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Phytoremediation: Green technology for heavy metal clean up from contaminated soils

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Abstract

Heavy metal concentration is being continuously increasing in the soil due to modern industrialization and anthropological activities. Heavy metals being nonbiodegradable and with long biological half-lives when entered the food chain, their concentrations get increase increasing with each trophic level due to biomagnification. This increased concentration of heavy metals also poses a threat to human life. A recent new solution to cope up with this problem is the use of green plants for the removal of toxic heavy metals from soil and convert it into harmless and reusable form by a process called phytoremediation. This technology has many advantages over conventional methods for the heavy metal clean up from the soil. Still, there are so many challenges to make this technology practically feasible and useful on a large scale. The present review focuses on the mechanisms of HM uptake, transport, and plant tolerance mechanisms to cope with heavy metals.

Keywords: Heavy metals, metal uptake, phytoremediation, translocation, tolerance mechanism

Introduction

Phytoremediation can be defined as a process in which green plants remove, sequester, or stabilize many organic and inorganic contaminants including heavy metals, radionuclides as well as for organic pollutants like polynuclear aromatic hydrocarbons, polychlorinated biphenyls, and pesticides to render them harmless (Greipsson, 2011) [25]. Heavy metals are difficult to remove from contaminated soils mainly because, being inorganic contaminants, they are bound to the soil matrix and cannot be easily mineralized. Thus heavy metal can be removed from contaminated soils by physical, chemical, and biological methods of remediation (Cunningham and Ow, 1996) [12]. As per now different physico-chemical and engineering, techniques have been developed and are being employed to remove toxic heavy metal ions from polluted soils and waters. They, however, are associated with several disadvantages which include a negative effect on soil properties and biodiversity, and also these techniques are quite expensive (Padmavathamma and Li, 2007) [43]. Phytoremediation technology offers an eco-friendly alternative to this problem. It is a novel, cost-effective, efficient, environment and eco-friendly, in situ applicable, remediation strategy (Vithanage *et al.*, 2012; Feng *et al.*, 2017) [75, 18]. Plants detoxify the soil contaminants without affecting the topsoil, hence conserving its utility and fertility. Also plants aid in increasing the fertility of the soil by the input of organic matters (Mench *et al.*, 2009) [38].

Hyperaccumulators plant species accumulate toxic heavy metals in above ground parts. These plants can take up large amounts of metals in their shoots without showing significant signs of toxicity. This makes hyperaccumulators ideal candidates for metal phytoremediation and phytomining. A hyperaccumulator plant can be distinguished from a non-hyperaccumulator by its capability to absorb and accumulate 50–100 times than nonaccumulators plants. Hyperaccumulators achieve a shoot-to-root metal concentration ratio called translocation factor (TF) of greater than one (Badr *et al.*, 2012) [4]. The other factor which is very important for phytoremediation and identification of hyperaccumulator species is bioconcentration factor. It is the ratio of metal ion concentration in plant tissue to the soil. About 0.2 % of all known plant species are classified as HM accumulators (Rascio and Navari-Izzo, 2011; Sarma, 2011) [48, 55]. The phytoremediation concept is gaining good public acceptance and can be applied over large scale field sites where other remedial measures are not cost-effective. This green technology for heavy metal detoxification is classified into five subgroups (Alkorta *et al.*, 2004; Thakur *et al.*, 2016) [2, 69] which areas: (a) phytoextraction: Metal translocation to shoots is a crucial biochemical process and is desirable in an effective phytoextraction because

