Phytoremediation of toxic metals in soils and wetlands: concepts and applications (Book Chapter)

Phytoremediation of toxic metals in soils and wetlands: concepts and applications

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Abstract:

Over centuries, industrial, mining and military activities, agriculture, farming, and waste practices have contaminated soils and wetlands in many countries with high concentrations of toxic metals. In addition to their negative effects on ecosystems and other natural resources, toxic metals pose a great danger to human health. Unlike organic compounds, metals cannot be degraded, and clean-up usually requires their removal. Most of the conventional remedial methods lost economic favor and public acceptance because they are expensive and cause degradation of soil fertility that subsequently results in adverse impacts on the ecosystem. Conventional methods of environmental remediation do not solve the problem; rather they merely transfer it to future generation. Obviously, there is an urgent need for alternative, cheap and efficient methods to clean-up sites contaminated with toxic metals.

Phytoremediation, a plant-based green technology, is cost effective, environmental friendly, aesthetically pleasing approach for the remediation of toxic metals. Due to its elegance and the extent of contaminated areas, phytoremediation approaches have already received significant scientific and commercial attention. Two approaches have been proposed for the phytoremediation of toxic metals from soils and wetlands: natural and induced phytoremediation. Natural phytoremediation refers to the use of hyper-accumulating plants and associated soil microbes, while the induced phytoremediation refers to the use chemicals, especially synthetic chelating ligands, for the increase of metal bioavailability and uptake in plants. Recently, genetically modified plants (GMPs) have been proposed to use in phytoremediation technology; however, this approach is being hindered by ideology-driven restrictive legislation over the use of GMPs. We will discuss the concepts and practical applications of phytoremediation technologies for the restoration of contaminated soils and wetlands.

Keywords: Phytoremediation, Heavy metals, Soil, Wetland.

1. Metal contamination of soils and wetlands and human health impact

Metals comprise about 75% of the known elements and have been used from the beginning of ancient human civilization. Since the beginning of the industrial age, metals have been emitted to and deposited in the environment (Sparks, 2005). In some cases, metals can be accumulated in terrestrial and aquatic environments in high concentrations and cause harm to living beings via ingestion of soil and/or dust, food, and water, inhalation of polluted air, and absorption via the skin from polluted soils, water, and air (Hillel, 2005). Increasing use of metals with population and economic growth, especially in the developing countries, may contribute to soil and water contamination causing the deterioration of environmental quality and posing threats to human health (Sparks, 2005).

Toxic metals can be derived from both natural and anthropogenic sources. Natural (geogenic) sources include rocks and minerals, and anthropogenic sources include agriculture (fertilizers, pesticides, herbicides, and animal manures), mining, smelting, and sewage sludge and scrap disposal (Adriano, 2001). Anthropogenic deposition is a major mechanism for toxic metal input in the environment. Soil is the major recipient of trace elements in terrestrial environment, while sediments are the major sink in aquatic environment. Leaching of toxic metals or transport via mobile colloids can contaminate groundwater. On the other hand, runoff and drainage of toxic metals via sediments can contaminate freshwater environment (Adriano, 2001, Hillel, 2005).

For many years across the world, industrial, mining, military, farming and waste management have contaminated large areas of soils and wetlands with high concentrations of toxic metals and organic pollutants (Peuke and Rennenberg, 2005a, Li et al., 2001, Del Río et al., 2002). The problem of soil and water contamination of toxic metals is becoming more and more serious with increasing industrialization and disturbance of natural biogeochemical cycles by human activities and climate change (Ali et al., 2013). For example, in Europe, an estimated 52 million hectares of land – more than 16% of the total land area of the continent – are affected by some level of soil degradation (Peuke and Rennenberg, 2005a). The largest and probably most heavily contaminated areas are found near

industrialized regions in north-western Europe, but many contaminated areas exist in the vicinity of major European cities (EEA, 2003). In China, over 20 million acres of farmland (almost one fifth of the total arable farmland area), has been contaminated by toxic metals, such as Sn, Cr, Pb, and Zn, causing approximately 10 million tons of crop losses per year (Wu et al., 2010). A total area of 2.9×10^6 hectares of degraded land has been produced in China as a result of mining and an additional mean area of 46700 ha of destroyed land is produced annually. These degraded lands almost completely lack vegetation due to serious pollution and ultimately cause severe soil erosion and off-site pollution (Xia, 2004). The Campine region in Belgium and the Netherlands with 700 km^2 is diffusely contaminated by atmospheric deposition of Cd, Zn and Pb (Meers et al., 2010).

Bioaccumulation of toxic elements in the food chain from contaminated soils and wetlands can be especially dangerous to human health. Toxic metals can enter the human body by either inhalation or ingestion (Islam et al., 2007). For the majority of people the main route of exposure to toxic metals is diet except for workers with high levels of occupational exposure (Sharma et al., 2008). Exposure to toxic metals through the food chain has been reported in many countries, particularly in developing countries, and received significant attention from government and non-government agencies (Åkesson et al., 2008, Al Jassir et al., 2005, Demirezen and Aksoy, 2006, Gulz et al., 2005). Concern over the accumulation of toxic metals in the food chain and environment has escalated in recent years. Once metals enter into biological systems they have the potential to disturb normal biochemical processes, and in extreme cases can be fatal (Pillay et al., 2003). Many countries have developed regulations for industries and other systems limiting discharges of pollutants into the environment in order to control the emission of trace elements and their subsequent health effects.

In fact, exposure to high content of toxic metals can cause significant adverse effects to humans, animals, microorganisms and plants (Wagner, 1993, Gaetke and Chow, 2003, Hernández-Ochoa et al., 2005, Bodar et al., 2005). Regarding their toxicities, the most problematic toxic metals are Hg, Cd, Pb, As, Cu, Zn, Sn, and Cr (Ghosh, 2010). Hg, Cd, Pb, and As are not essential for living organisms, while

Cu and Zn are essential metals. Exposure of humans to toxic metals may results in neurobehavioral disorders, such as fatigue, insomnia, decreased concentration, depression, irritability, sensory, and motor symptoms. Exposure to toxic metals may also cause developmental retardation, various types of cancers, kidney damage, autoimmunity, and even death in some instances of exposure to very high concentrations (Glover-Kerkvliet, 1995). For instance, at high concentration, Hg can damage vital organs, such as the lungs and kidneys, may cause foetal brain damage (Sharma, 2003). Accumulation of Cd in human bodies (principally in the kidney and liver) can cause renal dysfunction and bone disease (e.g., Itai-Itai in Japan) (Nordberg, 1996). Lead poisoning in children causes neurological damage leading to reduced intelligence, loss of short-term memory, learning disabilities, and coordination problems (Rai, 2008a). The effects of As include cardiovascular problems, skin cancer and other skin effects, peripheral neuropathy, and kidney damage (Hughes, 2002).

