Phytoremediation of Heavy Metals and Radionuclides: Sustainable Approach to Environmental Management

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Abstract

Heavy metals (HMs) and radionuclides pose a serious threat to human health because of their ubiquity, non-biodegradability, and long-term persistence in the environment. The presence of high amounts of these pollutants in soil has a detrimental influence on soil fertility, agricultural productivity, and yield. HMs and radionuclides contamination have been remediated using a variety of conventional approaches. However, these technologies have limitations, such as excessive cost, intensive labor, and alteration of the soil native microflora by affecting soil properties with the potential to pollute the environment with the release of secondary pollutants. As a result, switching to a more cost-effective and eco-friendly method is very desirable. Phytoremediation technology for HMs and radionuclides decontamination has been recognized as a novel, low-cost, and ecologically acceptable solution. The present chapter explains the major processes of phytoremediation, as well as the function of transgenic plants in increasing plant efficacy for HMs and radionuclides decontamination. The role of plant growth regulators (PGRs), beneficial microorganisms, arbuscular mycorrhizal fungi (AMF), and nanoparticles (NPs) in phytoremediation is also discussed.

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5.1 Introduction

Pollutants are substances that are found at higher concentrations in the environment than their natural abundance and have a negative impact on the ecosystem. Organic pollutants include benzene, toluene, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), dioxins, nitro-aromatics, dyes, polymers, pesticides, and chlorinated organics. Inorganic pollutants, on the other hand, comprise a variety of toxic heavy metals (HMs) and radionuclides. HMs are highly notorious contaminants because of their abundance, non-biodegradability, and long-term persistence in the environment. They include copper (Cu), cadmium (Cd), chromium (Cr), cobalt (Co), zinc (Zn), iron (Fe), nickel (Ni), mercury (Hg), lead (Pb), arsenic (As), aluminum (Al), silver (Ag), and platinum (Pt). HMs pollute the soil and water and have toxic, genotoxic, teratogenic, and mutagenic impacts on living organisms. Once accumulated in soils, these metals have an inverse effect on soil fertility and diminish agricultural production. Furthermore, even at low concentrations, they induce endocrine disruption and neurological problems. They are classified as priority pollutants by environmental protection agencies across the globe because they can pose serious health risks. Like HMs, radionuclides cannot be naturally or synthetically degraded. In addition, numerous studies have reported that cesium (^{37}Cs) and strontium (^{90}Sr) are not removed from the top 0.4 meters of soil even under high rainfall, and the migration rate from the top few centimeters of soil is slow. Therefore, radionuclides have become a threat to public health when exposed and/or deposited in the soil and water. Moreover, exposure to radioactivity is a common and natural phenomenon. For instance, exposure to cosmic radiation, radon (Rn) gas from rocks and soil, or potassium (^{40}K) through food.

Furthermore, elevated levels of these pollutants in soils have a negative impact on crop development, and yields by dissolving cell organelles and disrupting membranes, acting as genotoxic substances, disrupting physiological processes like photosynthesis, or inactivating respiration, protein synthesis, and carbohydrate metabolism. Hence, remediation of these pollutants has become a necessity to sustain a stable environment. Several traditional remediation approaches have been explored to remediate HMs and radionuclides contamination. However, these technologies are costly and hazardous with the potential to release secondary pollutants into the environment. Therefore, adaptation to an alternative, cost-effective, eco-friendly technology having high removal efficiency is highly desirable. Phytoremediation has been identified as an emerging, low-cost, and eco-sustainable approach to HMs and radionuclides decontamination (Sarma et al. 2021). Phytoremediation uses plants to remove, degrade, or detoxify toxic metals (Nedjimi 2021; Thakare et al. 2021). Phytoextraction, phytostabilization, phytovolatilization, phytodegradation, and rhizodegradation are types of phytoremediation techniques that have been utilized for soil decontamination. The present chapter discusses various sources and toxic effects of HMs and radionuclides, plant strategies for avoiding and/or tolerating hazardous metals, as well as the importance of genetic engineering (GE) in improving efficiency of phytoremediation. The role of plant growth regulators (PGRs), beneficial microorganisms, arbuscular mycorrhizal fungi (AMF), and nanoparticles (NPs) in assisting phytoremediation is also highlighted.

5.2 Heavy Metals (HMs) and Radionuclides

Heavy metals (HMs) are defined as elements with numerous metallic properties, i.e., ductility, conductivity, stability, ligand specificity, etc., an atomic number >20, and a density >5 g/cm^3. They are generally present in the environment at a trace level (<1 g/kg/ppb). HMs can also be classified into essential and non-essential HMs. Essential HMs consist of Co, Cr, Cu, Fe, Mn, Ni, and Zn and non-essential includes Pb, Cd, and Hg. In addition, according to their level of toxicity, they can also be grouped as extremely poisonous, moderately poisonous, and relatively less poisonous. Radionuclides, on the other hand, are a class of chemicals where the nucleus of the atom is unstable. Radionuclides achieve stability through changes in the nucleus (spontaneous fission, emission of alpha particles, or conversion of neutrons to protons or the reverse). The emission of radionuclides from nuclear power plants, as well as their subsequent mobility in the environment, is a subject of intense public concern. HMs and radionuclides are emitted from both natural as well as anthropogenic sources, such as automobile exhaust, smelting, warfare, electronic industries, agrochemical use, irrigation, waste disposal, fossil fuel consumption, nuclear plants, and nuclear weapons testing as shown in Table 5.1.

The accumulation of HMs in the soil causes severe health problems for plants, animals, and humans. According to the United States Environmental Protection Agency (USEPA), soil HM contamination has caused health issues for about ten million humans all over the world. As a result, HM accumulation in plants via the soil–root interface is a serious threat (Sakizadeha and Ghorbani 2017). The most well-known case of Hg poisoning is the Minamata disease in Japan. Another example of HM poisoning is the disaster in the Spanish national reserve. The water in the reservoir was polluted with traces of HMs, mineral sediment, and acidic chemicals. In addition, Hinckley water contamination is another most common example of Cr contamination in the world. Lead poisoning is also not uncommon and is probably the best example of an HM poisoning. It has been reported that 890,000 children aged 1–5 have elevated blood lead levels in the USA (Pirkle et al. 1998). The Kyshtym disaster (1957), Stationary Low-Power Reactor Number One, also known as SL-1 accident (1961), Three Mile Island accident (1979), Chernobyl accident (1986), and Fukushima Daiichi disaster (2011) are a few major nuclear disasters in history. The Chernobyl disaster in Ukraine is a common example. The Chernobyl accident happened in a dangerously constructed nuclear power reactor

