14 Bioremediation of Heavy Metals 14 for Sustainable Agriculture

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Abstract

Due to industrial revolution, pollution of agricultural lands by toxic pollutants has become a great concern, worldwide. Naturally, heavy metals are present in the earth's crust. There are several/many toxic pollutants that can be changed into various oxidation states easily and cause many deleterious effects in several physiological processes in plants. Plants growing in these polluted soils show a reduction in growth, performance, and yield. Therefore, there is an urgent need to realize the heavy metal-induced toxicity in plants and animals and the harmful effects caused by the consumption of contaminated foods in humans. Bioremediation is an effective, suitable, cost-effective, and non-disturbing method of soil remediation; it is useful for the treatment of heavy metal-polluted soils. Microorganisms and plants employ different mechanisms for the bioremediation of polluted soils. Several microorganisms have been successfully used to reduce the toxicity of heavy metals. These microbes encode several detoxification processes to modify toxic metallic ions to nontoxic elemental state. Using plants for the treatment of polluted soils is a more common approach in this regard. Combining microorganisms and plants, for bioremediation, ensures a more efficient cleanup of heavy metal-polluted soils. This chapter presents the review of a comprehensive study of literature about heavy metal-induced toxicity in plants and its detoxification processes to provoke for advance research in the field of sustainable agriculture.

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14.1 Introduction

14.1.1 Heavy Metals and Their Sources

Highly electronegative metals with a density greater than 5 g/cm³ are termed "heavy metals." Arsenic is an exception. Although it is lighter and nonmetallic, it resembles with heavy metals and exerts chemico-ecological effects (Duffus [1981\)](#page-12-0). All heavy metals have relatively smaller cationic site because of their heavier nucleus and increasingly compressed electrons. The "electron thirst" ability makes many of them to bond with those atoms having loosely held outer shell electron like sulfur, thus forming sulfides as in lead, tin, copper, and mercury, or prevents them from yielding electron-hungry elements like oxygen (oxidation) and preserving their "nobleness" as gold, platinum, silver, etc. It is the fundamental chaleophile character of the heavy metals that threatens the sulfide bridge between giant protein molecules in the living system. For instance, mercaptan or mercury poisoning, known as Minamata disease, is due to the entry of mercury into organisms (Kim and Choi [1995](#page-13-0); Harada et al. [1999](#page-12-1)). Lead, zinc, copper, cadmium, arsenic, mercury, and nickel, the ecologically more significant heavy metals, play similar disruptive role when they enter the body system in amounts higher than what is required. Heavy metals include the elements with atomic numbers greater than 20, excluding alkali metal, alkaline earths, lanthanoids, and actionoids. Metallic elements are intrinsic components of the environment. Although the natural background or metal fraction in air, soil, and plants is highly variable, there are some anomalous areas or high levels caused by anthropogenic pollution due to mining of metal-rich ores by metal-smelting industries. The anthropogenic sources of eight common heavy metals are shown in Table [14.1.](#page-1-0)

The natural sources of heavy metal contamination include the dried water droplets from oceans, dust particles from volcanoes, erosion from soil, weathering of rocks, and forest fires. Biodegradation of dead animals and plants also contribute

Source	Hg	As	Cd	Cr	Cu	Ph	Ni	Z _n
Mining and ore processing	$\ddot{}$	$\ddot{}$	$\ddot{}$		$^{+}$			$\ddot{}$
Metallurgy	$\ddot{}$	$\ddot{}$	$\ddot{}$	$^{+}$	$^{+}$	$+$	$+$	$+$
Chemical industry	$\ddot{}$	$\ddot{}$	$\ddot{}$	$^{+}$	$^{+}$	$\ddot{}$	-	$+$
Alloy industry	-		-	-	—	$+$	-	
Paint industry			$^{+}$	$^{+}$	-	$\ddot{}$		$+$
Glass industry	$\ddot{}$	$^{+}$	$\overline{}$			$\ddot{}$	-	
Paper and pulp mills	$+$	-	-	$^{+}$	$^{+}$	$+$	$+$	
Leather tanning	$\ddot{}$	$^{+}$		$^{+}$				$+$
Textile dyeing and printing	$\ddot{}$	$\ddot{}$	$^{+}$	-	$^{+}$	$\ddot{}$	$+$	$+$
Chemical fertilizer industry	$\ddot{}$	$^{+}$	$^{+}$	$^{+}$	$^{+}$	$+$	$+$	$+$
Chlor-alkali industry	$\ddot{}$	$\ddot{}$	$^{+}$	$^{+}$	—	$\ddot{}$	–	$+$
Petroleum refining	$+$	$\ddot{}$	$^{+}$	$^{+}$	$^{+}$	$+$	-	$+$
Coal burning	$\ddot{}$	$\ddot{}$	$\ddot{}$	$^{+}$	$^{+}$	$\ddot{}$		

