 Introduction

The progress of demographic population combined with high industrial development causes serious environmental hazards. For a long time, humans have supplemented great quantities of pollutants to the soil, water and atmosphere biotopes as a consequence of industrial activities, such as mining of ores, gas emission, pesticide application and municipal waste production [168]. These pollutants can be accumulated in food chains, causing harmful effects on plants, animals and humans (damage to the endocrine system, impact on immunity, neurological disorders and cancer) [134].

Chemical methods for HMs decontamination such as excavation, precipitation, heat treatment, electroremediation and chemical leaching are still costly and depend on the pollutant and soil characteristics [133]. The modification of soil properties (especially pH), risk of soil fertility loss, small-scale application and by-product generations are the main disadvantages and drawbacks of these techniques.

Phytoremediation is a green strategy that uses hyper-accumulator plants and their rhizospheric microorganisms to stabilize, transfer or degrade pollutants in soil, water and environment [107]. This technology is considered as well-efficient, cheap and adaptable with the environment [10, 136]. According to the soil conditions, pollutant and the species of plants used, five types of phytoremediation have been applied: *phytodegradation*, *phytofiltration*, *phytoextraction*, *phytostabilization* and *phytovolatilization*.

Plants were classified to be tolerant and/or hyper-accumulator to HMs when they show rapid growth, high biomass and are capable to extract and accumulate high amounts of HMs in their shoots, without signs of toxicity when grown in contaminated soils [96] (Table 3). Hence

this green technology can be very useful for remediation of HMs contaminated soils/agro-ecosystems.

Plant hyper-accumulators have received greater attention in recent decades, due to its potential to HMs contamination. However, there are some limitations for these plants to become efficient at large scale. These limitations need to be overcome by transgenic approach applications to improve HMs tolerance/accumulation of these plants [150].

This critical review describes the effective mechanisms of phytoremediation, the promising potential of hyper-accumulator plants and the biotechnological approach for HMs decontamination. Last, some recent innovative technologies for improving phytoremediation and future prospects like over expression of foreign genes in non-tolerant plants, nanoparticles (NPs) addition and phytoremediation assisted with phytohormones, microbial and AMF inoculation are presented.

2 Heavy metals (HMs)

In recent decade, considerable attention was given to HMs as potential environmental pollutants. The sources of environmental HMs contamination are due generally to mining extraction, combustion and industrial effluents. Arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), chromium (Cr) and aluminum (Al) are the principal HMs, which cause toxicity to both flora and fauna in soil ecosystems. Most of these metals are easily stocked in plants, enter in the food chain and get transferred to the humans, which cause serious disorders and diseases [12, 134]. Despite the fact that some of HMs such as iron (Fe), copper (Cu), selenium (Se) and zinc (Zn) are indispensable in small concentrations, but their accumulation at higher levels may become very toxic in the environment [11, 115] (Table 1).

Table 1 Occurrence of heavy metals in contaminated soils around the world

Heavy metals	Average value in soils (ppm DW)	Tolerable limit ^a	Country	References
Cd	14	3	China	Shi et al. [175]
	19	3	India	Tiwari et al. [188]
Pb	1988	100	China	Niu et al. [137]
	711	100	United Kingdom	Nabulo et al. [128]
Cu	448	100	China	Wang et al. [194]
	19581	100	Australia	Sacristán et al. [156]
Cr	590	100	China	Xu et al. [202]
	224	100	Germany	Shaheen et al. [170]
Zn	3800	300	China	Niu et al. [137]
	1200	300	Germany	Shaheen et al. [170]

^aKabata-Pendias [81]

The HMs availability in soil solution is determined by the nature of metal, soil characteristics (such as pH, clay and organic matter content) and exchange reactions (precipitation and adsorption–desorption processes) [130, 168].

The accumulation of high quantities of HMs may exchange essential ions by antagonism in chlorophyll and/or enzymes causing oxidative stress. Plant growth reduction and decline of photosynthetic activity are the main symptoms of HMs toxicity [174] (Fig. 1).

3 Hyper-accumulator species

In nature certain plant species can stock very high quantities of toxic HMs to levels which exceed the soil contents [120]. Plants growing in polluted soils exhibit several strategies to coping with the toxicity of HMs including preventing their accumulation, detoxification or metal excretion from the tissues [83]. The ability of hyper-accumulator plants to grow normally under high HMs levels is related to multiple biochemical pathways that enable to maintain metals concentrations at a lower level in the cytoplasm than in the soil, which protects the cytoplasmic organelles

from toxic effect of HMs (vacuolar compartmentalization) [134]. *Includer* plant species which have no exclusion strategy assimilate and translocate high quantities of HMs and stock them in their shoots without signs of toxicity [120].

Heavy metal tolerance is realized by sequestration/compartmentalization of metals within the different cell compartments (especially in vacuoles), away from the cytosol, which protects sensitive site from toxic effect of HMs and avoiding the inhibition of metabolic processes in the cytoplasm. A significant accumulation of organic solutes and amino acids (such as proline) has been reported that helps plants to grow in a polluted environment. The complexation of metals with these solutes is involved in the reduction of translocation of HMs to different sensitive plant parts [132].

Exposed to HMs plants have developed several mechanisms to detoxify the adverse effect of HMs to continue their normal growth and metabolic activities. Two principal strategies are employed by plants to protect their organs from toxic HMs [1] restricting the uptake of HMs and [2] accumulation with tolerance mechanism applications. In the first type, the level of HMs is reduced by limiting the absorption of metals by precipitation. However in the second strategy, the toxic HMs are sequestered or

Fig. 1 Heavy metal toxicity in plants and their tolerance strategies (uptake/translocation and detoxification)

compartmentalized within the cell especially in the vacuoles [36] (Fig. 2). Restriction of HMs absorption is related to rhizospheric microorganisms such as AMF and bacteria. These microorganisms can decrease HMs uptake into plant cells by liberating metal chelating agents, such as citric acid, oxalic acid and phenolic compounds [60]. Vacuolar compartmentalization is another mechanism applied to prevent the free circulation of HMs in the cytosol and sequestered them into a limited area to avoid cell damage [210]. Many plants cope with HMs by forming complexes with phytochelatins (PCs) and are transported into the vacuole as complex metal peptides [203].

