Original Research Article

An eco-friendly technique of phytoremediation

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Abstract

In recent years, the development of green biotechnology methods for detoxification of metal poisoning has become a major concern of researchers. The magnitude of pollution problem in our soils and water calls for immediate action. Heavy metals, including mercury, lead, chromium, arsenic, zinc, cadmium, uranium, selenium, and nickel are among the most toxic substances. The danger of heavy metals is aggravated by their almost indefinite persistence in the environment due to their immutable nature. Conventional methods for heavy metal removal from aqueous solution and soil are often not economical and having inadequate efficiencies at low metal concentrations. Number of plants have been reported that play a significant role in phytoremediation of heavy metals. There are certain plants that can accumulate as much as 40% of their weight as heavy metals without harm to themselves. Plants seem to be the best suitable system for detoxification of heavy metals. Biosorption by plants involve complex mechanisms, mainly ion exchange, chelation, adsorption by physical forces and ion entrapment in inter and intra fibrillar capillaries and spaces of the structural polysaccharide cell wall network. A better understanding of the biochemical processes involved in plant heavy metal uptake, transport and accumulation will certainly improve phytoremediation using modern genetic approaches. Phytoremediation is an eco-friendly and cost effective technique for the removal of heavy metal contamination from soil and water.

Key Words: Phytoremediation, Plants, Heavy Metals, Genetic approaches, Eco-friendly.

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INTRODUCTION

One of the most serious environmental problem across the world is heavy metal contamination of soil and water. Heavy metal contamination is a serious problem due to their toxicity to human, animals, plants and microbes (Singh *et al.*, 1997; Meagher, 2000; Chandra *et al.*, 2009). Heavy metal pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaustion and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), use of insecticides, pesticides, disposal of industrial and municipal wastes, and excessive use of fertilizers in Agriculture (McGrath *et al.*, 2001; Nriagu and Pacyna, 1988; Schalscha and Ahumada, 1998). Presence of heavy metals in soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed or degraded biologically but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001; Gisbert *et al.*, 2003). Therefore, heavy metal pollution poses a great potential risk to the environment and human health. Metal- contaminated substrates can be recovered using *exsitu* conventional methods such as soil washing, excavation, and reburial (Ghosh and Singh, 2005; Pilon-Smits, 2005). But most of the conventional methods are expensive and effect the soil fertility; this consequently causes negative effects on the ecosystem. To maintain good quality of soils and waters and keep them free from contamination, continuous efforts have been made to develop technologies that are easy to use, sustainable, ecological and economically feasible. Physicochemical methodologies have been widely used for treating polluted soil and water, especially at a small scale. However, they experience more difficulties for a large scale of remediation because of high costs and side effects. An alternative biological cleanup techniques such phytoremediation, which uses plants and their associated rhizosphere microorganisms to extract, degrade, or stabilize pollutants has gained increasing attention since last decade (Salt, Smith, and Raskin, 1998; Garbisu and

Alkorta, 2001; Pilon-Smits, 2005). Phytoremediation has been emerging as a cheaper technology. Lot of research have been conducted in this field. Numerous plant species have been identified and tested for their traits in the uptake and accumulation of different heavy metals. These species can be genetically engineered to improve their metal tolerance and metal-accumulating capacity. The present review paper emphases on different approaches of phytormediation.

Sources of heavy metals in the environment: Heavy metals can enter into the environment from both natural and anthropogenic sources. The natural processes that contribute towards heavy metals releases are volcanic activity, weathering processes of minerals and erosion. Whereas the anthropogenic activities concerning with heavy metals are smelting, electroplating, mining, pesticides, fertilizers, discharge from industries, dumping of sludge, and deposition of heavy metals through atmospheric processes.

Table 1: Gives anthropogenic sources of heavy metals in the environment.