Table 14.1 Anthropogenic sources of eight common heavy metals (Dixit et al. [2015\)](#page-12-2)

Fig. 14.1 The global biogeochemical cycling of mercury (https://people.uwec.edu [2014\)](#page-13-3)

significantly to background levels of metals in waters (Callender [2003](#page-12-3); Järup [2003;](#page-13-1) Wilson and Pyatt [2007](#page-14-0)). Many studies have reported different sources and spreading of heavy metals, especially mercury in the environment as shown in Fig. [14.1](#page-2-0).

14.1.2 Heavy Metals to Agricultural Soil

Agricultural soils have become a big reservoir of heavy metals due to the extensive usage of different agrochemicals like fungicides, herbicides, and phosphate fertilizers, organic manure, and decaying plant and animal residues (Uwah et al. [2009\)](#page-14-1). The use of sewage sludge and industrial waste water for irrigation further increases the concentration of heavy metals in agricultural soils (Sharma et al. [2007\)](#page-14-2). Agricultural runoff together with soil erosion is the potential source of heavy metals in aquatic bodies. The ecological equilibrium is mainly disrupted by heavy metalpolluted rhizospheric soils. In nature, soils constitute a large variety of metallic elements with different concentrations and as variable chemical species. Some metallic elements have no biological importance, while some elements, known as essential trace elements, have very important role in biological ecosystem. These essential metallic elements become toxic when crossing a certain concentration level. The relevance of concentrations and bioavailabilities of metallic elements in nature is indicated by their reaction with negatively charged soil particles (Benedetti et al. [1995\)](#page-12-4). In order to maintain essential metals available and at a certain concentration level, microorganisms living in rhizosphere constantly regulate their activities (Khan et al. [2000\)](#page-13-2). The soil microorganisms adapt physiological pathways and

proceed their evolution process under selection pressure imposed by heavy metals by taking into account the space and time variability of soils (Orcutt [2000\)](#page-13-4).

14.1.3 Phytotoxic Effects of Heavy Metals

Toxic levels of metals in soil may be caused by natural soil properties or by agricultural, manufacturing, mining, and waste disposal procedures. Metal toxicity is an important growth-limiting factor for plants in many acid soils below pH 5.0. Metal toxicity in certain crops is aggravated by high temperature (Foy et al. [1978](#page-12-5)).

Any heavy metal can be toxic at source level of solubility and has been observed to cause phytotoxicity. Heavy metals exist as inorganic compounds or are bound to organic matter clays or hydrous oxides in soils. Due to this precipitation and sorption, the toxicity of many metals such as Zn, Cu, and Ni has occurred frequently. Toxicity of Pb, Co, Be, As, and Cd occurs only under very unusual conditions. Other elements may be toxic in solution cultures but are not phytotoxic in soils even at very high levels (e.g., Cr, Ag, Sn, Ga, Ge). Lead and cadmium are of interest, not only because of phytotoxicity but because their uptake by plants move them into the food chain. Thus most research on toxicity of heavy metals has involved Zn, Cu, Ni, Cd, Pb, and Hg (Ashraf et al. [2016;](#page-12-6) Clemens and Ma [2016](#page-12-7); Gao et al. [2016;](#page-12-8) Versieren et al. [2016\)](#page-14-3).

Due to the presence of heavy metals in parent materials, toxic metals also occur naturally in soils. Geochemical studies such as the science of biogeochemistry and the art of biogeochemical prospecting have confirmed the presence of metal residues and the enrichment of metal in plants and soils over or near an ore deposit.

