Phytoremediation of Heavy Metals: The Use of Green Approaches to Clean the Environment

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10.1 Introduction

The rapid development of industrialization results in overall environmental contamination with persistent organic and inorganic wastes (Chaudhry et al. 1998). Among these, heavy metals are playing a vital role in polluting the environment. Heavy metals are present in soils as natural components or as a result of human activity, for example mine tailings, metal smelting, electroplating, gas exhausts, energy and fuel production, downwash from power lines, severe agricultural practices, and sludge dumping pollute the soil with large quantities of toxic metals (Seaward and Richardson 1990; Förstner 1995; Kumar et al. 1995; Srivastava 2007). A list of sources causing heavy metal pollution is shown in Table 10.1. These heavy metals have a relatively high density and are toxic or poisonous at low concentrations (Lenntech 2004; Duruibe et al. 2007). Heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). Industries such as mining, petroleum, coal, and garbage burning create heavy metal pollution in the environment, which cannot be easily degraded or destroyed. In a very small amount they enter our bodies via food, drinking water, and air (Duruibe et al. 2007). As a trace element, some heavy metals are needed in small concentration to maintain the metabolism of the human body (Garbisu and Alkorta 2003). However, at higher concentrations they can lead to poisoning (Alkorta et al. 2004). Heavy metals such as lead and mercury are never desirable in any amount in our body. Elevated levels of mercury can cause various health problems (Clarkson 1992). Mercury is a toxic heavy metal which has no known function in human biochemistry or physiology and does not occur naturally in living organisms (Ferner 2001; Nolan 2003; Young 2005; Duruibe et al. 2007). Monomethylmercury has detrimental effects on brain and the central nervous system in humans. However, fetal and postnatal exposures of this form of mercury resulted in abortion, congenital malformation, and other abnormalities in children. However, cadmium is a bio-persistent heavy metal, which once absorbed by an organism is deposited in the body for many years, as far as over decades for humans. In humans and animals, its excessive exposure leads to renal disfunction, lung diseases, bone defects, etc. (Levine and Muenke 1991; Gilbert-Barness 2010).

Various physicochemical methods have been applied to clean up the heavy metals from the environment but these methods are very expensive and cost-effective. Moreover, these methods when applied to the soil are of high impact but are detrimental to soil texture and fertility (Negri and Hinchman 1996; Chaudhry et al. 1998). Heavy metals are only transformed from one oxidation state or organic complex to another. Microorganisms can be used for the bioremediation of metals as they reduce metals in their detoxification mechanism (Garbisu and Alkorta 2001; Edwards et al. 2013).

Phytoremediation can prove to be an important strategy for the removal of the heavy metal from the environment. It is the study of using green plants for the removal of harmful environmental contaminants. This new technology offers a potentially cost-effective cleanup of contaminated groundwater, terrestrial soil, sediments, sludge, etc. Various studies have been carried out for the removal of the heavy metals from the contaminated soils by using phytoremediation strategies (Table 10.2) The purpose of this chapter is to explore the use of a new technology to remove heavy metals from those environments, where it is concentrated. Its toxicity has been enhanced and its mobility into sensitive organisms increased with the increase in heavy metal pollution in the environment. The present

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Table 10.1 Heavy metals and their sources of contamination

Sr. No.	Heavy metals	Sources	References
1	As	Timber treatment, paints and pesticides, semiconductors, petroleum refining, wood preservatives, animal feed additives, coal power plants, volcanoes, mining, and smelting	(Bissen and Frimmel 2003; Walsh et al. 1979)
2	Cu	Timber treatment, fertilizers, fungicides, electroplating industry, smelting and refining, mining, biosolids	(Liu et al. 2005)
3	Cd	Anthropogenic activities, smelting and refining, fossil fuel burning, application of phosphate fertilizers, sewage sludge	(Alloway 1995; Kabata-Pendias 2001)
4	Pb	Batteries, metal products, 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)
5	Cr	Timber treatment, leather tanning, pesticides and dyes, electroplating industry	(Knox et al. 1999; Gowd et al. 2010)
6	Hg	Fumigants and fertilizers, volcano eruptions, forest fire, emissions from industries producing caustic soda, coal, peat, and wood burning	(Lindqvist 1991)
7	Zn	Dyes, paints, timber treatment, fertilizers and mine tailings, electroplating industry, smelting and refining, mining	(Liu et al. 2005)
8	Ni	Alloys, batteries and mine tailings, volcanic eruptions, land fill, forest fire, bubble bursting, and gas exchange in ocean	(Knox et al. 1999)
9	Cd, Pb, and As	Over application of fertilizers and pesticides	(Atafar et al. 2010)
10	Pb	Commercial organic fertilizer	(Wang et al. 2013)
11	Cd, Cu, Ni, and Zn	Urban and industrial wastewater used in agricultural practices	(Hani and Pazira 2011)

Table 10.2 Plants used for phytoremediation of heavy metal contamination

Sr. No.	Plants used	Contaminants	References
1	*Trifolium alexandrinum*	Cd, Pb, Cu, and Zn	(Ali et al. 2012)
2	*Tithonia diversifolia* and *Helianthus annuus*	Pb and Zn	(Adesodun et al. 2010)
3	*Thlaspi caerulescens*	Zn, Cd, and Ni	(Assunção et al. 2003)
4	*Pteris cretica cv Mayii* (Moonlight fern) and *Pteris vittata* (Chinese brake fern)	As	(Baldwin and Butcher 2007)
5	*Alyssum* and *Thlaspi*	Ni	(Bani et al. 2010)
6	*Aspalathus linearis* (Rooibos tea)	Aluminum	(Kanu Sheku et al. 2013)
7	*Helianthus annuus* (sunflower)	Zinc and cadmium	(Marques et al. 2013)
8	*Pelargonium roseum*	(Ni), cadmium (Cd), or lead (Pb)	(Mahdieh et al. 2013)
9	*Brassica napus* and *Raphanus Sativus*	Cd, Cr, Cu, Ni, Pb, and Zn	(Marchiol et al. 2004)
10	*Thlaspi caerulescens*	Cd and Zn	(Perronnet et al. 2003)
11	*Pteris vittate* L.	Arsenic	(Ma et al. 2001; Tu et al. 2002)
12	*Solanum nigrum*	Cd	(Chen et al. 2014)

