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Phytoremediation as a novel approaches to revegetation of heavy metal in polluted soil

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Abstract

Heavy metal accumulated in soil with the progression of time, due to rapid industrialization and human intervention pose serious environmental issues and health hazards to bio systems. The persistence of heavy metal contaminants in soil due to non-biodegradable nature ultimately enter the food chain through crop plants, and eventually accumulate in many folds human body through bio magnification. Therefore, remediation of land contamination is of paramount importance. Phytoremediation is an eco-friendly approach that could be a successful mitigation measure to revegetate heavy metal-polluted soil in a cost-effective way. To improve the efficiency of phytoremediation, a better understanding of the mechanisms underlying heavy metal accumulation and tolerance in plant is indispensable. In this review, we describe the mechanisms up take of heavy metal by plants, translocation and detoxified in plants. We focus on the strategies applied to improve the efficiency of phytostabilization and phytoextraction, including the application of genetic engineering approach.

Keywords: Phytoremediation, hyper accumulation, phytochelatins, metallothioneins, xenobiotics, heavy metals

Introduction

The heavy metals contaminants released in the environment have increased enormously during the past decades due to rapid industrialization and urbanization, which raised significant concerns throughout the world (Ashraf *et al.*, 2019) [2]. Heavy metals are chemical elements with relatively high densities, atomic weights, and atomic numbers. The commonly occurring metalloid in nature includes cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), zinc (Zn), copper (Cu), nickel (Ni), and chromium (Cr). These heavy metals are generated from oil and gas industries (Pichtel, 2016) [51], use of phosphate fertilizers in agriculture (Rafique and Tariq, 2016) [52], sewage sludge (Farahat and Linderholm, 2015) [22], metal mining and smelting (Chen *et al.*, 2016) [12], pesticide application (Iqbal *et al.*, 2016) [36], electroplating, and fossil fuel burning (Muradoglu *et al.*, 2015) [49].

The persistence and non-biodegradable nature of heavy metals in soil pose long-term threat for the environment (Suman *et al.*, 2018) [58]. According to their role in biological systems, heavy metals can be grouped as essential and non-essential. Essential heavy metals such as Cu, Fe, Mn, Ni, and Zn are required for physiological and biochemical processes during plant life cycle (Cempel and Nikel, 2006) [11]; however, they may become toxic when present in excess. Non-essential heavy metals like Pb, Cd, As, and Hg are highly toxic with no known function in plants (Fasani *et al.*, 2018) [23] and may cause environmental pollution and severely affect a variety of physiological and biochemical processes in crop plants and reduce agricultural productivity (Clemens, 2006) [13]. They can enter into the food chain through crops and accumulate in the human body through biomagnification, thus posing a great threat to human health (Rehman *et al.*, 2017) [54]. Hence, it is necessary to take remedial measures to prevent heavy metals from entering into terrestrial, atmospheric, and aquatic environments, and mitigate the contaminated land (Hasan *et al.*, 2019) [33]. So far, there are a variety of remediation approaches that have been developed to reclaim heavy metal-contaminated soil. These measures are mainly based on mechanical or physio-chemical techniques, such as soil incineration, excavation and landfill, soil washing, solidification, and electric field application (DalCorso *et al.*, 2019) [14]. However, there are limited reports available on physicochemical approaches. Therefore; there is a need to develop cost-effective, efficient, and environment-friendly remediation technologies to reclaim heavy metal-contaminated soil.

Phytoremediation as alternate approach

Phytoremediation is a plant-based approach, to extract and remove elemental pollutants or lower their bioavailability in soil (Berti and Cunningham, 2000) [7]. Plants have the abilities to absorb ionic compounds in the soil even at low concentrations through their root system. Plants extend their root system into the soil matrix and establish rhizosphere ecosystem to accumulate heavy metals and modulate their bioavailability, thereby reclaiming the polluted soil and stabilizing soil fertility (DalCorso *et al*., 2019) [14].

