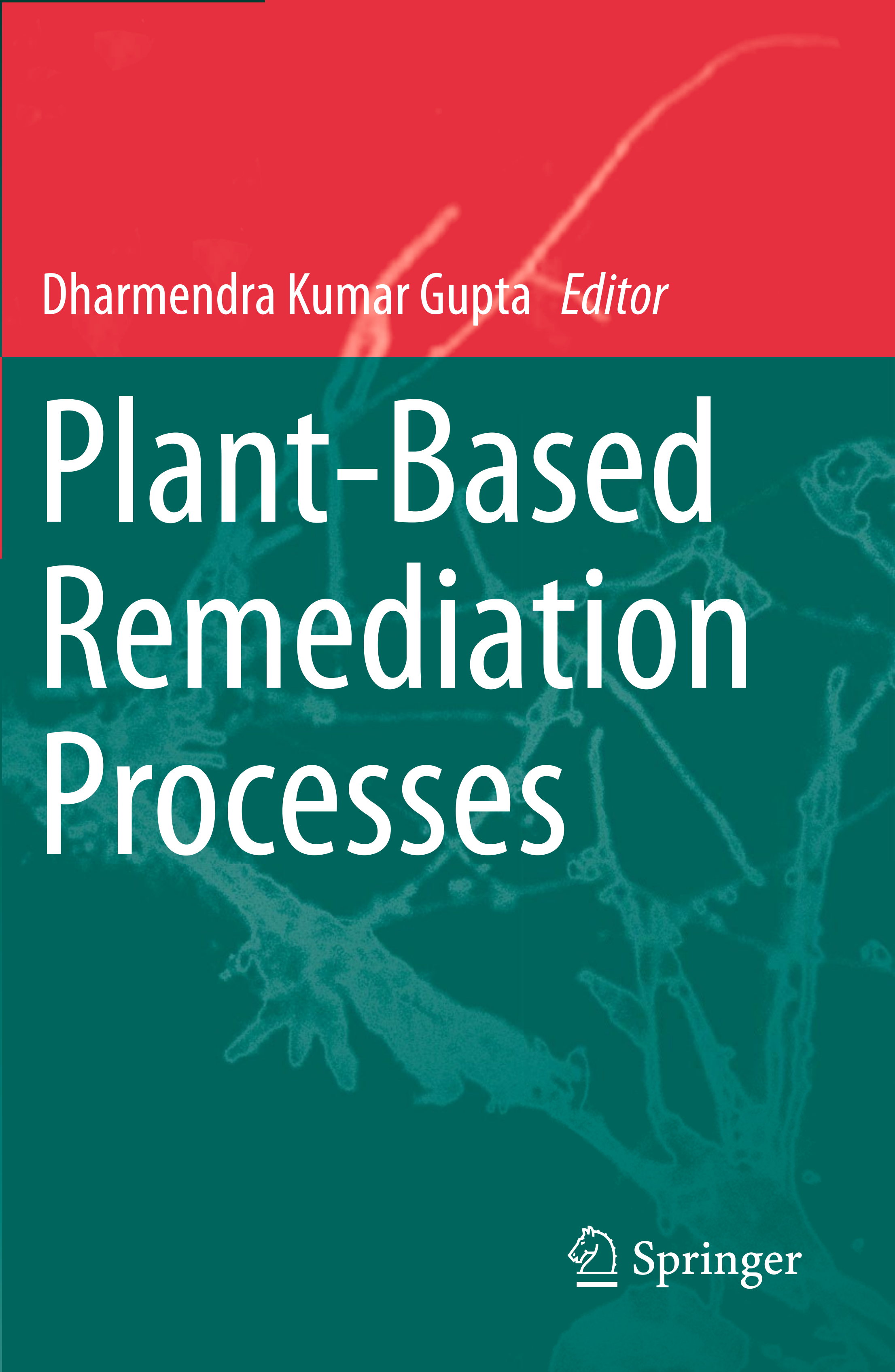

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Dharmendra Kumar Gupta *Editor*

Plant-Based Remediation Processes

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This book is dedicated to my beloved mother
Late Smt. Annapurna Gupta
1949–2011

Preface

The idea of cleaning up contaminated environments by using green plants is not new. About 300 years ago, plants were proposed to be used in the treatment of wastewater (Hartman 1975). At the end of the nineteenth century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented to accumulate high levels of metals in leaves (Baumann 1885). At present, there are about 420 species belonging to about 45 plant families which have been reported as hyperaccumulators of heavy metals (Cobbett 2003). Although the identification of new plant species with this property is still growing from field collections (Krämer 2003), only a few species have been tested in the laboratory to confirm their hyperaccumulating behaviors. The urgency to discover hyperaccumulators has shown several intriguing patterns (Baker and Whiting 2002). First, several plant families contain an inexplicably high number of hyperaccumulators: among those are Asteraceae, Brassicaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, and Violaceae, suggesting that several families and genera within them may be pre-adapted/predisposed to deal with high concentrations of metal. Second, there appears to be a disproportionately high percentage of hyperaccumulators in tropical regions.

