

Chapter 4

Plant Assisted Bioremediation of Heavy Metal Polluted Soils



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Abstract Industrial and anthropogenic activities are the major reason for heavy metal pollution. To date, thousands of hectares of farmland globally and in India specifically have been contaminated by heavy metals. This has adversely affected the crop productivity, soil microbial diversity and eventually deteriorated the soil quality. Soil quality is closely associated with crop quality, human health and welfare. Therefore, the remediation of these metal-polluted soils becomes imperative. Conventional remediation methods like precipitation, oxidation/reduction, filtration, evaporation and adsorption etc. are energy demanding or require a large number of chemical reagents and are associated with possible production of secondary pollutants. Fortunately, some microorganisms with the capability to induce resistance to heavy metals, and reduce or adsorb them in non-toxic form can be used for possible bioremediation

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of polluted soils, thus representing an economical and environment-friendly remediation method. These microbes detoxify the heavy metals, clean up the environment and increase the soil fertility, but, the adsorbed or converted metal still remains in the soil is the problem associated with it. Phytoremediation can be another option for detoxification of heavy metal polluted soils. However, phytoremediation alone has its limitations. Hence, the most effective way of remediation of heavy metal polluted soils is an integrated approach that involves both plants and microbes. Understanding the whole mechanism of plant assisted bioremediation along with bioavailability, uptake, translocation, sequestration and different defence mechanisms will help to develop heavy metal stress-resistant cultivars and highly efficient plant species for phytoremediation in harmony with microflora through genetic engineering technologies. Hence, this chapter will provide an understanding of plant assisted bioremediation, the fate of heavy metals in plant and soil, different plant defence mechanisms and potential microflora for plant assisted bioremediation.

Keywords Bioremediation · Heavy metal pollution · Microflora · Phytoextraction · Soil fertility

4.1 Introduction: Background of Heavy Metal Pollution

Heavy metals being toxic and bioaccumulative in nature, are environmental pollutants with prolonged persistence in the environment, thus leading to detrimental effects on floral wealth and human health (Rzymiski et al. 2014). They are metals possessing a specific density of more than 5 g cm^{-3} and have adverse impacts on the life and environment (Järup 2003). Some metals known as micronutrients, (copper, iron, manganese, molybdenum, nickel and zinc) play a vital role in the normal functioning of plant cells such as biosynthesis of nucleic acids, chlorophyll, carbohydrates, secondary metabolites, stress resistance and maintenance of biological membranes as well as overall growth of the plants (Rengel 2004). However, when their internal concentration transcends a certain threshold limit, they negatively influence plant growth and become toxic, forming a bell-shaped dose–response relationship (Marschner 1995). Moreover, the concentration of heavy metals is generally location-specific, subjected to the source of individual pollutants.

As per the World Health Organization (WHO), the common toxic ‘heavy metals’ of public health concern are arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), manganese (Mn), copper (Cu) and molybdenum (Mo). The standards for heavy metals in soil, plant and water as per Bureau of Indian standards (BIS) and WHO have been presented in Table 4.1.

Table 4.1 Normal and critical range of heavy metals in soil, plant and water

Heavy metal (s)	Normal range in soil (mg kg ⁻¹)	Critical soil total concs (mg kg ⁻¹)	Normal range in plant (mg kg ⁻¹)	Critical concentration in plants	Permissible limit in water (mg L ⁻¹)
Arsenic	0.1–40	20–50	0.02–7	5–20	0.01
Cadmium	0.01–2.0	3–8	0.1–2.4	5–3	0.003
Cobalt	0.5–65	25–50	0.02–1	15–50	0.05
Chromium	5–1500	75–100	0.03–14	5–30	0.05
Mercury	0.01–0.5	0.3–15	0.005–0.17	1–3	0.001
Nickel	2–750	100	0.02–5	10–100	0.02
Lead	2–300	100–400	0.2–20	30–300	0.01
Selenium	0.1–5	5–10	0.0001–0.2	5–30	0.01
Manganese	20–10,000	1500–3000	20–1000	300–500	0.3
Copper	2–250	60–125	5–20	20–100	1.5
Molybdenum	0.1–40	2–10	0.03–5	10–50	0.07

Data from Bowen (1979), Kabata-Pendias and Pendias (1984), BIS (2012)

4.1.1 World Status

Rapid industrialization and exponential increase in the human population has increased the discharge of massive loads of heavy metal pollutants in the environment (Zhang et al. 2020). Globally, around 500 M ha of our land resources are facing the problem of soil contamination ended up with higher concentrations of heavy metals compared to the regulatory levels (Liu et al. 2018). Industries and other human activities discharge approximately 2 million tons/day of sewage and effluents into the water bodies making them unfit for various agricultural and other activities. Fly ash dumping sites of coal-based thermal power stations are also a major source of heavy metal pollution around the world (Pandey and Singh 2010; Pandey 2020). In developing nations, the situation is more critical where about 90% of sewage and 70% of industrial wastes (generally untreated/partially treated) are being discharged into surface water resources (Anonymous 2010). Over the past few years, the annual global release of heavy metals has surpassed 0.2 lacs MT for Cadmium, 9.3 lacs MT for Copper, 7.83 lacs MT for lead and 1.35 lacs MT for Zinc (Thambavani and Prathipa 2012). Further, heavy metal poisoning has become a universal public health concern. Heavy metal pollution in soils also has tremendously impacted the global economy, which has annually been estimated to be beyond US\$10 billion.

4.1.2 Indian Status

The data available on the nature and extent of metal pollution and its impact assessment on the plant, soil and human health is not very conclusive in India. However, as per Indian central pollution control board (CPCB 2009), approximately 38,254 megaliters per day (MLD) of sewage and 25,000 MLD of untreated industrial wastewater generated from urban areas are released into the surface water bodies, wreaking degradation of the quality of water resources. Bhardwaj (2005) has estimated that by 2050, wastewater generation in India is going to be around 1,22,000 MLD. The utilization of such wastewater loaded surface water sources for irrigation purposes in agricultural fields has magnified the heavy metals concentrations in soils of agricultural fields particularly those situated in the vicinity of urban areas (Saha and Panwar 2013). However, the heavy metal accumulation in the soils will vary depending upon the source, concentration and duration of application (Rattan 2005). Usage of sewage water as irrigation for 20 years successively may result in significant accumulation of zinc (2.1 times), copper (1.7 times), iron (1.7 times), nickel (63.1%) and lead (29%) in the soils as compared to soils irrigated with tube well water (Simmons 2006). Such unrestricted transfer of heavy metals in arable land through wastewater irrigation will trigger more metal uptake by crops and will enter the food chain (Rattan 2002).