The advantages of using phytoremediation approaches include

1. **Economically feasible:** Phytoremediation is an autotrophic system powered by solar energy, therefore, simple to manage and the cost of installation and maintenance is low.
2. **Environment and eco-friendly:** It can reduce exposure of the pollutants to the environment and ecosystem.
3. **Applicability:** It can be applied over a large-scale field and can easily be disposed.
4. It prevents erosion and metal leaching through stabilizing heavy metals, reducing the risk of spreading of contaminants.
5. It can also improve soil fertility by releasing various organic matters to the soil (Jacob *et al*., 2018) [37].

Numerous Research investigations have been only focused to understand the underlying molecular mechanisms tolerance of heavy metals in past few decades and to develop techniques to improve efficiency of Phytoremediation. But, the current review describes, the mechanisms of uptake of heavy metals, translocation with in the plants and the detoxification strategies (avoidance and tolerance) adopted by plants in response to heavy metal have been discussed. The main objective is to overview the recent advances in developing phytoremediation techniques, including the strategies to improve heavy metal bioavailability, tolerance, and accumulation. This review also highlights the application of genetic engineering to improve plant performance during phytoremediation.

Mechanism of uptake of heavy metals and physiology of translocation

Heavy metal accumulation in plant involves series of step including

1) Mobilization

Heavy metal mostly exists as insoluble form in soil, which is not available to plants, in order to increase bioavailability plant releases a variety of root exudates, which in turn change rhizosphere pH and increase its solubility (Dalvi and Bhalerao, 2013) [15].

2) Uptake by roots

The uptake of heavy metals into roots occurs mainly through two pathways, apoplastic pathway (passive diffusion) and symplastic pathway (active transport against electrochemical potential gradients and concentration across the plasma membrane). The common uptake of heavy metals via symplastic pathway is an energy-dependent process mediated by metal ion carriers or complexing agents (Peer *et al*., 2005) [50].

After entering into root cells, heavy metal ions can form complexes with various chelators, such as organic acids. These formed complexes including carbonate, sulfate, and phosphate precipitate, are then immobilized in the extracellular space (Apoplastic cellular walls) or intracellular spaces (Symplastic compartments, such as vacuoles) (Ali *et al*., 2013) [1].

3) Loading in the xylem, root-to-shoot transport, cellular compartmentalization and sequestration:

The metal ions sequestered inside the vacuoles may transport into the stele and enter into the xylem stream via the root symplasm (Thakur *et al*., 2016) [60] and subsequently are translocated to the shoots through xylem vessels. Through apoplast or symplast, they are transported and distributed in leaves, where the ions are sequestered in extracellular compartments (cell walls) or plant vacuole, thereby preventing accumulation of free metal ions in cytosol (Tong *et al*., 2004) [61].

Transportation of heavy metals ions in plants

Heavy metal uptake and translocation in plants mediated by metal ion transporters (channel proteins or H^{+}-coupled carrier proteins located in the plasma membrane).They can transport specific metals across cellular membranes and mediate influx–efflux of metal translocation from roots to shoots (DalCorso *et al*., 2019) [14]. Besides transporters, complexing agents including organic acids and amino acids act as metal ligands to mediate chelation of heavy metal ions. For example, citrate is a major chelator for Fe and Ni in the xylem (Lee *et al*., 1977) [68], while Ni may also be chelated by histidine (Krämer *et al*., 1996) [40].

Metal transporters identified, so far, have been classified into several families, such as ZIP, HMAs, MTPs, and NRAMPs based on results of sequence homology.

ZIP family (ZRT–IRT-like proteins)

Members of this family involved in heavy metal accumulation processes including uptake and transport of many cations (e.g., Fe, Mn, and Zn) from root to shoot (Guerinot, 2000) [28]. For example, Zn hyperaccumulator *Thlaspi caerulescens* and *Arabidopsis halleri* roots have enhanced Zn uptake in comparison to non hyperaccumulator species, which is correlated with enhanced expression of some ZIP family members in hyperaccumulator (Assunção *et al*., 2001) [3].