Plant tolerance to heavy metals depends largely on plant efficiency in the uptake, translocation, and further sequestration of heavy metals in specialized tissues or in trichomes and organelles such as vacuoles. The uptake of metals depends on their bioavailability, and plants have evolved mechanisms to make micronutrients bioavailable. Some plants have developed resistance to high metal concentrations, basically by two mechanisms, avoidance and tolerance. The first mechanism involved exclusion of metals outside the roots, and the second mechanism consists basically in complexing the metals to avoid protein and enzyme inactivation. Some plants can also accumulate metals in their tissues at concentrations higher than those found in the soil, and these plants as referred as hyperaccumulators (Gupta and Sandalio 2012).

Given the nature and extent of contamination worldwide and the costs involved in remediation, recent years have seen a drive toward alternative yet effective technologies for the remediation of polluted sites. In this regard, bioremediation, typically referring to microbe-based cleanup, and phytoremediation, or plant-based cleanup, have generated much interest as effective low-cost and environmentally friendly technologies for the cleanup of a broad spectrum of hazardous organic and inorganic pollutants (Pilon-Smits 2005). Plant-based environmental remediation has been widely pursued by academic and industrial scientists as a favorable low-impact cleanup technology applicable in both developed and developing nations (Robinson et al. 2003). Physiological, biochemical, and molecular approaches are continually being applied to identify the underlying mechanisms of metal tolerance and hyperaccumulation (Lasat 2002). The drive to find genes underlying these unique biological properties is partly fueled by interest in using transgenic plants in phytoremediation (Pilon-Smits 2005). Interestingly, as transgenics are being tested in the field and the associated risks assessed, their use appears to be more accepted and less regulated than has been the case for transgenic crops (Pilon-Smits and Pilon 2002).

In last two decades phytoremediation work got so much attention from the scientists and researchers throughout the globe. The main purpose of this book is to present recent advances in the field, mainly on the use of green plants for remediation of various metal/metalloids. Other key features of the book are related to biomonitoring of heavy metal pollution, different amendments for higher uptake of toxic metals, transport of heavy metal in plants, mechanism of toxicity, and remediation through engineering plants. Some chapters are also dealt with transgenic as well as metallomics approaches for the remediation of heavy metal/metalloids. Some chapters are focusing on recent protocols for phytotechnological tools for metal contaminations. Overall the information compiled in this book will bring in-depth knowledge and advancement of phytoremediation technologies in recent years.

Dr. Dharmendra Kumar Gupta is personally thankful to the authors for contributing their time, knowledge, and enthusiasm to bring this book into shape.

Mol, Belgium Dr. Dharmendra Kumar Gupta

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Contents

Chapter 1
Phytoremediation Protocols: An Overview

Soumya Chatterjee, Anindita Mitra, Sibnarayan Datta, and Vijay Veer

1.1 Phytoremediation: An Introduction

Growth and development of any organism is always influenced by the environment. It is axiomatic that, plants do have unique characteristics to deal with wide-ranging of ambience that involve different fluctuating conditions like climate, temperature, moisture, and soil conditions (Norman 1962). Along with water, nutrients, and minerals essential for their growth, plants take up a diversity of natural and noxious compounds through their root system from soil and ground water. To survive with all such essential and nonessential components, plants use to develop diverse detoxification mechanisms within their system (Singer 2006). Microorganisms present in the rhizosphere region of plants have the ability to eliminate several contaminants from the surroundings by a range of enzymatic processes. Consequence with of their versatility, adaptability, and diversity in the environment, a number of microorganisms along with plants may be regarded as the excellent system to remediate most of the environmental contaminants, including organic and inorganic contaminants ones (Lovley 2003). Keeping in view of these attributes, plants may be regarded fundamentally as a "natural, solar powered pump and treat system" (Pilon-Smits 2005) for cleaning of contaminated sites leading to the concept of phytoremediation, a natural, esthetically pleasing, and low cost technology.

Phytoremediation (Ancient Greek: *phyto*-"plant," and Latin *remedium*-"restoring balance") describes the treatment of diverse environmental pollution problems. According to the recent definition presented by Landmeyer (2011), phytoremediation is the "application of plant-controlled interactions with groundwater and organic and

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inorganic molecules at contaminated sites to achieve site-specific remedial goals." Cleaning up of the environment through plants are rendered by direct uptake of the toxic chemical, followed by subsequent transformation, transport, and their accumulation in less toxic forms (Schnoor et al. 1995). In addition, plants support remediation process by releasing exudates and enzymes that induce microbial diversity at rhizosphere and biochemical activity in the bulk soil and mineralization (Macek et al. 2000).