4.2 Sources of Heavy Metal Pollution

Geogenic and anthropogenic activities are mainly responsible for heavy metal pollution in the environment. Geogenic processes such as biogenic, terrestrial, volcanic processes, erosion, leaching and meteoric are the main sources of heavy metals in the environment (Muradoglu et al. 2015). While, industrialization, urbanization and modernization of the agricultural sector are substantially contributed to the release of heavy metal pollutants into the surrounding which gets deposited on the soil through natural processes of sedimentation and precipitation. In addition, anthropogenic processes such as irrigation with sewage and industrial wastewater, mining activities, fly ash disposal, excessive application of pesticides and fertilizers, have disturbed the natural balance of geochemical cycles, which in turn has resulted in the entry of heavy metals into the soil (Zhang et al. 2011; Dixit et al. 2015). The major contributors of heavy metals in the environment are listed in Table 4.2.

4.3 Plant Assisted Bioremediation: Techniques/Strategies

Plant assisted bioremediation involves the symbiotic relationship between rhizospheric microorganisms and the plant roots (Kumar et al. 2017). The symbiotic relationship intensifies bioavailability of the heavy metals and stimulates absorption

capacity of the roots. Remediation of metal-polluted soil by soil microbes especially the rhizospheric population is known as rhizoremediation (Kuiper et al. 2004). Rhizoremediation involving plant growth-promoting rhizobia, mycorrhiza and other microorganisms is very efficient in promoting plant biomass and thus its efficiency to stabilize and remediate metal-polluted soil (Jing et al. 2007). Plant roots release exudates, may be enzymatic or non-enzymatic that modify the soil environment and habitat to numerous microorganisms. Rhizosphere plays a great role in the remediation of metal polluted soil. Heavy metals can only be transformed via several processes such as sorption, methylation, complexation or change in valence oxidation state, affecting their mobility and bioavailability. Microbes have an important role in the processes like carbon sequestration, plant growth, productivity and phytoremediation. Microorganisms (bacteria, fungi and microalgae) along with plants are the potential agents of bioremediation. They enhance the plant growth through different enzymatic activities, nitrogen fixation and reducing the ethylene production (Pandey and Singh 2019). Bacteria respond to the heavy metals and the molecules generated through oxidative stress in different ways. These are entrapped in the capsules, transported through heavy metals by the cell membrane, absorbed on the cell walls, precipitated or oxidized/reduced (Singh et al. 2010). The microbial response to heavy metals is important in harnessing them as potential candidates for remediation of metal polluted soils (Hemambika et al. 2011). Plant growth-promoting bacteria (PGPB) also known as growth-promoting agents are now assessed for their metal detoxifying potential in remediating metal-polluted soils (Ahemad 2014). Fungi are important as

Table 4.2 Major sources of heavy metals

Heavy metal (s)	Contributors of heavy metals in the environment
Arsenic	Volcanic eruptions, semiconductors, smelting coal mines, power plants, petroleum refining, metal adhesives, ammunition, wood preservatives, pesticides and herbicides, animal feed additives
Copper	Biosolids electroplating, mining activities, petroleum refining and smelting operations
Cadmium	Geogenic sources, metal smelting and refining process, combustion of fossil fuels, fertilizers, sewage sludge
Chromium	Sewage sludge, solid wastes, electroplating, tanning industries
Lead	Mining and smelting of metalliferous ores, leaded gasoline combustion, sewage and industrial waste, paints
Mercury	Volcanic eruptions, wild forest fires, emissions from industries producing caustic soda, combustion of coal, peat and wood
Selenium	Coal mining, oil refineries, fossil fuels, glass manufacturing industry, varnish and pigment formulation
Nickel	Volcanic eruptions, forest fire, landfilling operations, oceanic gaseous exchange, weathering of soils and geological processes
Zinc	Smelting and refining industries, mining operations, electroplating industry, bio solids

Source Lone et al. (2008)

these augment the phytoremediation by changing the bioavailability of metal through different ways like modifying the pH of the soil, production of different chelators, and controlling the redox reaction etc. (Ma et al. 2011a, b). Also, a high surface-to-volume ratio make bacteria a potential biosorbing agents. While, the plants absorb these metals and translocate them to various plant tissues and organs. Plants remediate the heavy metal polluted soil by adopting different techniques/strategies such as phytoextraction, phytostabilization, phytovolatilization, phytostimulation (Pandey and Bajpai 2019; Pathak et al. 2020). These are described as:

Phytoextraction: Phytoextraction is the process of the uptaking and storing of heavy metals from the soil by the plants (McGrath 1998). There are two fundamental ways of phytoextraction:

- **Natural:** The natural way of removal of heavy metals by the plants, also known as unassisted phytoremediation.
- **Assisted:** Microbes, plant hormones and chelating agents assist the plant in the remediation of heavy metal polluted soils (Malik et al. 2022).

Natural phytoremediation can be accomplished by either (1) hyperaccumulator plants or (2) genetic engineering of the plant with certain characteristics of hyperaccumulators for the accomplishment of phytoextraction (Chaney et al. 2005). The hyperaccumulator plants are the plants whose tissues can contain certain heavy metals from 1000 to 10,000 mg kg⁻¹ (Black 1995). They can collect and concentrate the heavy metals in the harvestable tissues, biomass without affecting the plant growth. The heavy metal concentration in the hyperaccumulator plants is approximately 100 times higher compared to the ordinary plants. It is approximately 1000 mg kg⁻¹ for arsenic and nickel, 100 mg kg⁻¹ for cadmium and 10,000 mg kg⁻¹ for zinc and manganese. The most prominent examples of hyperaccumulator plants are *Arabidopsis*, *Alyssum*, *Noccaea* and the members of Brassicaceae family.