HMAs

1. The P1B-type ATPases of heavy metal transporting ATPases (HMAs) transporter family are involved in the transport of heavy metals (such as Zn, Cd, Co, and Pb) and play a vital role in metal homeostasis and tolerance (Williams and Mills, 2005) [64].
2. HMA3, a vacuolar P1B-ATPase: involved in compartmentation of Zn, Cd, Co, and Pb by regulating their sequestration into the vacuole (Hanikenne and Baurain, 2014) [31].
3. Transporter of the family, HMA4: involved in long-distance root-to-shoot translocation of Zn and Cd (Verret *et al*., 2004) [69]. Over-expression of HMA4 enhanced Cd and Zn efflux from the root symplasm into the xylem vessels and promoted metal tolerance.

MTPs Family

Members of this family regulate metal homeostasis and involved in the translocation of metals (such as Zn and Ni) toward internal compartments and extracellular space (Gustin *et al*., 2011) [30]. MTP1, a vacuolar Zn^{2+}/H^+ antiporter, which localized at both vacuolar and plasma membrane, involved in Zn accumulation as well as Zn tolerance (Desbrosses-Fonrouge *et al*., 2005) [70]. Members of MTP1 family are also involved in Ni vacuolar storage in *Thlaspi goesingense* (Persans *et al*., 2001) [71].

The naturally resistant associated macrophage proteins (NRAMPs Family): Members of NRAMPs Family involved in the transport of many heavy metal ions including Cu^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+}, and Cd^{2+} (Bastow *et al*., 2018) [6].

AtNRAMP1 is localized in the plasma membrane and mediates Fe and Mn transport (Cailliatte *et al*., 2010) [10]. NRAMP3 and NRAMP4 are localized in the tonoplast and mediate the export of stored Fe from the vacuole in germinating seed (Bastow *et al*., 2018) [6].

Detoxification Mechanism

The Plants cope with the toxicity of heavy metals by the mechanism avoidance and tolerance and manage to maintain the cellular concentrations of heavy metals below the toxicity threshold levels (Hall, 2002) [73].

Avoidance

Avoidance mechanism acts as first line of defense against heavy metal uptake and works on strategy, limiting uptake and restrict movement of heavy metals into plant tissues through root cells by forming metal ion precipitation, root sorption and metal exclusion. Some of root exudates, such as of amino acids and organic acid, act as a heavy metal ligand to form stable heavy metal complexes in the rhizosphere and some of root exudates capable of changing pH of rhizosphere, leading to precipitation of heavy metals, thereby limiting their availability and lessening the toxicity (Dalvi and Bhalerao, 2013) [15]. Metal exclusion mechanism, excludes heavy metals by creating barriers between the root and the shoot system.Embedding the heavy metals in the plant cell walls is another heavy metal avoidance mechanism (Memon and Schröder, 2009) [47]. In Plant cell wall pectins consists of carboxylic groups of polygalacturonic acids, which are negatively charged andhave ability to bind heavy metals. Therefore, cell wall acts as a cation ex-changer to restrict entry of free heavy metal ions into the cells (Ernst *et al*., 2005) [21].

Tolerance

Tolerance mechanism acts as second line of defense to cope with toxicity of accumulated metal ion in the cytosol. At intracellular level various mechanisms such as inactivation, chelation, and compartmentalization of heavy metal ions plays role to detoxify heavy metal in order to minimize their toxic effects in cytosol (Manara, 2012) [44]. There are many organic and inorganic ligands in the cytoplasm that mediate heavy metal chelation. Among organic ligands, amino acids, phytochelatins (PCs), metallothioneins (MTs), and cell wall proteins/pectins/polyphenols plays major role (Gupta *et al*., 2013b) [29]. Organic acids within cells prevent the persistence of heavy metals as free ions in the cytoplasm by complexing and reducing their bioavailability to plants. For example, citrate mediates the chelation of Ni in *T. goesingense* leaves (Krämer *et al*., 2000) [41], while acetic and citric acids bind Cd in leaves of *Solanum nigrum* (Sun *et al*., 2006) [59]. In addition, malate is involved in chelation of Zn in *A. halleri* (Sarret *et al*., 2002) [55]. Heavy metal stress induces the accumulation of certain kinds of amino acid. For example, Cd can induce the production of cysteine in *Arabidopsis thaliana* (Domínguez−Solís *et al*., 2004) [18], while Ni hyperaccumulation induces histidine accumulation (Harper *et al*., 1999) [32]. High accumulation of proline is also induced by Cd, Pb, Zn, and Cu stress (Roy and Bera, 2002) [72]. These amino acids can detoxify heavy metals by chelating heavy metal ions within cells and xylem sap (Rai, 2002) [53].