Phytoremediation techniques are developing great interest because the method became an alternative to the conventional energy intensive, instrument, and chemical-based expensive restoration technologies of vast polluted areas of land and water (Azadpour and Matthews 1996; Garbisu et al. 2002; Vassilev et al. 2004; Padmavathiamma and Li 2007; Lone et al. 2008) and thus decontaminating the polluted environment by improving the utility, even of the marginal lands (Meagher 2000). The concept of cleaning pollutants using green living systems for environmental remediation is quite old. Nickel accumulation by the plant *Alyssum bertolonii* was first reported in 1948; however, the concept received momentum after the reports from the researcher Robert Brooks, of Massey University in New Zealand in 1977. Thereafter, widespread researches on the use of wetland plants, for treating heavy metals, radionuclide contaminated waters were initiated. After the nuclear disaster at Chernobyl, Ukraine, in 1986 *Phytotech* began using plants to decontaminate water and soil. This was to be proving ground for new technology. Iowa City used tree farms to clean landfills in 1989, after the results published from Phytotech experiments. In 1990, nitrogen-rich aquifer in New Jersey was managed by phytoremediation technology. The first *Living Machine* was designed and constructed in Europe during 1995, which lead to researching genetic engineering applications. Research proved that specific plants were capable of removing toxins and certain metals. The Department of Defense and EPA joined forces to develop plant-based cleanup approaches to large-scale cleanup projects (Rai and Pal 1999).

Phytoremediation of toxic elements like mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), cesium (Cs), and strontium (Sr) involves extraction and translocation of toxic cation or oxyanion to above ground tissues by plants for later harvest, converting the element to a less toxic chemical species (Meagher 2000). On the other hand, for organic pollutants, such as polychlorinated biphenyl (PCBs), dioxin, polycyclic aromatic hydrocarbons (PAH), trichloroethylene, the target of phytoremediation is to completely mineralize them into relatively nontoxic constituents, such as CO_2, nitrate, chlorine, and ammonia (Cunningham et al. 1996). Plants have several strategies (Fig. 1.1) for dealing with xenobiotics: phytostabilization, phytoextraction, phytovolatilization, rhizofiltration, phytodegradation, and phytostimulation (Salt et al. 1998; Fulekar et al. 2009; Marques et al. 2009). For soil phytoremediation, phytostabilization and phytoextraction are preferred (Salt et al. 1998).

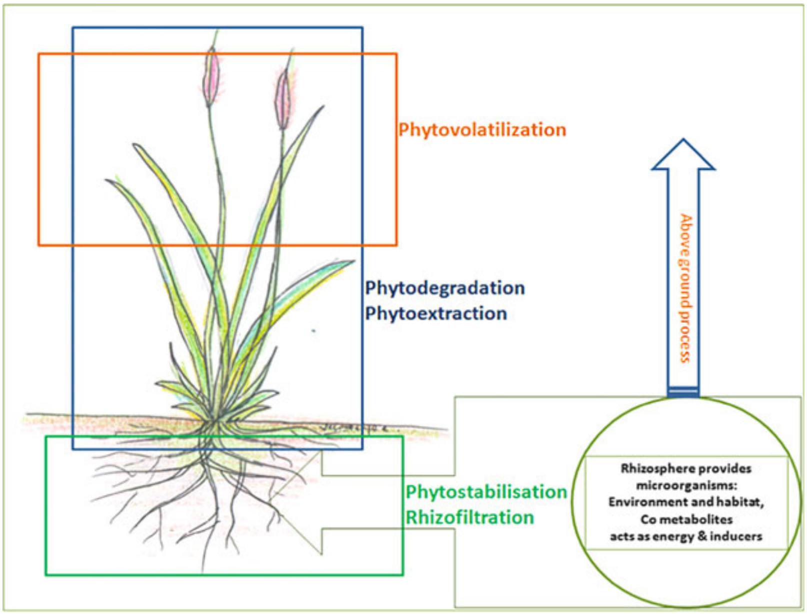

Fig. 1.1 Major processes of phytoremediation where root zone (rhizosphere) plays an important role in contaminant uptake and stabilization

1.2 Phytostabilization: Mobility Reduction of Contaminants

Phytostabilization is the process to reduce the mobility of contaminants in soil through adsorption onto roots, adsorption and accumulation by roots, or precipitation within the root zone. Vegetation are used to provide stabilization of migration of contaminants by leaching, erosion, or dispersion along with soil, water, or air to prevent pollution to ground water and surrounding environments (Ernst 2005). Plants suitable for phytostabilization should develop an extensive root system that provide good soil colonization, possess tolerance to the contaminant metals, ideally immobilize the contaminants in the rhizosphere (Kramer 2005), and endure drought and high temperature as well (Ernst 2005). This technique generally employs metal-tolerant varieties of grass species such as *Agrostis capillaris* and *Festuca rubra* (Kidd et al. 2009) but the leguminous species *Lupinus albus* also has been suggested as a good candidate for remediation of Cd and As-contaminated soil (Vazquez et al. 2006).