Phytostabilization: Phytostabilization involves complexation, precipitation, sorption or metal reduction (Ghosh and Singh 2005). Plants restrict the movement of the metals in the roots by the assimilation, aggregation, adsorption and precipitation. They also help to avoid movement of the metals through water, wind, drainage and dispersion of soil (USEPA 1999). The phytostabilization stabilizes the metal contaminant rather than translocating it to the edible parts, that in turn can reach human beings (Prasad and Freitas 2003). In this, there is the aggregation of metal by roots or root exudates that immobilize and lower the accessibility of the soil pollutants. Chromium and lead are toxic metals that are remediated by phytostabilization. The proficiency of the phytostabilization is increased by the addition of nutrients to soil viz. lime and phosphate. *Brassica juncea* has been reported to be an efficient Phytostabilizer as it accumulates chromium in the roots (Bluskov and Arocena 2005).

Phytovolatilization: The release of metal pollutants to the atmosphere by the plants in altered or unaltered form after metabolic and transpirational pull is called phytovolatilization (USEPA 1999). Selenium, arsenic and mercury are the main metal pollutants that can be remediated through phytovolatilization (Dietz and Schnoor 2001).

4.4 Significance of Plant Assisted Bioremediation of Heavy Metal in Agriculture

Agriculture is the main backbone of the Indian economy and socio-political stability. With approximately 7% of the growth, Indian economy is the 7th largest in the world. The contribution of agriculture and its allied sectors was 51.81% during 1950–51, which declined to 18.20% by 2013–2014 and now it is approximately 14.39% (2018–19). These figures are still higher than most of the countries. Soil quality is one of the main contributing factors for sustainable agriculture. Sustainability is defined as the living within the regenerative capacity of the biosphere (Wackernagel et al. 2002). Inappropriate agricultural management practices, excessive use of a large number of chemicals, insecticides, pesticides, sludge and manure are attributing declination of soil quality. Developmental activities such as industrialization, urbanization and transportation are competing with natural resources, and impacting soil quality and biodiversity (Godfray et al. 2010). Despite several adverse effects of industries on the environment, they are considered important because of the unlimited human desires. The dependency of agriculture and industries on each other and their impact on the environment and human life is depicted in Fig. 4.1. Different anthropogenic activities have become the main reason for the deterioration of natural resources (soil + water). In the long run, polluted soil and water will not be suitable to grow the food which will directly or indirectly impact the socio-economic condition of the country (Saha et al. 2017). In the agroecosystem, agriculture and industries are the main reason for

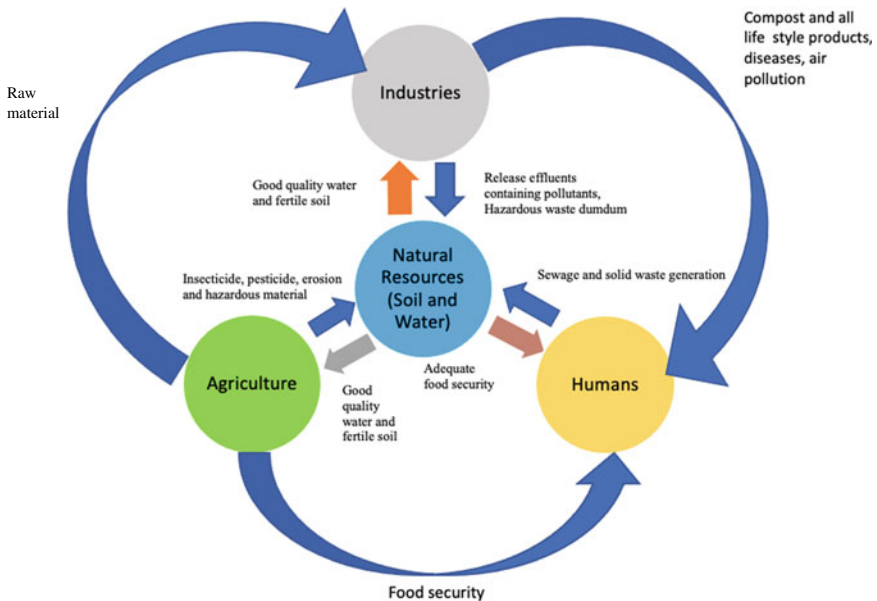


Fig. 4.1 Relationship of agriculture and industrial activities affecting natural resources and humans

soil pollution by heavy metals such as cadmium, chromium, lead, arsenic, nickel and mercury. Introduction of the above metals in the soil environment and their impact on soil is quite alarming, as:

- Entry of these metals in the agroecosystem results in degradation of soil structure and affects moisture retention in the soil profile.
- Heavy metals interact with soil components, microorganisms (nitrogen transformation, mineralization/immobilization etc.), root cells and affect the transformation of soil nutrients and their uptake.
- Build-up of salinity problem in the polluted soil along with the effect on water and nutrient uptake.
- Heavy metals lead to alkalinity development, result in more ammonia volatilization losses.
- The agronomic efficiency and partial factor productivity of polluted soil are normally lower than the unpolluted soil.
- The shelf life of the crops irrigated with industrial wastewater is lower than irrigated with fresh water.
- Heavy metal pollution results in huge ecological disturbances

4.5 Role of Microflora and Flora in Plant Assisted Bioremediation

Plant assisted bioremediation is an eco-friendly approach, encompassing the complex phenomenon of interaction between the microbes and plant genotype with its biotic and abiotic environment. The most important components of plant assisted bioremediation consist the in situ selection of genotypes and symbiotic microorganisms in the rhizosphere (having the capability of degrading the organic contaminants completely). Further, the identification of candidate genes and alleles linked with biochemical and physiological processes also has a key role in the development of a potential plant assisted bioremediation strategy. High levels of metal extraction and translocation to shoots and organic degradation are keys to develop an efficient phytoremediation measure. A most promising approach to substitute the costly remediation technologies is the use of plants assisted by microbes to clean up heavy metal polluted soils and water (Malik et al. 2022). Therefore, the selection of appropriate microbes and plant species is a prerequisite for effective remediation of heavy metal pollution.