Heavy metals	Sources
As	Semiconductors, petroleum refining, wood preservatives, animal feed additives, coal power plants, herbicides, volcanoes, mining and smelting (Nriagu, 1994; Walsh *et al*., 1979)
Cu	Electroplating industry, smelting and refining, mining, biosolids (Liu *et al*., 2005)
Cd	Geogenic sources (Baize, 1997), anthropogenic activities (Nriagu and Pacyna, 1988), metal smelting and refining, fossil fuel burning, application of phosphate fertilizers, sewage sludge (Alloway, 1995; Kabata-Pendias, 2001)
Cr	Electroplating industry, sludge, solid waste, tanneries (Knox *et al*., 1999)
Pb	Mining and smelting of metalliferous ores, burning of leaded gasoline, municipal sewage, industrial wastes enriched in Pb, paints (Gisbert *et al*., 2003; Seaward and Richardson, 1990)
Hg	Volcano eruptions, forest fire, emissions from industries producing caustic soda, coal, peat and wood burning (Lindqvist, 1991)
Se	Coal mining, oil refining, combustion of fossil fuels, glass manufacturing industry, chemical synthesis (e.g., varnish, pigment formulation)
Ni	Volcanic eruptions, land fill, forest fire, bubble bursting and gas exchange in ocean, weathering of soils and geological materials (Knox *et al*., 1999)
Zn	Electroplating industry, smelting and refining, mining, biosolids (Liu *et al*., 2005)

Harmful effects of heavy metals on human health: Heavy metals are posing adverse impact on plants and humans as they can access the food chain through contaminated soil and water. Heavy metals and metalloids cause harmful toxicological effects even if enter in very small amount. They have the tendency to cause oxidative stress as they produce free radicals, which can increase antioxidants inside the cell and results in damage or death of the cell. Further, they also have the ability to replace the enzymes or attach in place of enzymes affecting in the disruption of enzymes normal function. Different health issues are more likely to be concerned with the heavy metals accumulation and it also depends on their oxidation state and concentration of heavy metal.

Table 2: Shows harmful effects of heavy metals on human health

Element	Acute exposure *usually a day or less*	Chronic exposure *often months or years*
Cadmium	Pneumonitis (lung inflammation)	Lung cancer Osteomalacia (softening of bones) Proteinuria (excess protein in urine; possible kidney damage)
Mercury	Diarrhea Fever Vomiting	Stomatitis (inflammation of gums and mouth) Nausea Nephrotic syndrome (nonspecific kidney disorder), Neurasthenia (neurotic disorder), Parageusia (metallic taste),Pink Disease (pain and pink discoloration of hands and feet),Tremor
Lead	Encephalopathy (brain dysfunction) Nausea Vomiting	Anemia, Encephalopathy Nephropathy (kidney disease) Foot drop/wrist drop (palsy)
Chromium	Gastrointestinal hemorrhage (bleeding) Hemolysis (red blood cell destruction) Acute renal failure	Pulmonary fibrosis (lung scarring) Lung cancer
Arsenic	Nausea Vomiting Diarrhea Encephalopathy Multi-organ effects Arrhythmia Painful neuropathy	Diabetes Hypopigmentation/Hyperkeratosis Cancer

Techniques of phytoremediation: Different techniques have been introduced to use the potential of plants for the removal of hazardous compounds from contaminated water and soil. Schwitzguebel has explained various types of phytoremediation.