The introduction of Hg in plant systems has principle importance due to its application in fertilizers, herbicides, and seed disinfectants (Cavallini et al. [1999\)](#page-12-9). Few mercury species are being used on tree foliage as fungicides, and they can be transferred, relocated, and redistributed in plants. At the cellular and subcellular level, the processes by which metals may prove lethal include obstruction of biologically significant molecules (e.g., enzymes, polynucleotides), transportation of micronutrients, displacement or substitution of metal ions from biomolecules (e.g., Mg from chlorophyll), deformation and inactivation of enzymatic proteins, and compromise of cell membrane integrity. The possible processes causing Hg-induced phytotoxicity are modifications in the porosity of the cell membrane; high affinity for sulfhydryl (–SH) groups, phosphate groups, and reactive groups of adenosine diphosphate (ADP) or adenosine triphosphate (ATP); and displacement of essential ions and its capability in the disruption of several functions involving critical proteins (Patra and Sharma [2000;](#page-13-5) Patra et al. [2004\)](#page-13-6). Toxic Hg^{2} also disrupts the antioxidant defense mechanism by altering the modulation of intracellular nonprotein thiols (NPSH); reduced glutathione (GSH), which is a nonenzymatic antioxidant; ascorbate peroxidase (APX) and glutathione reductase (GR); and superoxide dismutase (SOD), an antioxidant enzyme (Ortega-Villasante et al. [2005](#page-13-7); Sparks [2005](#page-14-4); Israr et al. [2006;](#page-13-8) Calgaroto et al. [2010\)](#page-12-10).

The evidence of mercury phytotoxicity has been studied in various grain crops like *Oryza sativa* and *Triticum aestivum*. The primary effects of Hg compounds are on the embryo and secondary on endosperm. Hg compounds cause the breakdown of –SHsystem by interfering in biological systems resulting in the production of –S-Hg-Sbridge which may influence germination and embryo development (rich in SH ligands). In *Oryza sativa* and *Zea mays*, HgCl₂ is involved in the obstruction of primary root elongation as compared to shoots (Patra and Sharma [2000;](#page-13-5) Patra et al. [2004](#page-13-6)).

Hg influences both, light and dark reactions, of photosynthesis by substituting the central atom of chlorophyll (Mg) by Hg, in vivo, which is an important damaging mechanism. It also reduces the transpiration rate, water uptake, and chlorophyll synthesis. Toxic mercuric cations are involved in the loss of magnesium, potassium, manganese, and deposition of iron which lead to the modifications in cell membrane porosity (Boening [2000\)](#page-12-11). The cellular and molecular mechanisms that are involved in Hg-induced toxicity in plants are practically unknown due to scarce studies considering Hg genotoxicity. However, it has been shown that mercury can insert harmful genetic effects to different plant species (De Flora et al. [1994\)](#page-12-12).

In earlier experiments, multinucleated cells in the root tips of corn seedlings, exposed to solution of Ceresan (ethyl mercuric phosphate, a fungicide), resulted in the formation of polyploidy, aneuploidy, and c-tumors through c-mitosis (Kostoff [1939,](#page-13-9) [1940\)](#page-13-10). C-mitosis (colchicine treated), sister chromatid exchanges, chromosomal aberrations, and spindle alterations can be stimulated by several compounds at similar dosage, but butyl mercury bromide is most notable in this respect (FiskesjÖ [1969\)](#page-12-13). It has been reported that inorganic mercury poisoning in *Allium cepa* and *Allium sativum* resulted in the reduction of mitotic index in the root tip cells and an increment in chromosomal aberrations that depend on concentration and time of exposure. $HgCl₂$ was concluded as more cytotoxic as compared to mercurous chloride, and lowest effective concentration tested (LECT) was measured as 10 ppm. The greater tolerance of *A. sativum* than *A. cepa* was attributed to the presence of high levels of heterochromatin in the former and low amount of sulfur in the later (Patra and Sharma [2000](#page-13-5); Patra et al. [2004](#page-13-6)).