4.5.1 Potential Microbes Involved in Bioremediation

Microorganisms help in the uptake of heavy metals through both active (bioaccumulation) and passive (adsorption) modes. Microbes (bacteria, fungi and algae)

have been utilized to remediate the contaminated sites. The high surface to volume ratio, ubiquitous nature, the capability of growing in extreme conditions and active chemisorption sites make bacteria a potential candidate for bioremediation (Srivastava et al. 2015; Mosa et al. 2016). Higher absorption, uptake and recovery capacity of fungi make them suitable biosorbents for the remediation of toxic metals (Fu et al. 2012). Also, algae compared to other biosorbents produce high biomass. The high sorption capacity of algae and the presence of various metal binding chemical groups like hydroxyl, carboxyl, phosphate and amide make them a suitable contender for remediation of heavy metals (Abbas et al. 2014). The microorganisms involved in bioremediation of heavy metals are summarized in Table 4.3.

Numerous researchers have reported bacterial accumulation and sorption along with other plant growth promoting features responsible for the enhanced plant growth in polluted soils (Ma et al. 2011a, b; Kumar et al. 2009). Higher accumulation of heavy metals in plants without having any phytotoxicity is due to decreased internal availability of metals or metalloids and higher rhizospheric plant bioavailability (Deng et al. 2013a, b; Weyens et al. 2010). Nickel uptake by *Alyssum murale* was significantly enhanced by *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens* and *Microbacterium arabinogalactanolyticum* inoculation compared to the un-inoculated control (Abou-Shanab et al. 2003). Correspondingly, the inoculation of *Phaseolus vulgaris* with *Pseudomonas putida* KNP9 protected it from metal toxicity (lead and cadmium) and improved its growth with respect to controls (Tripathi et al. 2005). Therefore, the application of metal remediating plant growth-promoting bacteria (PGPB) along with plant growth promoting activities makes the remediation process more effective and efficient (Glick 2012). The utilization of the mining sites with the higher concentration of heavy metals is a global challenge to environmental sustainability (Ahirwal and Pandey 2021). In this direction, researchers demonstrated that the *Pseudomonas aeruginosa*-HMR1 removes heavy metals and exhibits plant growth-promoting attributes. Thus, the *P. aeruginosa*-HMR1 can be used for the restoration of mining lands for forestry, ornamental plants and agricultural purposes (Bhojiya et al. 2021).

Fungi have been found to have more tolerance to metals than bacteria (Deng and Cao 2017; Deng et al. 2013a). Fungi easily reach the microsites that are not accessible to the plant roots and thus can compete with other microbes for food and metal uptake. These protect the plant roots from directly interacting with metals and increase the soil hydrophobicity, thus hindering metal transport. Moreover, the extended mycelia formation by fungi also makes them suitable for bioremediation. In metal-polluted soils, various fungi like *Aspergillus*, *Trichoderma* and the arbuscular mycorrhizae (AM) have demonstrated the capacity to improve the phytoremediation process (Deng et al. 2011, 2013a). These fungi have high capability of immobilization of toxic/heavy metals by forming either the insoluble compounds, chelation or through biosorption. The fungal species and ecotype greatly affect phytoremediation efficiency. Some examples of bioremediation of heavy metals are given in Table 4.4.

Table 4.3 Potential microbes for remediation of heavy metal pollution

Microorganisms	Metal	Metal concentration (Initial) (mg L ⁻¹)	Efficiency (%) or Sorption capacity (mg g ⁻¹)	References
Bacteria				
<i>Bacillus laterosporus</i>	Cd	1000	159.5	Zouboulis et al. (2004)
<i>Bacillus licheniformis</i>	Cd	1000	142.7	Zouboulis et al. (2004)
<i>Desulfovibrio desulfuricans</i>	Cu	100	98.2	Kim et al. (2015)
<i>Acinetobacter sp.</i>	Cr	16	87%	Bhattacharya et al. (2019)
<i>Bacillus subtilis</i>	Cr	0.57	99.60%	Kim et al. (2015)
<i>Methylobacterium organophilum</i>	Pb		18%	Kim et al. (1996)
<i>Cellulosimicrobium sp. (KX710177)</i>	Pb	50	99.33%	Bharagava and Mishra (2018)
<i>Staphylococcus sp.</i>	Cu	100	98.20%	Kim et al. (2015)
<i>Flavobacterium sp.</i>	Cu	1.194	20.30%	Kumaran et al. (2011)
<i>Micrococcus sp.</i>		100	65.00%	Jafari et al. (2015)
<i>Enterobacter cloacae</i>	Pb	7.2	2.3	Kang et al. (2005)
<i>Pseudomonas aeruginosa</i>	Co	58.93	8.92	Kang et al. (2005)
	Ni	58.69	8.26	
<i>Pseudomonas sp.</i>	Cu	300	5.52	Rajkumar et al. (2008)
	Zn	275	3.66	
Fungi				
<i>Aspergillus niger</i>	Pb	100	34.4	Dursun et al. (2003)
	Cr(VI)	50	6.6	
<i>Phanerochaete chrysosporium</i>	Pb	100	88.16	Iqbal and Edyvean (2004)
	Zn	100	39.62	
<i>Rhizopus oryzae</i>	Cu	100	34	Fu et al. (2012)
<i>Sphaerotilus natans</i>	Cr	200	60%	Kumar et al. (2017)
<i>Saccharomyces cerevisiae (Y)</i>	Cr	570.25	95%	Benazir et al. (2010)
<i>Aspergillus versicolor</i>	Cu	50	29.06%	Tas et al. (2010)
Algae				

(continued)

Table 4.3 (continued)