technology of phytoremediation is centered on plants that have been genetically engineered with bacterial genes. These genetically engineered plants having these genes encode enzymes that catalyze the alteration of heavy metal electrochemical form especially in case of mercury. This new strategy is intended to allow the detoxification and controlled translocation of mercury from locations where it may threaten human health or the integrity of ecosystems. It has been predicted that as the field of genetic engineering advances, engineered organisms will replace mechanical tools for many applications, including in the remediation of environmental pollution. These "clean technologies" will result in reductions to the release of toxic substances so inexorably linked to industrial processes yet so toxic to organisms.

10.2 Processes of Phytoremediation

There are five different types of major processes involved in phytoremediation. These include phytoextraction, rhizofiltration, phytovolatilization, phytostabilization, and phytodegradation. A short overview of all these process can be seen in Fig. 10.1.

10.2.1 Phytoextraction

Phytoextraction is the use of plants to uptake contaminants into their biomass. In this process plants uptake the contaminants by roots and accumulate in the aerial parts or shoots of

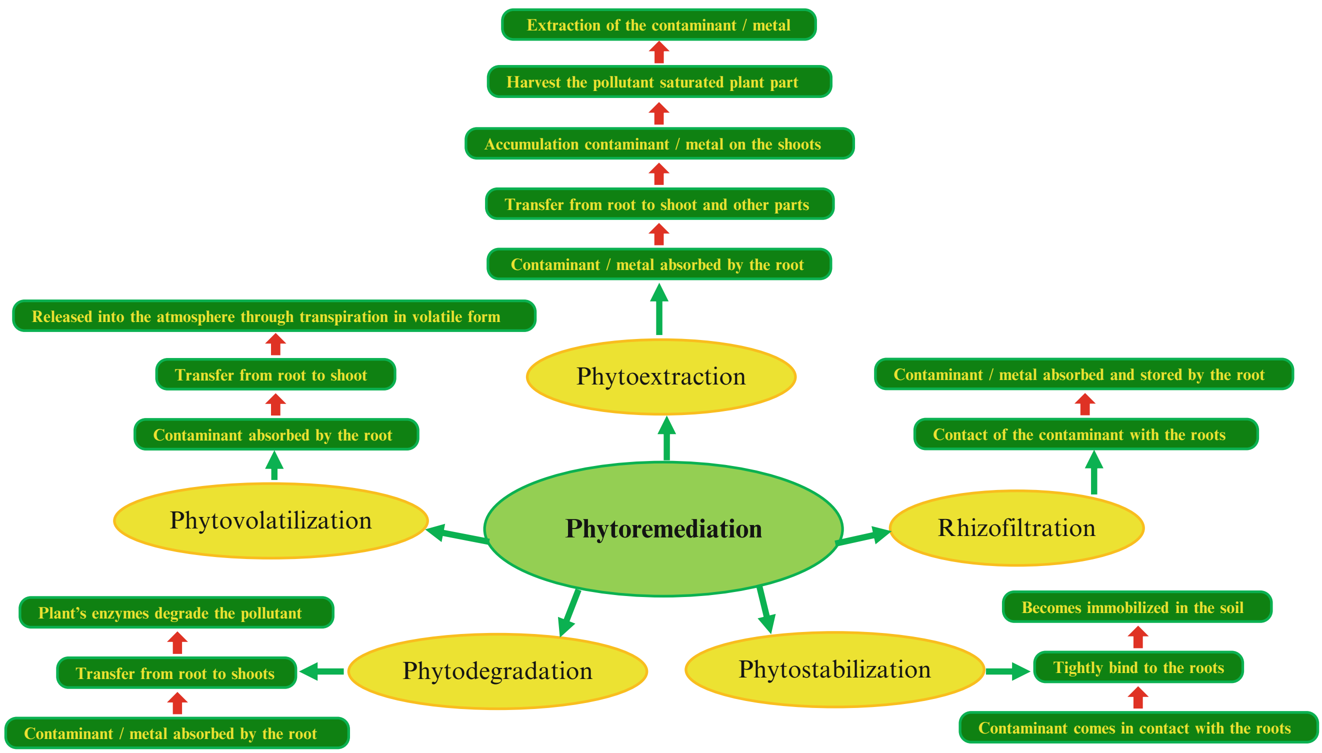

Fig. 10.1 Different processes of phytoremediation

the plant and finally it is harvested and disposed of (Vishnoi and Srivastava 2007). The plant-based remediation technology is one of the largest technologies to remediate the heavy metal pollution from the environment (Raskin et al. 1997). Phytoextraction can be natural and induced. In natural phytoextraction there is low biomass of hyperaccumulators and it may require decades to reduce the heavy metal concentration in soil, e.g., *Thlaspi caerulescens* (Mahmood 2010). In induced phytoextraction there is high-biomass of hypoaccumulators. Metal hyperaccumulation is triggered through soil amendments that increase the metal phyto availability and translocation from root to shoot e.g., sunflower, ryegrass, and various species of *Brassica* (Salt et al. 1995a). All plant species cannot be used for phytoremediation. Only the hyperaccumulating plants can be used for metal remediation. A plant that is able to take up more metals than normal plants is called a hyperaccumulator, which can absorb more heavy metals that are present in the contaminated soil. This process helps in the reduction of erosion and leaching of the soil. With successive cropping and harvesting, the levels of contaminants in the soil can be reduced (Vandenhove et al. 2001). Various studies emphasized to estimate the metal accumulation capacity of high-biomass plants that can be easily cultivated using agronomic practices. Particularly, on the evaluation of shoot metal-accumulation capacity of the cultivated *Brassica* (mustard) species. Certain varieties of *Brassica juncea* concentrated toxic heavy metals (Pb, Cu, and Ni) to a level up to several percent of their dried shoot biomass (Kumar et al. 1995). *Zea mays* and *Ambrosia artemisiifilia* were also identified as good accumulators of Pb (Huang and Cunningham 1996; Raskin et al. 1997).