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the harvest of root biomass is generally not feasible (Zacchini *et al.*, 2009) [84]. (b) Phytodegradation: Some plants can enhance microbial degradation of organic contaminants in the rhizosphere, implied in both soil and water. Recently, scientists have shown their interest in studying the phytodegradation of various organic pollutants including synthetic herbicides and insecticides. Some studies have reported the use of genetically modified plants for this purpose (Doty *et al.*, 2007) [15]. (c) Phytostabilization or phytoimmobilization is the use of certain plants for the stabilization of contaminants in contaminated soils (Singh, 2012) [65]. Phytostabilization limits the accumulation of heavy metals in biota and minimizes their leaching into underground waters. However, phytostabilization is not a permanent solution because the heavy metals remain in the soil; only their movement is limited. It is a management strategy for stabilizing (inactivating) potentially toxic contaminants (Vangronsveld *et al.*, 2009) [73]. (d) Phytovolatilization: It is the uptake of pollutants from the soil by plants, their conversion to volatile form and subsequent release into the atmosphere. This technique can be used for organic pollutants and some heavy metals like Hg and Se. limited by the fact that it does not remove the pollutant completely; only it is transferred from one segment (soil) to another (atmosphere) from where it can be redeposited. Phytovolatilization is the most controversial of phytoremediation technologies (Padmavathiamma and Li, 2007) [43]. (e) Rhizofiltration and rhizodegradation: Metal ions are uptaken and removed from contaminated water by plant roots, implied in surface water. (Thakur *et al.*, 2016) [69]. Rhizodegradation refers to the breakdown of organic pollutants in the soil by microorganisms in the rhizosphere (Mukhopadhyay and Maiti, 2010) [40]. The release of nutrients-containing exudates by plant roots provides carbon and nitrogen sources to the soil microbes and creates a nutrient-rich environment in which microbial activity is stimulated. In addition to secreting organic substrates for facilitating the growth and activities of rhizospheric microorganisms, plants also release certain enzymes capable of degrading organic contaminants in soils (Yadav *et al.*, 2010) [81]. Among these classes, phytoextraction, rhizofiltration, and phytostabilization are commercially important. A lot of research has been done on phytoremediation technology for detoxification of heavy metal contaminated soil, but there are still many knowledge gaps that must be addressed before the actual commercial field level utilization of this green technology. This review emphasizes various aspects including metal uptake from contaminated soils, metal ion translocation in plants, mechanisms of heavy metal tolerance in plants, significance, and challenges along with future perspectives for the success of green remediation technology.

Heavy metal uptake from contaminated soil

The concentrations of heavy metals in the environment is increasing continuously due to industrialization and anthropological activities (Govindasamy *et al.*, 2011) [24]. Among the heavy metal ions, some are more mobile and available for plant uptake, e.g., Cd and Zn, than others like Pb which are relatively immobile (Lasats, 2000) [33]. Plants have also evolved highly specific mechanisms to translocate and store micronutrients. These same mechanisms are also involved in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation. Thus, to make metal

ions available to be taken up by roots, they must be mobilized into the soil solution first. Root cell walls initially bind metal ions from the soil and thereafter *via* high-affinity binding sites and plasma membrane-localized transport systems; metal ions are taken up across the plasma membrane. Generally, uptake of metal ions occurs through secondary transporters such as channel proteins and/or H⁺-coupled carrier proteins. Uptake of cations through secondary transporters is facilitated by the membrane potential of the plasma membrane, which is negative on the inner side of the membrane (Hirsch *et al.*, 1998) [28]. The mobility of metal ions in soil mainly depends on the pH of soil and presence of chelating agents like metal concentrations, temperature, metal interaction, the addition of nutrients, and salinity also seem to play a minor role to influence the metal ions mobility in soil (Rieuwerts *et al.*, 1998) [52]. Besides, the solubility of metal ions in water is very low and they have a strong affinity toward soil particles and many other organic contaminants present in the soil. In this case, the use of soil microorganisms to make metal ions available for translocation is a beneficial technique. The soil microorganisms present in the rhizosphere and enzymes secreted by them also have a crucial role in controlling the availability of metal ions for absorption by roots (Burns and Dick, 2002) [7]. The mobile fraction of metal available in the soil which is ready for absorption by plants is known as the bioavailable portion. For the successful reclamation of metal-contaminated soil, it is very important to identify the bioavailable fraction from the total metal concentration present in soil (Olaniran *et al.*, 2013) [42]. After absorption by roots, the metal ions first come in contact with the cell wall which is an ion exchanger of low selectivity and affinity (Ghosh and Singh, 2005) [22]. The uptake of heavy metals into roots occurs either by passive diffusion through the cell membrane or by active transport against concentration and electrochemical potential gradients mediated by carriers. These carriers can be complexing agents, such as organic acids or proteins that bind to the metal species (Fergusson, 1990) [19].