In addition, soil amendments are indispensable to achieve a long-term phytostabilization such as (1) increasing soil pH to more than 5 by liming with $CaCO_3$ and/or $Ca(OH)_2$ (Mench et al. 1994), (2) immobilization of heavy metals by the application of soil additives such as compost (Vangronsveld et al. 1995), and (3) improving soil quality by fertilization (Li and Chaney 1998). The toxic elements, chiefly chromium and lead can be promisingly phytostabilized. Deep-rooted plants effectively reduce the highly toxic and soluble Cr^{6+} compounds to insoluble Cr^{3+}, which does not pose an environmental risk (James 1996). Chemical species of Pb in soil are usually somewhat bioavailable, whereas, chloropyromorphite, a Pb phosphate mineral is both extremely insoluble and non-bioavailable (Ma et al. 1995). The roots of *Agrostis capillaris* growing in highly contaminated Pb/Zn mine wastes are known to form pyromorphite from soil lead and phosphate by an unknown mechanism, thus minimizing the escape of lead movement (Cotter-Howells and Capom 1996). Advantage of using grass species for phytostabilization is that they bioaccumulate less metals in their shoots in comparison to dicot species, in this way minimizing exposure of wildlife to toxic elements (Pilon-Smits 2005).

1.3 Phytoextraction

Phytoextraction involves the cultivation of higher plants that concentrate and translocate soil contaminants in their above ground tissues that can be harvested at the end of the growth period (Salt et al. 1998). It is the most effective among several phytoremediation methods, although technical difficulties are there for its applications (Kramer 2005). Selection of suitable plant species is crucial for effective phytoextraction and biomass derived from shoot of a phytoremediator crop plant should be capable of depositing metal(oid) species at concentration 50–500 times higher than those in the contaminated soil substrate (Kramer 2005). The best-known natural hyperaccumulators plants are alpine pennycress (*Thlaspi caerulescens* L.) capable of hyperaccumulating Zn^{2+}, and occasionally Cd^{2+} and Ni^{2+} (Milner and Kochian 2008), the serpentine endemic shrub *Alyssum* sp., Indian mustard *Brassica juncea* (Brassicacea) and *Astragalus racemosus* (Leguminosae). The Asian stonecrop *Sedum alfredii* (Crassulaceae) has gained increased attention due to higher accumulation rate of Zn, Cd, and Pb (Lu et al. 2008; Deng et al. 2008). Plants ideal for phytoextraction besides having an inherent capacity to tolerate and hyperaccumulate metals should possess multiple traits like (1) high and fast growing biomass; (2) extensively branched root systems; (3) ability to grow outside their area of collection; (4) relatively easy to cultivate; and (5) possible repulsive to herbivores to avoid the escape of accumulated metals to the food chain (Seth 2012). Unfortunately, most of the naturally hyperaccumulating plants have slow growth, poor biomass, and often strong association with a specific habitat, therefore limiting the phytoextraction potential (Chaney et al. 2005). However, non-hyperaccumulator plants having higher growth rate and biomass could be modified or engineered to achieve the above-mentioned attributes. To increase the potential of phytoextraction, factors limiting trace element accumulation in plants have to be resolved, which may include mobilization of poorly available contaminant in the soil, root uptake, sequestration by metal-complex formation and deposition in vacuoles for detoxification within roots, translocation to symplast, efficient xylem loading, distribution and storage inside the aboveground organ and tissues, and eventually expulsion of accumulated metal to less metabolically active cells, e.g., trichomes (Clemens et al. 2002). Two approaches are currently being explored to improve or modify the metal accumulating plants: the conventional breeding and genetic engineering. Although a number of reports exist on successful crop breeding (Gleba et al. 1999; Dushenkov et al. 2002; Alkorta et al. 2004; Nehnevajova et al. 2007) yielding improved metal accumulator plants, the major constraint in developing such hybrid is sexual incompatibility between the taxa. Transgenic plants have opened new avenues in phytoremediation technology by expressing the desired gene and overcoming the limitations imposed by sexual incompatibility.

1.3.1 Transgenic Approaches to Develop Metal-Accumulating Plants

Metallophytes have distinct biological mechanisms that enable them to tolerate high tissue metal concentration. Recent progress in understanding the molecular basis of metal accumulation and tolerance by metallophytes has provided a strong scientific basis for creating transgenics that enhance phytoextraction potential. Some of the possible areas of genetic manipulation are outlined below:

- Metallothioneins (MT) and phytochelatins (PCs) are known as metal-chelating proteins, responsible for the detoxification and accumulation of metals (Hirata et al. 2005). Genetic manipulation of the plants for synthesis of metal chelators will improve the capability of plants for metal uptake by increasing the availability of such metals (Pilon-Smits and Pilon 2002; Clemens et al. 2002; Lee et al. 2003).
- Genes involved in metal uptake, translocation, and sequestration in plants are well studied. Introduction or overexpression of any of these genes into candidate plants (Table 1.1) could be a way to enhance the previously mentioned pathway in non-hyperaccumulators (Clemens et al. 2002). Transgenic plants overexpressing the genes encoding the enzymes for histidine biosynthesis and ACC deaminase, Hg^2-reductase, glutathione synthetase, arsenate reductase, and aldolase/aldehyde reductase, were shown to become more tolerant to the toxic levels of metals and carried out phytoextraction with increasing potential (Stearns et al. 2005; Thomas et al. 2003; Bennett et al. 2003; Shah and Nongkynrih 2007).
- The repression of an endogenous gene expression by inserting an antisense RNA can also result in enhanced metal uptake by plants (Shah and Nongkynrih 2007).
- The introduction of an additional metal-binding domain to the implemented protein further enhances the metal-binding capacity (Kotrba et al. 1999).
- Another promising approach is overexpressing the enzymes catalyzing rate-limiting steps. ATP sulfurylase (APS) is such a rate-limiting enzyme in the selenium detoxification processes. The overexpression of APS in transgenic *Brassica juncea* led to three times more uptake and accumulation of selenium in comparison to wild plants (Pilon-Smits et al. 1999).