After chelation, the complexes of ligands with heavy metals are actively transported from the cytosol into inactive compartments, such as vacuole where the complexes are stored without toxicity (Tong *et al*., 2004) [61]. Sequestration and vacuolar compartmentalization provide an effective protection against the detrimental effect of heavy metals by removing toxic heavy metal ions from sensitive sites of the cell where cell division and respiration occur, thereby reducing the interactions between heavy metal ions and cellular metabolic processes and avoiding damages to cell functions (Sheoran *et al*., 2011) [57]. Besides vacuoles, heavy metal ions can be sequestrated and compartmentalized into other locations, such as leaf petioles, leaf sheathes, and trichomes (Eapen and D'souza, 2005) [20], where heavy metals cause less damage to the plant. Heavy metals can also be translocated to old leaves and removed from the plant body by natural leaf shedding (Thakur *et al*., 2016) [60].

Under high levels of heavy metals stress, above-mentioned strategies are inadequate to detoxify the detrimental effects of heavy metals, the increased accumulation of metal ions in the cytoplasm trigger the production of reactive oxygen species (ROS). The excess production of ROS results in oxidative stress, which may cause disruption of cell homeostasis, inhibition of cellular processes, DNA damage, and protein oxidation (DalCorso *et al*., 2019) [14]. To cope with heavy metal-induced oxidative damage, plant cells activate the ROS-scavenging machinery by inducing antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR), as well as non-enzymatic antioxidant compounds including glutathione, flavonoids, carotenoids, ascorbate, and tocopherols (DalCorso *et al*., 2019) [14]. Hence, this anti-oxidative defense system of plants plays an important role in response to heavy metal stress.

Phytoremediation

Basically refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environment.

The plant species selected for phytoextraction should possess the following characteristics

1. High tolerance to the toxic effects of heavy metals
2. High extraction ability with accumulation of high levels of heavy metals in above ground parts.
3. Fast growing with high biomass production.
4. Abundant shoots and extensive root system
5. Good adaptation to prevailing environment, strong ability to grow in poor soils,
6. easy cultivation and harvest

7. Highly resistant to pathogens and pests, be repulsive to herbivores to avoid heavy metals entering into the food chain (Ali *et al*., 2013) [1].

Strategies that is applicable for the remediation of heavy metal-contaminated
Soils, including

1. Phytostabilization: Using plants to reduce heavy metal availability in soil.
2. Phytoextraction: Using plants to extract and remove heavy metals from soil.
3. Phytovolatilization: Using plants to absorb heavy metal from soil and release into the atmosphere as using hydroponically cultured plants to absorb or adsorb heavy metal ions from groundwater and aqueous waste (Marques *et al*., 2009) [45].

Phytostabilization

Phytostabilization is the use of metal-tolerant plant species to immobilize heavy metals below ground and decrease their bioavailability, thereby preventing their migration into the ecosystem and reducing the likelihood of metals entering into the food chain (Marques *et al*., 2009) [45]. Phytostabilization can occur through precipitation of heavy metals or reduction in metal valence in the rhizosphere, absorption, and sequestration within root tissues, or adsorption onto root cell walls (Gerhardt *et al*., 2017) [26]. hazardous biomass is not required when compared with phytoextraction (Wuana and Okieimen, 2011) [66]. The selection of appropriate plant species is crucial for phytostabilization. To improve phytostabilization efficiency, organic or inorganic amendments can be added to the contaminated soil. These soil amendments can alter metal specification; reduce heavy metal solubility and bioavailability by changing pH value and redox status of the soil (Burges *et al*., 2018) [9]. Moreover, the application of amendments can increase the organic matter content and essential nutrients of the soil and improve physicochemical and biological properties, which can benefit plant colonization and improve water1holding capacity