Metal ions translocation in plants

Metal hyperaccumulator plants translocate a very high concentration of metal ions into the shoot via symplastic movement through the xylem. Heavy metal ions enter into the xylem stream via root symplasm (Tester and Leigh, 2001) [66]. The apoplastic movement of the metal ions is possible only as noncationic metal chelates, because cell walls have comparatively high exchange capacity for cations (Raskin *et al.*, 1997) [49]. Thus, as the most of the metal ions are insoluble and unable to move on their own in the vascular system, they are immobilized in apoplastic and symplastic compartments after forming carbonate, sulphate, or phosphate precipitates (Raskin *et al.*, 1997; Garbisu and Alkorta, 2001) [49, 2]. Also, the metal ions sequestered inside the cell vacuoles may enter into the xylem stream via the stele. Thus, the entry of metals from the root into the xylem is mainly determined by three processes: metal ion sequestration into root cells, symplastic transport into the stele, and release into the xylem (Saxena and Misra, 2010) [56]. Metal ions are transported with the transpiration stream in the xylem from the roots to transpiring shoot (Page *et al.*, 2006) [44]. After the release into the root xylem, free or chelated ions flow with the xylem sap upwards. Important for the concentration of heavy metals in the transpiration stream are xylem loading in the roots, interactions with cell walls during acropetal transport and selective removal from the xylem sap (Reisen and feller,

2005)^[51]. In xylem, the transport of metal is mediated by membrane transport proteins. The transport of metal ions through the Casparian strip occurs by an energy-requiring active transport system (Cunningham and Berti, 1993)^[11]. Once inside a cell, the metal ions can move along a concentration gradient or through different types of cation channels in the cell membrane meant for other essential metal ions (Prasad, 2004)^[46]. Recent techniques like transporter identification by sequence comparison, regulation of gene activities in transgenic plants, several gene families have been identified which are likely to aid the transport of HM ions. Such Gene families are Zn-regulated transporter (ZRT), heavy metal ATPases, cation diffusion, natural resistance-associated macrophage proteins (Nramps), facilitators (CDFs), ZIP family and cation antiports (Williams *et al.*, 2000; Gaxiola *et al.*, 2002)^[78, 21]. The heavy metal ATPases (HMAs) possess eight transmembrane domains with large cytoplasmic loop (Williams *et al.*, 2000; Mills *et al.*, 2003)^[78, 39] which contains the phosphorylation site along with many highly conserved motifs (Hall and Williams, 2003)^[26]. The P_{1B}-type ATPases, known as heavy metal ATPases (HMAs), play an important role in metal transport in plants. Functional studies on the HMAs have shown these transporters to be divided into two subgroups based on their metal-substrate specificity: a copper (Cu)/silver (Ag) group and a zinc (Zn)/cobalt (Co)/Cd/lead (Pb) group (Williams, 2005)^[77]. The ATP-binding cassette (ABC transporters) superfamily is another important and diverse family of transmembrane proteins involved in a wide range of transport functions by utilizing energy from ATP hydrolysis (Martinoia *et al.*, 2002)^[35]. In plants, 13 subfamilies of this superfamily are identified, the multidrug resistance proteins (MDRs) being the largest other important subfamilies ABC transporter superfamily are multidrug resistance-associated protein homologs (MRPs), peroxisomal membrane protein homologs (PMPs), pleiotropic drug resistance homologs (PDRs), etc. (Rea, 2007)^[50]. They have a role in Mg-ATP hydrolysis-driven vacuolar sequestration of glutathione (GS) conjugates (Martinoia *et al.*, 1993)^[34]. The natural resistance-associated macrophage proteins (Nramps): Three different Nramps have been identified in yeasts. They mediate the uptake of many heavy metal ions (Cu²⁺, Mn²⁺, Co²⁺, Fe²⁺, and Cd²⁺) (Chen *et al.*, 1999)^[9]. The occurrence of Nramp in bacteria, yeast, and plants has been well documented in the literature (van der Zaal *et al.*, 1999)^[73]. For example, the analysis of the expression of AtNramp genes in Arabidopsis has established their role as constitutive metal transporters involved in the transport of Fe, Mn, and Cd (Thomine *et al.*, 2000)^[70]. The ZIP family of genes is involved in the transport of many cations. Different subfamilies of ZIP gene superfamily exhibit variations in terms of substrate and specificity. The 2 ZIP family members are identified in *Oryza sativa* and 15 in Arabidopsis (Mäser *et al.*, 2001)^[36]. The cloning of various ZIP/NRAMP transporter genes from plant species and other organisms has shown a wide range of metal specificity and sequences (Williams *et al.*, 2000)^[78]. These investigations of ZIP and NRAMP gene families suggested the existence of consensus regions on the amino acid sequences which could be responsible for the determination of the metal transport. The previous studies on phytoremediation clearly show that there is an array of transporters/pathways for the cellular uptake of diverse heavy metals.