Table 5.1 Sources of heavy metals (HMs) and radionuclides in the environment

Contaminant	Sources
Heavy metals	
Zinc (Zn)	Electroplating and smelting
Cadmium (Cd)	Smelting, incineration, fuel combustion, waste batteries, e-waste, and paint sludge
Copper (Cu)	Mining, electroplating, and smelting operations
Mercury (Hg)	Chlor-alkali plants, thermal power plants, electrical appliances, fluorescent lamps, and hospital waste
Chromium (Cr)	Mining, leather tanning, industrial coolants, and chromium salt manufacturers
Lead (Pb)	Lead-acid batteries, e-waste, coal-based thermal power plants, bangle industry, ceramics, paints, and smelting operations
Arsenic (As)	Geogenic/natural processes, smelting operations, thermal power plants, and fuel-burning
Cobalt (Co)	Volcanic emissions, weathering of rocks, and decomposition of plant waste
Nickel (Ni)	Smelting operations, battery industry, and thermal power plants
Manganese (Mn)	Mining, alloy production, goods processing, iron-manganese operations, welding, and agrochemical production
Iron (Fe)	Geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources
Aluminum (Al)	Mining and processing of aluminum ores or the production of aluminum metal, alloys, and compounds, coal-fired power plants and incinerators
Radionuclides	
Uranium ($^{235,\ 238}$U)	Mining/milling of uranium ores, geological repositories of nuclear waste, testing of nuclear weapons, and natural sources
Thorium (^{232}Th)	Natural, mining, milling and processing, phosphate fertilizer production, tin processing, industrial boilers, and military operations
Strontium ($^{89,\ 90}$Sr)	Spent nuclear fuel, nuclear accidents, nuclear fallout, nuclear fission, nuclear weapons testing, geological repository of nuclear waste, and radioactive storage leaking
Radium ($^{226,\ 228}$Ra)	Decay product of U and Th from mill tailing and production of phosphate fertilizers
Cobalt (^{60}Co)	Car, truck, and airplane exhausts, burning coal and oil, industrial processes, and nuclear medicines
Iodine (^{131}I)	Nuclear tests, fuel reprocessing, and spent nuclear fuel
Cesium (^{137}Cs)	Nuclear accidents and weapons testing
Carbon (^{14}C)	Natural and nuclear weapons explosions
Tritium (^{3}H)	Nuclear accidents and testing of nuclear weapons
Potassium (^{40}K)	Natural
Plutonium (^{239}Pu)	Geological repositories of nuclear waste, nuclear accidents, testing of nuclear weapons, and fuel reprocessing
Radon (^{220,226}Rn)	Decay product of U and Th from mill tailing

with a total meltdown of the core and 10 days of free emission of radionuclides into the atmosphere. In addition, nuclear disasters, such as Fukushima, have contaminated coastal ecosystems by dispersing radionuclides. Several amendments' applications, independently of their type and concentration, reduced their concentrations in the soil available fraction and the soil leachates. Any change in the concentration of these metals will either cause deficiency or will interfere with cellular functions, ultimately adversely affecting the growth of plants, as presented in Table 5.2.

5.3 Phytoremediation: An Environmental Tool for the Reclamation of Contaminated Sites

Phytoremediation is a broad concept that refers to a variety of processes involving plant–soil–atmosphere interactions. It is an emerging technology that involves the use of plants to extract, sequester, degrade, or immobilize pollutants from the soil and water. Potential plants for phytoremediation usually possess four important characteristics; (1) rapid growth and high biomass, (2) abstruse root system, (3) harvestable, and (4) accumulation of excessive concentration of pollutants in the shoots. The ability of plants to remove HMs and radionuclides from soils has been reported by many researchers. *Eichhornia crassipes* roots removed 54% of the initial U within 4 min of contact time (Bhainsa and D'Souza 2001). Entry et al. (2001) compared the potential of bahiagrass, Johnson grass, and switchgrass to accumulate ^{137}Cs and ^{90}Sr from contaminated soils in the presence and absence of either sphagnum peat or poultry litter amendments. Johnson grass growing on soil treated with chicken litter showed the highest accumulation of these radionuclides. Among three plants, viz., Indian mustard, redroot pigweed, and tepary bean, redroot pigweed showed the highest accumulation of ^{137}Cs and ^{90}Sr (Fuhrmann et al. 2002). Bystrzejewska-Piotrowska and Urban (2004) reported that onion plants (*Allium cepa*) may play an important role in the ^{137}Cs recycling by facilitating the transfer of fallout ^{137}Cs to the soil. Eapen et al. (2006) reported that *Calotropis gigantea* plants accumulated ^{90}Sr and ^{137}Cs more in their roots than in their shoots. Sasmaz and Sasmaz (2009) reported that *Astragalus gummifer* can be utilized to rehabilitate the soil contaminated by Sr. In another study, *Ocimum basilicum* seeds showed significant uptake of both ^{137}Cs and ^{90}Sr. The maximum adsorption capacity was 160 mg Cs g^{-1} and 247 mg Sr g^{-1} seed dry weight (Chakraborty et al. 2007). *Melastoma malabathricum* L. was reported to accumulate a relatively high range of Pb and As concentration (Selamat et al. 2014). In comparison to other plants, *Miscanthus floridulus* and *Cyperus iria* are reported to have the potential for phytoremediation of radionuclide ^{232}Th in the soil (Yan 2016). In another study, Bhat et al. (2016) reported that *Centella asiatica* can uptake and accumulate Fe significantly in the aerial parts. Silva et al. (2018) suggested that *Cassia alata* plants can be used for the phytoremediation of Cd. *Hypnum plumaeforme* has been described as a possible Rn pollution accumulator plant, as well as a possible indicator plant for Rn pollution monitoring (Zhang et al. 2019). Phytoremediation