Microorganisms	Metal	Metal concentration (Initial) (mg L ⁻¹)	Efficiency (%) or Sorption capacity (mg g ⁻¹)	References
<i>Codium vermilara</i>	Ni	147	13.2	Romera et al. (2007)
<i>Lessonia nigrescens</i>	Ar(V)	200	45.2	Hansen et al. (2006)
<i>Sargassum muticum</i>	Sb	10	5.5	Ungureanuet al. (2015)
<i>Spirogyra sp.</i>	Pb	200	140	Gupta and Rastogi (2008)
<i>Chlorella vulgaris</i>	Cu	50 mg dm ⁻³	97.70%	Goher et al. (2016)
<i>Spirulina sp.</i>	Cu	5	81.20%	Mane and Bhosle (2012)
<i>Nostoc sp.</i>	Pb	1	99.60%	Kumaran et al. (2011)
	Cd	1	95.40%	Kumaran et al. (2011)
	Ni	1	88.23%	Kumaran et al. (2011)

4.5.2 Potential Plants Involved in Bioremediation

The potential phytoextractive plant species has the ability to accumulate the high content of the metals into the aboveground biomass without showing any toxicity symptoms. Their potential of phytoextraction can be enhanced by the use of fast-growing hyperaccumulator tree species with extensive root systems, thus ensuring its economic and environmental feasibility. Remarkable genetic variability has been reported to exist among plants of Salicaceae species adapted to soil of varying level metal contaminants (Dickinson and Pulford 2005; Puschenreiter et al. 2010; Marmiroli et al. 2011; Yang et al. 2015). In addition to this, adoption of native fast growing tree species may provide us with a better possible solution. Some of the plants suitable for plant assisted bioremediation are given in Table 4.5.

4.6 Mechanism of Plant Assisted Bioremediation

The mechanism for the plant assisted bioremediation involves bioavailability, uptake, translocation, sequestration and different defence mechanism that can help to develop heavy metal stress-resistant cultivars and highly efficient plant species

Table 4.4 Plant assisted microflora involved in bioremediation of heavy metals

Microorganisms	Plant	Heavy metals	Role/effect	Reference
<i>Rhizobium sp. strains</i> E20-8 and NII-1	<i>Pisum sativum</i> L	Cd	Remediates Cd pollution through various mechanisms like cytoplasmic sequestration, periplasmic allocation, extracellular immobilization and biotransformation	Cardoso et al. (2018)
<i>Microbacterium</i> sp. NE1R5, <i>Curtobacterium</i> sp. NMIR1	<i>Brassica nigra</i>	As, Zn, Cu, Pb	Enhancement of seed germination and root development	Román-Ponce et al. (2017)
<i>Thiobacillus thiooxidans</i>	<i>Gladiolus grandiflorus</i> L	Cd, Pb	Uptake and accumulation of Cd and Pb increases along with enhanced root length, plant height and dry biomass	Mani et al. (2016)
<i>Pseudomonas brassicacearum</i> , <i>Rhizobium leguminosarum</i>	<i>Brassica juncea</i>	Zn	Attenuates metal toxicity and promotes metal chelation	Adediran et al. (2015)
<i>Pseudomonas sp.</i>	Soybean, mungbean, wheat	Ni, Cd, Cr	Promotion of growth	Gupta et al. (2002)
<i>Sinorhizobium</i> sp. Pb002	<i>Brassica juncea</i>	Pb	Lead phytoextraction efficiency is enhanced by <i>B. juncea</i> plants	Di Gregorio et al. (2006)
<i>Pseudomonas sp.</i> A3R3	<i>Alyssum serpyllifolium</i> , <i>Brassica juncea</i>	Ni	More biomass (<i>B. juncea</i>) and Ni content (<i>A. serpyllifolium</i>) in plants grown in Ni-stressed conditions	Ma et al. (2011a, b)
<i>Bradyrhizobium</i> sp. 750, <i>Pseudomonas cytitssp.</i> , <i>Ochrobactrum</i>	<i>Lupinus luteus</i>	Cu, Cd, Pb	Enhanced the accumulation of metals	Dary et al. (2010)
<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Ralstoniametallidurans</i>	Maize	Cr, Pb	Accelerated soil metal mobilization and increased uptake of Cr and Pb	Braud et al. (2009)
<i>Bacillus weihenstephanensis</i> strain SM3	<i>Helianthus annuus</i>	Ni, Cu, Zn	Accelerated Cu and Zn accumulation in plants, also increased the water soluble Ni, Cu and Zn concentrations in soil with their metal mobilizing potential	Rajkumar et al. (2008)
<i>Achromobacterxylooxidans</i> strain Ax10	<i>Brassica juncea</i>	Cu	Enhanced Cu uptake by plants	Ma et al. (2009)

(continued)

Table 4.4 (continued)

Microorganisms	Plant	Heavy metals	Role/effect	Reference
<i>Glomerales</i> species <i>Rhizophagus</i> <i>Funnelformis</i> <i>Claroideoglossum</i>	<i>Lactuca sativa</i> <i>Daucus carota</i>	Sb	Increased its uptake and accumulation in plants particularly in roots	Pierart et al. (2018)
<i>Funnelformis</i> mosseae, <i>Rhizophagus</i> irregularis, <i>Claroideoglossum</i> lamellosum	<i>Ricinus communis</i>	Cr(III), Cr(VI)	Reduction of Cr(VI) concentration in soils	Gil-Cardesa et al. (2018)
AM fungi	<i>Solanum melongena</i>	Pb, As, Cd	AM increased metal (loids) uptake and biomass	Chaturvedi et al. (2018)
<i>Glomus mosseae</i>	<i>Cajanus cajan</i>	Cd, Pb	Lead distribution pattern seems to be changed by fungal symbiont in extra radical hyphae of fungi, roots and shoots. Inoculation of fungal cultures in pigeon pea demonstrated the bioremediation potential by assisting it to grow in heavily metal-contaminated soils	Garg and Aggarwal (2011)
<i>Scleroderma citrinum</i>	<i>Pinus sylvestris</i> L	Zn, Cd, Pb	Reduction in translocation of Zn, Cd, or Pb from roots to shoots in pine seedlings	Krupa and Kozdrój (2007)
<i>Bacillus thuringiensis</i> GDB-1	<i>Alnus firma</i>	Cd, Ni, As, Cu, Pb, Zn	Promoted accumulation of metal(loids) (As, Cu, Pb, Ni and Zn)	Babu et al. (2013)

for phytoremediation in harmony with microflora through genetic engineering technologies.