A major setback to the improvement of phytoextraction technology is that the shoot metal accumulation in the hydroponically cultivated plants greatly exceeded the metal accumulation. This phenomenon is explained by the low bioavailability of heavy metals in soils (Cunningham et al. 1995). *Trifolium alexandrinum* effectively extracted the selected heavy metals from the simulated heavy metal-contaminated soil, as evident from the difference of heavy metal concentration values between control and experimental plants. *T. alexandrinum* has many advantages for phytoremediation. It produces considerable biomass and has a relatively short life cycle. It has resistance to prevailing environmental and climatic conditions and above all offers multiple harvests in a single growth period (Ali et al. 2012). Effect of EDTA on phytoremediation has also been studied. The seedling of *Brassica napus* was able to accumulate large quantity of heavy metals in the presence of EDTA. EDTA enhances shoot metals accumulation but does not affect plant growth (Zaier et al. 2010). Phytoextraction can be improved by inoculating some plant-growth beneficial bacterium *Phyllobacterium myrsinacearum* RC6b with plants, e.g., the plant species *Sedum plumbizincicola* affects plant growth and enhances Cd, Zn, and Pb uptake (Ma et al. 2013). They suggested that the metal mobilizing can be improved by using inoculants such as *P. myrsinacearum* RC6b for the multimetal polluted soils. Similarly, the biomass production and shoot Ni concentrations in *Alyssum serpyllifolium* subsp. *malacitanum* was found to be higher when inoculated with two bacterial strains LA44 and SBA82 of *Arthrobacter* than non-inoculated plants (Becerra-Castro et al. 2013). The phytoextraction efficiency can also be affected by fungicidal sprays, soil pH, planting density, and cropping period (Puschenreiter et al. 2013; Simmons et al. 2014).

Roots play a major role in drawing out elements from the soil and deliver to the shoots (Raskin et al. 1997). Scanty information is available on the mechanisms of mobilization, uptake, and transport of most environmentally hazardous heavy metals, such as Pb, Cd, Cu, Zn, U, Sr, and Cs. Before the plant accumulates metals from the environment, it must be mobilized into the environment. Various factors are involved in the mechanism of phytoextraction.

10.2.1.1 Phytoavailability of Metals

The first step of phytoextraction is the phytoavailability of metals in soil. The bioavalability of metals is increased in soil through several means (Ghosh et al. 2011). There are some factors involved by which the plant uptakes the heavy metals from soil (a) quantity factor (the total content of the potentially available metals in soil), (b) the intensity factor (the activity and ionic ratios of metals in the soil solution), and (c) reaction kinetics (the rate of transfer from soil to the liquid phase to the plant roots) (Brümmer et al. 1986). These metals make a complex structure with the soil. Several approaches have been studied and are accomplished in a number of ways. To chelate and solubilize the soil-bound metal some metal-chelating molecules can be secreted into the rhizosphere. Phytosiderophores are iron-chelating compounds, which have also been studied well in plants (Kinnersely 1993). Based on their ability, the phytosiderophores can chelate other heavy metals also other than iron (Meda et al. 2007). These phytosiderophores are released in response to iron deficiency and can mobilize Cu, Zn, and Mn from soil. The Cu toxicity in barley is a signal that activates phytosiderophores release by plant roots, whereas, phytosiderophores release is induced by Cu toxicity which is strongly attenuated by Cd toxicity (Kudo et al. 2013). Metal-chelating proteins called metallothioneins (Robinson et al. 1993) may also function as siderophores in plants. Certain metals induce the synthesis of these proteins in the plant cells. Metallothioneins can tightly bind with zinc, copper, cadmium, mercury, or silver reducing the availability of diffusible forms within the cells and therefore decreasing their toxic potential (Cherian and Goyer 1978). The contribution of phytosiderophores in toxic metal possession by the roots of phytoextracting plants remains largely unexplored. A Ni hyperaccumulator, *Alyssum lesbiacum*, uses histidine, an excellent Ni chelator, to acquire and transport Ni (Kramer et al. 1996). Phytosiderophores such as mugineic acid and avenic acid (which are exuded from roots of graminaceous plants in response to Fe and Zn deficiency) can mobilize Cu, Zn, and Mn (Römheld 1991). The Cd and P phytoavailability of kangkong (*Ipomoea aquatica* Forsk.) with Alfred stonecrop (*Sedum alfredii* Hance) can be induced while inoculated with arbuscular mycorrhizal fungi (Hu et al. 2013).