Table 5.2 Effects of heavy metals (HMs) and radionuclides on plants

Contaminant	Harmful effects
Zn	Excessive concentration of Zn hampers growth and development, metabolism and causes oxidative damage in plants. It also affects the catalytic efficiency of enzymes, which results in retarded growth and ultimately causes senescence
Cd	Elevated levels of Cd show symptoms of injury, i.e., chlorosis, inhibition of growth, root tips browning, and finally death. It might also reduce the absorption of nitrate and its transport from roots to shoots by inhibiting nitrate reductase activity. It can also induce lipid peroxidation, inhibit chlorophyll biosynthesis, and reduce the activity of enzymes that are involved in the fixation of CO_2
Cu	Cu in the soil is cytotoxic. Elevated concentrations of Cu cause oxidative stress and the development of reactive oxygen species (ROS). It can disturb metabolic pathways and also damage macromolecules. Cu causes leaf chlorosis and plant growth retardation
Hg	A high level of Hg^{2+} inhibits mitochondrial function and causes oxidative stress by activating the development of ROS. This ultimately leads to the disruption of biomembrane lipids and plant cellular metabolism
Cr	A high concentration of Cr affects the germination of seeds. It can also interfere with the process of photosynthesis, i.e., CO_2 fixation, electron transport, photo-phosphorylation, and enzyme activities
Pb	The toxic concentration adversely affects growth and photosynthetic processes by inhibiting the activity of carboxylation enzymes. It also inhibits elongation of roots and stems and expansion of leaves. Pb poisoning also impairs mineral nutrition by inhibiting enzyme activity, creating a water imbalance, altering membrane permeability, and disrupting mineral nutrition
As	Roots are generally the first tissue to be exposed to As, where the metalloid inhibits root extension and proliferation. As interferes with critical metabolic processes, which can lead to death. Antioxidant resistance systems are triggered by As exposure
Co	Crop dropping, suppression of greening, discolored veins, premature leaf closure, and decreased shoot weight are the toxic effects of Co
Ni	Excessive Ni^{2+} in the soil induces a variety of physiological changes and toxicity in plants, including chlorosis and necrosis. A high Ni^{2+} environment causes nutrient imbalance, which leads to cell membrane dysfunction
Mn	The accumulation of too much Mn in the leaves reduces the photosynthetic rate. Mn toxicity is characterized by necrotic brown spotting on leaves, petioles, and stems. The symptom is commonly known as "crinkle leaf." It is also linked to browning and chlorosis in these tissues. Excess Mn is said to block a Fe-related mechanism, preventing chlorophyll synthesis
Fe	The excess Fe^{2+} produces free radicals, which irreversibly destroy cellular structure and damage membranes, DNA, and proteins
Al	An elevated concentration of Al causes a reduction in plant growth, thickening of roots, root tip dieback, yellowing and purpling, wilting, loss of apical dominance, and sometimes loss of geotropism occurs. Al also reduces the performance of several enzymes such as ATPases
Radionuclides	An elevated concentration of U can cause macroscopic effects such as stunted growth and reduced biomass production. U can interact with macromolecules and can affect enzyme capacities and membrane permeability, inducing oxidative stress-related responses in plants. The higher concentration of Sr damages various processes of photosynthesis, such as energy absorption, energy transfer, and photosynthetic carbon assimilation, and induces oxidative stress

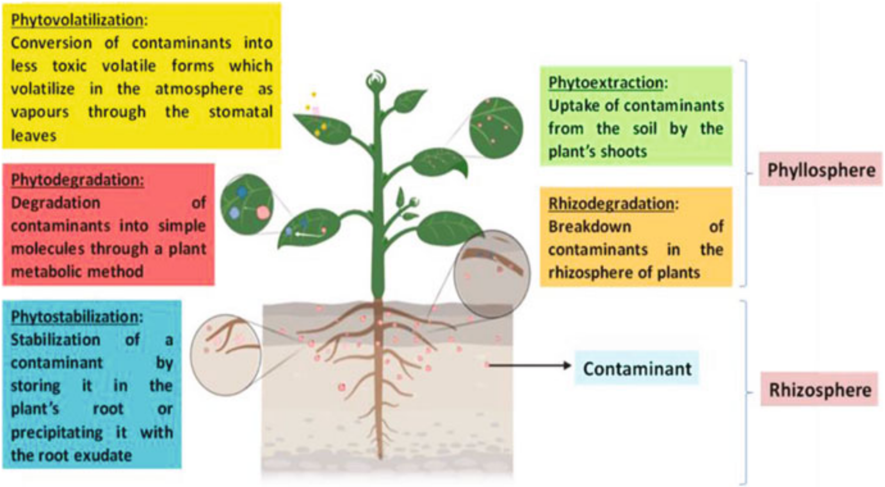

Fig. 5.1 An overview of the soil contaminant cleanup mechanisms

employs various techniques, such as phytoextraction, phytostabilization, phytovolatilization, phytodegradation, and rhizodegradation for the remediation of polluted soil as present in Fig. 5.1.

5.3.1 Phytoextraction

Plants can absorb nutrients from the soil naturally. The absorption of chemicals through the plant's root system and the accumulation of metal and radioactive contaminants from the soil in their shoots is known as phytoextraction. Phytoextraction is also called phytoaccumulation. The contaminants, as well as plant biomass containing metals and radionuclides, have been extracted during the post-harvest process (Raskin and Ensley 2000). Phytoextraction has been applied to many contaminants such as metals-Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, and Zn (Salt et al. 1995), metalloids-As and Se (Kumar et al. 1995), and radionuclides- ^{90}Sr, ^{95}Nb, ^{99}Tc, ^{106}Ru, ^{144}Ce, ^{226,228}Ra, ^{239,240}Pu, ^{241}Am, ^{228,230,232}Th, ^{244}Cm, and ^{237}Np (Nisbet and Shaw 1994; Kabata-Pendias and Pendias 1996). The ability of a plant to translocate and accumulate contaminants varies, depending on the plant species (Susarla et al. 2002).

5.3.2 Phytostabilization

The alternative approach is to slow down contaminant movement and stabilize the contaminant by storing it in the plant roots or precipitating it with root exudate. This method is best for dealing with radionuclides with short half-lives (Lee 2013) and metals such as Pb, As, Cd, Cr, Cu, and Zn (Etim 2012). Metals in the root zone can be stabilized by changing their oxidation state from soluble to insoluble by root-mediated precipitation. Metal-tolerant plants are used to restore vegetation at polluted sites, reducing the risk of contaminants migrating by wind erosion and transport of exposed surface soils, as well as pollution leaching into groundwater. Plants also help to prevent soil erosion and reduce the amount of water available in the environment through a thick root system.

5.3.3 Phytovolatilization

Phytovolatilization is the process of contaminants being absorbed and converted into less toxic volatile forms which are assimilated by the roots, translocated to the shoot, and then volatilized in the atmosphere as vapors through the stomatal leaves (Tollsten and Muller 1996; Raskin and Ensley 2000). Phytovolatilization can occur with contaminants present in the soil, sediment, or water. This approach has the benefit of converting the contaminant, mercuric ion, into a less toxic material. The downside is that Hg emitted into the atmosphere is likely to be recycled by precipitation and then redeposited in lakes and oceans. Phytovolatilization of radionuclides, which takes advantage of a plant's ability to transpire massive quantities of water, is currently being used for tritium (^3H) remediation. Tritium, a radioactive hydrogen isotope with a half-life of around 12 years, decays to stable He. However, since phytovolatilization requires the release of pollutants into the environment, a risk assessment of the effects on the ecosystem and human health may be required.