2. Remediation of metal-contaminated soils and wetlands

Low to medium range contamination of lands with toxic metals may induce their accumulation in the food chain. Therefore, strict environmental laws have been imposed in many countries to prevent any such occurrence of the toxic metals in foods by limiting the food production on contaminated lands. For example, a European Union Council Directive (EC, 1986) limited the concentrations of toxic metals in agricultural soils to be 3 mg kg^{-1} for Cd, 140 mg kg^{-1} for Cu, 75 mg kg^{-1} for Ni, 300 mg kg^{-1} for Pb, 300 mg kg^{-1} for Zn, and 1.5 mg kg^{-1} for Hg (Grčman et al., 2001). According to the directive, several million hectares of agricultural lands are considered polluted by toxic metals in Europe (Flathman and Lanza, 1998), and between 59 and 109 billion EUR is required to clean-up the contaminated sites using conventional techniques such as soil washing using particle size separation, chemical extraction with aqueous solutions of surfactants and mineral (EC, 2004). Such high costs for land restoration using traditional remediation methods make the clean-up of many sites unaffordable even in the more developed nations. For instance, in Germany, only one-third of the total contaminated

sites are cleaned up in soil remediation facilities while the remainder has been left untreated (Evangelou et al., 2007, Peuke and Rennenberg, 2005a). This does not solve the problem, rather transferring it to future generation.

Aquatic ecosystems (including wetlands) are used directly or indirectly as recipients of potentially toxic liquids and solids from domestic, agricultural and industrial wastes (Demirezen et al., 2007, Peng et al., 2008). Thus, wetland sediments are the major sink of toxic metals (Marchand et al., 2010). Clean-up of contaminated soils and wetlands is necessary to reduce the risk of metal toxicity to human and ecosystems. To date, different physical, chemical and biological approaches have been employed for this purpose. The conventional remediation methods include *in situ* vitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification, and stabilization of electro-kinetic systems (Sheoran et al., 2011, Wuana and Okieimen, 2011). Generally, the physical and chemical methods suffer from limitations like high cost, intensive labour, irreversible changes in soil properties and disturbance of native soil microflora. Chemical methods can also create secondary pollution problems. Therefore, there is an urgent need for alternative, cheap and efficient methods to clean up heavily contaminated soils and wetlands. To improve how contaminated sites are remediated there is a need to move beyond more traditional remediation practices and include some of the more novel remediation techniques like phytoremediation. Due to the potential of phytoremediation and the extent of contaminated sites, this technology has received significant scientific and commercial attention world-wide (McIntyre, 2003, Gleba et al., 1999, Meagher, 2000, Dietz and Schnoor, 2001, Rahman et al., 2007, Salt et al., 1998).

3. Phytoremediation – a green technology for the remediation of contaminated environment

3.1. Phytoremediation strategies for the restoration of contaminated soils and wetlands

Phytoremediation (from ancient Greek “phyto” meaning "plant", and Latin “remedium” meaning "restoring balance") defines the remediation of contaminated environment (soils and wetlands) based on the idea of using natural bioaccumulation abilities of plants without excavating the contaminant material. Phytoremediating plants are ideally fast growing, tolerant of toxic metals, and efficient at transferring toxic metals from roots to above ground biomass. Sometimes, rhizospheric microorganisms or chemicals (e.g., chelating ligands) are used to increase of metal bioavailability and uptake. Based on the natural abilities of the phytoremediating plants, restoration of contaminated soils and wetlands can be achieved by employing the following phytoremediation strategies –

- Phytoextraction
- Phytostabilization
- Phytovolatilization
- Phytodegradation
- Phytofiltration
- Phytotransformation/detoxification

3.1.1. Phytoextraction

Phytoextraction is the uptake of contaminants from soils or waters by plant roots and their translocation to the harvestable biomass (Sekara et al., 2005, Yoon et al., 2006, Rafati et al., 2011). Translocation of toxic metals form roots to harvestable biomass (shoots) is necessary for an effective phytoextraction protocol because the harvest of root biomass is generally not feasible due to the location of roots within the soil (Zacchini et al., 2009, Tangahu et al., 2011). In general, phytoextraction has been tried more often for extracting toxic metals than for organics. Some of the examples of phytoextraction are – sunflower (*Helianthus annuus*) (Marchiol et al., 2007) and Chinese brake fern (*Pteris vittata*) (Ma et al., 2001) for As; willow (*Salix viminalis*) Cd, Zn, Ni, Pb and Cu (Greger and Landberg, 1999, Borišev et al., 2009); Indian mustard (*Brassica juncea*) for Pb (Blaylock et al.,

1997). The main advantage of phytoextraction is environmental friendliness. Traditional methods that are used commonly used for cleaning up metal-contaminated soil disrupt soil structure and reduce soil productivity, whereas phytoextraction can clean up soil without causing harm to soil quality. Another benefit of phytoextraction is that it is less expensive than the traditional clean-up process.

Phytoextraction can be used for phytoremediation of toxic and hazardous metals as well as for phytomining of precious metals (such as Au, Pd, Tl and Pt) (Ali et al., 2013, Brooks et al., 1998). Phytomining has the potential to be an economically feasible solution to the disposal of used phytoremediation biomass. Plant biomass containing metals can be combusted to obtain energy and the remaining ash is considered as "bio-ore", which can be processed for the recovery or extraction of precious metals (Rascio and Navari-Izzo, 2011). An advantage of phytomining is that this is a cost-effective and ecofriendly option as compared to the conventional metal extraction methods (Ali et al., 2013, Rascio and Navari-Izzo, 2011). The commercial feasibility of phytomining, however, depends on many factors like phytoextraction efficiency of precious metals and current market value of the processed metals. In addition, this technique can only be used to extract metals from the rhizosphere. Phytomining has been commercially used for Ni and it was found to be less expensive than the conventional extraction methods for Ni (Robinson et al., 1997, Nicks and Chambers, 1998). Commercial phytomining has also been used for Au and Tl (Anderson et al., 1999).

The chemical composition and sorption properties of soil influence metal mobility and bioavailability (Kłos et al., 2012). The bioavailability of toxic metals in soils is a critical factor affecting the efficiency of phytoextraction of target metals. Low bioavailability is a major limiting factor for phytoextraction of contaminants such as Pb (Ali et al., 2013). Strong binding of toxic metals to soil particles or precipitation causes a significant fraction of the toxic metals non-bioavailable, and therefore, remain unavailable for uptake by phytoremediating plants (Sheoran et al., 2011). Based on bioavailability, toxic metals in soils can be categorised into three groups: readily bioavailable (Cd, Ni, Zn, As, Se, and Cu); moderately bioavailable (Co, Mn, and Fe) and least bioavailable (Pb, Cr, and U)

(Prasad, 2003). However, many plants have developed mechanisms for solubilizing heavy metals in soil such as the secretion of metal-mobilizing "phytosiderophores" into the rhizosphere by members of the Poaceae (Lone et al., 2008, Reichman and Parker, 2005), for solubilizing toxic metals in soil.

Phytoextraction is classified into natural and induced based on the bioaccumulation process of the plants species involved. Natural phytoextraction is based on the idea of the use of natural hyperaccumulators that have exceptionally high metal-accumulation ability and tolerance to toxic metals (Baker et al., 2000). In induced phytoextraction, a conditioning fluid containing a chelator or another agent is added to soil to increase metal solubility or mobilization so that the plants can absorb higher concentrations of metals.