Heavy metal detoxification mechanism in plants

Heavy metal detoxification is the key prerequisite for the successful implementation of phytoremediation. Plants are

equipped with multiple means of HM detoxification by which plants try to keep the cellular concentrations of HMs below the toxicity threshold levels (Hall, 2002; Sharma and Dietz, 2006)^[27, 62]. Plants use two different defense strategies to prevent the accumulation of excess metal concentrations in the cytoplasm, first is avoidance and another one is tolerance. According to Verkleij and Schat (1990)^[74], avoidance refers to the ability of plants to hinder excessive metal uptake. On the other hand, tolerance refers to the ability to cope with the accumulated metal ions by using different mechanisms. These mechanisms depend upon the metal involved and its concentration, plant species, organs, and developmental stage (Navari-Izzo and Quartacci, 2001)^[41]. Plants avoid toxic metal ion accumulation in the cytoplasm by preventing metal ion transport across the plasma membrane and altering membrane permeability, changing metal-cell wall binding capability, increasing the exudation of metal-chelating substances, and stimulating the efflux pumping (Yang *et al.*, 2005)^[82]. Embedding the toxic metals in the plant cell walls is another distinct mechanism of metal tolerance and accumulation by plants (Memon and Schröder, 2009)^[37]. The plasma membrane is the primary site of heavy metal toxicity. Plants tend to control the entry of heavy metal ions at the plasma membrane level as its failure may lead to the disruption of normal cellular functions and ultimately the whole plant functions (Dietz *et al.*, 1999)^[14]. Toxic effects of heavy metal ions on the plasma membrane include K⁺ leakage, oxidation, and cross-linking of protein thiols and inhibited the activity of membrane proteins (Hall, 2002)^[27] and alteration of membrane lipid composition and fluidity. Active efflux mechanisms of plasma membrane prevent the accumulation of metal ions into the cytosol. Avoiding the entry of heavy metal cations in the cytoplasm and preventing the development of oxidative stress are the major strategies employed by metal-tolerant plants (Dietz *et al.*, 1999)^[14].