On the basis of metal concentration in their tissues, plant species can be divided into 3 groups [1] HM accumulator species, [2] HM indicator species and [3] HM *excluder* species [14]. The accumulator species; plants are able to uptake very high contents of HMs (in shoots or roots) exceeding the levels in the soil. The indicator species accumulate HMs that exceed the concentration in the soil. The *excluder* species restrict the entry of HMs to the roots and/or the translocation to the shoots [83]. However, species are classified tolerant, if they can propagate on contaminated soil with levels of HMs that are no tolerable (toxic) to other vegetation. Indicator and hyper-accumulator species are tolerant, but tolerant species are not obligatory indicators or hyper-accumulators [147].

Plants suitable for phytoremediation have four important characteristics: [1] rapid growth and high biomass [2] profound root system, [3] easily harvestable and [4] accumulation of high levels of HMs in shoots. Hyper-accumulator plants are generally relatively rare in the nature

and are widespread in contaminated soils, signifying that hyper-accumulation is an important eco-physiological trait to HMs resistance and one of the indicators of toxin adaptation [111]. Metal hyper-accumulator plants can store exceptionally high contents of HMs (> 1000 ppm). In contrast, the rate of these metals accumulation does not exceed 10 ppm in non-accumulator plants or sensible plants [100].

Hyper-accumulator plants assimilate high amounts of HMs in their above-ground parts during normal growth and reproduction [132]. Baker and Brooks [14] determined the threshold concentrations for metals hyper-accumulated in plants as Cd = 100 ppm dry weigh (DW), Ni, Cu, Co, Pb = 1000 ppm DW and Zn, Mn = 10,000 ppm DW (Table 2).

Table 2 Threshold for definition of hyper-accumulator plant species [14]

Trace element	Threshold for hyper-accumulator plants (ppm DW)
Mercury (Hg)	1000
Selenium (Se)	1000
Cadmium (Cd)	100
Copper (Cu)	1000
Cobalt (Co)	1000
Nickel (Ni)	1000
Chromium (Cr)	1000
Lead (Pb)	1000
Zinc (Zn)	10,000
Manganese (Mn)	10,000

Fig. 2 Principal biochemical and molecular strategies involved in heavy metal accumulation

However, these levels were far higher than those found in non-accumulator species. Over 500 species have been qualified as hyper-accumulators, including mainly from the families such as Brassicaceae, Asteraceae, Amaranthaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae and Euphorbiaceae (Table 3). Many of these hyper-accumulator plants are particularly well represented in the family Brassicaceae [143].

4 Uptake, translocation and detoxification of HMs

Heavy metal accumulation in plant depends to metals, solubility, translocation, plant species and variety [100]. HMs in the soil solution can pass through the epidermis,

then through the endodermis and finally reaching the xylem sap. The HMs can be stocked into plant organs, transformed or be volatilized in atmosphere through stomatal leaves [85, 146].

Plants uptake HMs from the rhizospheric system by two mechanisms: [1] symplastic pathway via plasma membrane of cell roots by specific ion channels or [2] by apoplastic pathway between cell wall (intercellular compartment) [65, 168]. Several kinds of metal transporters which are involved in HMs homeostasis were identified in the plasma membrane such as the Zn/Fe permeases (ZIP), the natural resistance-associated macrophage proteins (Nramp), the cation exchangers (CAXs), cation diffusion facilitators (CDF) and ATP-binding cassettes (ABC) [163].

Generally, metal bioavailability was lower when soil pH, clay content or organic matter were higher. However,

Table 3 Some potential species for phytoremediation

Species	HMs	Reference
<i>Acacia nilotica</i>	Cd	Shabir et al. [166]
<i>Atriplex lentiformis</i>	Cd	Eissa and Abeer [48]
<i>Atriplex halimus</i> subsp. <i>schweinfurthii</i>	Cd	Nedjimi and Daoud [132]
<i>Atriplex halimus</i>	Zn	Lutts et al. [109]
<i>Brassica Juncea</i>	As	Ko et al. [91]
<i>Berkheya coddii</i>	Ni	Robinson et al. [153]
<i>Boehmeria nivea</i>	Cd	Pan et al. [140]
<i>Canna indica</i>	Cd	Solanki et al. [178]
<i>Dalbergia sissoo</i>	Cu/Ni	Kalam et al. [84]
<i>Genipa americana</i>	Cr	Santana et al. [160]
<i>Lactuca sativa</i>	Cu	Shams et al. [172]
<i>Haumaniastrum robertii</i>	Co	Kabeya et al. [80]
<i>Helianthus annuus</i>	Cu/Pb	Forte and Mutiti [53]
<i>Hydrocotyle ranunculoides</i>	Cu/Zn	Demarco et al. [45]
<i>Lagerstroemia indica</i>	Cd	Wang et al. [195]
<i>Lathyrus sativus</i>	Cd/Pb	Abdelkrim et al. [1]
<i>Ludwigia peploides</i>	Pb/Zn	Fernández San Juan et al. [52]
<i>Luzula campestris</i>	Pb	Elekes [49]
<i>Medicago sativa</i>	Pb	López et al. [108]
<i>Nicotiana tabacum</i>	Cu	Evangelou et al. [50]
<i>Noccaea caerulea</i>	Zn/Cd/Ni	Kozhevnikova et al. [95]
<i>Pistia stratiotes</i>	Ag	Odjegba and Fasidi [138]
<i>Pistia</i> sp.	Cr	Mondal and Nayek [125]
<i>Pteris vittata</i>	As	Wang et al. [193]
<i>Salix viminalis</i>	Ni	Korzeniowska and Stanislaw-ska-Glubiak [93]
<i>Sedum alfredii</i>	Cd	Wu et al. [201]
<i>Solanum nigrum</i>	Cd	Wei et al. [199]
<i>Spartina</i> sp.	Hg	Tian et al. [186]
<i>Suaeda fruticosa</i>	Cd/Cu	Bankaji et al. [16]
<i>Thlaspi caerulescens</i>	Zn/Pb/Cd	Banasova et al. [15]
<i>Usnea amblyoclada</i>	Pb	Carreras et al. [28]
<i>Vetiveria zizanioides</i>	Cd/Cu	Kumar et al. [97]
<i>Youngia japonica</i>	Cd	Yu et al. [207]