5.3.4 Phytodegradation

Phytodegradation, also known as phytotransformation, is the degradation of organic contaminants into simple molecules through a plant metabolic method. Contaminant-metabolizing enzymes formed by plants can be released into the rhizosphere, where they may continue to work in contaminant transformation. Dehydrogenase, nitrogenase, laccase, and nitrilase are examples of plant-formed enzymes in plant sediments and soils and released by roots (Schnoor et al. 1995). The plant degrades the organic contaminant and uses it for its own purposes. Plants can pick up nitrate and integrate it into proteins or other nitrogen-containing compounds or it can be converted to nitrogen gas. Some organic contaminants, such as chlorinated solvents, herbicides, 2,4,6-trinitrotoluene, and trichloroethylene, are remedied through phytodegradation.

5.3.5 Rhizodegradation

Rhizodegradation is a term that describes the breakdown of pollutants in the rhizosphere of plants. Plants provide habitats for bacteria and mycorrhizal fungi to work together to degrade pollutants. The bacteria flourished in the rhizosphere, causes the contaminant to degrade. Plant exudates, such as sugar, amino acids, enzymes, and other components increase the microbial population (Shahzad et al. 2015). The rate of rhizodegradation can be accelerated by soil characteristics such as aeration and moisture content (Kirk et al. 2005). Organic chemicals such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), chlorinated solvents, pesticides, polychlorinated biphenyls (PCBs), benzene, toluene, ethylbenzene, and xylenes are removed through rhizodegradation. Table 5.3 presents various phytoremediation techniques employed for the remediation of HMs and radionuclides.

5.4 Plants Strategies Towards Metals

Plants tolerant to the presence of high concentrations of metals in the soil are classified as metallophytes. To cope with the toxicity of high amounts of elements in the soil, metallophytes exhibit two major strategies, viz., exclusion and accumulation (Baker 1981). In exclusion, plants resist the translocation of metals to their tissues. The metal excluder restricts the amount of metal translocated from roots to shoots, thus maintaining low levels of metal concentration in the aerial sections of the plants. Exclusion involves modification of the pH in the rhizosphere by secretion of organic acids from roots which bind to the metals and decrease their bioavailability. Other mechanisms involve the accumulation of metals in cell walls. However, beyond a certain threshold dose, this mechanism usually breaks down and the metal is taken up by the roots. In *Silene paradoxa*, the generation of metal-excluding root cell walls was suggested to be one of the factors contributing to low Cu accumulation and thus limiting the Cu uptake by the root cells by decreasing their pectin concentration in the cell wall and increasing pectin methylation, thus preventing the binding of Cu (Colzi et al. 2012). Seregin et al. (2003) reported maize as an excluder plant, with its root system acting as a barrier, restricting Ni uptake by above-ground organs. In another study, Wei et al. (2005) reported *Oenothera biennis* and *Commelina communis* as Cd excluders and *Taraxacum mongolicum* as a Zn excluder.

In accumulation, metals are accumulated in a non-toxic form in the upper plant parts at both low and high concentrations. Plants can be distinguished as indicators, accumulators, and hyperaccumulators based on their ability to accumulate metals in their tissues. Indicator plants sequester metals in the above-ground aerial tissue, but the level of metal within their tissue reflects those in the surrounding soil. These plants are of biological and ecological importance since they are pollution indicators. Accumulator plant species can accumulate greater metal concentrations in the aerial portions of the plant with a shoot/root ratio of >1 (Baker 1981). Hyperaccumulator plants can accumulate extraordinarily high amounts of metals in the aerial organs, far

Table 5.3 Remediation of heavy metals (HMs) and radionuclides by different types of phytoremediation

Type	Plant species	HMs/ radionuclides	References
Phytoextraction	*Brassica juncea* and *Brassica chinensis*	U	Huang et al. (1998)
Phytovolatilization	*Arabidopsis thaliana*	Hg	Rugh et al. (1996)
Phytovolatilization	*Liriodendron tulipifera*	Hg	Rugh et al. (1998)
Phytovolatilization	*Arabidopsis* and *Brassica juncea*	Se	LeDuc et al. (2004)
Phytoextraction	*Nyssa sylvatica* and *Liquidambar styraciflua*	^{238}U and ^{232}Th	Hinton et al. (2005)
Phytoextraction	*Rumex crispus*	Zn and Cd	Zhuang et al. (2007)
Phytovolatilization	*Brassica juncea*	Hg	Moreno et al. (2008)
Phytostabilization	*Atriplex halimus subsp. schweinfurthii*	Cd	Nedjimi and Daoud (2009)
Phytoextraction	*Calotropis procera*	Pb and Cd	D'Souza et al. (2010)
Phytoextraction	*Catharanthus roseus*	^{137}Cs	Fulekar et al. (2010)
Phytoextraction	*Salix* spp. and *Helianthus annuus*	U	Mihalik et al. (2010)
Phytoextraction	*Raphanus sativus*	^{88}Sr and ^{133}Cs	Wang et al. (2012)
Phytostabilization	*Solanum nigrum*	Cd	Khan et al. (2014)
Phytostabilization	*Vigna radiata*	Cd	Prapagdee et al. (2014)
Phytoextraction	*Pteris vittata*	As	Lei et al. (2016)
Phytostabilization	*Hibiscus cannabinus*	Cd	Chen et al. (2017)
Phytostabilization	*Leptochloa fusca*	U and Pb	Ahsan et al. (2017)
Phytostabilization	*Helianthus annuus* cv. Zaria	Cd	Shahabivand et al. (2017)
Phytostabilization	*Canavalia ensiformis*	Cu	Santana et al. (2018)
Phytoextraction	*Chlorophytum laxum* R. Br	Cd	Chuaphasuk and Prapagdee (2019)
Phytoextraction	*Lepidium sativum*	Hg	Smolinska (2019)
Phytoextraction	*Rhizophora apiculata*	Mn	Khan et al. (2020)
Phytoextraction	*Vetiveria zizanioides*	U	Pentyala and Eapen (2020)

above the levels found in the majority of species, without experiencing phytotoxic effects. These plants have a high rate of metal uptake, a faster root-to-shoot translocation, and a better ability to detoxify and sequester toxic metal in their leaves (Rascio and Navari-Izzo 2011). The criterion for hyperaccumulators of Co, Cu, Cr, Pb, and Ni are plants containing over 1000 μg/g of any of these elements in the dry matter; for Mn and Zn, the criterion is 10,000 μg/g (Baker and Brooks 1989). The fate of hyperaccumulation depends on the plant species, soil physicochemical properties such as pH, cation exchange capacity (CEC), organic matter content, electrical conductivity (EC), and metal concentration in the soil. Hyperaccumulators are excellent models for studying metal control, including the physiology of metal intake, transport, and sequestration, as well as evolution and adaption in harsh settings. Above-ground parts assimilate high amounts of metal as compared to ground parts in hyperaccumulator plants. Species belonging to the family Brassicaceae, Asteraceae, Amaranthaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae, and Euphorbiaceae have been qualified as hyperaccumulators (Table 5.4). However, high metal specificity, lower biomass production with specific ecology and requirements in terms of climate, soil characteristics, water regime, are some of the obstacles to hyperaccumulator plants based remediation technology.