Heavy metal ion chelation and compartmentalization

Plants employ multiple strategies for cellular management of toxic metal concentrations (Hall *et al.*, 2003; Sharma and Dietz, 2006)^[26, 62]. They range from cytosolic chelation of heavy metal ions through different ligands to sequestration into different cellular compartments. The main organic compounds involved in metal ion chelation are phytochelatins (PCs), metallothioneins (MTs), ferritins, organic acids, amino acids, and cell wall proteins (Hall, 2002; Sharma and Dietz, 2006)^[27, 62] whereas the main inorganic compounds are phosphates and silicates (Bourg and Loch, 1995)^[6]. These metal-chelating compounds reduce heavy-metal-induced phytotoxicity by reducing the free metal ion concentrations through chelation (Salt *et al.*, 1998)^[53]. The resulting metal-chelator complex is transported actively from the cytosol across the tonoplast into the vacuoles. The vacuoles are the last destination for these metal-chelator complexes as these complexes are removed from the cytoplasm by efflux of ions into vacuoles (Hall, 2002)^[27]. This is achieved by the increased ability to transport metals into the vacuoles. For example, overexpression of a Zn transporter (ZAT) has been shown to increase the Zn tolerance by its sequestration into the vacuoles. Yeast mutant (hmt1) incapable of accumulating the Cd-PC complex was found to be Cd-sensitive (Salt *et al.*, 1998)^[53]. Vacuoles evidently appear to be the final destination for HMs either as free ion or as metal complexes. Barley roots accumulated elevated transcript levels of two vacuolar ATPase subunits namely VHA-E and VHA-C under Cd and Fe stress although the VHA-E protein level remained

unchanged (Sharma *et al.*, 2004) ^[63]. Different compartmentalization strategies are also employed by plants other than metal ion sequestration into vacuoles. These include the heavy metal ion sequestration in the apoplast or specialized cell types, e.g., epidermal cells, mesophyll cells, and trichomes (Eapen and D'souza, 2005) ^[16]. Another effective way to get rid of excess ions is the translocation of metals into old leaves, which are then removed as a result of natural leaf shedding (Ernst *et al.*, 1992) ^[17].

Role of amino acids in the heavy metal tolerance mechanism

Amino acids and derivatives can chelate metal conferring in plants resistant to toxic levels of metal ions. Amino acids, particularly proline and histidine, have been found to chelate metal ions in cells as well as in the xylem sap (Sharma and Dietz, 2006) ^[62]. Histidine is considered to be important in Ni tolerance (Kramer *et al.*, 1996; Callahan *et al.*, 2006) ^[32, 8]. Further, the plant responds to different toxic HM ions by an elevated synthesis of free proline (Bassi and Sharma, 1993; Schat *et al.*, 1997) ^[5, 57]. Proline might contribute to HM detoxification in multiple ways. These involve proline dependent protection of enzymes against metal toxicity, acting as an antioxidant (Kaul *et al.*, 2008) ^[31] and as a metal chelator (Sharma and Dietz, 2006) ^[62]. Changes in the histidine content have functional significance in metal stress tolerance. Proline has been reported to accumulate under heavy metal stress (Yusuf *et al.*, 2016) ^[83]. The functions of proline include a major role in adjustment to osmotic stresses, maintaining the water balance as it stabilizes the subcellular structures, and has a role in free radical scavenging (Sharma and Dietz, 2006) ^[62]. Accumulation of proline has also been suggested to be related to the changes in the water status of the plants under heavy metal stress and could have a possible role in heavy metal tolerance. Besides, proline has also been reported to have a role as a free radical scavenger, having been demonstrated in many *in vitro* assay systems Kaul *et al.*, 2008) ^[31]. At high metal concentrations, the avoidance mechanism of plants does not work instead, metal ion concentrations in the cytoplasm increased causing the formation of free radicals that leads to oxidative stress. Thereafter, plants need to undergo some biochemical changes to defend against oxidative stress. The degree of heavy-metal-induced cell damage depends on the rate of free radical and reactive oxygen species and the efficiency of detoxification and repair mechanisms.