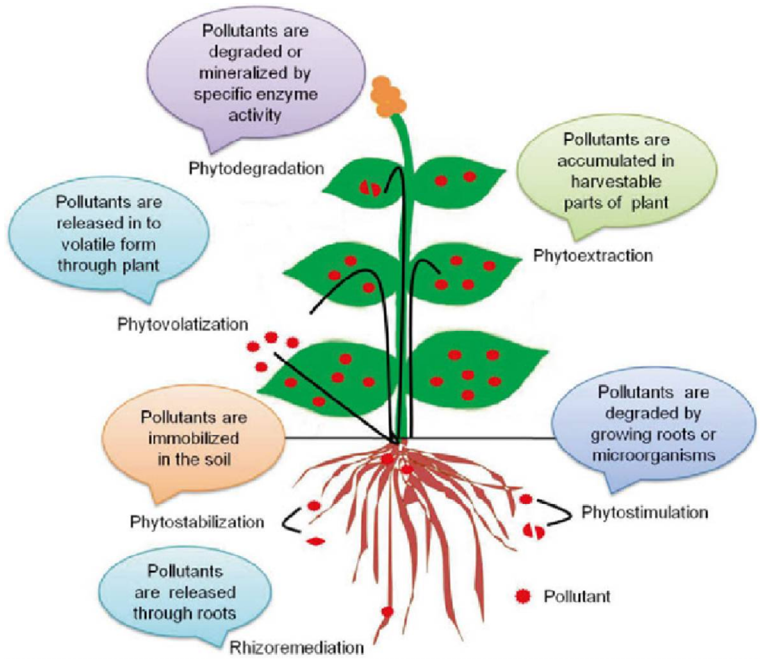

Figure 1:

Phytoextraction: (phytoabsorption, phytosequestration or phytoaccumulation) In Phytoextraction, the contaminants are being taken up by the plant roots from the contaminated soil and water and then they are accumulated in the biomass above the ground in shoots. In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and are cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the above-ground stems, in leaves and other parts of shoots. After sufficient plant growth and metal accumulation, the above-ground parts of the plant are harvested, a process referred to as phytomining. Phytomining is a "green" technology that uses metal hyperaccumulating plant species to extract metals from soil, harvest the biomass and burn it to produce bio-ore (Brooks *et al*., 1998; Robinson *et al*., 2009). This results in the permanent removal of metals from the site. Phytoextraction is a long-term remediation effort which requires many cropping cycles to reduce metal concentrations (Kumar *et al*., 1995). Phytotransformation (phytodegradation): It is the breakdown of organic contaminants sequestered by plants through metabolic processes within the plant or by the effect of compounds, such as enzymes, produced by the plant. The organic contaminants are degraded into simpler compounds that are integrated with plant tissue, which in turn, foster plant growth. Remediation of a site by phytotransformation is dependent on direct uptake of contaminants from the media and accumulation in the vegetation.

Phytostimulation: This process includes the stimulation of enzymes present in the rhizosphere which can lead to the bioremediation using microbes or fungal degradation by releasing exudates.

Phytovolatilization: It is the technique in which the pollutants are taken up by the plants from the soil, and then converted into the volatile form and then released in the atmosphere.

Rhizofiltration (Phytofiltration): It is used to inhibit the organic pollutants in waste water and surface water from mixing into the water streams or groundwater using plants for filtration purpose as they can absorb or adsorb the pollutants. Due to phytofiltration, the movement of contaminants in the soil minimized.

Phytostabilization (phytoimmobilization): In this technique, the contaminants are immobilized in the soil by using different plants having the ability to stabilize the pollutants. By using this technique, the mobility of the contaminants will be reduced which lead to the reduction of bioavailability of these pollutants. This then help in reducing the migration of pollutants into the groundwater and preventing their ability to enter into the food chain. In this process, heavy metals are immobilized by their sorption into the roots, reducing their valence number and then making them complex and immobilize in the rhizosphere. Metals having different valences also tend to have different toxicity. By releasing redox enzymes, several plants convert different hazardous metals into a relatively less toxic state and leads to the decrease of metal stress and its damages. In the process the contaminants are not really removed from the soil but their movement in the soil and leaching down the groundwater can be minimized. So, it cannot be claimed that this is the permanent solution for the removal of contaminants from the soil but it can aid other different processes and can be used to manage the pollutants. The pollutants from the atmosphere can also be re deposited into the soil after some time.It is a controversial method for treating contaminated soils and water.

Potential Strategies for Phytoremediation: Plants have been used for phytoremediation of toxic metals and metalloids but this practice has been slow and largely rendered unproductive due to phytotoxicity of heavy metals to plants (Dhankher *et al*., 2011). Natural hyperaccumulators of heavy metals are also available. But, they lack the critical biomass required for effective phytoremediation and are also limited to particular geo-climatic conditions. Genetic engineering approaches to improve plant tolerance and the accumulation of toxic metals embraces great potential for phytoremediation. In addition, several recent studies employing omics technologies including genomics, transcriptomics, proteomics, and metabolomics have been carried out to reveal the genetic determinants and pathways involved in heavy metal and metalloid tolerance in plants. Biotechnological approaches are currently being used for the phytoremediation of heavy metals and metalloids such as mercury (Hg), cadmium (Cd), lead (Pb), selenium (Se), copper (Cu), and arsenic (As). Three main biotechnological approaches are being used to engineer plants for phytoremediation of heavy metals and metalloids) (1) manipulating transporter genes and uptake systems; (2) increasing ligand production; (3) transformation of metals and metalloids to less toxic and volatile forms (Kotrba *et al*., 2009).