metal bioavailability was enhanced by low pH soil and root exudate secretion [36]. In addition, rhizospheric microorganisms and mycorrhiza associated with roots can also favor the HMs uptake by plants [100]. The sorption and desorption of HMs can be associated with soil characteristics such as pH and OM. The soil pH is one of the most important factors that directly affect the HMs bioavailability. At high pH (basic soil), HMs tend to be adsorbed in colloids due to high soil retention capacity, which decreases their mobility. In contrast, the availability of HMs increases at low pH (acid soil) [7]. Organic matter amendments negatively affected the HMs bioavailability in soil and consequently decreased the HMs bioaccumulation in plants due to the tendency of HMs to form complexes with organic ligands (formation of organometallic complexes) [26, 168].

Heavy metal translocation in plants is carried out by vascular tissues (xylem and phloem) and is associated with water relations (transpiration). The flow of HMs by xylem sap from the root to the shoot is mostly controlled by two mechanisms: root hydraulic conductance and leaf transpiration [99, 132]. Hyper-accumulator plants have a high capacity to accumulate HMs, translocate and load them in roots and shoots. HMs translocation from the roots to the shoots for the purpose of harvesting is one of the principal aims of phytoremediation [83].

One of the typical consequences of HMs toxicity in plant species is the production of reactive oxygen species (ROS) causing oxidative stress to plants. These ROS include superoxide radical (O_2^-), hydroxyl radical ($OH^\cdot$) and hydrogen peroxide (H_2O_2). In that situation, plants possess some anti-oxidative defense systems to scavenge toxic free radicals such as: β -carotene, glutathione, superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) [114]. In addition, some plants possess specialized anatomical structures to eliminate metals from their tissues. In this case, the excess of metals can excrete through salt glands in the form of non-toxic crystals. This method is called phytoexcretion [112]. Once loaded into the different tissues of shoot, the toxic HMs are sequestered or compartmentalized in the vacuoles [190] or volatilized across the stomata's [189].

5 Mechanisms of phytoremediation

Except for essential elements such as Cu, Fe, Mg, Mo, Mn, Se and Zn, which are indispensable for plant growth, some plants have the capacity to accumulate HMs (Ag, Cd, Hg and Pb) which have no recognized biological functions. However, high accumulation of these HMs can be toxic to most flora. There are five categories of phytoremediation

technologies applied in cleanup of contaminated soils (Table 4). These include:

5.1 Phytoextraction

Phytoextraction is the method of planting species that are known to accumulate maximum amounts of pollutants (more than 0.1% of DW) in their shoots. The criteria of selection of these plants can be based on degree of translocation of the contaminant from the roots to shoot [183]. This strategy can be divided into 2 methods: *continuous* phytoextraction and *induced* phytoextraction. *Continuous* phytoextraction uses endemic plants with natural abilities to accumulate high contents of HMs (hyper-accumulators). *Induced* phytoextraction enhanced plant metals accumulation by addition of chemical substances like chelates [117]. Phytoextraction is enhanced by plants with high growth rate (large quantity of plant biomass) and deeper root system. Begonia et al. [19] show that coffee weed (*Sesbania exaltata*) was successful to removing Pb from contaminated soils. Fourati et al. [54] demonstrated that Ni was accumulated in higher level ($1050 \mu\text{g g}^{-1}$ DW) in the aboveground part of *Sesuvium portulacastrum*. Jacobs et al. [76] found Zn concentration in leaves of *Noccaea caerulea* exceeding 300 g Cd ha^{-1} after 2 months before transplantation in the field conditions. Ghazaryan et al. [57] compared the capacity of *Melilotus officinalis* and *Amaranthus retroflexus* in remedying contaminated soils by Cu and Mo. The results showed that *A. retroflexus* has a preference of Cu and Mo accumulation in shoot, while *M. officinalis* has a preference of Zn storage in roots. Recently Yang et al. [205] examined the ability of three Napier grass varieties (*Pennisetum purpureum*) to Cd and Zn uptake in field conditions and found that *P. purpureum* cv. *Guiminyin* accumulates the maximum contents of Cd (197.5 g ha^{-1}) and Zn (5023.9 g ha^{-1}) in their shoots. Khalid et al. [87] assessed the phytoextraction capacity of *Alternanthera bettzickiana* to Ni and Cu by pot experiments and found after 8 weeks of treatments that this species accumulates 2 times more Cu in shoots as compared to control.

5.2 Phytodegradation/phytotransformation

Phytodegradation or phytotransformation is the degradation of pollutants assimilated by plants through metabolic processes, or the degradation of pollutants outside the plant through enzymes produced by roots (like dehalogenases, nitroreductases and peroxidases) [164]. Genetically modified yellow poplar (*Liriodendron tulipifera*) can grow in tissue culture with higher mercury concentrations and transform it from highly toxic mercury Hg^{2+} to much less toxic form Hg^0 [155]. Through this method,