Table 1.1 Selected examples of genetically engineered plants with the respective gene transferred, gene product, gene source, and improved trait

Gene	Product	Source	Target plant species	Observed effect	References
MT1A	Metallothionein	Mouse	*Nicotiana tabacum*	Cd tolerance	Pan et al. (1994)
TaPCS	Phytochelatin synthase	Wheat	*Nicotiana glauca*	Pb accumulation	Gisbert et al. (2003)
APs	ATP-sulfurylase	*Arabidopsis thaliana*	*Brassica juncea*	Se hyperaccumulation	Banuelos et al. (2005)
merA18	Hg(II) reductase	Gram − ve bacteria	*Liriodendron tulipifera*	Hg tolerance and volatilization	Rugh et al. (1998)
merB	Organomercurial lyase	Gram − ve bacteria	*Arabidopsis thaliana*	Hg tolerance and volatilization	Rugh et al. (1996)
arsC	Arsenate reductase	*Escherichia coli*	*Brassica juncea*	As tolerance	Dhankher et al. (2002)
GSH1	Glutathione synthetase	*Saccharomyces cerevisiae*	*Arabidopsis thaliana*	Increased accumulation of Cd and As	Guo et al. (2008)

- Another strategy for increasing the efficiency of phytoextraction involves increase in the metal translocation to shoots by increasing plant transpiration (Gleba et al. 1998).
- According to Raskin (1996), transgenic plants could be developed to secrete metal selective ligands (phytosiderophores or chelating agents) into the rhizosphere, which could specifically solubilize the toxic elements (Ma and Nomoto 1996).

1.3.2 Phytoextraction with Endophytic Microbes

Researchers carried out several experiments on the application of endophytic bacteria and mycorrhizal fungi in the phytoextraction of pollutants (Doty 2008). Endophytes are the symbiotic microbes inhabiting in the internal plant tissue and are able to facilitate plant growth and increase resistance of plants against pathogen and drought (Taghavi et al. 2010). It has been recently reported that the endophytic symbiotic bacteria *Methylbacterium populum* that lives within poplar can mineralize 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (VanAken 2009). However, the success rate of phytoextraction of heavy metals using endophytic bacteria remains slow because of the lack of proper strains with heavy metal resistance and detoxification capacities (Luo et al. 2011). Besides endophytes, the arbuscular mycorrhizal (AM) fungi are also known to be involved in the uptake of elements into plants (Doty 2008) and are reported to be present in mutualistic association in the roots of plants growing on markedly contaminated soil (Khade and Adholeya 2009; Javaid 2011; Miransari 2011). Therefore, mycorrhizal fungi can be applied for significant phytoextraction by improving several attributes like increased metal tolerance, increased biomass production, and greater metal concentration in plant tissue (Vamerali et al. 2010). In brief, the goal of phytoextraction is to reduce the presence of trace elements in soils through their uptake and accumulation by plants; in contrast, phytostabilization aims to minimize the mobile and bioavailable fraction of metals by combining the use of metal-tolerant plants and soil amendments and thus reduces leaching through soil. In both processes the “mobility and bioavailability of trace elements in the soil—particularly in the rhizosphere where root uptake and exclusion takes place—is a critical factor affecting their outcome and success” (Kidd et al. 2009).

1.4 Phytovolatilization

A variant of phytoextraction is phytovolatilization, where the contaminant is not primarily concentrated in aboveground tissues, but instead transformed by the plant into evaporable and less toxic form before releasing into the atmosphere (Kramer 2005). It is not a direct clean up method rather a dispersal technology of the

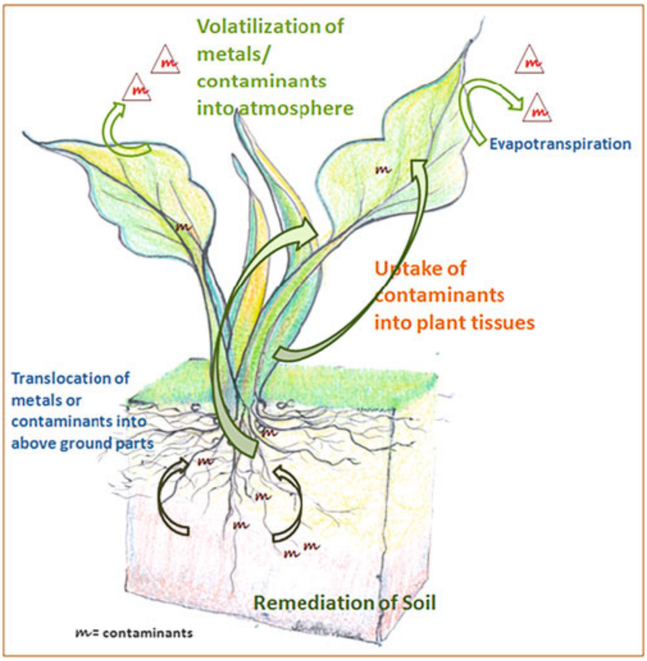

Fig. 1.2 Schematic representation of phytovolatilization where metals are volatilized by the process of evapotranspiration by plants

contaminants. Phytovolatilization is very much promising for mercury (Hg) and selenium (Se) in which metals are converted to a volatile form for release and dilution into the atmosphere (Bhargava et al. 2012). This method is advantageous over other phytoremediation methods as it removes metal(loid) from a site without the need of harvest/disposal of contaminated plants (Fig. 1.2).