Manipulating Transporter Genes and Uptake System: Amended metal tolerance and accumulation has been achieved in different plant species by manipulating metal transporter genes.For example, *Arabidopsis thaliana* overexpressing yeast YCF1 (Yeast Cadmium Factor 1) resulted in enhanced tolerance to Pb(II) and Cd(II) and accumulated higher amounts of these metals in plants (Song *et al*., 2003). YCF1 is involved in Cd transport into vacuoles by conjugation with glutathione (GSH). Overexpression of full length *Nicotiana tabacum* plasma membrane channel protein (*NtCBP4*) showed Pb^{2+} hypersensitivity and enhanced Pb^{2+} accumulation in the transgenic plants. The overexpression of a truncated version of *NtCBP4* produced by deletion of its C-terminal, calmodulin-binding domain and part of the putative cyclic nucleotide-binding domain exhibited improved tolerance to Pb^{2+} and less accumulation of Pb^{2+} (Sunkar *et al*., 2000). Furthermore, T-DNA mutants of the *Arabidopsis*CNGC1 gene (cyclic nucleotide-gated ion channel 1), which encodes a homologous protein to *NtCBP4*, also conferred Pb^{2+} tolerance. These results suggest that NtCBP4 and AtCNGC1 play a role in the Pb^{2+}transport pathway (Sunkar *et al*., 2000; Zeng *et al*., 2015). Tobacco plants expressing *CAX2* (calcium exchanger 2) accumulated more Ca^{2+}, Cd^{2+}, and Mn^{2+} and were more tolerant to elevated Mn^{2+} levels. Expression of *CAX2* in tobacco increased Cd^{2+} and Mn^{2+} transport in isolated root tonoplast vesicles (Hirschi *et al*., 2000). Arsenic (As) is a highly toxic metalloid which is classified as a group I carcinogen for humans. Inorganic As forms, arsenate (AsV) and arsenite (AsIII), are common in the environment and more toxic than its organic forms (Dhankher *et al*., 2011). Arsenate is a phosphate analog taken up via phosphate uptake systems in plants (Catarecha *et al*., 2007; Zhao *et al*., 2009). Recent studies have shown that arsenite is transported in plants by members of the aquaporins family (Bienert *et al*., 2008; Zhao *et al*., 2009; Mosa *et al*., 2012). Strategies of developing transgenic plants for arsenic (As) phytoremediation include enhancing plant uptake for phytoextraction, decreasing plant uptake, improving the plants' tolerance to As contamination, and increased methylation for enhanced food safety; these are reviewed in depth by Zhu and Rosen (2009) and Dhankher *et al*. (2011). Other metalloids like antimonite (SbIII), Silicon (Si) and Boron (B) have been reported to be transported through aquaporin channel proteins (Bienert *et al*., 2008; Mosa *et al*., 2011, 2016; Mosa, 2012; Kumar K *et al*., 2014). Recently, genome-wide expression analysis of rice roots exposed to different heavy metals and metalloids; As(V), Cd, Cr(VI), and Pb, revealed several differentially expressed common as well as unique genes (Dubey *et al*., 2014). Interestingly, genes belonging to different transporter families such as major facilitator superfamily antiporter were identified (Dubey *et al*., 2014). Furthermore, Cu tolerant genes have been identified in the *Paeonia ostii* plant using the *de novo* transcriptome sequencing approach (Wang *et al*., 2016).