HMs/radionuclides can be transferred by apoplastic and symplastic channels through the roots, stems, and leaves (Song et al. 2017). Acidification of the rhizosphere via plasma membrane proton pumps and release of ligands capable of chelating the metal allows plants to desorb metals from the soil matrix. The metal ion can be transferred through the root in a radial fashion. Before reaching the xylem for transport to the shoot, the metal passes through the epidermis, cortex, casparian strip in the endodermis, and the pericycle of the roots. Once metal reaches the xylem, it is transported to the leaves by the flow of xylem sap, where it crosses a membrane to enter the leaf tissues. Once metal penetrates the leaf tissues, it can be sequestered in numerous subcellular compartments, such as the cell wall, cytosol, and vacuole, or volatilized through the stomata. Cellular compartmentation of metals in leaves varies between hyperaccumulator species. Kupper et al. (1999) demonstrated that Zn was sequestered predominantly in the epidermal vacuoles in *Thlaspi caerulescens* leaves instead of its mesophyll cells. However, in another study, *Arabidopsis halleri* preferentially accumulated Zn in its mesophyll cells as compared to epidermal cells (Kupper et al. 2000).

5.5 Phytoremediation by Transgenic Plants

Genetic engineering (GE) is used as an efficient method for evaluation and a better understanding of various important steps at the molecular level for improving plant tolerance to various environmental stresses and metal toxicity. A gene from a foreign source, such as a plant species, bacteria, or animals, is transferred and incorporated into the genome of a target plant. The foreign gene inherited after DNA recombination confers unique traits to the plants. GE can significantly improve metal absorption, transport, oxidation, and sequestration. Important crop plants like maize, rice, and sorghum are frequently grown in acidic soils where Al toxicity is a major issue. Overproduction of citrate resulted in Al tolerance in transgenic *Nicotiana tabacum* and *Carica papaya* plants. This study demonstrates that organic acid excretion is a mechanism of Al tolerance in higher plants (de la Fuente et al. 1997). *Arabidopsis thaliana* expressing *merBpe* that encodes for organomercurial lyase (MerB) grew vigorously at a wide range of concentrations of monomethylmercuric chloride and phenylmercuric acetate (Bizily et al. 1999). *Arabidopsis thaliana* expressing *merBpe*

Table 5.4 Potential hyperaccumulator species

HMs	Hyperaccumulator species	References
Zn	*Sedum alfredii*	Yang et al. (2002)
	Potentilla griffithii	Qiu et al. (2006)
	Thlaspi caerulescens	Banasova et al. (2008)
	Justicia procumbens	Phaenark et al. (2009)
Cd	*Sedum alfredii*	Ni and Wei (2003)
	Viola baoshanensis	Liu et al. (2004)
	Thlaspi caerulescens	Banasova et al. (2008)
	Chromolaena odoratum, *Gynura pseudochina*, *Impatiens violaeflora,* and *Justicia procumbens*	Phaenark et al. (2009)
	Lonicera japonica	Liu et al. (2009)
	Prosopis laevigata	Buendia-Gongalez et al. (2010)
	Coronopus didymus	Sidhu et al. (2017)
	Vetiveria zizanioides	Kumar et al. (2018)
Cu	*Helianthus annuus* and *Hydrangea paniculata*	Forte and Mutiti (2017)
	Lactuca sativa	Shams et al. (2019)
Hg	*Mentha arvensis*	Manikandan et al. (2015)
Cr	*Leersia hexandra*	Zhang et al. (2007)
	Prosopis laevigata	Buendia-Gongalez et al. (2010)
	Iris ensata	Usman et al. (2012)
	Nopalea cochenillifera	Adki et al. (2013)
Pb	*Sesbania drummondii*	Sahi et al. (2002)
	Helianthus annuus	Boonyapookana et al. (2005)
	Colocasia esculenta	Islam et al. (2016)
	Hydrangea paniculata	Forte and Mutiti (2017)
As	*Pteris vittata*	Ma et al. (2001)
	Pteris cretica, *Pteris longifolia*, and *Pteris umbrosa*	Zhao et al. (2002)
	Pityrogramma calomelanos	Francesconi et al. (2002)
	Lemma gibba	Mkandawire and Dudel (2005)
Co	*Haumaniastrum robertii* and *Haumaniastrum katangense*	Kabeya et al. (2018)
Ni	*Sebertia acuminata*	Jaffre et al. (1976)
	Berkheya coddii	Robinson et al. (1997a)
	Alyssum bertolonii	Robinson et al. (1997b)
	Streptanthus polygaloides	Reeves et al. (1981)
Mn	*Austromyrtus bidwillii*	Bidwell et al. (2002)
	Phytolacca acinosa	Xue et al. (2004)
	Schima superba	Yang et al. (2008)
	Phytolacca americana	Pollard et al. (2009)
Fe	*Imperata cylindrica*	Rodriguez et al. (2005)
	Centella asiatica	Bhat et al. (2016)

may be used to degrade methylmercury at polluted sites and sequester Hg(II). Expression of *CAX2* (calcium exchanger 2) in *Nicotiana tabacum* accumulated more Ca^{2+}, Cd^{2+}, and Mn^{2+} and was more tolerant to elevated Mn^{2+} levels. The expression of *CAX2* also increased Cd^{2+} and Mn^{2+} transport in isolated root tonoplast vesicles. These findings imply that *CAX2* has a broad substrate range and maybe a key component in improving plant ion tolerance (Hirschi et al. 2000). *Arabidopsis thaliana* plants expressing *Escherichia coli* arsenate reductase (*arsC*) and γ-glutamylcysteine synthetase (γ-ECS) genes enhanced As tolerance and hyperaccumulation of As in above-ground parts (Dhankher et al. 2002). Pilon et al. (2003) expressed a mouse (*Mus musculus*) Se-Cys lyase (SL) in the cytosol or chloroplasts of *Arabidopsis* to direct Se flow away from incorporation into proteins. SL specifically catalyzes the decomposition of Se-Cys into elemental Se and alanine. The transgenics showed SL activities up to two-fold in cytosolic lines and six-fold in chloroplastic lines compared to wild-type plants. Se incorporation into proteins was reduced two-fold in both types of SL transgenics, indicating that the approach successfully redirected Se flow in the plant. Enhanced shoot Se concentrations up to 1.5-fold were shown in both the cytosolic as well as chloroplastic lines.