10.2.1.2 Uptake of Metals by the Roots

The absorption of metals into roots can occur by means of symplastic and apoplastic pathways (Tandy et al. 2006; Lu et al. 2009). In contrast to the apoplastic pathway in which metal ions or metal–chelate complex enters the root through intercellular spaces, the symplastic pathway is an energy-dependent process mediated by specific or generic metal ion carriers or channels. Plant roots can solubilize soil-bound toxic metals by acidifying their soil environment with protons extruded from the roots. A similar mechanism has been observed for Fe mobilization in some Fe-deficient dicotyledonous plants (Crowley et al. 1991). The soil-bound metal ions are reduced by the roots by some enzymes known as reductases bound to the plasma membrane results in the metal availability. For example in case of a pea plant deficient in Fe or Cu, it shows an increased capability to reduce Fe^{3+} and Cu^{2+} and which later on fastens increase in uptake of Cu, Mn, Fe, and Mg from the soil (Welch et al. 1993). The mycorrhizal fungi associated with the roots and root-colonizing bacteria also shows increase in the bioavailability of metals. It is believed that the rhizospheric microorganisms help the plant to uptake the mineral nutrients such as Fe (Crowley et al. 1991), Mn (Barber and Lee 1974), and Cd (Salt et al. 1995b). In a recent report by Lindblom et al. (2014), two rhizosphere fungi *Alternaria seleniiphila* (A1) and *Aspergillus leporis* (AS117) inoculated with selenium (Se) hyperaccumulator *Stanleya pinnata* and non-hyperaccumulator *Stanleya elata* were studied. They concluded that rhizosphere fungi affect the growth and Se and/or S accumulation in these plant species. But some metal ions such as Ca^{2+} and Mg^{2+} that are present at higher concentrations in the soil solution do not require mobilization as they enter the roots through any of the extracellular (apoplastic) or intracellular (symplastic) pathways (Clarkson and Luttge 1989). Recently, a new Mn-hyperaccumulating plant species *Celosia argentea* Linn. has been reported (Liu et al. 2014), which shows higher Mn accumulatation and tolerance level. They found that as the Mn supply level ranged from 2.5 (control) to 400 mg/L, the biomass and the relative growth rate of *C. argentea* were insignificantly changed. In one of the study conducted by Foster and Miklavcic (2014) on the uptake and transport of ions via differentiated root tissues a physical model was proposed. This model indicates both the forced diffusion and convection by the transpiration stream.

The reducing diffusive permeabilities result in altering ion concentration profiles in the pericycle and vascular cylinder regions. However, the increased convective reflectivities affect predominantly ion concentrations in the cortex and endodermis tissues. They concluded that the ion fluxes and accumulation rates are predicted by the self-consistent electric field that arises from ion separation.

10.2.1.3 Transportion from Root to Shoot

In non-hyperaccumulator plants the metal is generally stored within the root cells and is not available for the xylem loading. Whereas, in case of hyperaccumulators roots efficiently transport metals to the shoots, e.g., in case of *Sedum alfredii* ecotype, xylem plays an important role in Cd uptake as compared to the non-hyperaccumulating ecotypes (Lu et al. 2009). The translocation of Cd uptake and Cd phytoextraction has been recently studied by Hu et al. (2013) in another species of *Sedum plumbizincicola*. In this study they found that the rate of Cd uptake was more from roots to the shoots when NO^{3-} treatment was given. For the translocation of metals from roots to shoot via xylem, firstly, they must have to cross the Casparian band on endodermis, which is a water-impervious barrier that blocks the apoplastic flux of metals from the root cortex to the stele. Therefore, to cross this barrier and to reach the xylem, metals must move symplastically. The xylem loading process is mediated by membrane transport proteins (Huang and Van Steveninck 1989; Clemens et al. 2002). However, in metal accumulators, xylem loading as well as translocation to shoot is facilitated by complexing of metal with low-molecular weight chelators (LMWCs), e.g., organic acids (Senden et al. 1995), phytochelatins (Przemeck and Haase 1991), and histidine (Krämer et al. 1996). The metal translocation patterns of important heavy metals such as Cr, Ni, Cu, Cd, and Pb in plant species *Solanum melongena* has recently been studied by Wiseman et al. (2014). They examined tissue patterns of metal (Cr, Ni, Cu, Cd, and Pb) concentrations associated with elemental deposition and soil-to-root and root-to-shoot transfers. They concluded that copper easily translocates to roots in waterlogging soils as compared to Cd which has highest soil-to-root and root-to-shoot translocation. Metal chelators and transporters regulate metal homeostasis in plants. Studies have been carried out on HMA2 gene characterization from various plants for their potential application in phytoremediation. The membrane transporter protein helps the plants to become metal-resistant and metal-hyperaccumulator. Whereas, the other gene HMA3 contributes towards metal detoxification by Cd sequestration into the vacuole and the HMA4 gene triggers the process of metal hyperaccumulation. Both the genes HMA2 and HMA4 play an important role during root-to-shoot metal translocation (Park and Ahn 2014). The uptake of gold nanoparticles (AuNPs) followed by translocation and transport into plant cells in case of poplar plants (*Populus deltoides* × *nigra*, DN-34) has been recently studied and found that these gold nanoparticles accumulated in the plasmodesma of the phloem complex in root cells (Zhai et al. 2014).

10.2.1.4 Metal Unloading, Trafficking, and Storage in Leaves

Metal is transported to the apoplast (free diffusional space outside the plasma membrane) of leaves from where it is distributed within the leaf tissue via apoplast or transporters-mediated uptake by symplast (inner side of the plasma membrane in which water (and low-molecular-weight solutes) can freely diffuse) (Mahmood 2010). At any point of the transport pathway metals make a complex with organic ligands and thus the metal converts into a less toxic form (Peers et al. 2005). Metals are sequestered in extracellular or subcellular compartments of the leaves. About 35 % of the Cd taken up by *T. caerulescens* was found in the cell walls and the apoplast in leaves (Cosio et al. 2005), whereas in Ni hyperaccumulator *Thlaspi geosingense*, Ni is sequestered in the cell wall as well as in vacuoles (Krämer et al. 2000; Mahmood 2010). Leaf trichomes may be the major sequestering sites for Cd in *Brassica juncea* (Salt et al. 1995b); for Ni in *Alyssum lesbiacum* (Krämer et al. 1997); and for Zn in *Arabidopsis halleri* (Küpper et al. 2000). Different approaches have been envisaged by Clemens et al. (2002) for engineering the plant metal homeostasis network to increase the metal accumulation in plants. For example, keeping in view the importance of vacuoles as the metal storage organelle, engineering tonoplast transporters in specific cell types might enhance the metal accumulation capability. Alternatively, creation of artificial metal sinks in shoots via expression of the cell wall proteins with high-affinity metal binding sites might be explored to increase the metal demand in shoots thus enhancing the accumulation in leaves (Clemens et al. 2002). Metal translocation can also be affected by fertilizer treatment. While working on cadmium translocation in *Oryza sativa* Sebastian and Prasad (2014) they found that ammonium phosphate–sulfur fertilization affects the shoot growth. Due to fertilizer treatment an increase in photosynthetic pigments was recorded that altered the activity of antioxidant enzymes which ultimately results in steady photosynthetic rate.