4.6.1 Bioavailability

It is defined as a part of the total elemental concentration available to plants that determines the uptake and accumulation of heavy metal ions in plants. Heavy metals exist in soils with several degrees of fractions i.e. soil solution form, soluble metal complexes and free metal ions forms. Several factors that determine the bioavailability of heavy metal elements are environmental conditions (moisture, temperature and oxidation state), soil properties (pH and organic matter) and enhanced biological activity by microbes (Yang et al. 2012; Bravin et al. 2012). These factors regulate the release of heavy metals into the soil and influence the plant uptake from soils. Environmental factors like high temperature enhance the physical, chemical and biological activities in soil–plant system, while precipitation and rainfall are known

Table 4.5 Potential plants for remediation of heavy metal pollution from soil

Plant species	Metal	Reference
<i>Alyssum spp.</i> , <i>Phyllanthus serpentines</i> , <i>Isatispinnatiloba</i> , <i>Berkheyacoddii</i>	Nickel (Ni)	Li et al. (2003), Bani et al. (2010), Chaney et al. (2010), Mesjasz-Przybyłowicz et al. (2004), Altinözlü et al. (2012)
<i>Azolla pinnata</i> , <i>Solanum photeinocarpum</i> , <i>Thlaspicaerulescens</i> , <i>Rorippaglobosa</i> , <i>Turnip landraces</i> , <i>Prosopis laevigata</i>	Cadmium (Cd)	Rai (2008), Zhang et al. (2011), Lombi et al. (2001), Wei et al. (2008), Li et al. (2016), Buendía-González et al. (2010)
<i>Pteris spp.</i> , <i>Corrigiolatelephiiifolia</i> , <i>Eleocharis acicularis</i> , <i>Azolla carobiniana</i>	Arsenic (As)	Srivastava et al. (2006), Sakakibara et al. (2011), Garcia-Salgado et al. (2012)
<i>Eleocharis acicularis</i> , <i>Aeolanthusbiformifolius</i> , <i>Ipomoea alpine</i> , <i>Haumaniastrumkatangense</i> , <i>Pteris vittata</i>	Copper (Cu)	Sakakibara et al. (2011), Chaney et al. (2010), Mitch (2002), Sheoran et al. (2009), Wang et al. (2012)
<i>Pteris vittata</i>	Chromium (Cr)	Kalve et al. (2011)
<i>Thlaspicaerulescens</i> , <i>Eleocharis acicularis</i> , <i>Thlaspicalaminare</i> , <i>Deschampsiaespitosa</i>	Zinc (Zn)	Cunningham and Ow (1996), Sakakibara et al. (2011), Sheoran et al. (2009), Kucharski et al. (2005)
<i>Medicago sativa</i> , <i>Brassica spp.</i> , <i>Thlaspirotundifolium</i> , <i>Helianthus annuus</i> , <i>Euphorbia cheiradenia</i> , <i>Betula occidentalis</i> , <i>Deschampsiaespitosa</i>	Lead (Pb)	Koptsik (2014), Cunningham and Ow (1996), Chehregani and Malayeri (2007), Kucharski et al. (2005)
<i>Lecythisollaria</i> , <i>Astragalus racemosus</i>	Selenium (Se)	Marques et al. (2009)
<i>Schima superba</i> , <i>Macadamia neurophylla</i> , <i>Maytenusbureaviana</i> , <i>Alyxiarubricaulis</i>	Manganese (Mn)	Yang et al. (2012), Sheoran et al. (2009), Marques et al. (2009), Chaney et al. (2010)
<i>Achillea millefolium</i> , <i>Marrubium vulgare</i> , <i>Rumex induratus</i> , <i>Hordeum spp.</i> , <i>Festuca rubra</i> , <i>Helianthus tuberosus</i> , <i>Poa pratensis</i> , <i>Armoracia lapathifolia</i> , <i>Brassica juncea</i>	Mercury (Hg)	Wang et al. (2012), Rodriguez et al. (2003), Sas-Nowosielska et al. (2008)

to accelerate plant growth and development. High soil moisture content regulates the movement of water-soluble trace elements during bioremediation. Soil properties, viz., pH, organic matter/organic carbon and cation exchange capacity (CEC) are the important factors that control the bioavailability of cations in soil. Soils with higher organic matter and high pH will form complex with heavy metals more firmly and become less available to plants for uptake and accumulation. Acidification of the rhizosphere is considered to increase the metal accumulation potential of plants raised on heavy metal contaminated soils. At acidic pH, heavy metals are found in free ionic forms and are more bioavailable, but at the basic pH metals form insoluble metal complexes with phosphates and carbonates (Sandarin and Hoffman 2007; Rensing and Maier 2003). Biological activities within the soil–plant system alter the bioavailability of metal elements. Microbes in the rhizosphere can produce chelating compounds, enhance the key nutrient uptake and also the availability of soil heavy metals (Rajkumar et al. 2012). Some plants secrete the organic components that form soluble complexes with heavy metal ions in soils. These soluble complex formations promote the mobility of heavy metals in soils. Yang et al. (2012) reported that root exudates include various organic acids and amino acids viz., oxalic acid, citric acid, tartaric acid, succinic acid, aspartic acid and glutamic acid, that form heavy metal soluble complexes and increase the mobility of Cd, Cu, Zn and Pb in soils (Fig. 4.2).