Phytoextraction

Phytoextraction is the use of plants to take up contaminants from soil or water, and translocate and accumulate those contaminants in their above ground biomass (Jacob *et al*., 2018) [37]. In recent times, phytoextraction is the most important phytoremediation technique for reclamation of heavy metals and metalloids from the polluted soil (Sarwar *et al*., 2017) [56].

Unlike phytostabilization, by which plants only temporarily contain heavy metals, and these heavy metals still remain belowground, phytoextraction is a permanent solution for the removal of heavy metals from polluted soil. Therefore, it is more suitable for commercial application.

Steps involved in phytoextraction

1. Mobilization of heavy metals in rhizosphere.
2. Uptake of heavy metals by plant roots
3. Translocation of heavy metal ions from roots to aerial parts of plant
4. (iv)Sequestration and compartmentation of heavy metal ions in plant tissues (Ali *et al*., 2013) [1].

The efficiency of phyto extraction relies on factors such as plant selection, plant performance, heavy metal bio availability, soil, and rhizosphere properties.

Therefore, the strategies to improve phytoextraction efficiency are developed in light of those aspects and are discussed below.

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3. Fast growing with high biomass production,
4. Abundant shoots and extensive root system
5. Good adaptation to prevailing environment, strong ability to grow in poor soils, easy cultivation and harvest.
6. Highly resistant to pathogens and pests, be repulsive to herbivores to avoid heavy metals entering into the food chain (Ali *et al*., 2013) [1].

Among these characteristics, metal-accumulating capacities and above ground biomasses are the key factors that determine the phytoextraction potential of a plant species. Therefore, two different strategies for plant selection are being employed:

1. The use of hyperaccumulator plants, which can accumulate heavy metals in aboveground parts to a greater extent
2. The use of plants with high above ground biomass production, which may have lower metal-accumulating capacities, but overall accumulation of heavy metals is comparable to that of hyper accumulators (Ali *et al*., 2013) [1].

Hyper accumulator

Hyperaccumulators are plant species capable of accumulating very high levels of heavy metals in their above ground parts without phytotoxicity symptoms (van der Ent *et al*., 2013) [62]. Currently, more than 450 plant species from at least 45 angiosperm families have been identified as metal hyper accumulators so far (Suman *et al*., 2018) [58], ranging from annual herbs to perennial shrubs and trees, such as Brassicaceae, Fabaceae, Euphorbiaceae, Asterraceae, Lamiaceae, and Scrophulariaceae families (Dushenkov, 2003) [19]. Some species can even accumulate more than two elements, such as *Sedum alfredii*, which can hyperaccumulate Zn, Pb, and Cd (Yang *et al*., 2004) [67]. However, using edible crops for phytoremediation should be avoided as heavy metals can accumulate in edible parts of the plant and thus enter into the food chain by human or animal consumption, raising concerns on human health. Hence, selection of the non-edible hyper-accumulators is a key for efficiency and safe phytoremediation of heavy

Criteria to be followed in selection of hyper accumulator

1. The shoot-to-root ratio of heavy metal concentration is greater than 1, which is a sign of efficient ability to transport metals from roots to shoots (Marques *et al*., 2009) [45].
2. The shoot-to-soil ratio of heavy metal concentration is greater than 1, indicating a higher capability to take up heavy metals from soil (McGrath and Zhao, 2003) [46]
3. The concentration of the metal in the shoot is higher than 10 mg/kg for Hg, 100 mg/kg for Cd and Se, 1,000 mg/kg

for Co, Cu, Cr, Ni, and Pb, and 10,000 mg/kg for Zn and Mn (Baker and Brooks, 1989) [4].