Table 4 Some mechanisms of phytoremediation

Phytoremediation	HMs	Species	References
Phytoextraction	Cu	<i>Alternanthera bettzickiana</i>	Khalid et al. [87]
Phytoextraction	Cd/Zn	<i>Pennisetum purpureum</i>	Yang et al. [205]
Phytoextraction	Cu	<i>Boehmeria nivea</i>	Rehman et al. [152]
Phytoextraction	Cd	<i>Atriplex nummularia</i>	Nedjimi [135]
Phytoextraction	Cd	<i>Atriplex lentiformis</i>	Eissa [47]
Phytoextraction	Ni	<i>Sesuvium portulacastrum</i>	Fourati et al. [54]
Phytoextraction	Zn	<i>Halimione portulacoides</i>	Cambrollé et al. [27]
Phytoextraction	Cd	<i>Sesuvium portulacastrum</i>	Ghnaya al. [58]
Phytoextraction	Pb	<i>Tamarix smyrnensis</i>	Kadukova et al. [82]
Phytoextraction	Cu	<i>Sedum alfredii</i>	Liu et al. [104]
Phytoextraction	Zn/Pb	<i>Armeria arenaria</i>	Frérot et al. [55]
Phytoextraction	Cd	<i>Sedum alfredii</i>	Zhou and Qiu [212]
Phytoextraction	Ni/Zn	<i>Cannabis sativa</i>	Meers et al. [119]
Phytoextraction	Pb	<i>Pelargonium hortorum</i>	Manzoor et al. [113]
Phytoextraction	Cd	<i>Quercus robur</i>	Sozoniuk et al. [182]
Phytoextraction	Cd/Cu	<i>Salix alba</i>	Mataruga et al. [116]
Phytostabilization	Cd	<i>Acanthus ilicifolius</i>	Shackira and Puthur [167]
Phytostabilization	Zn/Pb	<i>Pistacia lentiscus</i>	Concas et al. [37]
Phytostabilization	Cd	<i>Atriplex halimus</i>	Nedjimi and Daoud [132]
Phytostabilization	Pb	<i>Brassica juncea</i>	Meyers et al. [121]
Phytodegradation	PAHs	<i>Rhizophora mangle</i>	Sampaio et al. [159]
Phytodegradation	MCP	<i>Liriope muscari</i>	Dash and Osborne [43]
Phytodegradation	PAHs	<i>Rhizophora mangle</i>	Sampaio et al. [159]
Phytodegradation	TCE	<i>Salix viminalis</i>	Clausen et al. [35]
Phytodegradation	TNT	<i>Myriophyllum spicatum</i>	Hughes et al. [72]
Rhizofiltration	Cr/Zn	<i>Salvinia molesta</i>	Kodituwakku and Yatawara [92]
Rhizofiltration	As	<i>Salvinia molesta</i>	da Silva et al. [38]
Rhizofiltration	U	<i>Phragmites australis</i>	Wang and Dudel [197]
Rhizofiltration	Cr	<i>Genipa americana</i>	Santana et al. [160]
Phytovolatilization	As	<i>Arundo donax</i>	Guarino et al. [56]
Phytovolatilization	Hg	<i>Brassica juncea</i>	Moreno et al. [126]
Phytovolatilization	Se	<i>Stanleya pinnata</i>	Parker et al. [142]

plants are able to transform pollutants into non-hazardous components. Das et al. [41] demonstrated that *Vetiveria zizanioides* plants were capable to clean up 97% of TNT from the soil. Hannink et al. [66] indicated that *Nicotiana tabacum* contributed to the degradation of TNT through *NfsI* nitroreductase enzyme produced by roots. Just and Schnoor [79] revealed that *Populus deltoids* plants were able to transform RDX into metabolic components.

Some plants can provide best environments for association of bacteria and mycorrhizae to develop and degrade toxins. The components of this degradation are volatilized or incorporated into soil matrix [44]. The plant exudates production such as sugars and organic acids increase fungi and bacterial populations [171]. Rhizodegradation can be enhanced by favorable soil characteristics such as soil aeration and moisture content [90]. Recently, Papadopoulos and Zalidis [141] carried out experiment culture to remedy

the wetland contaminated with Terbutylazine (TER) and found that rhizomes of *Typha latifolia* were a promising species to TER phytodegradation. Sampaio et al. [159] demonstrated that *Rhizophora mangle* mangrove associated with plant growth-promoting rhizobacteria (*Pseudomonas aeruginosa* and *Bacillus* sp.) was able to degrade polycyclic aromatic hydrocarbons (PAHs) in contaminated sediment.

5.3 Phytovolatilization

Phytovolatilization is the process of uptake of pollutants and converts it to less harmful volatile forms. In this method, the pollutants are assimilated by the roots, translocated to the shoot and volatilized in atmosphere through the stomatal leaves [189]. Bañuelos et al. [17] suggested that Indian mustard (*Brassica napus*) could be successful

in the phytovolatilization of Se from soil. Bizily et al. [20] showed that *Arabidopsis thaliana* converted organic Hg salts to the volatile elemental form. *Brassica juncea* grown hydroponically was reported to remove up to 95% of Hg by both phytovolatilization and plant accumulation [124]. Ashraf et al. [10] show that transgenic *Nicotiana tabacum* carrying the *merA* gene, was able to eliminate Hg from contaminated soils. Recently Guarino et al. [56] reported that *Arundo donax* assisted by plant growth-promoting rhizobacteria (*Stenotrophomonas maltophilia* and *Rhizobacterium* sp.) tended to volatilized arsenic when plants were grown in sterile garden soil amended with 2, 10 and 20 mgL⁻¹ of NaAsO₂.

5.4 Rhizofiltration

Rhizofiltration is a technique to eliminate pollutants from water and liquid waste by the precipitation of toxins onto surface roots or the absorption of soluble pollutants into the roots [46]. Fibrous root system and large surface areas of roots play a significant role in this technique [158]. The plants able to take up a large quantity of water from the soil are appropriate for this purpose [183]. Amaya-Chavez et al. [6] have demonstrated that *Typha latifolia* could be efficacious to remove methyl parathion (MeP) from hydromorphic soils. Yang et al. [204] found that bean species (*Phaseolus vulgaris*) efficiently removes uranium (Ur) and cesium (Cs) from groundwater. Oustriere et al. [139] indicated that *Arundo donax* is an effective Poaceae to rhizofiltrate Cu from constructed wetlands. Recently Kodituwakku and Yatawara [92] demonstrated that *Eichhornia crassipes*, *Salvinia molesta* and *Pistia stratiotes* are promising terrestrial candidates for removing heavy metals (Cu, Cr, Cd, Ni and Zn) from industrial sewage sludge.