Increasing Ligand Production: There are several reports of using Cys-rich peptides such as MTs, PCs, and GSH as metal-binding ligands for the detoxification or accumulation of heavy metals For example, *A. thaliana* over expressing a MT gene, PsMTA from pea (*Pisum sativum*), showed increased Cu^{2+} accumulation in roots (Evans *et al*., 1992). When the *E. coli gshII* gene encoding GSH synthetase (GS) was overexpressed in the cytosol of Indian mustard (*Brassica juncea*), transgenic plants demonstrated enhanced tolerance to and accumulated significantly more Cd than wild-type (WT) plants (Liang Zhu *et al*., 1999). Shrub tobacco overexpressing the wheat *TaPCS1* gene encoding PC synthase increased its tolerance to Pb and Cd significantly; transgenic seedlings grown in soil containing 1572 ppm Pb accumulated double the amount of Pb than WT plants (Gisbert *et al*., 2003). Arsenic (As) tolerance in plants can also be enhanced by modifying GSH and PCs. When two bacterial genes, *E. coli* arsenate reductase (*arsC*) and γ-glutamylcysteine synthetase (*γ-ECS*), were co-expressed in *Arabidopsis*, the double transgenic plants grown in the presence of 125 μM sodium arsenate accumulated threefold more As in the aboveground biomass and showed almost 17-fold higher biomass than WT plants (Dhankher *et al*., 2002). Constitutive overexpression of *AtPCS1* in *A. thaliana*showed enhanced tolerance to arsenate but failed to enhance As accumulation (Li *et al*., 2004). Similarly, overexpression of AtPCS1 in *B. juncea* showed a moderate increase in tolerance to arsenate, but not As accumulation in shoots (Gasic and Korban, 2007). These studies showed that manipulation of genes for increasing the production of metal chelation agents hold great potential for improving heavy metal and metalloid tolerance and accumulation in plants. Recently, the *de novo* sequencing approach for radish (*Raphanus sativus L.*) root under Cd stress has been used to identify differentially expressed genes and microRNAs (miRNAs) involved in Cd-responsive regulatory pathways. Different candidate genes were suggested to play a major role on Cd accumulation and detoxification, particularly those encoding for MTs, PCs, and GSHs as well as other genes belonging to ABC transporters and zinc iron permease (ZIPs) (Xu *et al*., 2015). Similarly, RNA-Seq and *de novo*transcriptome analysis showed that 1561 unigenes were down-regulated and 1424 unigenes were up-regulated, respectively, in radish roots under chromium stress (Xie Y. *et al*., 2015). Here, several transcription factors such as Cr stress-responsive genes involved in signal transduction, chelate compounds and antioxidant biosynthesis have been proposed (Xie P. *et al*., 2015). Furthermore, the degradome sequencing approach has been used to identify miRNAs and their target genes under Pb stress in *Platanus acerifolia* tree plants (Wang *et al*., 2015).

Transformation of Metals and Metalloids to Less Toxic and Volatile Forms: Several research groups have focused their efforts on developing phytoremediation strategies for Se and Hg using biotechnological approaches employing the conversion of these metals to less toxic and volatile forms. Selenium (Se) is an essential micronutrient for many organisms. However, in excess, it is very toxic and is a worldwide environmental pollutant (Zwolak and Zaporowska, 2012). Se occurs naturally in soil, and is chemically similar to sulfur (S). Therefore, plants uptake the inorganic and organic forms of Se via S transporters and metabolize them to volatile forms through S assimilation pathways to relatively non-toxic forms, such as dimethylselenide (DMSe). As reviewed by Pilon-Smits and LeDuc (2009), biotechnological strategies that have been used for selenium phytoremediation have focused on enhancing Se tolerance, accumulation, and volatilization. A constitutive overexpression of *A. thaliana* ATP sulfurylase (APS), converting selenate to selenite, in *Brassica juncea* showed enhanced reduction of selenate to organic Se forms in the APS overexpressed plants, whereas WT plants accumulated mainly selenate. The APS transgenic plants showed enhanced tolerance to selenate as compared to WT plants (Pilon-Smits *et al*., 1999). The overexpression of the SMT gene from the Se hyperaccumulator *Astragalus bisulcatus* in *A. thaliana* and *B. juncea* improved the tolerance of transgenic plants to selenium and enhanced Se accumulation in shoots. The transgenic plants also increased Se volatilization rates (LeDuc *et al*., 2004). Mercury is liberated into the environment mainly in inorganic forms of mercury, elemental metallic Hg(0) or ionic Hg(II (Ruiz and Daniell, 2009). Strategies used for mercury phytoremediation have employed two bacterial genes; merA, encoding mercuric ion reductase, and merB, encoding organomercurial lyase, to convert mercury into less toxic forms (Meagher, 2000; Dhankher *et al*., 2011). Various plant species such as *A. thaliana* (Rugh *et al*., 1996), yellow poplar (Rugh *et al*., 1998), cottonwood (Che *et al*., 2003), rice (Heaton *et al*., 2003), and tobacco (Heaton *et al*., 2005) constitutively expressing modified *merA* were resistant to levels up to 25–250 μM $HgCl_2$ and exhibited significant levels of Hg(0) volatilization as compared to control plants. Similarly, expression of a modified bacterial *merB* in *Arabidopsis* showed a significant resistance to high levels of monomethylmercuric chloride and phenylmercuric acetate relative to control plants (Bizily *et al*., 1999). *Arabidopsis* plants expressing both genes, *merA* and *merB*, grow on 50-fold higher methylmercury concentrations than WT plants and up to 10-fold higher concentrations than plants that express *merB* alone. Transgenic plants were also seen to detoxify organic mercury by converting it to volatile and significantly less toxic elemental mercury (Bizily *et al*., 2000).