For other metals which are beneficial to plants, concentration in small amount of these metals in the soil could actually improve plant growth and development. However, at higher concentrations of these metals, reduction in plant growth had been recorded. Uptake of low amount of heavy metals increased in plant growth, nutrient content, biochemical content, and antioxidant enzyme activities for plant. Improvements in growth and physiology of cluster beans have also been reported by Manivasagaperumal et al. [\(2011](#page-13-11)) at medium Zn concentration of the soil solution. On the other hand, excess concentration of Zn has adverse effects on plant growth. It is also reported that the combination of Pb and Cu at both high concentration and low concentration resulted in a rapid and complete death of the leaves and stem of *Lythrum salicaria* (Brennan and Shelley [1999\)](#page-12-14). Some plants are able to tolerate these metals through three mechanisms: (i) exclusion of heavy metal in the shoot over a wide range of soil concentrations, (ii) inclusion of heavy metal in the shoot reflecting those in the soil solution through a linear relationship, and (iii) bioaccumulation of metals in the shoot and roots of plants at both low and high soil concentrations.

14.1.4 Remediation of Heavy Metals by Microorganisms

Soil microorganisms are involved in interaction with soil constituents and roots of plant (Attitalla et al. [2004](#page-12-15); Khan et al. [2009\)](#page-13-12). The regions on the surface of roots and those around them are nutrient rich, and because of the availability of nutrients, the activities of microorganisms are higher in the rhizosphere, as compared to other areas of plants (Dessaux et al. [2009\)](#page-12-16). Plant growth-promoting bacteria present in soil are group of different bacteria involved in improving plant growth while directly and indirectly bioremediating heavy metals like Hg, Cd and Co, etc. (Hayat et al. [2010](#page-13-13); Yu et al. [2014\)](#page-14-5). The direct effect depends on the production of hormones, nutrient availability, and increase in plant defense processes against pathogens (Choudhary [2011\)](#page-12-17). Indole acetic acid improves plant root growth and supply of phosphorus to plants (Marschner et al. [2011\)](#page-13-14). *Bacillus* and *Paenibacillus* sp. have the ability to produce spores, and as spores are resistant, so these bacteria can be more persistent in soil environment (Nicholson et al. [2000;](#page-13-15) Lal and Tabacchioni [2009\)](#page-13-16). Soil microbes convert the insoluble form of phosphate into its soluble form and make it available for the plants to promote their growth (Rodríguez et al. [2006\)](#page-14-6). Bacteria that are involved in phosphate solubilization and nitrogen fixation can be used in biofertilizers (Cakmakci et al. [2007\)](#page-12-18). Some bacteria such as *Bacillus* and *Rhodococcus* are reported to be involved in the siderophore production (Tian et al. [2009\)](#page-14-7). Microorganisms that are present in rhizospheric environment improve the plant growth and directly or indirectly involved in yield increase of the plant (Dimkpa et al. [2009\)](#page-12-19).

Bacillus is involved in growth promotion of plants by producing auxin and siderophore (Kumar et al. [2012\)](#page-13-17). Beneficial microorganisms can be used as biofertilizers, hence minimizing the use of chemical fertilizers. The usage of microorganisms as a biofertilizer is cost-effective, reduces pollution caused by chemical fertilizers, and helps to preserve the natural environment (Stefan et al. [2008\)](#page-14-8).

Release of heavy metals from natural sources and anthropogenic sources poses a major menace to the soil environment (de Oliveira et al. [2001](#page-12-20)). Generally, heavy metals cannot be degraded by biological mechanisms and exist in the environment to an indefinite extent. After their accumulation in the soils, the lethal heavy metals adversely influence the soil microflora, including plant growth-promoting rhizobacteria (PGPR) in the rhizosphere, and their physiological processes. Furthermore, the elevated concentrations of heavy metals and their uptake by plants also pose adverse effects on plant growth (Han et al. [2006](#page-12-21)), symbiotic relationships, and ultimately crop yields by disrupting cell organelles and disintegrating the membranes, serving as genotoxic substance that disrupts photosynthetic and respiration processes (Piehler et al. [1999;](#page-13-18) Perez-Sanz et al. [2012](#page-13-19)). Therefore, the bioremediation of heavy metal-polluted sites has become an urgent need, as these lands have covered large areas which have been interpreted inapplicable for sustainable agriculture.