Eapen et al. (2003) developed hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor* by *Agrobacterium rhizogenes* mediated genetic transformation. The stable, transformed root systems of *B. juncea* and *C. amaranticolor* uptake 20–23% and 13% of U from the solution containing up to 5000 mM concentration, respectively. Wangeline et al. (2004) reported that Indian mustard [*Brassica juncea* (L.) Czern.] transgenics overexpressing ATP sulfurylase were more tolerant to As(III), As(V), Cd, Cu, Hg, and Zn, but less tolerant to Mo and V than the wild-type. LeDuc et al. (2004) overexpressed the gene encoding selenocysteine methyltransferase (SMT) from *Astragalus bisulcatus* in *Arabidopsis* and *B. juncea*. SMT transgenic seedlings tolerated Se, particularly selenite, producing three- to seven-fold greater biomass and three-fold longer root lengths. A significant increase in Se accumulation and volatilization was also observed in SMT plants. To enhance the phytoextraction capacity of *Linum usitatissimum* L., the linseed breeding line AGT 917 was engineered to constitutively express the genetic fusion of the *α*-domain of mammalian metallothionein 1a (*α*MT1a) and the *β*-glucuronidase *gus* gene. The stem of the *αMT1/2* line contained an average of 3.3 and 1.9 times higher levels of Cd than stems of the corresponding AGT 917 when grown in soils amended with Cd at 20 and 360 mg kg^{-1} (Vrbova et al. 2013). In another study, expression of the bacterial Hg transporter *MerE* promoted the transport and accumulation of methylmercury in transgenic *Arabidopsis*, which may be a useful method for improving the efficacy of plants to facilitate the phytoremediation of methylmercury pollution (Sone et al. 2013). Transgenic plants enhancing the phytoremediation of HMs are depicted in Table 5.5.

Table 5.5 Genes introduced into plants for improved phytoremediation of heavy metals (HMs)

Target plant species	Gene introduced	Effect	References
Brassica napus L. and *Nicotiana tabacum* L.	MT-II (human metallothionein-II)	The growth of transformed seedlings was unaffected by up to 100 μM $CdCl_2$	Misra and Gedamu (1989)
Nicotiana tabacum cv. NC89	MT-I (mouse metallothionein-I)	The growth of transformed plants was unaffected by up to 200 μM Cd	Pan et al. (1994)
Arabidopsis thaliana	*merApe9* (mercuric ion reductase)	Transgenic *merApe9* seedlings evolved considerable amounts of Hg^0 relative to control plants. Plants were also resistant to toxic levels of Au^{3+}	Rugh et al. (1996)
Liriodendron tulipifera	*merA18* (mercuric ion reductase)	*merA18* plants conferred resistance to toxic, ionic Hg and released elemental Hg ten times the rate of wild-type plantlets	Rugh et al. (1998)
Brassica juncea	*gshI* (γ-glutamylcysteine synthetase)	Transgenic plants accumulated more Cd and showed increased tolerance than wild-type plants	Zhu et al. (1999)
Nicotiana tabacum	*RCS1* (cysteine synthase)	Transgenics showed up to three-fold higher activity of cysteine synthase and exhibited enhanced Cd tolerance	Harada et al. (2001)
Populus deltoides	*merA9* and *merA18* (mercuric ion reductase)	Transgenic *merA9* and *merA18* plants evolved two- to four-fold the amount of elemental Hg and accumulated significantly higher biomass than wild-type plantlets	Che et al. (2003)
Nicotiana glauca R. Graham	*TaPCS1* (phytochelatin synthase)	Transformed seedlings showed increased tolerance for Pb and Cd	Gisbert et al. (2003)
Arabidopsis thaliana	*YCF1* (yeast cadmium factor 1)	Enhanced tolerance and accumulation of Pb and Cd	Song et al. (2003)
Arabidopsis thaliana	SMT (selenocysteine methyltransferase)	Selenite tolerance and foliar Se accumulation were significantly improved	Ellis et al. (2004)
Arabidopsis thaliana	*AsMT2b* (metallothionein)	Showed stronger Cd tolerance and higher Cd accumulation	Zhang et al. (2006)
Nicotiana glauca	*TaPCS1* (phytochelatin synthase)	Overexpressed gene showed a greater accumulation of Zn, Pb, and Cd	Martinez et al. (2006)
Brassica juncea	*AtPCS1* (phytochelatin synthase)	Significantly higher tolerance to Cd and As was exhibited by transgenic plants	Gasic and Korban (2007)

Arabidopsis thaliana	*GSH1* and *AsPCS1* (γ-glutamylcysteine synthetase and phytochelatin synthase)	Increased tolerance and accumulation of Cd and As in dual-gene transgenic lines were observed	Guo et al. (2008)
Nicotiana tabacum	*tzn*1 (zinc transporter)	Transgenic plants showed enhanced accumulation of Zn (up to 11 times) compared to control plants	Dixit et al. (2010)
Agrostis stolonifera	*PaGCS* (γ-glutamylcysteine synthetase)	Transgenic lines accumulated more Cd^{2+} and phytochelatins (PCs) than the wild-type line	Zhao et al. (2010)
Nicotiana tabacum	*AtPCS1* and *CePCS* (phytochelatin synthase)	Transformants accumulated more As both in roots and leaves	Wojas et al. (2010)
Brassica juncea	*YCF1* (yeast cadmium factor 1)	Transformed seedlings were found to be 1.3–1.6 times more resistant to Cd stress and 1.2–1.4 times more resistant to Pb stress than wild-type plants	Bhuiyan et al. (2011)
Nicotiana tabacum	*tcu*-1 (Cu transporter)	Exhibited higher acquisition of Cu (up to 3.1 times)	Singh et al. (2011)
Nicotiana tabacum	Met(GluCys)6Gly (phytochelatin synthase)	Significantly enhanced Cd accumulation in shoots than control	Postrigan et al. (2012)
Populus alba × *Populus tremula* var. *glandulosa*	*ScYCF1* (yeast cadmium factor 1)	Transgenic poplar plants exhibited enhanced growth, reduced toxicity symptoms, and increased Cd content in the aerial tissue compared to the non-transgenic lines. Plants also accumulated increased amounts of Cd, Zn, and Pb in the roots	Shim et al. (2013)
Nicotiana tabacum	*ScMTII* (metallothionein)	Transgenic tobacco plants accumulated 3.5–4.5-fold more Cd above the threshold level of 100 mg Cd kg^{-1}	Daghan et al. (2013)
Beta vulgaris L.	*StGCS-GS* (γ-glutamylcysteine synthetase-glutathione synthetase)	Transgenic sugar beets accumulated more Cd, Zn, and Cu ions in the shoots and exhibited increased biomass, root length, and relative growth compared to the wild-type	Liu et al. (2015)
Populus tremula × *Populus alba*	*γ-ECS* (γ-glutamylcysteine synthetase)	Exhibited greater Cd accumulation in the aerial parts than wild-type plants in response to Cd^{2+} exposure	He et al. (2015)
Nicotiana tabacum var. Sumsun	*AtACR2* (arsenic reductase 2)	Accumulated higher amount of As in the roots as compared to the wild-type	Nahar et al. (2017)