CONCLUSION AND FUTURE ASPECTS

Phytoremediation is an eco-friendly 'green-clean' technology that has tremendous potential to be utilized in the cleaning up of heavy metals and organic pollutants. Plants can uptake, detoxify, translocate, and accumulate heavy metals in the aboveground biomass, which has to be then harvested for metal recovery. Despite tremendous potential for the application of phytoremediation in the cleaning up of contaminated soil, sediment, and water, it has not been commercialized and used extensively on a large scale. There are many reports of heavy metal uptake, detoxification, and accumulation but most of these were described at the laboratory scale in model plants (Dhankher *et al.*, 2011; Hossain *et al.*, 2012; Ovečka and Takáč, 2014. Furthermore, progress toward commercializing the phytoremediation of heavy metals has been hampered due to a lack of complete understanding of the metal uptake process from soil to roots, translocation from roots to shoots and accumulation in the biomass tissues. Several recent studies have endeavored to unravel the mechanism of heavy metal and metalloid transport and accumulation in plants using transcriptomic and proteomics approaches (Cvjetko *et al.*, 2014). Additionally, metabolomic analysis can help to identify the metabolites associated with heavy metal and metalloid stresses, which can be further mapped to its metabolic pathways to identify the related candidate genes (Kumar A *et al.*, 2014). Despite recent progresses in biotechnological applications and the availability of complete genome sequences of several plants species, the potential of phytoremediation has still not been fully exploited for the successful application of this technology on a commercial scale for the cleaning of contaminated soil and water. Another major factor for the lack of progress in this area is inadequate funding for phytoremediation research. In future, efforts should be made to develop strategies to improve the tolerance, uptake, and hyperaccumulation of heavy metals using genomic and metabolic engineering approaches. Additionally, efforts should be made to develop breeding programs to improve the biomass and growth habits of natural hyperaccumulators and breed those traits into non-food, high biomass, fast growing plants for commercial phytoremediation of heavy metals. This is a low cost and eco-friendly means of reclaiming heavy metal contaminated soils (Rajakaruna *et al.*, 2006). The cost of growing crops is minimal compared to those of soil removal and replacement. As biological processes are ultimately solar-driven, phytoremediation is on average ten-fold cheaper than engineering-based remediation methods (Marques *et al.*, 2009). A limitation of phytoremediation is that the plant roots have to be able to reach the pollutant and act on it. Therefore, the soil characteristics, toxicity level, and climate have to be suitable to plant growth. Additionally, the pollutant must not only be within physical reach of the roots, it must be bio-available for absorption as well (Pilon-Smits and Freeman, 2006). This is the fast, innovative, emerging, ecofriendly and cost effective alternate to the conventional remedial method. Further research is required to observe the economic and ecological aptitudes of Phytoremediation.

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