Amin and Latif ([2015\)](#page-11-0) have provided a comprehensive study of literature about Hg-induced toxicity in plants and its detoxification processes to provoke the advance research in this field. Two extensively studied bioremediation systems based on clustered genes on *Mer* operon and also *Met* gene allow microorganisms to detoxify

 Hg^{2} into volatile Hg^{0} and to precipitate it into nontoxic HgS by encoding mercuric reductase and also sulfhydrylase (SHLase) enzymes, respectively (Ray et al. [1989;](#page-14-9) Ono et al. [1991](#page-13-20), [1996](#page-13-21)). Detoxification mechanisms that employ different microbes to take off environmental contaminants have obtained a profound interest in the recent years (Gupta and Ali [2004](#page-12-22)).

Many bacterial and yeast genera are being commonly used in the bioremediation of heavy metals (Patra and Sharma [2000;](#page-13-5) Patra et al. [2004](#page-13-6)). Rafique et al. [\(2015](#page-14-10)) have reported species of *Cronobacter*, *Pseudomonas*, and *Bacillus* which are capable to bioremediate mercury up to 95% in mercuric chloride supplemented in YEM medium. The ability of mercury-resistant nitrogen-fixing bacteria (NFB) to remediate it from the synthetic medium, containing 20 μ g/ml HgCl₂, was determined. Figure [14.2](#page-6-0) indicates that *Cronobacter* species (ZM12 and ZM36) are more efficient to remove mercury from the medium as compared to *Pseudomonas* (ZM24, ZM45, and ZM50) and *Bacillus* sp. (ZM2, ZM40, and ZM57). It is also clear from the observations that H2S producing NFB with minimum zone of inhibition on Hg amended agar plates are more resistant to mercury and remediate up to 95% of total mercury supplemented in synthetic YEM medium.

Tariq et al. [\(2015](#page-14-11)) have also reported *Pseudomonas* spp. on the basis of biochemical characterization and single-sequence repeat (SSR) phylogenetic analysis that possess dual characteristics such as detoxification of mercury pollutants and fixation of atmospheric nitrogen (N_2) . The phylogenetic tree was constructed in order to check the percentage homology of different *Pseudomonas* species which

Fig. 14.2 Correlation between zone of inhibition (mm) by well-plate method (*dotted bars*) and removal of mercury (μg/ml) in culture medium after 36 h incubation at 37 °C quantified by dithizone method (*black bars*). The *p* < 0.05 was calculated by ANOVA, and different letters indicate significant difference between means of each treatments calculated by DMRT at probability level 0.05

Fig. 14.3 Genetic diversity in mercury-resistant *Pseudomonas* species using SSR (GACA)₄: (a) Gel electrophoresis (**b**) Dendrogram constructed by using SSR banding pattern

showed that strains SZ-2, SZ-10, SZ-20, and SZ-29 (cluster 1) showed highest homology (100%) with others on the basis of banding pattern while strain SZ-16 showed 81.45% similarity with strains of cluster 1. Similarly, strains SZ-30, SZ-6, and SZ-8 showed 71%, 46%, and 44% homology with strains of cluster 1 and SZ-16, respectively (Fig. [14.3](#page-7-0)).

Agronomic strategy for using these microorganisms is helpful for obtaining sustainable agriculture. No doubt, a continued work in this area of research is needed to explore the potential of PGPRs and their ecological, genetic, and biochemical relationships in habitat.

Among yeasts, *Candida xylopsoci* and *Pichia kudriavzevii* have the potential to detoxify mercury by 95% and 94.5%, respectively, from enriched medium containing mercury (Amin and Latif [2011](#page-11-1)).

The study suggests that both strains may have significant biotechnological role in the treatment of contaminants, containing mercury, before they discharge into the soil environment to make it friendly for living organisms. In another study, Amin and Latif [\(2013](#page-11-2)) have reported that immobilization of yeast cells responsible for the detoxification of mercury has numerous advantages over free suspended culture (Fig. [14.4\)](#page-8-0). The immobilization of yeast cells has advantages over the free cells such as the reuses of entrapped yeast strains to remediate mercury remain constant after using multiple times (Fig. [14.5](#page-8-1)).