1.4.1 Detoxification of Mercury by Plants

The most spectacular achievements of biotechnology in phytoremediation were the engineering of plants capable of removing methyl-Hg from contaminated soil (Rugh et al. 1996; Brunner et al. 2008). The purpose is achieved by the introduction of bacterial *mer*A and *mer*B genes into several plant species including *Arabidopsis*, tobacco, poplar, rice, and cottonwood (Rugh et al. 1996; Bizily et al. 2000; Heaton et al. 2003; Czako et al. 2006; Lyyra et al. 2007). The *mer*A gene encodes an NADPH-dependent mercuric ion reductase which converts Hg^{2+} to nontoxic volatile metallic Hg^0 and *mer*B encodes organomercurial lyase liberating Hg^{2+} from organomercurial compounds $R\text{-}Hg^+$ (Silver and Phung 2005). Transgenic *A. thaliana* (Rugh et al. 1996; Yang et al. 2003), *Nicotiana tabacum* (Ruiz et al.

2003), *Oryza sativa* (Heaton et al. 2003), yellow poplar *L. tulipifera* (Rugh et al. 1998), overexpressing bacterial *mer*A and/or *mer*B become more tolerant to Hg^{2+} and R-Hg^{+} and release 10 times higher elemental Hg as compared to nontransformed plants. It has been reported that transgenic plants in which *MerB* is targeted in the endoplasmic reticulum rather than cytoplasm, release mercury in tenfold higher volatile form (Bizily et al. 2003).

1.4.2 Detoxification of Selenium by Plants

Two pathways dominate in the natural detoxification of selenium (Se) in plants. In most species, selenium is most toxic after metabolization into analogues of amino acid cysteine and methionine. Selenium hyperaccumulating plant species have a specific enzyme, selenocysteine methyltransferase (SMT) which is responsible for converting selenate into methyl selenocysteine (MetSeCys), ultimately incorporated into the proteins and thus resulting in hyperaccumulation of selenium. In a second detoxification mechanism, selenate can be metabolized into dimethylselenide (DMSe) which is 100 times less toxic than selenate and selenite in soil and volatilized from leaves and roots (Terry et al. 2000). Transgenic Indian mustard (*Brassica juncea* L.) transformed with the SMT gene from Se-hyperaccumulator *Astragalus bisulcatus* releases a higher DMSe in addition to an improved Se accumulation and tolerance in comparison to the control plants (LeDuc et al. 2004).

1.5 Rhizofiltration

This phytoremediation method can be defined as the use of aquatic plants, either floating or submerged to absorb, concentrate, and remove hazardous compounds particularly heavy metals or radionuclides from aqueous environment by their roots (January et al. 2008; Eapen et al. 2003) (Fig. 1.3). A suitable plant for rhizofiltration should have larger root system through which toxic metals are taken up from solution over an extended period. Such plant should be capable of producing up to 1.5 kg (dry weight) of roots per month per m^2 of water surface (Dushenkov et al. 1997). Rhizofiltration usually involves in hydroponically cultivated plants in a stationary or moving aqueous system wherein the plant roots absorb pollutants from the water (Salt et al. 1995). Candidate plant for rhizofiltration includes the Indian mustard (B*rassica juncea*), sunflower (*Helianthus annuus*), and corn (*Zea mays*) (Brooks and Robinson 1998). Success of rhizofiltration greatly depends on the physicochemical characteristics of the plants, which may favor the process of bio-adsorption (Olguın and Sanchez-Galvan 2012).

Dushenkov et al. (1995) reported that within 24 h, submerged roots of sunflower plants were able to substantially reduce the levels of Cd, Cr, Cu, Mn, Ni, Pb, Sr, U, and Zn in water bringing metal concentration close to or below the discharge limit.