In the past decade, chelant-enhanced phytoextraction has received much attention from the scientific community. Chelants, when added to soil, are capable of forming soluble complexes with both 'labile' and 'non-labile' metal in the soil solution via desorption of sorbed species and dissolution of precipitated compounds (Norvell, 1984). Re-precipitation and re-sorption of metals are prevented by the chelant-metal-complex formation, and the metals become bioavailable (Salt et al., 1995a). The drawback of metal phytoextraction due to limited bioavailability of metals is minimized after the unearthing that the translocation of metals from soil to plants can be increased to maximum with the addition of certain chelants (Blaylock and Haung, 1999). However, the soil properties and the nature of the applied chelant determine the amounts of bioavailable metals in soil matrix (Kos and Lestan, 2004, Tandy et al., 2004, Luo et al., 2005). Stability constants, K_s, of chelant-metal-complexes are the decisive feature to select a chelant or rank different chelants for the extraction of metals from the metal-contaminated soil. The chemical characteristics of the chelant itself and the metal speciation in the soil matrix also influence the effectiveness of different chelant in the separation process (Elliott and Brown, 1989, Luo et al., 2005, Huang et al., 1997).

A wide range of synthetic chelants have been tested for chelant-induced phytoextraction with aminopolycarboxylate chelants (APCs) among the most used chelant type. Typically used APCs for

metal phytoextraction include Ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), N-(hydroxyethyl)ethylenediaminetriacetic acid (HEDTA), nitrilotriacetic acid (NTA), S,S-ethylenediaminedisuccinic acid (S,S-EDDS), methylglycinediacetic acid (MGDA), glutamic acid diacetic acid (GLDA) . In particular, EDTA has most often been utilized among the APCs, since EDTA forms strong water-soluble chelant complexes with most toxic metals (Egli, 2001, Nowack and VanBriesen, 2005, Leštan et al., 2008, Salt et al., 1998). Despite the success of the use of EDTA in phytoextraction of toxic metals, the enhanced mobility of the metals in soil by EDTA and their potential risk of leaching are important concerns (Cooper et al., 1999, Wu et al., 2004, Sun et al., 2001). Several studies showed that EDTA enhanced the leaching of heavy metals during the phytoextraction process (Sun et al., 2001, Wu et al., 2004, Grčman et al., 2001, Kedziorek et al., 1998). For example, Wu et al. (2004) found that the mobility and leaching of Cu, Zn and Cd increased significantly during the EDTA-enhanced phytoextraction process. Increased mobility and leaching of Zn, Cu, Cd and Pb during the EDTA-enhanced phytoextraction process has also been reported by Sun et al. (2001).

In addition to enhanced mobility and leaching of heavy metals by EDTA, the persistence of metal-EDTA complexes in contaminated soils and their effects on soil microbial community are important drawbacks of this technology. Several studies have indicated that EDTA-metal complexes are resistant to microbial degradation (Nörtemann, 1999, Oviedo and Rodríguez, 2003). Palumbo et al. (1994) found that the bacterial ability to degrade EDTA is rare, since they could not obtain degrading consortia from places polluted with the chelate. Other studies also found EDTA to be slowly biodegraded to CO_2 in soil, with only 6.7% degraded after 4 weeks and a lower rate of degradation in the subsoil than in surface soil (Tiedje, 1975). Means et al. (1980) reported that the EDTA degradation rate was not rapid enough, even under optimal laboratory conditions, to stop disquiet about its release into the environment. Therefore, biodegradable chelating ligands can be alternatives to the EDTA for the phytoextraction of toxic metals.

The method of chelant application significantly affects the efficiency of the phytoextraction process. The chelant can be applied to the soil matrix either in a single dose after the optimum growth of the accumulator crop, or in small multiple doses gradually during the growth cycle. Studies showed that application of chelant in multiple doses is more effective than that of single dose (Wenzel et al., 2003). Phytoextraction efficiency can also be improved by the combined application of different chelants to the metal-contaminated soil (Leštan et al., 2008). For example, Blaylock et al. (1997) showed that the application of EDTA and acetic acid results in a two-fold accumulation of Pb in Indian mustard shoots compared with the application of EDTA alone. Luo et al. (2006) also found that the combined application of EDTA and EDDS results in a higher level of efficiency in the phytoextraction of Cu, Pb, Zn and Cd than could be obtained by the application of either chelant alone.

Although phytoextraction of toxic metals can be achieved by using hyperaccumulators, the technology may not be applicable for remediating sites with multiple contaminants. Wu et al. (2006) proposed a solution to the phytoremediation of soils with multiple contaminants using a combination of microbe-plant symbiosis within the plant rhizosphere. They showed that inoculation of sunflower roots with the engineered rhizobacterium resulted in a marked decrease in Cd phytotoxicity and a 40% increase in Cd accumulation in the plant root. Owing to the significantly improved growth characteristics of both the rhizobacterium and plant, they proposed the use of a metal-binding peptide (EC20) in a rhizobacterium (*Pseudomonas putida*) with organic-degrading capabilities as a promising strategy to remediate mixed organic-metal-contaminated sites.

3.1.2. Phytostabilization

Phytostabilization is the immobilization of a contaminant in soil through adsorption onto roots, absorption and accumulation by roots, or precipitation within the root zone of plants (Brunner et al., 2008). Unlike phytoextraction, phytostabilization focuses mainly on sequestering pollutants in soil near the roots. This technique is used to reduce the mobility and bioavailability of pollutants in the

environment, thus preventing their leaching in groundwater and entry into the food chain (Erakhrumen and Agbontalor, 2007).

Phytostabilization occurs through contaminant accumulation in plant tissue and in the soil around the roots because of changes in the chemistry of the contaminants, which become insoluble and/or immobilized on soil components. Plants used for phytostabilization will need to be tolerant of the metals present in the particular site, but the accumulation of metals in their aerial parts may be positively disadvantageous. If the objective of phytostabilization is purely to prevent erosion and improve the visual amenity of a derelict site, then the accumulation of metals in the plants may be irrelevant (Macnair et al., 2000).

Metal immobilizing chemicals (soil amendments) are used for phytostabilization technology to improve soil conditions for plant growth and to reduce the chemical mobility in soil and toxicity of the metals to biota (Vangronsveld et al., 2009). Different soil amendments are used in phytostabilization technology for different toxic metals. For example, the most promising amendments for phytostabilization of Pb are phosphate materials, materials containing hydrous iron oxides, steel shot, inorganic clay minerals, and organic material (Cunningham and R. Berti, 2000). By excreting special proteins and/or redox enzymes, certain plant species can convert metals to relatively less bioavailable forms and decrease possible metal bioavailability and toxicity to biota. For example, Cr(III) is less mobile and toxic than Cr(VI), and the reduction of Cr(VI) to Cr(III) can be a strategy of phytostabilization (Wu et al., 2010).

Phytostabilization technology influences the mobility of toxic metals in soils in different ways:

- The amendments directly alter the soil conditions (acidic and/or alkaline conditions, organic matter, oxygen levels) in the rhizosphere that influence metal mobility;
- Proteins and/or enzymes are released by the roots into the rhizosphere soil, leading to precipitation and immobilization of the toxic metals either in the soil or on the root surface;
- The toxic metals are taken up by the plants and sequestered in the root system;

- The surface of the soil is vegetated, and the vegetation acts as a barrier to erosion and exposure of the contaminated soil to wind, water, and direct contact with humans or animals.

An example of the application phytostabilization technology is the use of vegetation cap to stabilize and contain mine tailings (Mendez and Maier, 2008, Conesa et al., 2007).