5.5 Phytostabilization

In this strategy, pollutants are stored by plant roots or precipitated by root exudates [144]. Thus this method decreases the movement of the pollutants and avoids their migration to groundwater.

A study by Nedjimi and Daoud [132] showed that *Atriplex halimus* (Aramanthaceae family) a local north African halophyte was a promising candidate for phytostabilization of Cd. The same authors found that this species accumulated high concentrations of Cd in roots compared to shoots (606.51 and 217.52 ppm DW, respectively). Morphology and depth of roots were the principal characteristics of plants suitable for phytostabilization. Maximum contact with the soil matrix was provided by numerous fine root system extents throughout the soil (high surface area of the fibrous roots). However, root depth

varies significantly according to plant species, moisture, soil texture, calcareous encrust, dry conditions and soil amendments.

Through the excretion of root exudates, some plants species can modify metal solubility and/or mobility. However, other species sequester large amount of HMs in their roots [23]. Al Chami et al. [5] compared the remedy effects of *Sorghum bicolor* and *Carthamus tinctorius* on Ni, Pb and Zn by pot experiments and indicated that these species were able to uptake these heavy metals. Furthermore, roots accumulated more metals than leaves. *Erica australis* possesses the ability to uptake Cu, Cd and Pb via its roots without any sign of damage and suggest as a phytostabilizer species [123]. Bacchetta et al. [13] indicated that Zn, Cd and Pb uptake was restricted largely in root tissue of *Helichrysum microphyllum* which is suitable for phytostabilization.

Recently Manzoor et al. [113] selected *Stigmatocarpum criniflorum* and *Pelargonium hortorum* as tested species under Pb stress conditions and quantified their extraction ability. The results exhibited that the aboveground part of *P. hortorum* accumulates more Pb in its roots than *S. criniflorum*. *Quercus robur* was found to be the best accumulator of Cd in roots and thus a promising candidate for phytostabilization of Cd in polluted soils [183]. In field conditions, Mataruga et al. [116] proved that *Salix alba* was an useful tree to Cd and Cu phytostabilization in the riparian soils of the Sava River.

6 Biotechnological processes

In the recent decades, genetic engineering approaches have been applied to ameliorate the efficacy of plants as candidates for HMs decontamination. Plants have been modified genetically using hyper-accumulator plant and bacterial genes, which are known by their great accumulation, degradation capacity or transformation properties [146]. Many researches have emphasized transgenic solutions to enhance phytoremediation. This approach is based on introduction of genes to improve tolerance and hyper-accumulation of toxic HMs into larger plants with rapid growth rate [94]. Preferably, the spontaneous plant species can be used which are adapted to growing in the local climate. This technique can also be employed to increase biomass, metal storage capacity and hyper-accumulation of multiple HMs [144]. Morphology and depth of roots are also essential factors for the selection of suitable plant species with shallow roots would be preferable for the remediation of surface-contaminated soils, whereas species with deep roots would be appropriate for more profound soils [100]. For phytoextraction strategy the species used should produce rapidly a large quantity of green

foliage and be easily harvested, preferably several times/year [203]. However, in the nature, most native species hyper-accumulators are herb or shrub plants with small green biomass, and the works and researches are being made to create transgenic varieties with high biomass [162]. A large root volume, high foliage biomass, great transpiration rate with high metal assimilation and high exudates production were the principal characteristics of plants useful for phytoremediation. The period time necessary for soil remediation was reduced by fast growth rate and/or large biomass [157]. In the recent decades the comprehension of metal hyper-accumulation eco-physiology of plants has improved by the development of molecular tools such as HMs transporters, enzymes production and metal-detoxifying chelators [192].

Genetic engineering or transgenic transformation of plants played an important role to improve the phytoremediation aptitudes of plants toward the elimination or detoxification of HMs in the environment. This method based on the overexpression of particular genes implicated to enhance the plant tolerance by extraction, translocation and sequestration of HMs or by convert the toxic metals into a less toxic forms [4]. For example, Cai et al. [25] suggest that expression of rice *OsHMA3* decreases shoot Cd accumulation in transgenic tobacco. The co-expression of *OsLCT1*, *OsHMA2* and *OsZIP3* transporters increases the uptake, translocation and potential oxidative stress of Cd and Zn in *Oryza sativa* [187]. Khan et al. [89] identified two novel rice genes HPP (heavy metal-associated plant protein) and HIPP (heavy metal-associated isoprenylated plant protein) tolerant to Cu, Zn, Cd and Mn. In transgenic tobacco, Gouiaa and Khoudi [62] demonstrated that expression of vacuolar proton pump (*V-PPase*) with a Na/

proton antiporter (*NHX1* transporter) enhances Cu tolerance and accumulation in transgenic tobacco. Recently, Liu et al. [106] found that metallothionein *PpMT2* gene involved in HMs tolerance of *Physcomitrella patens* could be used as a potential gene in transgenic *Arabidopsis* plants.

7 New insights and innovative technologies for improving phytoremediation

7.1 Microbial-assisted phytoremediation (PGPR)

Bioremediation implicates the use of plant growth-promoting bacteria (PGPB) that are able to colonize the rhizospheric system and stimulate the growth and mineral nutrition of plants. These bacteria have a potential to degrade toxic contaminants or to convert them to less harmful forms [191]. Several PGPB have been reported to enhance the phytoremediation capacity of plants by allow the roots to uptake HMs. These bacteria play a key role in HMs decontamination by secreting different substances such as siderophores (chelators) and organic acids, which enhance the bioavailability of HMs by decreasing the soil pH [34]. Others bacteria have been reported to secrete polymeric compounds such as polysaccharides and glomalin which contribute to phytostabilization of HMs by reducing their mobility [151]. Some PGPR play a vital role in the phytoremediation processes by various ways including (a) improvement the detoxification rates of plants, (b) enhancement of enzymes root secretion leading to accelerated pollutant degradation or (c) soil pH modification