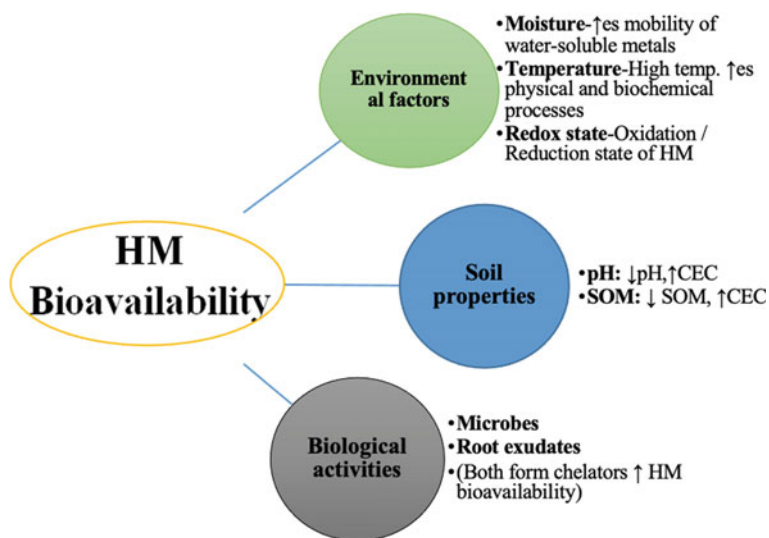


Fig. 4.2 Bioavailability of the heavy metals (HM) to plants

4.6.2 Plant Uptake

The movement of heavy metals in soils depends upon precipitation, redox potential, absorption/adsorption and its complexation/methylation responses mediated by microbes along with plants (Kumar et al. 2017). The mechanism of plant metal uptake, rejection, translocation and sequestration is specific and highly variable within the plant varieties (Lone et al. 2008). Plants adopt two main strategies to combat heavy metal stress by either reduce metal uptake or increase vacuolar sequestration. The heavy metal is bioactivated by the root microbe's interaction first which leads to root absorption and further compartmentalization.

(i) Bioactivation of metals by root-microbe interaction

Several studies depicted the positive interaction of microorganisms with plant species in the rhizosphere (Dakora and Phillips 2002; Kuiper et al 2004). Plant growth promoting rhizobacteria increase the bioavailability of metal ions by dissolving them via changing the chemical properties (pH, redox state, organic matter) of soils in the rhizosphere and modify the heavy metal speciation in the root zone (Jing et al. 2007). They solubilize the ions like phosphate, siderophore and increase acid production (Kumar et al. 2017). During heavy metal stress, mycorrhizae release natural acids that enhance zinc solubility and its mobility, ultimately playing a significant role to strengthen plant survival rate (Giasson et al. 2008).

Bacterial endophytes are considered to be beneficial for host plants usually during stress conditions, because they regulate the plant growth promoting mechanisms like phytohormone production by activating enzymes viz., 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, ethylene and Indole acetic acid (IAA) (Hardoim et al. 2008; Rajkumar et al. 2012). Endophytes also known to enhance nitrogen fixation and phosphate availability in rhizosphere, hence helps to recover the plant during heavy metal (HM) stress conditions (Kuklinsky-Sobral et al. 2004).

(ii) Root absorption and compartmentalization

The transport of nutrients and heavy metals from soils to plant roots occurs via symplastic and apoplastic transport. In symplastic transport heavy metals enter the root cells through the plasma membrane of the endodermis of the root. While in apoplastic transport, it enters the root apoplast via spacing within the cells. Generally, heavy metals and nutrient ions cross the membranes only with the aid of naturally occurring membrane transport proteins (Fig. 4.3). The abundance of these proteins depends upon tissue type and environmental conditions. If a small amount of nutrients is present in soils, then the plant requires high-affinity transporters for uptake; whereas if the nutrients in the soil are present in high concentrations (e.g. agricultural soils with fertilizers), then low-affinity transporters would be more useful for plant uptake (Cailliatte et al. 2010).

Several transporter families have been reported in plants such as heavy metal ATPase (HMA), natural resistance and macrophage proteins (NRAMP), Zrt, Irt-like proteins (ZIP) etc. (Table 4.6). In the cytosol, toxic metals rapidly bind to chelators

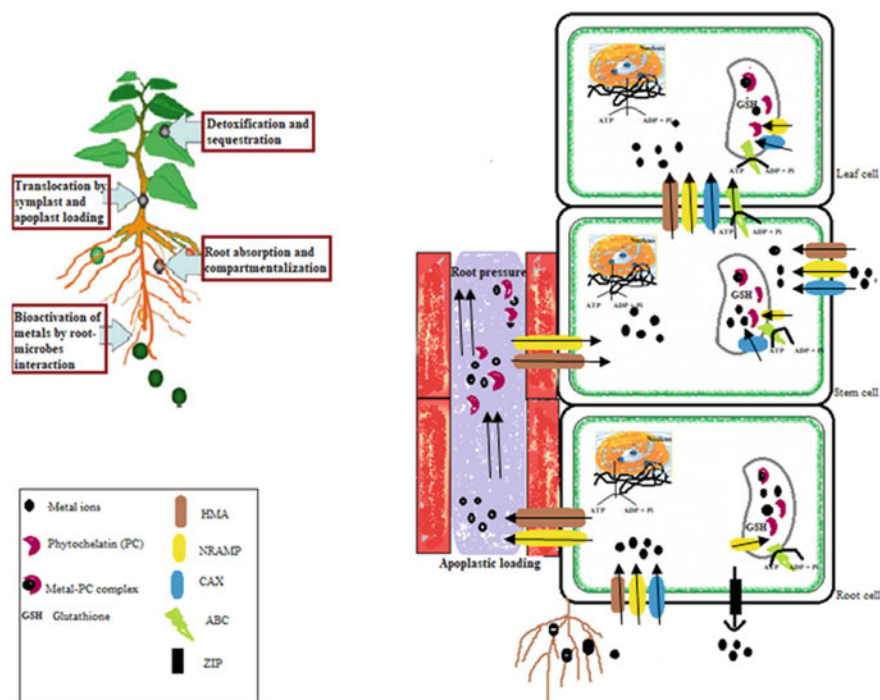


Fig. 4.3 Uptake of heavy metal and its compartmentalization in various plant part through different transporter proteins

and are transferred to the vacuole for sequestration. Ingle et al. (2005) observed that histidine is involved in Ni-chelation in root cells and helps plant to tolerate Ni toxicity. Cr (III) in root cells is chelated with acetate and sequestered in the vacuole (Bluskov and Arocena 2005).