Fig. 14.5 Repeated use of immobilized *Candida xylopsoci* (Z-HS51) and *C. rugosa* (Z-HS 13) to check the potential of mercury remediation (four constitutive cycles). Reduction in mercury concentration (μg/ml) is shown by *bars*, and *encircled* one shows the comparison of immobilized beads with free cells of *C. xylopsoci* for the remediation of mercury from the culture medium supplemented with 20 μ g/ml HgCl₂

The most important finding of this study is that no residues remain in the medium because they redissolved at the end. The same strategy can be applied in any polluted reservoir because the immobilized cells never lose their ability to reduce the pollutants from the environment and also there would not be any need to dispose entrapped microorganism from the bioreactor because they redissolve within the system. The immobilization does not affect the shelf life of microbes but provides favorable microenvironmental conditions for the organisms, protects against harsh environment, improves genetic stability, and can be transferred easily and safely at any time and place.

Thus, by applying these microorganisms as a biofertilizer to heavy metalcontaminated soils, the toxicity of heavy metal can be reduced resulting in the enhancement of soil fertility and crop productivity which aids in sustainable agriculture.

14.1.5 Remediation of Heavy Metals by Plants

Phytoremediation uses different types of green plants to clean up hazardous waste from contaminated soil polluted by heavy metals. It is an important form of bioremediation and is suitable for pollutants that cover a large area and are within the root zone of the plant (Padmavathiamma and Li [2007\)](#page-13-22). There are different remediating mechanisms of heavy metals by plants.

14.1.5.1 Phytoextraction

Phytoextraction is primarily being used for the remediation of heavy metal-polluted rhizospheric soils. In this treatment, specific plant species, also known as higher accumulator, absorb and precipitate the higher concentrations of heavy metals from polluted soils and accumulate them into their aerial parts. Padmavathiamma and Li [\(2007](#page-13-22)) found that some plants have a great potential to extract the concentrated heavy metals into their roots and translocate them into their aerial parts which results in the production of increased plant biomass. Plants used for phytoextraction usually have the following characteristics: rapid growth rate, high biomass, extensive root system, and ability to take high amounts of heavy metals. Generally, there are different criteria being used for hyperaccumulators:

- 1. The concentration of metal in the shoot must be higher than 0.1% for Al, As, Co, Cr, Cu, Ni, and Se, higher than 0.01% for Cd, and higher than 1.0% for Zn.
- 2. The ratio of shoot to root concentration must be consistently higher than 1; this indicates the capability to transport metals from roots to shoots, the existence of hypertolerance ability, and the degree of plant metal uptake.

In most cases, plants absorb metals that are readily available in the soil solution. Some metals are present in soil in soluble forms for plant uptake, whereas others occur as insoluble precipitates and are thus unavailable for plant uptake.

14.1.5.2 Phytostabilization

Phytostabilization is also being used for the treatment of heavy metal-polluted soils, sediments, and sludges. By this method, heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn) are being remediated by plant roots which limit the heavy metals in the rhizosphere via mobility and bioavailability mechanisms (Sharma and Sharma [1993\)](#page-14-12). The plants prohibit the root epidermis via soil matrix and act as barrier which results in the decrease of water percolation and also prevent direct contact with the polluted rhizosphere. It may also prevent the soil from reducing their bioavailability through erosion, leaching, and distribution of the toxic heavy metal to other areas. It is helpful in the treatment of contaminated land areas affected by mining activities. Plants help stabilize the soil through their root systems that prevent leaching, and hence erosion, via reduction of water percolation through the soil. Plants used for phytostabilization should have the following characteristics: dense rooting system, ability to tolerate soil conditions, ease of establishment and maintenance under field conditions, rapid growth to provide adequate ground coverage, longevity, and ability to self-propagate. Soil and organic amendments are used for contaminated soil to reduce the toxicity of heavy metals, increase availability of nutrients for plant growth, and improve the physical properties of soil. Several studies have suggested that phytostabilization may detoxify metal toxicity by converting soluble oxidation state to an insoluble oxidation state of metal, e.g., plants have converted available toxic Cr (VI) to unavailable and less toxic Cr (III) (Salt et al. [1995\)](#page-14-13).