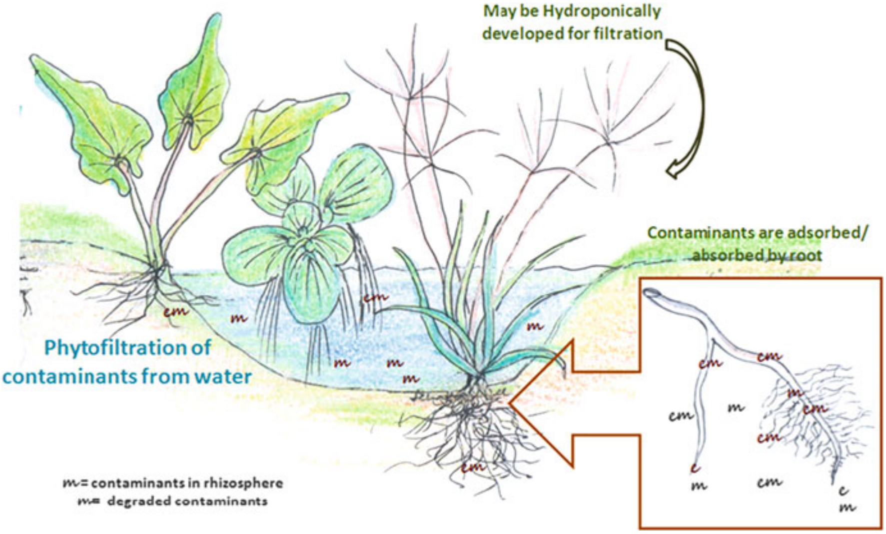

Fig. 1.3 Schematic representation of rhizofiltration where contaminants are adsorbed from water by wetland plants

Because this method is especially effective in situation involving large volume of water and relatively low concentration of contaminants, it is particularly applicable to radionuclide-contaminated water (Dushenkov et al. 1997). In a similar test carried out in Astabula, Ohio, it was found that, within 24 h, submerged roots of sunflower plants incredibly reduced the uranium level from a range of 100–400 ng mL^{-1} in contaminated water bodies to below the EPA standard level of 20 ng mL^{-1} (Cooney 1996). Several physicochemical technologies may also be executed for removal of toxic metal from wastewater such as chemical precipitation, ion exchange, adsorption, membrane filtration, photocatalytic degradation, and electrochemical method (Fu and Wang 2011). Disadvantages of these methods are high cost and disposal problem, making difficult their application in large scale. On the contrary, rhizofiltration offers a cost effective and eco-friendly alternative for the removal of contaminants from water (Rai 2012).

1.6 Phytodegradation

This method is also known as phytotransformation that refers to uptake of contaminants with the subsequent breakdown, mineralization or metabolization by plants itself through various internal enzymatic reaction and metabolic processes (Salt et al. 1998; Spaczynski et al. 2012). Subsequently many of these uptaken substances may even be metabolized into CO_2 and H_2O by enzyme complexes involved in the plant metabolic cycle (Mc Cutcheon and Schnoor 2003). The ideal plant for use of phytodegradation should have (1) highly developed root system that has the ability to secret a considerable amount of enzyme for degradation of the xenobiotics, (2) tolerance to the xenobiotics at a concentration found in soil, (3) fast growth, and (4) a relatively high biomass (Wang and Chen 2007). The enzymes secreted from plant root into soil include laccases, dehalogenase, nitroreductase, nitrilases, and peroxidases (Carreira and Wolfe 1996; Schnoor et al. 1995; Duran and Esposito 2002; Jansen et al. 2004; Wang et al. 2004). In a field test reported by Wolfe et al. (1993), plant-derived enzymes nitroreductases and laccases showed significant degradation of TNT, dinitromonoaminotoluene, mononitrodiaminotoluene and triaminotoluene. Another study reported the degradation of various nitroaromatic compounds by nitroreductase secreted by plants (Boyajian and Carreira 1997). In another report, laccases have been shown to be useful for the degradation of a variety of persistent environmental pollutants including alkenes, bisphenol A, and synthetic dyes (Mayer and Staples 2002). The presence of plant-derived enzymes capable of degrading environmentally hazardous xenobiotics thus can be successfully exploited for the development of future phytoremediation strategies (Salt et al. 1998).

1.7 Phytostimulation

It is also called rhizospheric biodegradation and is based on the secretion by plants in root exudates which support the growth and metabolic activities of diverse fungal and bacterial communities in the rhizosphere capable of degrading varied pollutants (Anderson et al. 1994). The secreted enzymes can transform the chemicals in the rhizosphere; therefore, the plants do not need to take up the pollutants for detoxification (Fig. 1.4). Plants are able to increase the abundance of soil microflora in the rhizosphere by 1–4 orders of magnitude compared to the surrounding bulk soil and these microflora show greater range of metabolic capabilities than the microbes in the surrounding loose soil (Walton et al. 1994; Salt et al. 1998). Some plants such as mulberry (*Morus rubra*) preferentially harbor PCB degrading microbes in the rhizosphere (Wenzel et al. 1999). Rhizospheric microorganisms may also decontaminate areas by volatilizing pollutants such as polynuclear aromatic hydrocarbons (PAH) or by increasing the production of humic substances from organic pollutants (Cunningham et al. 1996; Dec and Bollag 1994).