High biomass producing crops, such as Helianthus annuus, Cannabis sativa, Nicotiana tabacum, and Zea mays, have been reported to effffectively remove heavy metals from contaminated soil through phytoextraction (Herzig *et al*., 2014) [34]. Grasses can also be used for phytoextraction because of their short life cycle, high growth rate, more biomass production, and high tolerance to abiotic stresses (Malik *et al*., 2010) [43].

Phytovolatilization

Phytovolatilization is a phytoremediation strategy using plants to take up pollutants from soil, convert these toxic elements into less toxic volatile form, and subsequently release them into the atmosphere by plant transpiration process via the leaves or foliage system. This approach can be applied for detoxification of organic pollutants and some heavy metals like Se, Hg, and As **(**Mahar *et al*., 2016) [42]. Brassicaceae family is good volatilizers of Se, such as *Brassica juncea* (Banuelos *et al*., 1990) [5]. Inorganic Se is first assimilated into the organic selenoamino acids selenocysteine (SeCys) and selenomethionine (SeMet). SeMet is bio methylated to form dimethylselenide (DMSe), which is volatile and can be dispersed into the air with less toxicity compared with inorganic Se.

The advantage of phytovolatilization compared with other phytoremediation strategies is that heavy metal (metalloid) contaminants are removed from the site and dispersed as gaseous compounds, without any need for plant harvesting and disposal. However, as a remedial strategy, phytovolatilization does not remove the pollutants completely-the pollutants are still in the environment. It only transfers pollutants from soil to atmosphere, where the toxic volatile compounds will contaminate the ambient air. Moreover, they may be redeposited to the soil by precipitation (Vangronsveld *et al*., 2009) [63]. Thus, a risk assessment is required before its application in the field.

Phytofiltration

Phytofiltration is the use of plant roots (Rhizofiltration), shoots (Caulofifiltration), or seedlings (Blastofiltration) to remove pollutants from contaminated surface waters or waste waters (Mesjasz-Przybyłowicz *et al*., 2004) [48]. In this method heavy metals are either adsorbed onto the root surface or absorbed by the roots. Root exudates can change rhizosphere pH, which leads to the precipitation of heavy metals on plant roots (Javed *et al*., 2019) [38], further minimizing movement of heavy metals to underground water.

Ideally, plants used for rhizofiltration should have a dense root system, high biomass production, and be tolerant to heavy metal. Both terrestrial and aquatic plants can be used for rhizofiltration. For remediation of wetland water, aquatic species such as hyacinth, azolla, duckweed, cattail, and poplar are commonly used due to their high accumulation of heavy metals, high tolerance, or fast growth and high biomass production (Hooda, 2007) [35]. Terrestrial plants such as Indian mustard (*B. juncea*) and sunflflower (*H. annuus*) have longer and hairy root system compared with aquatic plants. They also show good capacities to accumulate heavy metals during rhizofiltration (Dhanwal *et al*., 2017) [17].

Crop selection for Phytoremediation

Plant selected for Phytoremediation should overcome limitations

Such as slow growing, which limit rapid and large-scale applications of these plants and adaptation to a variety of environmental conditions like nutrient-poor soils (Gerhardt *et al*., 2017) [26]. Hence, to minimize these limitations, a strategy is developed through modifying and improving certain traits of these plants to ensure their ability for effective phytoremediation.

Traditional breeding (plant hybridization) or genetic engineering (creation of transgenic plants) are employed to either improve growth rate and biomass of hyperaccumulator or introduce hyperaccumulation traits to fast growth, high biomass plants (DalCorso *et al*., 2019) [14]. Brewer *et al*. (1999) [8] used electrofusion to fuse protoplasts isolated from the Zn hyperaccumulator *T. caerulescens* and *Brassica napus*. The selected hybrids (somatic hybrid), which have enhanced hyperaccumulation capability and tolerance derived from *T. caerulescens* and higher biomass production derived from *B. napus* (Brewer *et al*., 1999) [8], showed the ability to accumulate high levels of Zn and Cd.