The molecular mechanisms for heavy metal adaptation has been well studied in some model plants such as *A. halleri* or *Thlaspi/Noccaea* spp. (Becher et al. 2004; Dräger et al. 2004; Hanikenne et al. 2008; Plessl et al. 2010; van de Mortel et al. 2006). A network of transporters tightly controls uptake into roots, xylem loading, and vacuolar sequestration (Broadley et al. 2007; Verbruggen et al. 2009). Although these transporters are thought to balance the concentration of essential metals such as Zn, they also unselectively transport

Table 10.3 Plant species used for the rhizofiltration of heavy metal contaminants

Sr. No.	Contaminants	Plant species	References
1	Cd and Pb	*Brassica juncea*	(Qiu et al. 2014)
2	Sb	*Cynodon dactylon*	(Xue et al. 2014)
3	Pb	*Oxycaryum cubense* (Poep. & Kunth) Palla	(Alves et al. 2014)
4	Pb	*Azolla pinnata*	(Thayaparan et al. 2013)
5	Al, Fe, and Mn	*Pistia stratiotes* L.	(Veselý et al. 2012)
6	As	*Cynara cardunculus*	(Llugany et al. 2012)
7	Pb	*Carex pendula*	(Yadav et al. 2011)
8	Cd	*Setaria italica* (L.) Beauv.	(Chiang et al. 2011)
9	Cd and Pb	*Pistia stratiotes* L., *Salvinia auriculata* Aubl., *Salvinia minima* Baker, and *Azolla filiculoides* Lam	(Veselý et al. 2011)
10	Cu, Ni, and Zn	*Eichhornia crassipes* (Mart.) Solms	(Hammad 2011)
11	Al, Fe, Zn, and Pb	*Typha domingensis*	(Hegazy et al. 2011)
12	Mn	*Cnidoscolus multilobus*, *Platanus mexicana*, *Solanum diversifolium*, *Asclepius curassavica* L., and *Pluchea sympitifolia*	(Juárez-Santillán et al. 2010)
13	U (Uranium)	*Helianthus annuus* L. and *Phaseolus vulgaris* L. var. *vulgaris*	(Lee and Yang 2010)

toxic elements such as Cd (Mendoza-Cozatl et al. 2011; Verbruggen et al. 2009). Inside the cells, metals are chelated with small molecules such as the low molecular weight, cysteine-rich metallothioneins or non-translationally synthesized, glutathione-derived phytochelatins (Cobbett and Goldsbrough 2002). Remarkable similarity in copy number expansion and transcriptional regulation was found for the xylem loading transporter HEAVY METAL ATPASE 4 (HMA4) in *A. halleri* and *N. caerulescens*, indicating parallel evolutionary pathways in these two Brassicaceae species (Hanikenne et al. 2008; Ó Lochlainn et al. 2011). Moreover, HMA4 was recently found to be involved in maintenance of Zn homeostasis also in poplar (Adams et al. 2011). This example of cross-species functionality suggests that well-studied pathways might also act in *S. caprea* metal tolerance.

10.2.2 Rhizofiltration

Plant roots absorb or adsorb, concentrate, and precipitate toxic metals from contaminated sites (waste water, surface water). Both terrestrial and aquatic plants show these type of activities (Yadav et al. 2011). In rhizofiltration it is the root system of plants that interacts with the contaminants or polluted site for making that area pollution free (Krishna et al. 2012). It is a potential technique for the removal of wide range of organic and inorganic contaminants, and it also reduces the bioavailability of the contaminant in the food chain. During the rhizofiltration, the contaminant remains on/within the root. The different plant species that have been used for rhizofiltration so far are listed in Table 10.3. These contaminants are to be taken up and translocated into other portions of the plant by the roots, which depends on the contaminant, its concentration, and the plant species. This mechanism is supported by the synthesis of certain chemicals within the roots, which cause heavy metals to rise in plant body. The precipitation of the metals/contaminants on the root surfaces is due to the presence of some internal factors within the soil such as root exudates and pH (Day et al. 2010; Krishna et al. 2012). As the plants absorb metals contaminants from the soil, roots or whole plants are harvested for disposal (Prasad and Freitas 2003). Various exudates such as simple phenolics and other organic acids are released during root decay, which results in change of metal speciation (Ernst 1996). This leads to the increased precipitation of the metals. The organic compounds in the root exudates can stimulate microbial growth in the rhizosphere (Pivetz 2001). Genes plays an important role in the plants to make it efficient for metal accumulation. For example glutathione and organic acids metabolism pathways play a key role in making the plant metal tolerant. Other environmental factors such as light, temperature, and pH also affect metal accumulation efficiency (Rawat et al. 2012). Rhizofiltration can be done in situ i.e. in surface water bodies and ex situ by means of engineered tanks having system of contaminated water and the plants. Both the systems require an understanding of the contaminant speciation and interactions of all contaminants and nutrients (Terry and Banuelos 2000; Akpor et al. 2014). The hydroponically cultivated roots of terrestrial plants are found to be more effective than the normal plant-based systems. For an ideal rhizofiltration mechanism, a plant should have rapidly growing roots that have the ability to remediate toxic metals in soluble form. For example some varieties of sunflower and *B. juncea* have high efficiency for rhizofiltration (Dushenkov et al. 1995). For the improvement of the rhizofiltration, attempts have been made to grow young plant seedlings in aquaculture for removing heavy metals. From the last few years studies have been conducted on the ability of plant roots to tolerate, remove, and degrade pollutants. The roots degrade the contaminants by releasing root exudates and some oxidoreductive enzymes such as peroxidases and laccases (Agostini et al. 2013). Due to the root's environmental compatibility and cost-effectiveness it has great potential to remediate contaminated soils and groundwater. So, research has been carried out to develop genetically engineered roots for the remediation of the polluted sites especially organic pollutants and heavy metals. By using this technology, hairy roots can be produced to increase the phytoremediation efficiency. However, with the help of the rhizospheric bacteria this efficiency can be used to improve more tolerance level to pollutants (Zhou et al. 2013). Recently, Al-Shalabi and Doran (2013) has studied hairy root efficiency for hyperaccumulation of Cd and Ni in plants.