Heavy metal tolerance mechanism in plants

Heavy metals induce oxidative stress by stimulating the generation of ROS and concomitantly suppressing the cellular antioxidative defense (Schutzendubel and Polle, 2002; Sharma and Dietz, 2009) ^[58, 61]. These ROS are scavenged by low molecular weight antioxidative metabolites e.g., glutathione, ascorbic acid, α -tocopherol and antioxidative enzymes e.g., catalase, ascorbate peroxidase, and superoxide dismutase. However, under different stress conditions, the free radical generation exceeds the overall cellular antioxidative potential leading to oxidative stress, which contributes to adverse effects on plant growth (Sharma and Kumar, 2015) ^[60]. Plants employ different strategies to cope with the adverse impacts imposed by heavy metals. Toxic concentrations of metals influence the growth and metabolism by interfering with the conformation of proteins mainly the enzymes but also the transporters and regulatory proteins. Such interference is due to the strong binding affinity of

metals ions to -SH and carboxyl group (Van- Assche and clijster, 1990) ^[71]. Overproduction of ROS, resulting in the peroxidation of many vital constituents of the cell. Strongly redox ions such as Cu^{2+} and Hg^{2+} are capable of initiating the peroxidation of lipids components of a membrane system. Data from diverse biochemical and metabolic approaches have established a firm link between cellular redox imbalance and heavy metal toxicity (Sharma and Dietz, 2009) ^[61]. The amount of lipid peroxidation enhanced by the increasing concentration of HMs such as Cu (Wang, 2004) ^[76] disrupts the membrane which leads to leakage of enzymes and proteins associated with membranes.

In this way, plants have an efficient defense system comprising a set of enzymatic as well as non-enzymatic antioxidants. The largest variety of enzymatic antioxidants consisting of superoxide dismutase (SOD). It catalyzes the dismutation of superoxide anion ($\text{O}_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) and oxygen (O_2). The metal-cofactor dependent isoforms of SOD are located in different cell compartments (Dalton, 1995) ^[13]. Other antioxidants are peroxidase (POD), catalase (CAT) and glutathione-*s*-transferase (GST) which may efficiently convert the superoxide radicals into hydrogen peroxide and subsequently water and oxygen whereas low molecular weight non-enzymatic antioxidants consisting the proline, ascorbic acid and glutathione may directly detoxify the ROS (Yadav *et al.*, 2014; Singh and Parsad, 2014) ^[80, 64]. These two groups of antioxidants may professionally quench a wide range of toxic oxygen derivatives and prevent the cells from oxidative stress. Depending upon their localization at the different compartments of the cell, their quenching mechanism also differs. In this way, SODs are a group of metalloenzymes that accelerate the conversion of superoxide radical (SOR , $\text{O}_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) whereas CAT, guaiacol peroxidase (GPX), and a variety of general PODs catalyze the breakdown of H_2O_2 (Gill and Tuteja, 2010) ^[23]. Several studies report that under stress condition proline acts as an osmolyte and may increase antioxidant enzymes to minimize the adverse effect of oxidative stress. Similarly, exogenous application of proline reduces phytotoxic effects of selenium by minimizing oxidative stress and also improves growth in *Phaseolus vulgaris* L. Seedlings (Aggarwal *et al.*, 2011) ^[1]. Glutathione and ascorbate also have a role in ROS elimination as they act as an electron donor for a group of peroxidases involved in H_2O_2 scavenging. Besides, the role of glutathione is also evidenced as a chelator and signaling component under metal-stressed conditions (Jozefczak *et al.*, 2012) ^[30].