Table 5 Some plant growth-promoting bacteria (PGRB)-assisted phytoremediation

Bacteria	HMs	Host species	References
<i>Arthrobacter</i> sp.	Cd	<i>Glycine max</i>	Rojjanateeranaj et al. [154]
<i>Arthrobacter</i> sp. FM-1	Cd	<i>Polygonum hydropiper</i>	Li et al. [103]
<i>Bacillus cereus</i>	Cr/Fe	<i>Vetiveria zizanioides</i>	Nayak et al. [131]
<i>Bacillus XZM</i>	As	<i>Vallisneria denseserrulata</i>	Irshad et al. [75]
<i>Chaetomium cupreum</i>	Al	<i>Miscanthus sinensis</i>	Haruma et al. [68]
<i>Cupriavidus basilensis</i>	As	<i>Pteris vittata</i>	Yang et al. [206]
<i>Enterobacter</i> sp. strain EG16	Cd/Fe	<i>Hibiscus cannabinus</i>	Chen et al. [34]
<i>Mesorhizobium loti</i> HZ76	Pb/Zn	<i>Robinia pseudoacacia</i>	Fan et al. [51]
<i>Microbacterium arborescens</i>	U/Pb	<i>Leptochloa fusca</i>	Ahsan et al. [3]
<i>Paxillus involutus</i>	Pb	<i>Populus canescens</i>	Szuba et al. [184]
<i>Pseudomonas</i> sp. strain Lk9	Cd	<i>Solanum nigrum</i>	Chen et al. [32]
<i>Pseudomonas thivervalensis</i>	Cd	<i>Brassica napus</i>	Chen et al. [31]
<i>Pseudomonas libanensis</i>	Ni	<i>Helianthus annuus</i>	Ma et al. [110]
<i>Pseudomonas fluorescens</i>	Sb	<i>Trifolium repens</i>	Daryabeigi et al. [40]
<i>Simplicillium chinense</i>	Cd/Pb	<i>Phragmites communis</i>	Jin et al. [78]
<i>Streptomyces</i> sp.	Zn/Cd/Pb	<i>Salix dasyclados</i>	Złoch et al. [214]

[107]. Thus, many strains of bacteria were found to increase HM tolerance of plants (Table 5).

For example, *Arthrobacter* inoculated to *Ocimum gratissimum* inducing the phytoextraction of Cd by roots [148]. Guo and Chi [64] show that PGRB *Bradyrhizobium* sp. can alleviate growth and promote Cd uptake of *Lolium multiflorum* and *Glycine max* seedlings in Cd-contaminated soil. Ike et al. [74] demonstrated that symbiosis between leguminous plant species and rhizobia with the two genes (*MTL4* and *PCS*) enhances the bioremediation of Cd. Szuba et al. [184] revealed that Pb-tolerant *Paxillus involutus* strain can alleviate growth and promote Pb tolerance of *Populus canescens* seedlings under in vitro culture. Likewise, three bacterial endophytes, namely *Pantoea stewartii* ASI11, *Enterobacter* sp. HU38 and *Microbacterium arborescens* HU33, increase *Leptochloa fusca* plants phytostabilization in soils contaminated with U and Pb [3]. *Mesorhizobium loti* HZ76 and associated bacterial community improve *Robinia pseudoacacia* growth and enhance its phytoremediation capacity [51]. Inoculation of *Vallisneria denseserrulata* plants with *Bacillus* XZM strain enhances significantly the detoxification efficiency of As [75]. Yang et al. [206] found that *Pteris vittata* accumulates about 170% of As in its organs when inoculated with *Cupriavidus basilensis* strain r507.

7.2 AMF inoculation-assisted phytoremediation

The arbuscular mycorrhizal fungi (AMF) are a symbiotic fungi association with roots host plants to increasing the phytoavailability of phosphorus [209]. Two strategies were adopted by AMF to HMs decontamination: (a) immobilization of HMs by production of chelating agents and adsorption to fungal cell walls, (b) phytoextraction of HMs by improving plant growth and increase HMs uptake in the rhizosphere by modifying the chemical composition of root exudate and/or reducing the soil pH [24]. For example, the inoculation of *Cassia italica* by AMF significantly enhanced the Cd tolerance by preventing its translocation to aerial parts [67]. Symbiosis between *Festuca arundinacea* plants and *Glomus mosseae* fungi improves both Ni translocation and expression of ABC transporter and metallothionein genes [165]. Inoculation with AMF significantly enhances growth, phosphorus pool and HMs uptake in maize plants (*Zea mays*) grown in soil polluted with Sr and Cd [29]. Abdelhameed and Metwally [2] investigated the response of *Trigonella foenum-graecum* plants inoculated with AMF under different concentrations of Cd (0, 2.25 and 6.25 mM CdCl₂) and found that this symbiosis becomes a promising tools to Cd phytostabilization. Armendariz et al. [9] reported that soybean (*Glycine max*) plants inoculated *Bradyrhizobium japonicum*

E109 and *Azospirillum brasilense* Az39, exhibit better tolerance against As stress. Shahabivand et al. [169] confirmed the ability of endophytic fungus *Piriformospora indica* to alleviate Cd toxicity by improving the physiological status in sunflower (*Helianthus annuus* L.) seedlings. Likewise recently Rahman et al. [149] demonstrated that exogenous inoculation of *Artemisia annua* by *Piriformospora indica* can confer high degree of tolerance against As stress.

7.3 Earthworm-assisted phytoremediation

Earthworms known as 'ecosystem engineers' are the main group of soil macroinvertebrates. They play a vital role in organic matter decomposition, nutrient cycling and amelioration of soil conditions [173]. By secretion of some organic acid such as fulvic and humic acids through their gut microflora, earthworms contribute to decrease the pH of soil which enhances the nutrient and HMs bioavailability in rhizosphere [101, 198]. For example, Wang et al. [198] demonstrated that integration of earthworms in culture medium enhances the phytoremediation capacity of Cd in *Solanum nigrum*. Bongoua-Devisme et al. [22] revealed that *Pontoscolex corethrurus* can alleviate Cr and Ni tolerance of *Acacia mangium*. Likewise, *Rhizoglossum clarum* integration increases the phytoextraction capacity of *Canavalia ensiformis* plants in sandy soil contaminated with Cu [161]. Incorporation of *Brassica juncea* plants with *Eisenia fetida* earthworm enhances significantly the detoxification efficiency of Cd [86]. Addition of vermicompost using *Ensenia Andrei* to HM-contaminated soil increases the ability of black oat (*Avena strigosa* Schreb) plants to remove Cd, Cr and Pb [70].