10.2.3 Phytovolatilization

It involves the use of plants to uptake the contaminants from the soil and transforming them into volatile form and released into the atmosphere through transpiration (Ghosh and Singh 2005). Plants take up organic and inorganic contaminants with water and pass on to the leaves and volatilize into the atmosphere (Mueller et al. 1999). Mercury is the first metal that has been removed by phytovolatilization. The mercuric ion is transformed into less toxic elemental mercury (Henry 2000). Transgenic technology has been applied by inserting an altered mercuric ion reductase gene (*merA*) into *Arabidopsis thaliana*, for the production of a mercury-resistant transgenic plants that volatilized mercury into the atmosphere (Rugh et al. 1996). Some of the other toxic metals such as Se, As, and Hg can be biomethylated to form volatile molecules and liberated into the atmosphere. Phytovolatilization has also been done by using plant–microbe interactions for the volatilization of Se from soils (Karlson and Frankenberger 1989). *Brassica juncea* has been identified as an efficient plant for removal of Se from soils (Bauelos and Meek 1990). The plant species *Pteris vittata* L. (Chinese Brake fern) has been reported as an arsenic (As) hyperaccumulator that can also accumulate a large amount of Se. Some anti-oxidative enzymes such as catalase, ascorbate peroxidase, and peroxidase contribute towards hyperaccumulation of Se (Feng and Wei 2012). Some chemicals such as organochlorines (OCs), 1,4-dichlorobenzene (DCB), 1,2,4-trichlorobenzene (TCB), and γ-hexachlorocyclohexane (γHCH) are persistent chemicals in the environment. Their uptake depends mostly on their hydrophobicity, solubility, and volatility. The uptake of organochlorines (OCs) has been studied in *Phragmites australis* under hydroponic conditions (San Miguel et al. 2013).

Studies have been carried out also on some other volatile organic compounds (VOCs) such as 1,4-dioxane. It has been found that dioxane (2.5 μg/L) was effectively removed by using phytovolatilization (Ferro et al. 2013).

10.2.4 Phytostabilization

Phytostabilization is the process in which plants immobilize the contaminants in the soil or ground water using absorption, adsorption onto the surface of the roots, or by the formation of insoluble compounds. This process reduces the mobility of contaminants and ultimately prevents their migration into the groundwater or into the air (Soudek et al. 2012). It depends on the ability of the roots to limit contaminant's mobility in the soil (Berti and Cunningham 2000). It decreases the amount of water percolation through the soil matrix, forms hazardous leachate. It helps in preventing soil erosion and prevents spreading of toxic metal to other areas. It is not a process of removal of metal contaminants from the sites, but more the stabilization and reduction of the contamination. For an efficient phytostabilization system a plant needs a dense root system (Cunningham and Ow 1996). *Sorghum bicolor* L is one of the plant species which is able to accumulate large quantities of metals in shoots grown in hydroponic conditions. Heavy metals such as Cd and Zn were found to be accumulated primarily in roots. But as the concentration of the metals increased in the solution their transfer to the shoots increased (Soudek et al. 2012). Similarly, in copper-contaminated soil, *Oenothera glazioviana* had high tolerance to copper and shows low upward transportation capacity of copper. Therefore, this plant has a great potential for the phytostabilization of copper from the copper-contaminated soils and a high commercial value without risk to human health (Guo et al. 2014). Other plants such as *Sesbania virgata* have also been reported as excellent phytostabilizers for metals such as copper, zinc, and chromium from the metal-contaminated soils. The main accumulation of heavy metals appeared in plant roots, and more Zn is removed from soils. When supplied in a mixture of Cu and Zn, *Sesbania* plants absorb the highest concentrations of these metals. In contrast, Cr was more absorbed in the individual treatments (Branzini et al. 2012). In one of the study conducted on *B. juncea* by Pérez-Esteban et al. (2013), phytostabilization ability can be enhanced by the addition of manure in the contaminated soil.