Genetic engineering as a promising technique

For improving phytoremediation abilities of plants toward heavy metal genetically modify plants, a foreign source of gene from an organism, such as a plant species or even bacteria or animals, is transferred and inserted into the genome of a target plant. After DNA recombination, the foreign gene is inherited and confers specific traits to the plants.

Genetic Engineering advantages

- Modify plants with desirable traits for phytoremediation in a much shorter time.
- Genetic engineering can even transfer desirable genes from hyperaccumulator to sexually incompatible plant species, which is impossible to achieve through traditional breeding methods such as crossing (Marques *et al*., 2009) [45].
- Genetic engineering is used to develop transgenic plants with the desired traits has shown attractive prospects in the field of phytoremediation.
- Fast-growing, high-biomass plants are engineered either to enhance tolerance against heavy metals or to increase heavy metal-accumulation ability, which are the key properties of hyperaccumulators.
- Heavy metal tolerance to enhance antioxidant activity (Kzminska *et al*. 2018) [39], can be achieved by overexpression of genes involved in antioxidant machinery. and overexpress genes that are involved in the uptake, translocation, and sequestration of heavy metals. (Das *et al*., 2016) [16]. Hence, genes encoding heavy metal/metalloid transporters can be transferred and overexpressed in target plants to improve heavy metal accumulation. These genes encode metal ion transporters including ZIP, MTP, MATE, and HMA family members, which are discussed previously. As metal chelators act as metal1binding ligands to improve heavy metal bio availability, promote heavy metal uptake and root-to-shoot translocation, as well as mediate intracellular sequestration of heavy metal ions in organelles, it is a promising strategy to increasing heavy metal

accumulation by promoting the production of metal chelators via genetic engineering. By overexpression of genes encoding natural chelators, heavy metal uptake and translocation can be improved (Wu *et al*., 2010) [65].

Although genetic engineering approach has shown attractive prospects on improving plant performance in phytoremediation of heavy metals, there are also a few setbacks that remain. As the mechanisms of detoxification and accumulation of heavy metals are very complicated and involve a number of genes, genetic manipulation of multiple genes to improve desired traits is time and effort consuming and usually not successful. Another issue is that genetically modified plants are difficult to gain approval for field testing in some areas of the world due to the risk raised on food and ecosystem safety. Therefore, alternative approaches are required to improve plant performance in phytoextraction once genetic engineering is impracticable.

Conclusion

Phytoremediation has been proven to be a promising technique for revegetation of heavy metal-polluted soil and shows a variety of advantages compared with other physicochemical techniques. The application of heavy metal hyperaccumulators is the most straightforward approach and hundreds of hyperaccumulator plants have been identified so far. However, phytoremediation with these natural hyperaccumulators has a few limitations, as it is a time-consuming process, which takes a very long time to clean-up heavy metal-contaminated soil, particularly in moderately and highly contaminated sites. This may partially be due to slow growth rate and low biomass production of these hyperaccumulators. Therefore, improving plant performance is a critical step for developing high effective phytoremediation. Fortunately, genetic engineering approach has been emerging as a powerful tool to modify plants with desired traits such as fast grow, high biomass production, high heavy metal tolerance and accumulation, and good adaption to various climatic and geological conditions. Hence, good understanding of the mechanisms of heavy metal uptake, translocation, and detoxification in plants, and identification and characterization of different molecules and signaling pathway will be of great importance for the design of ideal plant species for phytoremediation via genetic engineering. Genes involved in heavy metal uptake, translocation, sequestration, and tolerance can be manipulated to improve either heavy metal accumulation or tolerance in plants. In addition, chelating agents and microorganisms can be used either to increase heavy metal bio availability, which facilitates heavy metal accumulation in plants, or to improve soil health and further promote plant growth and fitness. Practically, single approach is neither possible nor sufficient for affective clean-up of heavy metal-polluted soil. The combination of different approaches, including genetic engineering, microbe-assisted and chelate assisted approaches, is essential for highly effective and exhaustive phytoremediation in the future.

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