Phytostabilization technology for the remediation of metal-contaminated soils has both the advantage and disadvantage. Advantages of this technology include –

- This technology reduces the mobility, and therefore the risk, of toxic metals without removing them from their location.
- This technology does not generate secondary contamination that needs treatment.
- Usually this technology enhances the soil fertility. It may combine treatment with ecosystem restoration.

Disadvantages of phytostabilization technology may include –

- The contaminants are left in place, so the site must be monitored perpetually to make sure the stabilizing conditions continue.
- If the contaminant concentrations are very high, toxic effects may prevent the growth of plants until extensive amendments application reduce their bioavailability to plants.
- If soil additives are used, they may need to be periodically reapplied to maintain the effectiveness of the immobilization.

3.1.3. Phytovolatilization

Phytovolatilization is the uptake of pollutants from soil by plants, their conversion to volatile form and subsequent release into the atmosphere (Ali et al., 2013). This method can be used for organic pollutants and some toxic metals like Hg, Se, and As that have gaseous forms.

There is some evidence that certain plant species have the ability to accumulate Hg both from the atmospheric and soil sources; however, no plant species with Hg hyperaccumulating properties has

been identified (Raskin and D., 2000). Therefore, transgenic plants such as Arabidopsis (*Arabidopsis thaliana* L.) and tobacco (*Nicotiana tobacum*) containing bacterial mercuric ion reductase (*merA*) and organomercurial lyase (*merB*) genes, responsible for detoxifying methyl-mercury, have been investigated for their ability of Hg phytovolatilization (Heaton et al., 1998, Bizily et al., 1999). The advantage of this technology is that the plant may be transform toxic methyl-mercury to a less toxic volatile elemental Hg. However, the important limitation of Hg phytovolatilization is that the released elemental Hg into the atmosphere is likely to be recycled by precipitation and then redeposit back into ecosystem (Henry, 2000).

Bacteria containing the As (III) S-adenosylmethionine methyltransferase (*arsM*) gene were able to sequentially methylate toxic inorganic As to less toxic pentavalent methylated arsenicals such as methylarsenate (MAs(V)), dimethylarsenate (DMAs(V)) and trimethylarsine oxide (TMAs(III)) (Qin et al., 2009, Qin et al., 2006). The phytovolatilization of the final product, gaseous TMAs(III), could remove arsenic from polluted water and soil using engineered hyperaccumulator such as Chinese fern *Pteris vittata* (Sakakibara et al., 2010, Zhu and Rosen, 2009).

The major disadvantage of phytovolatilization is that it does not remove the pollutants completely from the environment; rather it transfers the pollutants from soils/waters to atmosphere from where it can be re-deposited through atmospheric precipitation. Therefore, the use of phytovolatilization for the remediation of environmental contaminants remains controversial (Padmavathiamma and Li, 2007).

3.1.4. Phytodegradation

Phytodegradation refers to the microbial breakdown of pollutants, particularly organic pollutants, in the rhizosphere (Mukhopadhyay and Maiti, 2010, Newman and Reynolds, 2004). The main reason for the enhanced degradation of organic pollutants in the rhizosphere compared to the bulk soil? is likely the increase in the numbers and metabolic activities of the microbes in the rhizosphere. Plants can stimulate microbial activity by 10-100 times higher in the rhizosphere compared to the bulk soil by

the secretion of exudates containing substances such as carbohydrates, amino acids, flavonoids (Ali et al., 2013). The release of nutrient-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 that facilitate the growth and activity of rhizospheric microbes, plants also release enzymes that are directly capable of degrading organic contaminants in rhizosphere (Kuiper et al., 2004, Yadav et al., 2010).

3.1.5. Phytofiltration

Phytofiltration (also known as rhizofiltration), which is related to phytoextraction, is the removal of pollutants from contaminated wetlands by aquatic plants (Mukhopadhyay and Maiti, 2010, Dushenkov et al., 1995). Gardea-Torresdey et al. (2004) reviewed phytofiltration technology for the removal of toxic metals form contaminants from aqueous effluents. Phytofiltration may be rhizofiltration (use of plant roots) or blastofiltration (use of seedlings) or caulofiltration (use of excised plant shoots) (Mesjasz-przybyłowicz et al., 2004).

The phytofiltration of toxic metals from contaminated waters using aquatic plants has been extensively studied (Selvapathy and Sreedhar, 1991, Sen and Bhattacharyya, 1993, Low et al., 1994, Alam et al., 1995, Ingole and Ting, 2002, Sen and Mondal, 1990a, Dushenkov et al., 1995). This cleanup process involves biosorption and accumulation of pollutants. Many aquatic plants (floating and sub-merged) have been investigated for the remediation of wastewater contaminated with Cu(II), Cd(II) and Hg(II) (Sen and Mondal, 1987a, Selvapathy and Sreedhar, 1991, Alam et al., 1995). Water fern (*Salvinia natans* L.) is a free floating freshwater macrophyte that has been tested for remediation of Hg(II) (Sen and Mondal, 1987a), and Cu(II) (Sen and Mondal, 1990a) and As(V) (Rahman et al., 2008c). Other examples of phytofiltration of toxic metals by aquatic plants are - *Medicago sativa* (Alfalfa) for Cd, Cr Pb, and Zn (Gardea-Torresdey et al., 1998), ferns (*Pteris vittata* and *Pteris cretica*) for As (Huang et al., 2004), Yellow burrhead (*Limnocharis flava*) for Cd (Abhilash et al., 2009), water

hyacinth (*Eichchornia crassipes*) for Cd and Zn (Hasan et al., 2007), and duckweed (*Spirodela polyrhiza*) for As (Rahman et al., 2007) .

3.1.6. Phytotransformation/detoxification

Phytotransformation of toxic metals is not a direct remediation technique, rather it reduce/detoxify the toxicity of toxic metals to the organisms. There are some microbes (e.g., bacteria, phytoplankton, fungi, etc.) that in the soil and aquatic environment that have the ability/mechanisms to transform more toxic forms of the toxic metals and metalloids to their less toxic form (Summers and Silver, 1978, Raab and Feldmann, 2003, Bender et al., 1995). This detoxification process of microbes is considered as a promising method for bioremediation of heavy metals and metalloids.

Higher plants also have detoxification mechanisms of toxic metals (Zenk, 1996). A set of toxic-metal-complexing peptides has been isolated from plants and plant suspension cultures. The structure of these peptides was established as (γ-glutamic acid-cysteine)$_n$-glycine, and are called phytochelatins (PC) (Zenk, 1996, Cobbett and Goldsbrough, 2002). The biosynthesis of PCs proceeds by metal activation of a constitutive enzyme that uses glutathione (-GSH) as a substrate. In a recent review, Rahman and Hassler (2014) discussed the rules of PCs and GSH in As resistance and detoxification by photosynthetic organisms. Other studies also reported the phytotransformation/detoxification of toxic metals by plants and photosynthetic organisms (Cobbett and Goldsbrough, 2002, Lytle et al., 1998, Ow et al., 1998). However, more research and knowledge on the natural detoxification mechanisms of toxic metals by plants are required to improve plant's performance in removing these toxicants from the environment.