14.1.5.3 Phytovolatilization

Phytovolatilization is a method being used for the treatment of heavy metal-polluted soils. In this remediation process, plants take up heavy metals from rhizosphere and transform their metallic forms into volatile forms which are then released into the atmosphere by transformation. The growing trees and other plants may use the xylem vessels for passing heavy metal contaminants from rhizosphere toward the leaves where transformation from toxic to nontoxic forms may occur and then may finally volatilize into the atmosphere. It is basically used for As, Hg, and Se which exist as gaseous species in the contaminated soil. In the recent years, scientists have found natural and genetically modified plants which have capability to absorb toxic forms of these metals and then biologically converting them into gaseous states for releasing them into the atmosphere. Phytovolatilization is a controversial technology in the field of phytoremediation because Hg and Se are toxic so there is uncertainty about the biosafety of these elements into the atmosphere (Suszcynsky and Shann [1995](#page-14-14); Sakakibara et al. [2010\)](#page-14-15). In Se phytovolatilization, the gaseous Se is produced from inorganic or organic Se compounds (McGrath et al. [2002\)](#page-13-23). Moreover, Se pollution is a worldwide problem, so its volatilization into the atmosphere is an attractive phytoremediation technology. Furthermore, many researchers have made considerable efforts to inert mercuric ion $(Hg⁺²)$ reductase into plants for Hg-volatilization (Rugh et al. [1998;](#page-14-16) Bizily et al. [1999](#page-12-23)).

14.2 Remediation of Heavy Metals by Combination of Plants and Microbes

The combined use of both microorganisms and plants for the remediation of polluted soils results in a faster and more efficient cleanup of the polluted area. Mycorrhizal fungi have been used in several remediation techniques of heavy metalpolluted soils. Increased mycorrhizal efficiency have resulted into decreased metal accumulation and increased the growth of white clover growing in heavy metal (Zn)-polluted soil.

Phytoextraction is the best method for the accumulation of heavy metals in plants, and other methods improve phytostabilization through metal immobilization and reduction of metal concentration in plants (Abhilash et al. [2012](#page-11-3)).

In general, the benefits derived from mycorrhizal associations, which range from increased nutrient and water acquisition to the provision of a stable soil, for plant growth and increase in plant resistance to diseases are believed to aid the survival of plants growing in polluted soils and thus help in the vegetation and revegetation of remediated soils. In addition of certain species of mycorrhizal fungi, arbuscular mycorrhizal fungi can be more sensitive to pollutants compared to plants. Other microorganisms apart from mycorrhizal fungi have also been used in conjunction with plants for the remediation of heavy metal-polluted soils. Most of these microbes are the PGPR that are usually found in the rhizosphere. Several microbes stimulate plant growth by some mechanisms such as production of phytohormones, siderophores, and other chelating agents specific for enzyme activity, supplying nutrients, N fixation, and reduction of ethylene production to encourage root growth (Divya and Kumar [2011\)](#page-12-24).

Enhanced accumulation of heavy metals such as Cd and Ni by hyperaccumulators (*Brassica juncea* and *Brassica napus*) has been observed when the plants were inoculated with *Bacillus* spp. (Khalid and Tinsley [1980](#page-13-24)). On the other hand, increased plant growth due to reduction in the accumulation of Cd and Ni in the shoot and root tissues of tomato plant was observed when it was inoculated with *Methylobacterium oryzae*.

14.3 Conclusion

This chapter reveals that heavy metals are hazardous contaminants associated with serious problems in plants and animals because they can be easily spread through many ecosystems. Unfortunately, very less knowledge is available about phytotoxicity caused by heavy metals, processes by which heavy metals are absorbed by plant cells and detoxification mechanisms by which they are modified from toxic to nontoxic form in soil through microorganisms. Although plants attribute a significant role as the base of several trophic levels in food chain, particularly of humankind subsistence and thriftiness, it is an urgent necessity to upgrade the knowledge about the mechanisms of heavy metal uptake by plants, its phytotoxicity, and bioremediation mechanisms of these pollutants. Combining both plants and microorganisms in bioremediation increases the efficiency of remediation. The literature presented here provides a worthy rootage for other scientists engaged in research on heavy metal-induced phytotoxicity and its modification or bioremediation processes to stimulate foster research in this field.

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