7.4 Phytohormone-assisted phytoremediation

Plant growth regulators (PGR)-assisted phytoremediation is a procedure that could improve the HMs accumulation in plant tissues. Four principal kinds of plant hormones that may be beneficial for this method were identified: auxins (IAA), cytokinins, gibberellins and abscisic acid (ABA). Many reports have revealed that these phytohormones positively improve the degree of accumulation of HMs and enhance the growth and HMs tolerance of plants. It is well known that the exogenous addition of phytohormones during the earlier growth stage can help plants to escape toxicity upon exposure to HMs. For example addition of 0.05 M auxin offers a promising strategy to improve *Arabidopsis thaliana* tolerance against Cd with minor damages [208]. Exogenous application of 10 and 100 mM IAA in nutrient solution alleviates the deleterious effects of Cd-stressed *Trigonella foenum-graecum* by inhibiting the uptake of Cd and regulation of ascorbate-glutathione cycle [18]. Ji et al. [77] revealed that application of 10, 100

and 1000 mgL⁻¹ gibberellic acid 3 (GA3) can significantly increase biomass and phytoremediation efficiency of *Solanum nigrum*. Recently Song et al. [180] demonstrated that the growth enhancement effect of supplemental ABA on Zn-stressed *Vitis vinifera* was due to the expression of ZIP and detoxification-related genes. Similarly, Leng et al. [102] found that supplementation of 5, 10 and 15 µM ABA alleviates adverse effects of Cd on the growth of mung bean (*Vigna radiate*) plants. This promoting effect of ABA on growth was associated with protection of membrane lipid peroxidation and the modulation of antioxidative defense systems.

7.5 Nanoparticles-assisted phytoremediation

Nanoparticles (NPs) addition is a new innovative method to enhance the removal efficiency of HMs [213]. Thus, these particles can increase the phytoremediation capacity by diverse strategies including: (a) interaction with HMs by adsorption/redox reactions, (b) stimulation of plant growth or (c) facilitate the HMs phytoremediation [179]. The chemical interaction showed that nanoparticles can help plants to stabilize HMs by electrostatic adsorption. Promotion of plant growth by NPs can be achieved by rhizospheric microbes and fungi. Several works demonstrated the beneficial impact of nanoparticles to increasing the phytoremediation. In this respect, Khan and Bano [88] reported that growth and phytoextraction capability of maize plants were modulated by addition of Ag nanoparticles (AgNPs) with plant growth-promoting rhizobacteria (PGPR). Supplementations of Cd-polluted soil with nano-TiO₂ particles were reported to enhance the removal potential of Cd in soybean plants (*Glycine max*) [177]. The exogenous addition of salicylic acid nanoparticles (SANPs) during the earlier growth stage can improve *Isatis cappadocica* phytoremediation against As [181]. Likewise, Gong

et al. [61] and Huang et al. [71] found that application of nanoscale zero-valent iron (nZVI) improves the antioxidative system and phytoextraction potential of Cd and Pb, respectively, in *Boehmeria nivea* and *Lolium perenne*. Recently it has been demonstrated that the combination between some nanoparticles such as hydroxyapatite, hematite and maghemite NPs, reduces the bioavailability of HMs in mining soils [8]. Hussain et al. [73] found that radish (*Raphanus sativus*) exhibits better Pb accumulation and antioxidative response when treated with thidiazuron (TDZ) growth regulator and magnesium oxide (MgO) nanoparticles.

7.6 Transgenic approaches

Transgenic plants are the species genetically modified—by DNA manipulation and genome transformation—to integrate new genes, which does not exist naturally in the species to enhance the uptake and translocation of HMs [150]. Nowadays, engineered transgenic approaches are considered as a main research field in biotechnology for improving phytoremediation. The overexpression of genes was applied to reduce the stress imposed by HMs and promote the phytoremediation capacity of plants [107]. Hyper-accumulator plants can additionally be improved through genetic approaches/molecular mechanisms to overcome some limitations of phytoremediation. Therefore, recently, progresses in biotechnology through gene expression are investigated intensively to enhance phytoremediation processes.

Genes isolated from bacteria, fungi or plants involved in sequestration and degradation of HMs were introduced into tolerate plant species (Table 6). Therefore, two strategies have been pursued: (a) overexpression of genes responsible of HMs hyper-accumulation, (b) introduction of genes from other organisms such as bacteria,

Table 6 Some genes introduced into plants and the impacts of their expression on HMs tolerance

Bacteria	Genes	Target Plant	Enzymes expressed	Beneficial effects	References
<i>Escherichia coli</i>	ECS genes	poplar	g-glutamylcysteine synthetase	Enhanced Cd flux and detoxification	He et al. [69]
<i>Caenorhabditis elegans</i>	AtPCS1 and CePCS	<i>Nicotiana tabacum</i>	Phytochelatin synthase	Increased As accumulation	Wojas et al. [200]
<i>Pseudomonas fluorescens</i>	SaNramp1	<i>Sedum alfredii</i>	iron-regulated transporter	Enhanced Zn uptake	Wang et al. [197]
<i>Saccharomyces cerevisiae</i>	gsh1 and AsPCS1	<i>Arabidopsis thaliana</i>	Glutathione synthase and phytochelatin synthase	Enhanced Cd and As tolerance	Guo et al. [63]
<i>Saccharomyces cerevisiae</i>	ScMTII gene	<i>Nicotiana tabacum</i>	Metallothionein transporter	Enhanced Cd and Zn accumulation	Daghan et al. [39]
<i>Streptococcus thermophilus</i>	STGCS-GS	<i>Beta vulgaris</i>	g-Glutamylcysteine synthetase and glutathione synthetase	Enhanced Cd Cu and Zn tolerance	Liu et al. [105]

fungi or other plants. The main objective of this transgenic approach is to acquire plants with high capacity to tolerate, accumulate or degrade HMs [59]. This approach could also lead to obtain plants with suitable agronomic properties such as high green biomass and deep root system, with fast growth in different pedo-climatic conditions [150].