10.2.5 Phytodegradation

Phytodegradation is the uptake and degradation of contaminants within the plant, or the degradation of contaminants in the soil, ground water, or surface water, by enzymes. This process involves the use of plants with associated microorganisms to degrade organic pollutants, such as 2,4,6-trinitrotoluene (TNT) and polychlorinated biphenyls, herbicides, and pesticides so that they can be converted from toxic form to nontoxic form (Lee 2013; Kukreja and Goutam 2013). Hybrid poplars are capable of degrading trichloroethylene, which is one of the most common pollutants (Newman et al. 1997). Some enzymes such as dehalogenase, peroxidase, nitroreductase, laccase, and nitrilase produced by the plants also helps in degradation of pollutants (Schnoor et al. 1995; Morikawa and Erkin 2003; Boyajian and Carreira 1997). Kagalkar et al. (2011) biodegrades the triphenylmethane dye Malachite Green by using cell suspension cultures of *Blumea malcolmii* Hook. This degradation was occurred due to the induction of enzymes such as laccase, veratryl alcohol oxidase, and DCIP reductase. The textile dye Red RB and Black B has also been achieved by using water hyacinth (*Eichhornia crassipes*) (Muthunarayanan et al. 2011). Plants such as *Hydrilla verticillata* and *Myriophyllum verticillatum* are efficient in degrading chemical contaminants such as bisphenol A(BPA) within the concentration of 1–20 mg/L (Zhang et al. 2011). Recently it has been reported that some chemical contaminants such as polycyclic aromatic hydrocarbons (PAHs), which are present in the terrestrial environment can be degraded by using a water hyacinth (*Eichhornia crassipes*) in combination with some chemicals such as sodium sulfate (Na_2SO_4), sodium nitrate ($NaNO_3$), and sodium phosphate (Na_3PO_4) (Ukiwe et al. 2013). They resulted that 99.4 % (pH 2.0) of acenaphthrene and 90.4 % (pH 4.0) of acenaphthrene was degraded after using $NaNO_3$ and Na_2SO_4 with *E. crassipes*, respectively.

10.3 Improvement of Phytoremediation Efficiency of Plants

10.3.1 Plant–Microbe Interactions to Enhance the Phytoremediation Efficiency of Plants

As the microbes are the first organisms which come in contact with the contaminated sites therefore they have to develop their own mechanism to grow in such sites and become tolerant to these pollutants. These microbes play an important role in degradation of the complex chemical compounds to the simpler chemicals which can be easily absorbed by the plant systems. Some bacteria have stress-tolerant genes, which make them resistant towards the heavy metals and some bacteria have enzymes such as metal oxidases and reductases to make them tolerant against these contaminants. To improve the phytoremediation efficiency of the plants researchers have made efforts by using the plant and soil–microbe interactions (Table 10.4). They selected biodegradative bacteria, plant growth-promoting bacteria, and other bacterial strains that resist soil pollutants (Wenzel and Jockwer 1999; Glick 2003). As most of the mineral nutrients are taken up by the plants through the rhizosphere where these microbes interacts with the plant root surface (Dakora and Phillips 2002). The root exudates provide source of carbon for the microbes and also take part in direct detoxification by forming chelates with metal ions (Bashan et al. 2008). Rhizosphere has a large quantity of microbes and has high metabolic activity (Anderson et al. 1993). The rate of exudation is increased by the presence of essential microorganisms in the rhizosphere and promoted by the uptake and assimilation of certain nutrients (Gardner et al. 1983). Various plant growth promoting rhizobacteria (PGPR) hydrolyse 1-aminocyclopropane-1-carboxylate (ACC) which is a precursor of the plant hormone ethylene

Table 10.4 Plant–microbe interaction used for heavy metal phytoremediation

Sr. No.	Name of metals	Associated plants	Associated microbes	References
1	Cd, Pb, and Zn	*Brassica napus*	*Enterobacter* sp. and *Klebsiella* sp.	(Jing et al. 2014)
2	Cd, Zn	Yellow lupine plants	*Rhizobium* sp., *Pseudomonas* sp., *Clavibacter* sp.	(Weyens et al. 2014)
3	Cd and Pb	*Brassica juncea*	*Enterobacter* sp	Qiu et al. 2014
4	Cd, Pb, and Zn	*Brassica napus*	*Enterobacter* sp. and *Klebsiella* sp.	(Jing et al. 2014)
5	Cd, Zn	*Helianthus annuus*	*Ralstonia eutropha* and *Chrysiobacterium humi*	(Marques et al. 2013)
6	Multimetal contaminants	*Agrostis capillaris* and *Festuca rubra*	Bacterial consortium	(Langella et al. 2014)
7	Cd	*Trifolium repens; Solanum nigrum*	Coinoculation of *Brevibacillus* sp. and AM Fungus; *Pseudomonas* sp. Lk9	(Vivas et al. 2003; Chen et al. 2014)
8	Cr (VI)	*Cicer arietinum*	*Kocuria flava*	(Singh et al. 2014)
9	As	*Pteris vittata*	Mycorrhization *Glomus mosseae* or *Gigaspora margarita*	(Trotta et al. 2006)
10	Ni	*Brassica campestris*	*Kluyvera ascorbata* SUD165	(Burd et al. 1998)

Table 10.5 Various transgenic plants raised by using various gene/genes to improve the heavy metal phytoremediation efficiency

Sr. No.	Gene	Source of gene	Target plant	Heavy metal	References
1	*gcsgs*	*Enterobacter* sp.	*Brassica juncea*	Cd and Pb	(Qiu et al. 2014)
2	P450 2E1	Human	alfalfa plants	Hg	(Zhang et al. 2013)
4	*ScMTII*	*Saccharomyces cerevisiae*	*N. tabacum*	Cd and Zn	(Daghan et al. 2013)
5	*ScYCF1*	*Saccharomyces cerevisiae*	*Populus alba*	Cd, Zn, and Pb	(Shim et al. 2013)
6	*PvACR3*	*Pteris vittata*	*Arabidopsis thaliana*	As	(Chen et al. 2013)
7	*TaVP1*	*N. tabacum*	*Arabidopsis thaliana*	Cd	(Khoudi et al. 2013)
8	*YCF1* and AsPCS1	Garlic and baker's yeast	*Arabidopsis thaliana*	As and Cd	(Guo et al. 2012)
9	APS1	*A. thaliana*	*Brassica juncea*	Se and Cd	(Kubachka et al. 2007)
10	OASTL	*A. thaliana*	*Arabidopsis*	Cd	(Dominguez-Solis et al. 2004)
11	SMT	*Astragalus bisulcatus*	*Arabidopsis* and *Brassica juncea*	Se	(LeDuc et al. 2004)
12	gshI and gshII and APSI	*E. coli*	*Arabidopsis thaliana* and *Brassica juncea*	As and Cd	(Bennett et al. 2003)
13	TaPCS1	Wheat	*Nicotiana. glauca*	Pb, Cd, Zn, Cu, and Ni	(Gisbert et al. 2003)
14	HisCUP1	Yeast	*Nicotiana tabacum*	Cd	(Thomas et al. 2003)
15	NtCBP4	*N. tabacum*	*N. tabacum*	Ni, Pb, and Ni	(Sunkar et al. 2000)