5.6 Plant Growth Regulators (PGRs) Facilitated Phytoremediation

Plant growth regulators (PGRs) are organic substances that regulate increased plant tolerance to abiotic stress by stimulating expression of the genes associated with antioxidant activity, modulation of cellular redox homeostasis, and alteration in transcription element activities. PGRs include auxins, gibberellins, cytokinins, ethylene, abscisic acid, salicylic acid, jasmonates, brassinosteroids, and strigolactones (Bulak et al. 2014). The exogenous application of indole acetic acid alleviated the negative effect of Cr on growth, protein, nitrogen content, and nitrogen metabolism, and led to a decrease in oxidative injuries caused by Cr (Gangwar and Singh 2011). In *A. thaliana*, 5 μM of gibberellic acid was reported to alleviate Cd toxicity by reducing Cd uptake and lipid peroxidation (Zhu et al. 2012). Ali et al. (2015) reported that the application of gibberellic acid-3 enhanced the length, fresh and dry weight of shoots and roots as well as grain yield of mungbean in the Ni contaminated soils. Application of gibberellic acid-3 significantly increased the biomass of *Solanum nigrum* by 56% and increased Cd concentrations in the shoot by 16% at 1000 mg L^{-1} (Ji et al. 2015). The exogenous abscisic acid can decrease Zn concentrations in *Populus* x *canescens* tissues by modulating the transcript levels of key genes involved in Zn uptake and detoxification, and by activating the antioxidative defense system (Shi et al. 2015). In another study, the addition of exogenous abscisic acid enhanced the tolerance of grapevine (*Vitis vinifera* L.) to excess Zn due to the expression of both *VviZIP* genes and detoxification-related genes (Song et al. 2019). The application of different PGRs (indole-3-acetic acid, indole-3-butyric acid, diethyl aminoethyl hexanoate, 6-benzylaminopurine, 1-naphthylacetic acid, abscisic acid, 2,4-dichlorophenoxyacetic acid, ethrel, brassinolide, gibberellic acid-3, and compound sodium nitrophenolate) enhanced the growth of *Amaranthus hypochondriacus* L. and the phytoextraction efficiency of Cd. However, the application of indole-3-butyric acid or diethyl aminoethyl hexanoate was reported to fix more Cd in upper and lower epidermal cells (Sun et al. 2019). Zhang et al. (2020) reported increased tolerance of tea plants to Cd stress on exogenous application of indole acetic acid (10 μM). Gong et al. (2020) reported that the exogenous application of indole-3-acetic acid reduced the malondialdehyde (MDA) concentrations in Cu stressed seedlings and increased biomass, proline content, and the activities of antioxidant enzymes. Thus, indole-3-acetic acid alleviated Cu toxicity and enhanced Cu tolerance in spinach seedlings.

5.7 Microbial Facilitated Phytoremediation

Beneficial microorganisms associated with plants enhance the efficiency of the phytoremediation process either by altering the metal accumulation in plant tissues or by conferring plant metal tolerance and/or enhancing plant biomass production. These microorganisms influence metal uptake through translocation, transformation, chelation, immobilization, solubilization, precipitation, volatilization, and complexation of metal, ultimately facilitating phytoremediation. Siderophores producing microorganisms inhabiting the rhizosphere are believed to play an important role in HM and radionuclide phytoextraction. Siderophores solubilize unavailable forms of HM and radionuclide bearing minerals by complexation reaction. The production of siderophores by *Streptomyces tendae* F4 has been reported to enhance uptake of Cd in sunflower (Dimkpa et al. 2009). Microbial production of other metabolites such as organic acids (Sayer et al. 1999; Saravanan et al. 2007), biosurfactants (Juwarkar et al. 2007; Sonowal et al. 2022), hormones (Ma et al. 2008), and extracellular polymeric substances such as exopolysaccharides and lipopolysaccharides (Joshi and Juwarkar 2009) also contribute to phytoremediation. Through oxidation or reduction reactions, several plant-associated microorganisms can alter HM and radionuclide mobility. A significant increase in the mobility of Cu, Cd, Hg, and Zn by >90% was reported when co-inoculated with Fe-reducing bacteria and the Fe/S oxidizing bacteria (Beolchini et al. 2009).

Many researchers have reported improved phytoremediation efficiency with plant-associated microorganisms. Chen et al. (2013) suggested that two metal-resistant and plant growth-promoting bacteria, viz., *Burkholderia* sp. J62 and *Pseudomonas thivervalensis* Y-1-3-9, promoted the growth and Cd uptake of *Brassica napus*. The study indicates there might be potential for developing an effective plant–microbe partnership for phytoextraction of Cd from heavily Cd contaminated soils. In another study, inoculation of *Pseudomonas* sp. Lk9 significantly increased shoot dry biomass (14%) and accumulated Cd (46.6%), Zn (16.4%), and Cu (16.0%) in aerial parts of *Solanum nigrum* L. compared to uninoculated plants. This symbiotic association between *S. nigrum* L. and *Pseudomonas* sp. Lk9 also resulted in a significant increase in the soil microbial biomass C (39.2%) and acid phosphatase activity (28.6%.) (Chen et al. 2014). Soil inoculation with *Arthrobacter* sp. TISTR 2220 enhanced Cd accumulation in the roots, above-ground tissues, and whole plant of *Ocimum gratissimum* L. by 1.2-fold, 1.4-fold, and 1.1-fold, respectively. This synergistic use of *Arthrobacter* sp. with *O. gratissimum* L. could be a feasible economic and environmental option for the reclamation of Cd polluted areas (Prapagdee and Khonsue 2015). Szuba et al. (2017) reported that *Paxillus involutus* accumulated Pb in the roots and stems of *Populus* × *canescens* trees, thus improving the host plant growth. Inoculation of *Leptochloa fusca* (L.) Kunth with endophytic bacterial consortia (*Pantoea stewartii* ASI11, *Enterobacter* sp. HU38, and *Microbacterium arborescens* HU33) resulted in a 22–51% increase in root length, 25–62% increase in shoot height, 10–21% increase in chlorophyll content, and 17–59% more plant biomass in U and Pb contaminated soils. Enhanced metal uptake capacity by 53–88% for U and 58–97% for Pb was also observed (Ahsan et al. 2017).