4. Tolerance and detoxification of toxic metals in plants

4.1. Mechanisms of heavy metal uptake in plants

Plants uptake heavy metals from soil solution and waters into their roots, and then a fraction of the heavy metal ions are stored in the roots while the rest are translocated to the aboveground parts primarily through xylem vessels (Prasad, 2004, Jabeen et al., 2009). The uptake of heavy metal ions from soil solution by plants' roots and subsequent translocation to the shoots and vacuoles is controlled and regulated by a variety of molecules. Some molecules are involved in the cross-membrane transport of the heavy metal ions and others are involved in their complexation with chelating compounds and subsequent sequestration in the vacuoles (Ali et al., 2013, Tong et al., 2004). Uptake of heavy metal ions into plant's roots is mediated by several classes of specialized transporter proteins (channel proteins) in the plasma membrane (Seth, 2012). These include the CPx-type heavy metal ATPases, the natural resistance-associated macrophage (Nramp) family of proteins, action diffusion facilitator (CDF) family proteins, and zinc-iron permease (ZIP) family proteins (Williams et al., 2000). The ZIP family proteins contribute to the uptake of Zn^{2+} and Fe^{2+} (Clemens, 2001), while CPx-type heavy metal ATPases have been involved in the transport of essential as well as potentially toxic metals like Cu, Zn, Cd and Pb across the cell membranes (Williams et al., 2000). The Nramp family proteins play an important role in transport of divalent metal ions into the plant's roots (Seth, 2012).

4.2. Mechanisms of metal tolerance and detoxification in plants

Plants have a range of potential cellular mechanisms in order to tolerate and detoxify heavy metal stress. These include metal binding to cell walls, exudation of metal chelating compounds and a network of processes that take up metals, chelate them and transport these complexes to above-ground tissues where they are sequestered into vacuoles (Peuke and Rennenberg, 2005a). Based on these mechanisms, plants can be classified into two groups – (i) non-accumulators that achieve metal tolerance by preventing toxic metals uptake into roots cells passively through binding the metal ions onto the cell walls; (ii) accumulators that evolved specific mechanisms for high levels of metal

accumulation and detoxification in cells. The second group of plants uptake heavy metals in shoots, and sequester them in cellular vacuoles to remove excess metal ions from the cytosol to reduce their interactions with cellular metabolic processes (Assunção et al., 2003).

The hyperaccumulating or metal-tolerant plant species such as *Silene vulgaris*, *Thlaspi caerulescens*, *Alyssum lesbiacum*, *Arabidopsis halleri* and *Brassica* spp. have been investigated by several researchers (Clemens et al., 2002, Kraemer, 2003). The ability of these plants to accumulate high concentrations of metals was observed for both essential nutrients, such as Cu, Fe, Zn, and Se, as well as non-essential metals, such as Cd, Hg, Pb, Al, and As (Salt et al., 1998, Meagher, 2000, Clemens et al., 2002, McGrath and Zhao, 2003). Metal concentrations in the shoots of accumulating plants can be 100–1000-fold higher than in non-accumulating plants (Peuke and Rennenberg, 2005a). Frequent generation of free radicals are taking place during heavy metal stress in accumulating plants and it leads to oxidative stress. Plants have been developed a good tolerance mechanisms against these heavy metal induced oxidative stress via significant synthesis of antioxidants and chelating compounds (Mishra et al., 2006, Seth et al., 2007, Seth et al., 2008). The transformation of toxic forms to less harmful forms is also an approach to detoxifying heavy metals, particularly As, Hg, Fe, Se and Cr, which exist in a variety of cationic and oxyanionic species and thio- and organo-metallic forms (Meagher, 2000, Guerinot and Salt, 2001).

In antioxidant system, plants have shown significant synthesis of various types of antioxidants subjected to metal stress, indicating a possible role in defense mechanisms (Cobbett, 2000, Mishra et al., 2006, Seth et al., 2007). The functional significance of a compound as antioxidant achieved through different mechanisms, such as metal chelation, activated oxygen species scavenging, recycling of other antioxidant, inhibition of lipid per oxidation and repair of damaged DNA molecules caused by oxidative stress (Allen, 1995, Seth et al., 2008). Among these, scavenging and/or removal of free radicals are the most likely mechanisms for antioxidants mediated tolerance strategy in plants (Seth,

2012). Several enzymatic antioxidants such as super oxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) are major types of reactive oxygen species (ROS)-scavenger that play significant roles in these mechanisms (Mishra et al., 2006). Other than enzymatic antioxidant, some non-enzymatic antioxidants such as cysteine, non-protein thiols (NP-SH), ascorbic acid and -GSH are also playing a very important role in plant resistance against oxidative stress (Seth et al., 2007, Hammond-Kosack and Jones, 1996).

Chelating peptides, most notably metallothioneins (MTs) and PCs, have a significant role in the detoxification of metals, and their synthesis in the plant is induced by exposure of root cells to heavy metals (Rauser, 1999, Cobbett and Goldsbrough, 2002, Cobbett, 2000, Hall, 2002). These cysteine-rich polypeptides exploit the property of heavy metals to bind to the thiol-groups of proteins - one of the toxic effects of heavy metals - for detoxification. Metallothioneins are S-rich proteins of 60-80 amino acids that contain 9-16 cysteine residues and are found in plants, animals and some prokaryotes (Cobbett and Goldsbrough, 2002, Cobbett, 2000, Rauser, 1999). Phytochelatins are a family of γ-glutamylcysteine oligopeptides with glycine or other amino acids at the carboxy-terminal end, in which γ-Glu-Cys units are repeated 2-11 times. They are synthesized from GSH and its derivates by phytochelatine synthase in the presence of heavy metal ions (Cobbett, 2000). It is reported that cells and tissues exposed to a range of heavy metal ions, such as Cd, Ni, Cu, Zn, Ag, Hg, and Pb are rapidly synthesized PCs, with the fact that Cd was the strongest inducer for PCs synthesis (Rauser, 1995, Yang and Yang, 2001, Pinto et al., 2003). The functional significance of PCs can be attributed due to the presence of thiol groups (-SH) which make co-ordination bond with toxic metal ions (Seth, 2012), which are then sequestered into the cellular vacuoles.

Sequestration of heavy metal ions in cellular vacuoles is an important detoxification/tolerance mechanism in metal hyperaccumulators (Tong et al., 2004). Complexation to low molecular weight organic chelators such as organic acids (malate, citrate), amino acids (O-acetylserine, histidine) and

nicotinamine have also shown to play significant roles in metal detoxification/tolerance in plants (Salt et al., 1995b, Clemens, 2001, Cobbett and Goldsbrough, 2002, Hall, 2002, Kraemer, 2003).