due to the presence of an enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Arshad et al. 2007). The application of microbes for metal solubilization from the polluted sites is a potential approach for increasing metal bioavailability to the plants, e.g., some bacterial strains such as *Proteus* sp., *Bacillus* sp., *Clostridium* sp., *Alcaligenes* sp., and *Coccobacillus* sp. have been studied earlier for remediation of cadmium from the environment (Venkatesan et al. 2011). The phytoremediation will be more effective if bacteria can degrade the soil pollutant as well as promote the growth of plants. Recently similar efforts have been done by working on the spinach. In which the plant–microbe interaction in soil contaminated with Cd showed improved the spinach growth and Cd uptake as compared to control (Ali et al. 2013). Similar studies have been carried out by taking some plants like *Alyssum murale, Brassica napus*, and *Thlaspi caerulescens* inoculated with rhizobacteria for the removal of Ni, Cd, Zn, respectively from the contaminated sites (Abou-Shanab et al 2006; Sheng and Xia 2006; Gonzaga et al. 2006).

10.3.2 Transgenic Technology to Enhance the Phytoremediation Efficiency of Plants

As the phytoremediation of pollutants is a slow process and accumulation of toxic metabolites also leads to the cycling of these metabolites into the food chain. From the last few decades, work has been carried out to develop transgenic plants to overcome the inbuilt constraints of plant detoxification capabilities. So transgenic technology is the new approach for phytoremediation, which enhances metal uptake, transport, and accumulation as well as plant tolerance capacity to abiotic stresses (Karenlampi et al. 2000). A list of the gene/genes used to raise the transgenic plants is listed in Table 10.5. In this way, *Nicotiana tabacum* was the first transgenic plant that shows the ability to tolerate heavy metal stress. In which the metallothionein gene was taken from a yeast that gives tolerance to cadmium, and *Arabidopsis thaliana* that overexpressed a mercuric ion reductase gene for higher tolerance to mercury (Eapen and D'Souza 2005). Similarly, transgenic alfalfa plants pKHCG co-expressing human CYP2E1 and glutathione S-transferase (GST) genes were developed for the phytoremediation of heavy metals and organic polluted soils. These plants showed tolerance to a mixture of cadmium (Cd) and trichloroethylene (TCE) and metabolized by the introduction of GST and CYP2E1 in combination (Zhang et al. 2013). Earlier, Bañuelos et al. (2005) has developed Indian mustard (*Brassica juncea* (L.) Czern.) lines by introducing overexpressed genes encoding the enzymes adenosine triphosphate sulfurylase (APS), ç-glutamyl-cysteine synthetase (ECS), and glutathione synthetase (GS) to improve their ability to remove selenium (Se). They found that these lines accumulate more Se in their leaves than wild type. Metal tolerance can also be significantly increased by overexpressing some proteins involved in intracellular metal sequestration (Eapen and D'Souza 2005). According to Kiyono et al. (2012) when *Arabidopsis* was introduced with a bacterial *merC* gene from the Tn*21*-encoded *mer* operon resulted in more resistant to cadmium than the wild type and accumulated significantly more cadmium. Similarly, transposon TnMERI1 of *Bacillus megaterium* strain MB1 was used to make the transgenic *Arabidpsis* for the expression of a specific mercuric ion binding protein (MerP) to increase the tolerance and accumulation capacity for mercury, cadmium, and lead (Hsieh et al. 2009).

The root-colonizing bacterium *Pseudomonas fluorescens* has been engineered to express XplA gene to degrade explosive chemicals like Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the rhizosphere (Lorenz et al. 2013). The overexpression of *AsPCS1* and *YCF1* genes in transgenic *Arabidopsis thaliana* leads to increased tolerance and accumulation of heavy metals and metalloids and found to be more tolerant to arsenic and cadmium (Guo et al. 2012). The transgenic white poplar plants plant obtained by the transfer of PsMTa1 gene from *Pisum sativum* for a metallothionein-like protein shows resistance to heavy metal, surviving high concentrations of $CuCl_2$ than the wild type (Balestrazzi et al. 2009). There are some specific genes which are induced by the presence of particular toxic chemicals in the environment are known as "pollutant-responsive elements" (Soleimani et al. 2011). The barley promoter gene HvhsplT in the presence of heavy metals fused to reporter gene was used to make a transformed tobacco plant which could be used as a bioindicator for monitoring heavy metal pollution (Mociardini et al. 1998).

10.4 Conclusion

Phytoremediation is a cost-effective technique for the removal of heavy metals from the contaminated soils/sites. During the last two decades a large number of researchers have worked on phytoremediation using plants, microorganisms, plant–microbe interactions, and transgenic plants. Nowadays biotechnology is a powerful tool used in phytoremediation to improve the metal uptake efficiencies of the plants, but it is limited to the lab conditions or at a very small scale. The studies reviewed in this chapter have remarkably contributed towards our knowledge on various phytoremediation strategies. Moreover, the application of transgenic technology and plant-microbe interactions are feasible strategies for the improvement of plants for heavy metal tolerance, their accumulation in the plant parts and also to metabolize the heavy metal pollutants. Hence, it is better to create or find an appropriate plant system for environmental cleanup.

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