Advantages and limitation of phytoremediation

The advantages of this technology are: (a) Aesthetically pleasing (b) Less disruptive than current techniques. (c) The effectiveness of contaminant reduction. (d) Low cost. (e) Applicable to a wide range of contaminants. (f) Environmental friendly method and (g) enhanced regulatory and public acceptance (Ghosh and Singh, 2005) ^[22]. Despite the above advantages of this green technology, all aspects of phytoremediation are still not fully understood. Before the implementation of this technology on commercial-scale several limitations associated with this technology should be overcome. Although this green technology is a promising approach for remediation of heavy metal-contaminated soils, it also suffers from some limitations (Ramamurthy and Memarian, 2012) ^[47] These limitations must be taken into account before the implementation of the phytoremediation commercially on a large scale which includes (a) Long time

required for clean-up. (b) slow growth rate and low biomass of metal hyperaccumulators. (c) limited bioavailability of the contaminants in the soil. (d) It applies to sites with low to moderate levels of metal contamination. (e) climatic conditions and soil health are also limiting factors, (f) the introduction of non-indigenous species for phytoremediation purposes may affect biodiversity.

Future prospective

Phytoremediation is a relatively recent field of research and application. Most of the studies to date are limited to laboratory and greenhouse scale studies and only a few studies have been conducted to test the efficiency of phytoremediation in the actual field. Results in the actual field can be different from those in laboratory or greenhouse conditions (Ji *et al.*, 2011) [29]. It is of great significance to get information on plant response right from seed germination, seedling growth as these are the most sensitive stages when facing any type of external stress. Plants that are used for phytoremediation have to face stress right from the early stages of growth (Thakur and Sharma, 2015) [68]. Also, plants grown on contaminated soils have to face multiple metal stress rather than that of a single metal. Thus ions of different metals will compete for binding sites of soil particles or plant metal transporters (Clemens *et al.*, 1998) [10]. Thus, understanding the response mechanism of a plant of interest under multiple metal stress is also a very important aspect of the successful realization of phytoremediation in field conditions. Research is in progress to identify genes coding for a hyperaccumulation of specific heavy metals in plants. Identification and successful transformation of such genes to other suitable plants make it possible to develop tolerant plants for phytoremediation. Transgenic plants could be developed to secrete metal selective ligands into the rhizosphere, which could specifically solubilize elements of phytoremediation interest (Thakur, 2006) [67]. Assuncao *et al.*, (2010) [3] proposed that in transgenic species *Thlaspi caerulescens* developed by using bacterial gene *ArsC* from *E. Coli* reduction of arsenate takes place which is an important detoxification mechanism for As. Seth, 2012 [59] reviewed that bacterial gene *merA* is responsible for encoding mercuric ion reductase and *merB* encoding organomercurial lyase in transgenic plants improved plant tolerance against Hg. An understanding of the coordination chemistry of metals within plant tissues will help researchers to finely tune the process of phytoremediation (Saraswat and Rai, 2011) [54]. Nonhyperaccumulator plants producing more aboveground biomass should be studied for Synthetic chelating agents including ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), and ethylene glycol tetraacetic acid (AGTA) can be successfully used to enhance metal bioavailability and thus uptake by plants (Pereira *et al.*, 2010) [45]. In spite of the many challenges, this green remediation technology has great potential for removal of toxic heavy metals from the contaminated soil.

Conclusion

This green technology for heavy metal removal from contaminated soil involves many factors for its successful commercial application. It includes the growth of plants in the contaminated soils and associated tolerance mechanism, nature of heavy metal ions in the contaminated soils, and soil properties. Further studies need to concentrate on all factors that either limit or enhance the uptake from contaminated soils, translocation of heavy metal ions and their sequestration

within the plant. Research needs to be focused on genetically modified plant species as these have higher biomass and faster growth potential. Hyperaccumulation genes should be targeted to make this technology more reliable. Research in phytoremediation is interdisciplinary in nature and requires background knowledge in soil chemistry, plant biology, ecology and soil microbiology as well as environmental engineering. With the current trend of integration of scientific knowledge of this green technology, many challenging questions about the commercial application of phytoremediation will be answered in the future.

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