5. Phytoremediation of toxic metals using transgenic plants

The efficiency of a remediation strategies depends on many issues including biotic (e.g., the plant species concerned) and abiotic (e.g., bioavailability of the heavy metals, speciation of the heavy metals, physico-chemical conditions of the contaminated sites, level of contamination) factors. The abiotic factors can be changed to conditions favourable for a phytoremediation strategy. For example, bioavailability of heavy metals has been reported to be increased by chelating ligands (Blaylock et al., 1997, Evangelou et al., 2007). The main challenge of an effective phytoremediation strategy for the removal of heavy metals from contaminated sites is the choice of a potential plant species that has desirable characteristics such as fast growth rate, high above-ground biomass, widely distributed root systems, tolerance to the toxic effects of the target heavy metals, adaptive to the target sites, and easy to harvest (Adesodun et al., 2010, Sakakibara et al., 2011, Ali et al., 2013). It is difficult to find a plant species with all of these characteristics. However, some researchers proposed that the phytoextraction potential of a plant species should be determined mainly by two key factors – (i) shoot metal concentration and (ii) shoot biomass (Li et al., 2010). Other researchers proposed hyperaccumulation and hypertolerance as more important characteristics than shoot biomass for a phytoremediation strategy (Chaney et al., 1997).

Most scientific and commercial interest in phytoremediation now focuses on phytoextraction and phytodegradation, which use selected plant species grown on contaminated sites. In phytoextraction, the plant species are harvested to remove the plants together with the pollutants that have accumulated in their tissues. Two different approaches have been tested for phytoextraction of heavy metals (Robinson et al., 1998, Tlustoš et al., 2006).

1. The use of hyperaccumulating plant species. In this technique, I idea is to use hyperaccumulators that produce comparatively less aboveground biomass but accumulate high amount of the target heavy metals.
2. The use of high biomass producing plant species. This technique aims to use plant species which is not a hyperaccumulator but produce more aboveground biomass than the hyperaccumulators so that overall metal accumulation in the aboveground biomass is comparable to that of hyperaccumulators.

However, in selecting a plant species for phytoremediation based on different desired characteristics discussed above, it should be carefully considered that use of hyperaccumulators will yield a metal-rich, low-volume biomass, which is economical and easy to handle in case of both metal recovery and safe disposal. On the other hand, use of non-accumulators will yield a metal-poor, large-volume biomass, which will be uneconomical to process for recovery of metals and also costly to safely dispose. However, high biomass yielding plants are usually not hyperaccumulators. Use of genetically modified plants (GMPs) has been proposed to be a solution to overcome the limitations of fast growing non-hyperaccumulators (Rugh et al., 1998, Pilon-Smits and Pilon, 2002, Cunningham and Ow, 1996, Bennett et al., 2003).

Unlike plant growth, which depends on numerous genetic and non-genetic factors, the accumulation of heavy metals is controlled by only a few gene loci and is more easily accessible for genetic manipulation (Clemens et al., 2002). Therefore, phytoremediation strategies that have been put into consideration are the genetic manipulation of GSH and PC production in plant tissues (Song et al., 2003, Noctor et al., 1998, Cobbett, 2000, Yadav, 2010).

Initial experiments with transgenic plants have shown that they are indeed efficient in drawing metals from heavily contaminated soils (Rugh et al., 1998, Cherian and Oliveira, 2005, Tong et al., 2004). Trees are probably the best-suited plants for transgenic approaches to improve the heavy-metal accumulation. Tree biotechnology is thus becoming an increasingly important tool for the remediation

of contaminated environments (Peuke and Rennenberg, 2005b). Fast-growing trees, such as *Populus* spp., are good candidates for phytoremediation due to their extensive root systems, high rates of water uptake and transpiration that is helpful in efficient transport of contaminants from roots to shoot, rapid growth and large biomass production (Rugh et al., 1998, Taghavi et al., 2005). Poplars can be grown in a wide range of climatic conditions and are used with increasing frequency in 'short-rotation forestry' systems for pulp and paper production. This raises the possibility of using plantations of transgenic poplars across several multiyear cycles to remove heavy metals from contaminated soils (Robinson et al., 2000, Peuke and Rennenberg, 2005a). In addition, a dense tree cover would also prevent erosion and the spread of contaminated soil by wind. After the first planting, the costs for field management are relatively low and the products (biomass/wood) can be used for the production of electricity and heat by burning in wood power stations. Another important point is that it is very unlikely that poplars will enter the human food chain or end up as feedstock for animals.

The transformation of grey poplar trees (*Populus tremula* x *P. alba*) to overexpress γ-ECS from *Escherichia coli* resulted in higher levels of GSH and its precursor γ-L-glutamyl-L-cysteine compared with wild type (Noctor et al., 1998), and an elevated capacity for PC production. These new transgenic trees have been shown a high potential for the uptake and detoxification of heavy metals (Peuke and Rennenberg, 2005b). Results from preliminary trials showed that the transgenic poplars are genetically stable and there are no indications so far of any impact on the environment. The transgenic trees have a higher capacity than wild-type trees for accumulating heavy metals on the heavily contaminated sites (Peuke and Rennenberg, 2005a).

Despite this and other advantages, the progress and application of GMPs in phytoremediation technology to tackle widespread environmental contamination problems is being hampered by ideology-driven, restrictive legislation over the use and release of GMPs in Europe, and many other countries (Peuke and Rennenberg, 2005a). However, if genetic engineering is eventually successful in producing plants that are able to restore/remediate contaminated sites without any potential impact on

the environment in general and in particular on agriculture and human health, then we may also see a better public acceptance of GMPs in the future.

6. Phytoremediation of toxic metals by aquatic plants

Phytoremediation of the heavy metals can be achieved by aquatic plants since the process involves biosorption and bioaccumulation of the soluble and bioavailable metals from water (Brooks and Robinson, 1998). The aquatic plants can be floating, emergent, and submerged. The floating aquatic plants accumulate metals by their roots from water, while the submerged plants accumulate metals from the sediments by their roots and from the water by their shoots (Rahman and Hasegawa, 2011, Rahman et al., 2011).

Provably, Hutchinson (1975) reviewed, for the first time, the ability of aquatic macrophytes to concentrate elements from the aquatic environment and described that the levels of toxic elements in these plants were at least an order of magnitude higher than that in the supporting aqueous medium. Later on, Outridge and Noller (1991) reviewed the accumulation of toxic trace elements by aquatic vascular plants and discussed the pathways and rates of elemental uptake and excretion, environmental factors that control uptake of elements, and the significance of trace elements uptake for the field of wastewater treatment and biomonitoring of pollutants. To date, numerous papers have been published in leading international journals on different aspects of biogeochemistry, mechanisms and uptake of toxic metals by different aquatic macrophytes. The aim of these studies was to develop an efficient and cost-effective phytoremediation technology. A list of aquatic plants that have been studied for the phytoremediation of toxic metals is listed in Table 1.

Microspora and *Lemna minor* were studied for Pb and Ni phytoremediation (Axtell et al., 2003). Five common aquatic plant species (*Typha latifolia, Myriophyllum exalbescens, Potamogeton epihydrus, Sparganium angustifolium* and *Sparganium multipedunculatum*) were tested for Al phytoremediation (Gallon et al., 2004). Parrot feather (*Myriophyllum aquaticum*), creeping primrose

(*Ludwigina palustris*), and water mint (*Mentha aquatic*) have shown to remove Fe, Zn, Cu, and Hg from contaminated water effectively (Kamal et al., 2004). *L. minor* was reported to accumulate Cu and Cd from contaminated wastewater (Kara, 2004, Hou et al., 2007). The submerged aquatic plant *Myriophyllum spicatum* L. was found to be efficient for metal-contaminated industrial wastewater treatment (Lesage et al., 2007). The aquatic plants *Rorippa nasturtium-aquaticum* (L.) and *Mentha* spp. have been reported to accumulate significant amount of As from contaminated freshwater (Robinson et al., 2006). Based on the outcomes of many studies, aquatic plants have been used for the remediation of contaminated constructed wetlands.