Two bacterial gene enzymes, namely mercuric ion reductase (*merA*) and organomercurial lyase (*merB*), were involved to enhance the Hg detoxification. The organic form of Hg was converted to less toxic ionic form Hg^{2+} by *merB* protonolysis. However Hg^{2+} was converted to volatilize from Hg^0 by *merA* reduction [21]. It has been demonstrated that *SbMT-2* gene isolated from *Salicornia brachiata* confers HMs (Cu, Zn and Cd) tolerance and modulates ROS scavenging in transgenic *Nicotiana tabacum* [30]. Macrophage protein (*Nramp*) was found to play a significant role of HMs decontamination. For example overexpressed *SaNramp6* isolated from *Sedum alfredii* improved significantly Cd accumulation in transgenic *Arabidopsis thaliana* [33]. Transgenic tobacco combined with *OsMTP1* protein gene from *Oryza sativa* cv. IR64 remarkably improved its tolerance against Cd [42]. Likewise, the *AtACR2* gene (arsenic reductase 2) isolated from *Arabidopsis thaliana* can be a promising tool of As decontamination in transgenic tobacco [129].

Vacuolar HMs compartmentation is found to be the major molecular process for HMs detoxification through a large number of protein transporters such as COPT5-transporter; VIT-transporter, H^+ -ATPase; Na^+/H^+ antiporter [211]. In hyper-accumulator *Sedum plumbizincicola* (Crasulaceae), P1B-type ATPase (HMA4 and HMA2) transporters were overexpressed under the Cd stress [145]. Meena et al. [118] investigated the response of tomato (*Solanum lycopersicum*) to different concentrations of CdCl_2 (0, 50, 100, 150, 200 and 250 μM) and found that the most contents of Cd were compartmented in the vacuoles due principally to high NRAMP3 transporter protein activity.

Phytochelatins (PCs), glutathione (GSH) and metallothioneins (MTs) play an essential role in HMs decontamination through chelating of toxic metal and transferred to vacuoles as low (LMW) or high molecular weight (HMW) [185]. The expression of genes of some enzymes such as glutathione synthase (GS) and phytochelatin synthase (PCS) involved in PCs biosynthetic pathway has contributed to enhance tolerance and detoxification of HMs [176]. The overexpression of *Arabidopsis* ATP sulfurylase gene and transferred them to *Medicago sativa* enhanced its tolerance against Cd [98].

8 Future prospects and research needs

Phytoremediation is one of the most promising techniques for the eco-rehabilitation of polluted sites, but further investigations and research are also necessary to enhance our knowledge in efficient phytoremediation of HMs. To avoid any failure during field cultures, it has become an essential requirement to tend toward future ways clarify mechanisms, metabolites and genes using latest omic techniques. These methods can aid in defining new metabolites and trails implicated in stabilization or extraction of HMs by hyper-accumulator plants.

Utilization of hyper-accumulator shrubs and plants to remove HMs from soil requires novel strategies for its progress. This can be accomplished either by finding and valorizing a vast diversity of new hyper-accumulator species or by genetic engineering. Multiplication and intensification of hyper-accumulator plants with higher translocation rates; high green biomass; or depth root system provide research about phenotypic differences between tolerant plants.

Overexpression of foreign genes in non-tolerant plants for HMs depollution of soil is practicable. Although several plants and shrubs were established to clean up HMs through genetic engineering, no perfect model can be established for HMs detoxification until the obtainability of whole genome data is certified.

Urgent works about the role of plant hormone such as IAA, GA cytokine were also needed to understand their role to increase potential of HMs detoxification plants.

Plant–microorganism interaction (bacteria, fungi) was also an effective approach to uptake and translocation of HMs in plants. These approaches can aid in discovering novel metabolites and trails contributed in degradation of contaminants through plant–microorganism interactions [122]. An additional advance technique is the study of hologenome of the microorganism of plants which could be applied in manipulation of microbial niches to increase resistance against HMs contamination [127]. Nanoremediation is a novel technology assisted by microbial cells integration to enhance remediation process more effectiveness to removing HMs from high contaminated soils [179, 213]. However, the relation between the molecular approaches of phytoremediation and nanoparticles needs to be clearly elucidated to expand the prospect of polluted soil remediation.

Also appropriate environmental management practices such as using metal chelators (phytochelatins and metallothioneins) need to be valorized to enhance HMs accumulation. Finally the success and reliability of phytoremediation can be achieved through contribution and coordination of farmers, local communities, researchers,

industrial sectors and environment authorities through educational programs to insure the long-term sustainability of this friendly green technology.

9 Conclusion

Heavy metal pollution is a global concern and a major health threat worldwide. Phytoremediation is a low-costly, social acceptable and environmentally friendly technology compared to other chemical methods of HMs decontamination. This technique applied various mechanisms, which include HMs uptake (phytoextraction), breakdown and transformation of HMs (phytodegradation), emission in atmosphere (phytovolatilization) and their stabilization in the root system (phytostabilization). To enhance the phytoremediation potential of the plants, various bioremediation approaches like genetic engineering, transgenic transformation, application of phytoremediation assisted with phytohormones, microbes, AMF inoculation and nanoparticles (NPs) addition have been widely used. More extensive research under field conditions, selection of the most useful plants, determination of new genes and development of transgenic plants will be useful to increase the understanding of the metabolic activities involved in HMs tolerance in hyper-accumulator plants and unlock new directions for phytoremediation.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the author.

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