Piriformospora indica enhanced growth, Chl a, Chl b, proline content and showed the ability to immobilize Cd in the root and reduce Cd concentrations in the stem and leaves. This alleviated metal toxicity in the *Helianthus annuus* cv. Zaria plants, and also resulted in phytostabilization of Cd polluted soils (Shahabivand et al. 2017). Inoculation of three metallotolerant siderophore-producing *Streptomyces* sp. B1-B3 strains significantly stimulated plant biomass, reduced oxidative stress, and enhanced the uptake and bioaccumulation of Zn, Cd, and Pb in *Salix dasyclados* L (Zloch et al. 2017). *Bacillus cereus* (T1B3) removed Cr^{6+} (82%), Fe (92%), Mn (67%), Zn (36%), Cd (31%), Cu (25%), and Ni (43%) in the HM amended extract medium. Results indicated that inoculating the native hyperaccumulator *Vetiveria zizanioides* with the T1B3 strain improves the phytoremediation efficiency of *V. zizanioides* (Nayak et al. 2018). Jin et al. (2019) reported that *Simplicillium chinense* QD10 significantly enhanced the phytoextraction of Cd and Pb by *Phragmites communis*. Irshad et al. (2019) reported higher As uptake and removal efficiency by *Vallisneria denseserrulata* and the indigenous *Bacillus* sp. XZM partnership. *Enterobacter* sp. FM-1, a potent bioaugmentation agent, facilitated Mn and Cd phytoextraction in *Polygonum hydropiper* L. and *Polygonum lapathifolium* L. (Li et al. 2020). *Cupriavidus basilensis* strain r507 showed excellent As tolerance, rapid arsenite oxidation ability, and strong colonization of *Pteris vittata*. Inoculation of *P. vittata* with strain r507 accumulated As (up to 171%), suggesting the feasibility of co-cultivating hyperaccumulators with facilitator bacteria for practical As phytoremediation (Yang et al. 2020).

5.8 Arbuscular Mycorrhizal Fungi (AMF) Facilitated Phytoremediation

Arbuscular Mycorrhizal Fungi (AMF) increases tolerance to HMs and radionuclides, improves acquisition of water and nutrients, and results in the establishment of plants in contaminated soil (Thakare et al. 2021). AMF improves phytoremediation via chelation/complexation, compartmentation in vacuoles, metal retention in vesicules, arbuscules, spore and cell walls, and production of glomalin (Cabral et al. 2015). Entry et al. (1999) reported the accumulation of ^{137}Cs and ^{90}Sr from the contaminated soil by bahiagrass (*Paspalum notatum*), Johnson grass (*Sorghum halpense*), and switchgrass (*Panicum virgatum*) after inoculation with *Glomus mosseae* and *Glomus intraradices*. Arriagada et al. (2004) reported that combined inoculation of *Glomus deserticola* and *Trichoderma koningii* resulted in the highest accumulation of Cd in the stem of the eucalyptus plant.

The AM association helped *Phyllanthus niruri* and *Paspalum vaginatum* plants to survive in a disturbed ecosystem by enhancing the uptake and recycling of radionuclides, particularly ^{137}Cs and ^{90}Sr (Selvaraj et al. 2004). Wang et al. (2005) reported that inoculation of an AM fungal consortium consisting of *Gigaspora margarita* ZJ37, *Gigaspora decipiens* ZJ38, *Scutellospora gilmori* ZJ39, *Acaulospora* spp., and *Glomus* spp., increased not only the shoot biomass but also the uptake of Cu, Zn, Pb, and Cd into the shoots of *Elsholtzia splendens* Nakai ex F. Maekawa. Hashem et al. (2016) reported that AMF inoculation mitigated the Cd stress tolerance of *Cassia italica* Mill by reducing lipid peroxidation and enhancing the antioxidant activity. *Trigonella foenum graecum* plants accumulated high concentrations of Cd in their root systems from AMF inoculation. Furthermore, AMF colonization diminished the negative effects of Cd on plant development by increasing antioxidant enzyme activity, soluble protein content, and decreasing malondialdehyde (MDA) content (Abdelhameed and Metwally 2019). Thus, AMF presents a viable strategy to remediate and reclaim sites contaminated with HMs and radionuclides.

5.9 Nanoparticles (NPs) Facilitated Phytoremediation

The use of nanotechnology in conjunction with phytoremediation is progressing rapidly. A nanoparticle's size typically falls between 1 and 100 nanometers. The ability of nanoparticles (NPs) to penetrate within plants and translocate from roots to other areas of the plants is largely determined by their size. Owing to their small size and large surface area, NPs can penetrate the contaminant zone where other particles are unable to, enabling NPs to have a wider range of applications. NPs cause physiological and morphological changes in plants and the plants' response strongly depend on the NPs type, dose, and speciation as well as on the plant species involved. NPs raise the pH of the soil and adsorb metal, reducing its mobility and bioavailability. NPs also boost the plant defense system by regulating the metal transport genes, promoting the synthesis of protective agents, and scavenging reactive oxygen species (ROS) (Zhou et al. 2020; Prasad et al. 2017).

Singh and Lee (2016) observed that an application of 300 mg/kg of nano-titanium dioxide (TiO_2) particles significantly enhanced the Cd uptake (507.6 μg/g) by soybean plants (*Glycine max*) from contaminated soil. In another study, the application of 5 g/kg nano-hydroxyapatite (NHAP) to Pb contaminated soils significantly increased the ryegrass biomass (Jin et al. 2016). Souri et al. (2017) reported a significant beneficial effect of salicylic acid nanoparticles (SANPs) on the growth and phytoremediation efficiency of *Isatis cappadocica* against As toxicity. The maximum As accumulation in the shoots and roots reached 705 mg/kg and 1188 mg/kg, respectively. Gong et al. (2017) studied the effect of 100, 500, and 1000 mg/kg starch stabilized nZVI (S-nZVI) particles on the Cd accumulation in *Boehmeria nivea* (L.) Gaudich (ramie). The addition of S-nZVI particles increased the Cd accumulation in the roots, stems, and leaves by 16–50%, 29–52%, and 31–73%, respectively. Huang et al. (2018) observed maximum accumulation of Pb (1175.40 μg/pot) in ryegrass (*Lolium perenne*) with the treatment of 100 mg/kg nZVI. However, decreased Pb accumulation was reported in high concentrations of nZVI (1000 and 2000 mg/kg). In another study, thidiazuron (TDZ) growth regulator and magnesium oxide (MgO) nanoparticles increased plant growth, phenolic and flavonoid contents, free radical scavenging activity, and Pb phytoaccumulation by radish (*Raphanus sativus* L.) (Hussain et al. 2019).

5.10 Conclusion

Phytoremediation is a cost-effective plant-based approach for the reclamation of HM and radionuclide polluted sites that has a high level of public acceptance. Plants can also be genetically modified to achieve desirable traits such as rapid growth, high biomass output, high metal tolerance and accumulation, and strong adaptation to a variety of climatic and geological settings. The prospect of using transgenic plants to clean up contaminated sites has been thoroughly investigated and many plant species harboring transgenes of various origins and presumptive functions have been surveyed. Furthermore, PGRs, plant-associated microorganisms, AMF, and NPs also boost phytoremediation efficiency.

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