6.1. Constructed wetlands for phytoremediation of toxic metals

Wetlands are often considered sinks for contaminants, and there are many cases in which wetland plants are utilized for removal of pollutants, including metals. Constructed wetlands offer a cost-effective and technically feasible method and have proven effective and successful in remediation of heavy metal pollution (Weis and Weis, 2004, Williams, 2002). Aquatic macrophytes have been shown to play important roles in wetland biogeochemistry through their active and passive circulation of elements including heavy metals (Weis and Weis, 2004). Active uptake into the wetland plant tissues may promote phytofiltration and immobilization of heavy metals in plant tissues, as seen in constructed wetlands for wastewater treatment and in the use of wetland plants in phytoremediation. Aquatic macrophytes are more suitable for restoration of water quality of wetlands due to their faster growth rate and relatively higher ability of pollutant uptake than terrestrial plants (Ali et al., 2013, Sood et al., 2012).

Water hyacinth (*Eichhornia crassipes*) has been one of the widely studied wetland plants for the phytoremediation of heavy metals in constructed wetlands (Jayaweera et al., 2007, Jayaweera et al., 2008, Zhu et al., 1999, Liao and Chang, 2004). It is a fast growing and easily adaptable to various

aquatic conditions floating plant with a well-developed fibrous root system and large biomass that can accumulate significant amount of heavy metals from water (Liao and Chang, 2004).

Cheng et al. (2002) investigated a twin-shaped constructed wetland comprising a vertical flow (inflow) chamber with *Cyperus alternifolius* followed by a reverse-vertical flow (outflow) chamber with *Villarsia exaltata* for phytoremediation of artificial wastewater polluted by heavy metals. Results showed that the system was very effective in removing toxic heavy metals from wastewater. From a field study with twelve emergent-rooted wetland plant species including different populations of *Leersia hexandra*, *Juncus effusus* and *Equisetum ramosisti*, Deng et al. (2004) proposed that these plants can be used in constructed wetlands for effective removal of toxic metals like Pb, Zn, Cu and Cd. Removal of Cu, Ni and Zn by *Phragmites australis* using a horizontal subsurface flow constructed wetland model for domestic wastewater treatment was studied by Galletti et al. (2010). Several other studies showed that engineered/constructed wetlands can be an effective model for phytoremediation of toxic metals (Liu et al., 2007, Liu et al., 2010, Rai, 2008a, Sobolewski, 1999, Yang and Ye, 2009, Zhang et al., 2010).

7. Conclusion

Over the past two decades, phytoremediation technology has become increasingly popular and has been employed to restore sites including soils and wetlands contaminated with toxic metals. While this technology has the advantages that environmental concerns may be treated without harming the ecosystems; one major disadvantage of phytoremediation is that it requires relatively longer time compared to traditional physical or chemicals methods as the process is dependent on a plant's ability to grow and thrive in an environment that is not ideal for normal plant growth. There are other limitations (listed below) of phytoremediation approaches for the restoration of contaminated soils and wetlands, which need to be considered for commercial application of this technology –

- Long operational time required for clean-up.

- Phytoremediation efficiency of most metal hyperaccumulators is usually limited by their slow growth rate and biomass production.
- Difficulty in mobilization (bioavailability) of tightly bound fraction of metal ions from soil.
- Phytoremediation is limited to the surface area and depth occupied by the roots of the hyperaccumulators.
- With plant-based systems of remediation, especially in the case of chemically-induced phytoremediation, it is not possible to completely prevent the leaching of contaminants into the groundwater.
- It is ideal for sites with low to moderate levels of metal contamination. In heavily contaminated sites, high concentrations of toxic metals can hamper the normal growth of the hyperaccumulators.
- The survival of the hyperaccumulating plants is affected by the toxicity of the contaminants and the general condition of the soil. Therefore, this approach may not be applicable an environment where the conditions are completely unfavorable for plant growth.
- There is a risk of food chain contamination in case of mismanagement and lack of proper care. Bioaccumulation of contaminants, especially the toxic metals, into primary producers (e.g., phytoplankton) can be passed on to the higher trophic levels of the food chain. Therefore, safe disposal of the used plants or organisms is required.

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Table 1: Aquatic plants studied for the phytoremediation of toxic elements from wetlands. This table was previously published in Rahman and Hasegawa (2011), and reused here with the permission of the publisher.

Common Name	Scientific Name	Trace Elements	References
Duckweed	*Lemna gibba* L.	As, U, Zn	(Mkandawire and Dudel, 2005, Mkandawire et al., 2004a, Mkandawire et al., 2004b, Fritioff and Greger, 2003)
Lesser duckweed	*Lemna minor* L.	As, Zn, Cu, Hg	(Alvarado et al., 2008, Fritioff and Greger, 2003, Kara, 2004, Miretzky et al., 2004, Mishra et al., 2008, Robinson et al., 2005)
Star duckweed	*Lemna trisulca* L.	Zn	(Huebert and Shay, 1992)
Water hyacinth	*Eichhornia crassipes*	As, Fe, Cu, Zn, Pb, Cd, Cr, Ni, Hg	(Alvarado et al., 2008, Vesk et al., 1999, Wolverton and McDonald, 1978, Chandra and Kulshreshtha, 2004, Cordes et al., 2000, Delgado et al., 1993, Dixit and Dhote, 2010, Espinoza-Quiñones et al., 2008, Junior et al., 2008, Mishra et al., 2008, Odjegba and Fasidi, 2007, Muramoto and Oki, 1983)
Water-starwort	*Callitriche cophocarpa*	Cr(V)	(Augustynowicz et al., 2010)
Petries starwort	*Callitriche petriei*	As	(Robinson et al., 2005)
Common reed	*Phragmites australis*	Cr, Cu, Ni, Pb, S, V, Zn, Cd	(Baldantoni et al., 2009, Deng et al., 2004, Ghassemzadeh et al., 2008)
Butterfly fern	*Salvinia rotundifolia*	Pb(II)	(Banerjee and Sarker, 1997, Dhir, 2009)
	Salvinia natans	As, Ni, Cu, Hg(II)	(Rahman et al., 2008c, Sen and Bhattacharyya, 1993, Sen and Mondal, 1987b, Sen and Mondal, 1990b)
	Salvinia minima	As, Pb, Cd, Cr	(Sanchez-Galvan et al., 2008, Hoffmann et al., 2004, Olguin et al., 2003)
	Salvinia herzogii	Cd, Cr	(Maine et al., 2004, Suñe et al., 2007)
Eared watermoss	*Salvinia auriculata*	Zn, Hg, Cr	(Wolff et al., 2009, Molisani et al., 2006, Espinoza-Quiñones et al., 2008)
Greater duckweed	*Spirodela intermedia*	Cu, Zn, Mn,Cr, Pb	(Miretzky et al., 2004)
	Spirodela polyrhiza L.	As, Hg	(Rahman et al., 2007, Rahman et al., 2008b, Mishra et al., 2008)
Indian/Sacred lotus	*Nelymbium speciosum*	Cr, Cu, Ba, Ti, Co, Pb	(Vardanyan and Ingole, 2006)
	Ludwigia perennis L.		(Vardanyan and Ingole, 2006)
Arrowhead	*Sagittaria sagittiflia* L.		(Vardanyan and Ingole, 2006)
	Nymphoides ceristatum		(Vardanyan and Ingole, 2006)
Shoreline seapurslane	*Sasuvium portulacastrum* L.		(Vardanyan and Ingole, 2006)
-	*Nymphae stellata*		(Vardanyan and Ingole, 2006)
Water spinach	*Ipomoea aquatica*	As, Cd, Pb, Hg, Cu, Zn	(Wang et al., 2008, Göthberg et al., 2002, Gothberg et al., 2004, Hu et al., 2008, Lee et al., 1991)
Eelgrass/Eelweed	*Vallisneria spiralis L.*	Cu, Cd, Hg	(Wang et al., 2010, Rai and Tripathi, 2009)