1.7.1 Genetically Modified Plants for Improved Phytostimulation

The most promising approach of rhizospheric phytodegradation is the production of transgenic plants targeted for secreting the enzymes or factors involved in phase I and phase II detoxification process in plants (Spaczynski et al. 2012). Xenobiotics, such as PCB, various herbicides, and explosives can be successfully degraded by

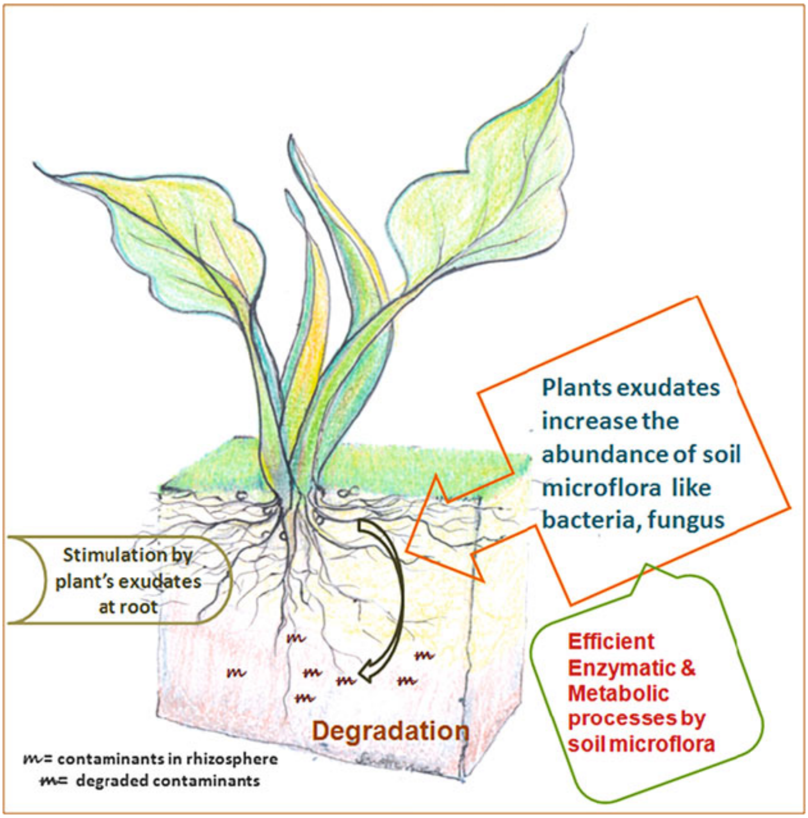

Fig. 1.4 Schematic representation of phytostimulation where plant exudates stimulate the microflora of root zone to degrade contaminants

phytostimulation. In the past decades, many successful attempts have been made with transgenic plants. Some of which are listed below:

- Mammalian cytochrome P450 gene inserted into the plants as *Nicotiana tabacum*, *Solanum tuberosum*, *Oryza sativa*, and *Arabidopsis thaliana* exhibited increased tolerance to herbicides mainly atrazine and simazine and showed a marked increase in the capability of metabolism of various xenobiotics (Doty et al. 2000; Eapen et al. 2007).
- Transgenic Indian mustard (*B. juncea*) expressing glutathione transferase (GSTs), a phase II cellular detoxification gene, shows increased tolerance to atrazine, metachlor, phenanthrene, and 1-chloro-2,4, dinitrobenzene (Flocco et al. 2004). Overexpression of GST genes enhances the potential for phytodegradation of herbicides (Kawahigashi 2009).
- Rhizodegradation of pollutant bisphenol A and PCB was efficiently carried out by transgenic tobacco plants inoculated with the gene coding laccase obtained from a fungus *Coriolus versicolor* (Sonoki et al. 2005).

- Transgenic plants are reported to remove explosives residue successfully from soil contaminated by highly toxic and mutagenic nitroglycerin, TNT, RDX, aminodinitrotoluene (Hannink et al. 2001; Rylott et al. 2006).
- *Arabidopsis thaliana* transformed with an extradiol dioxygenase gene remove 2,3- dihydroxybiphenol with high efficiency (Uchida et al. 2005).

1.8 Concluding Remarks

Phytoremediation techniques exploit the unique, selective, and naturally occurring uptake capabilities of plant root system, together with the translocation, bioaccumulation, or detoxifying abilities of the entire plant body. There are increasing number of reports suggesting that phytoremediation should become the technology of choice for remediation due to its cost efficiency and ease of implementation. Although phytoremediation techniques are successfully used in many contaminated sites in some developed countries, this technology is still in its infancy and yet to be applied commercially. In the last decades, a number of research projects have been carried out regarding production of suitable transgenic plant to increase potential phytoremediation in different countries but never has been implemented in the real contaminated sites. Restriction over field release of such genetically manipulated plants includes increased invasiveness and decreased genetic diversity of native plants due to interbreeding. Application of sterile clones may solve the problem (Abhilash et al. 2009). Another major procedural constriction is the insufficiency of knowledge regarding the specific enzyme involved in the detoxification of different pollutants by plants. Therefore, increased understanding of the enzymatic process involved in plant detoxification of diverse xenobiotics is necessary to provide information on which gene should be engineered and that will open new gateway for manipulating plant with superior remediation potential. In addition, agronomic improvement ranging from traditional crop management techniques (use of pesticides, soil amendments, fertilizer, etc.) to some precise phytoremediation approaches such as application of plants combined with microorganisms for efficient contaminant extraction (rhizoremediation) and improving metal solubility in soil by using suitable chelating agents is suggested for significant progress of phytoremediation capabilities.

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