Esthwaite waterweed	*Hydrilla verticillata*	As, Pb, Zn, Cr	(Dixit and Dhote, 2010, Lee et al., 1991)
Mosquito fern	*Azolla caroliniana*	As	(Zhang et al., 2008)
Water fern	*Azolla filiculoides* *Azolla pinnata*	As, Hg, Cd	(Zhang et al., 2008, Rahman et al., 2008a, Rai and Tripathi, 2009, Rai, 2008b)
Elephant's ear	*Colocasia esculenta*	Cd, Cu, Pb, Zn	(Cardwell et al., 2002)
Umbrella sedge	*Cyperus eragrostis*		(Cardwell et al., 2002)
Spike rush	*Eleocharis equisitina*		(Cardwell et al., 2002)
Parrot's feather	*Myriophyllum aquaticum*		(Cardwell et al., 2002)
Miriophyllum	*Myriophyllum propinquum*	As	(Robinson et al., 2005)
Water lily	*Nymphaea violacea*	Cd, Cu, Pb, Zn	(Cardwell et al., 2002)
	Nymphaea aurora	Cd	(Schor-Fumbarov et al., 2003)
Marshwort	*Nymphoides germinata*	Cd, Cu, Pb, Zn	(Cardwell et al., 2002)
Knotweeds	*Persicaria attenuatum*		(Cardwell et al., 2002)
-	*Persicaria orientalis*		(Cardwell et al., 2002)
-	*Persicaria subsessilis*		(Cardwell et al., 2002)
-	*Potamogeton orchreatus*	As	(Robinson et al., 2005)
Willow smartweed	*Persicaria lapathifolium*	Cd, Cu, Pb, Zn	(Cardwell et al., 2002)
-	*Potamogeton javanicus*		(Cardwell et al., 2002)
Fennel pondweed	*Potamogeton pectinatus*	Cd, Pb, Cr, Ni, Zn, Cu	(Demirezen and Aksoy, 2004)
Curled dock	*Rumex crispus*	Cd, Cu, Pb, Zn	(Cardwell et al., 2002)
River clubrush	*Schoenoplectus validus*		(Cardwell et al., 2002)
Cumbungi	*Typha domingensis*		(Cardwell et al., 2002)
Cumbung	*Typha orientalis*		(Cardwell et al., 2002)
Lesser Bulrush	*Typha angustifolia*	Cd, Pb, Cr, Ni, Zn, Cu	(Chandra and Kulshreshtha, 2004, Demirezen and Aksoy, 2004)
Bulrush	*Typha latifolia*	Cr, As, Zn, Pb, Cd, Cu. Ni	(Chandra and Kulshreshtha, 2004, Ye et al., 1998, Blute et al., 2004, Ye et al., 1997, Deng et al., 2004, Hozhina et al., 2001, Pratas et al., 2007, Sasmaz et al., 2008)
Waterweed/Pondweed	*Elodea canadensis*	As, Pb, Cr, Zn, Cu, Cd	(Chandra and Kulshreshtha, 2004, Dogan et al., 2009, Fritioff and Greger, 2003, Mal et al., 2002, Mayes et al., 1977, Robinson et al., 2005)
Brazilian Waterweed	*Veronica aquatica*	As	(Robinson et al., 2005)
Water-milfoil	*Myriophyllum spicatum*	Co, Cr, Cu, Pb, Zn, Ni	(Chandra and Kulshreshtha, 2004, Keskinkan et al., 2003, Lesage et al., 2007)
Fragrant water lily	*Nymphaea odorata*	Cr	(Chandra and Kulshreshtha, 2004)
Pickerelweed	*Pontederia cordata*		(Chandra and Kulshreshtha, 2004)
Tape grass/Eel grass	*Vallisneria spiralis*	Hg	(Gupta and Chandra, 1998)
Wild celery	*Vallisneria americana*	Cr	(Chandra and Kulshreshtha, 2004)
-	*Nymphaea spontanea*	Cr(VI)	(Choo et al., 2006)

Shichito matgrass	*Cyperus malaccensis* Lam.	Pb, Zn, Cu, Cd	(Deng et al., 2004)
Swamp rice grass	*Leersia hexandra* Swartz.		(Deng et al., 2004)
Burma reed	*Neyraudia reynaudiana*		(Deng et al., 2004)
Flagroot	*Acorus calamus* L.		(Deng et al., 2004)
-	*Eleocharis valleculosa*		(Deng et al., 2004)
Water pepper	*Polygonum hydropiper*	As	(Robinson et al., 2005)
Reed canary grass	*Phalaris arundinacea* L.	Pb, Zn, Cu, Cd	(Deng et al., 2004)
-	*Equisetum ramosisti* Desf.		(Deng et al., 2004)
Soft rush	*Juncus effusus* L.		(Deng et al., 2004)
-	*Polypogon fugax* Steud.		(Deng et al., 2004)
-	*Egeria densa*	As	(Robinson et al., 2005)
Alligatorweed	*Althernanthera philoxeroides*	As, Pb	(Elayan, 1999)
Water lettuce	*Pistia stratiotes*	As, Cr, Pb, Ag, Cd, Cu, Hg, Ni, Zn	(Espinoza-Quiñones et al., 2008, Espinoza-Quiñones et al., 2009, Lee et al., 1991, Maine et al., 2004, Miretzky et al., 2004)
Floating pondweed	*Potamogeton natans*	Zn, Cu, Cd, Pb	(Fritioff and Greger, 2003, Fritioff and Greger, 2006)
Willow moss	*Fontinalis antipyretica*	Cu, Zn	(Goncalves and Boaventura, 1998) (Martins and Boaventura, 2002)
Needle spikerush	*Eleocharis acicularis*	As, In, Ag, Pb, Cu, Cd, Zn, Sb, Ni, Mg	(Ha et al., 2011, Ha et al., 2009a, Ha et al., 2009b)
Rigid hornwort	*Ceratophyllum demersum*	As, Pb, Zn, Cu	(Keskinkan et al., 2004, Robinson et al., 2005)
New Zealand watercress	*Lepidium sativum* L.	As	(Robinson et al., 2003)
-	*Najas indica*	Pb	(Singh et al., 2010)
Watercresses	*Nasturtium officinale*	Cu, Zn, Ni	(Kara, 2005)
Curly waterweed	*Lagarosiphon major*	As	(Robinson et al., 2005)