4.6.3 Translocation

Heavy metal transporters are required for translocation of metallic ions from root symplast to xylem apoplast due to endodermal barrier (casparian strips) in the root. The translocation of heavy metal ions depends on two factors: root pressure and leaf transpiration (Kumar et al. 2017).

(i) Root symplast to apoplast through xylem tissues

Xylem loading of metals from root symplast is an important phenomenon making the plant to tolerate heavy metal toxicity instead of promoting its accumulation in root cells that would inactivate the enzymes involved in metabolic processes. Cation

Table 4.6 Metal transporters involved in heavy metal uptake, transport and sequestration during phytoremediation (Bhargava et al. 2012)

Transporter family	Transporter gene	Plant species	Metal transported	References
Natural resistance-associated macrophage proteins (NRAMP)	<i>nramp1</i> <i>nramp1-3</i> <i>nramp4</i>	<i>Malus baccata</i> <i>Lycopersicon esculentum</i> <i>Thlaspi japonicum</i>	Fe (Iron) Fe (Iron) Fe (Iron)	Xiao et al. (2008), Berezcky et al. (2003), Mizuno et al. (2005)
Fe-regulated transporter (IRT)	<i>irt1</i> <i>irt1-2</i> <i>irt1-2</i>	<i>A. thaliana</i> <i>T. caerulea</i> <i>L. esculentum</i>	Fe (Iron) Fe (Iron) Fe (Iron)	Kerkeb et al. (2008), Schikora et al. (2006), Plaza et al. (2007), Berezcky et al. (2003)
Zn-regulated transporter (ZRT)	<i>Zip</i> <i>zip1-12</i> <i>zip4</i> <i>znt1-2</i>	<i>Medicago truncatula</i> <i>A. thaliana</i> <i>O. sativa</i> <i>T. caerulea</i>	Zn (Zinc) Zn (Zinc) Zn (Zinc) Zn (Zinc)	Lopez-Millan et al. (2004), Roosens et al. (2008), Ishimaru et al. (2005), Van de Mortel et al. (2006)
P-type ATPase	<i>hma9</i> <i>hma8</i> <i>hma3</i> <i>hma4</i>	<i>Oryza sativa</i> <i>Glycine max</i> <i>A. thaliana</i> <i>A. halleri</i>	Cu (Copper), Zn (Zinc), Cd (Cadmium), Cu (Copper) Co (Copper), Zn (Zinc), Cd (Cadmium), Pb (Lead), Cd (Cadmium)	Lee et al. (2007), Bernal et al. (2007), Morel et al. (2008), Courbot et al. (2007)
Copper transporter	<i>copt1</i>	<i>A. thaliana</i>	Cu (Copper)	Sancenon et al. (2004), Andres-Colas et al. (2010)
Yellow stripe-like (YSL)	<i>Ysl3</i> <i>Ysl2</i>	<i>T. caerulea</i> <i>A. thaliana</i>	Fe (Iron), Ni (Nickel) Fe (Iron), Cu (Copper)	Gendre et al. (2006), DiDonato et al. (2004)
Cation diffusion facilitator (CDF)	<i>mtp1</i> <i>mtp1</i> <i>mtp1</i> <i>mtp1</i>	<i>Thlaspi goesingense</i> <i>A. thaliana</i> <i>A. halleri</i> <i>Nicotiana tabacum</i>	Zn (Zinc), Ni (Nickel) Zn (Zinc) Zn (Zinc) Zn (Zinc), Co (Cobalt)	Kim et al. (2004), Kawachi et al. (2008), Willems et al. (2007), Shingu et al. (2005)

diffusion facilitator (CDF) type of proteins conveys a broad array of metal divalent ions from cytoplasm toward the outer cell parts and even within the subcellular compartments (Hanikenne et al. 2005). HMA2 proteins are energy-dependent transporters, despite having selective nature they also get activated by analogue metal ions. Hussain et al. (2004) isolated HMA2 and HMA4 transporters in *Arabidopsis* for Zn transportation within cellular compartments and homeostasis. Milner and Kochain (2008) deciphered the importance of HMA2 and HMA4 genes in metal loading into the xylem.

(ii) *Root apoplast to aerial (stem and leaves) tissues*

Hyper accumulator plants rapidly translocate the absorbed metal ions from the root to the above-ground parts, while non-accumulators accumulate heavy metals only in their root portions. Heavy metals can be stored in root vacuoles. Due to the limited space and high heavy metal concentration in the soil matrix, it gets translocated to shoot tissue where sequestration and detoxification rate is comparatively high (Kumar et al. 2017). Generally, metals are stored in only chelated form but are transported from one cellular compartment to other in free ionic state according to the selectivity of transporter proteins (Ortiz et al. 1995). Research experiments showed that hyperaccumulator plants accumulate high concentration of heavy metals in stem and leaf vacuoles than the root tissues. In the leaf tissues, high amount of metals accumulates in epidermal tissues compared to the cortical and vascular tissues (Kupper et al. 2001; Kumar et al. 2017).

4.6.4 Sequestration/Detoxification

To cope up with heavy metal stress, plants adapt different survival strategies like compartmentalization, exclusion, complexation and synthesis of binding proteins (metallothioneins and phytochelatins). Heavy metal toxicity inside the plant cell gets detoxified by complex formation and compartmentalization to make them less available to metabolic active sites. Organic acids, glutathione precursor of phytochelatins and metallothioneins play a significant role in detoxification/sequestration. Phytochelatins (PC) have an imperative role to detox cadmium in fungi and plants through conjugation. Glutathione enhances the PC synthesis and thus more PC-metal complex formation in the vacuole which ultimately enhances cadmium tolerance in plants (Lee et al. 2003).

In plants, different heavy metal ions (Cu, Hg, Zn, Pb, Cd) stimulate the enzyme, γ -glutamyl-cysteinyl dipeptidyl transpeptidase (PC synthase) for phytochelatin synthesis which results in glutathione conversion (GSH) to phytochelatin (Fig. 4.4). These phytochelatins are produced from glutathione (GSH) through oxidation and reduction reactions. The metal ion binds to cysteine sulfhydryl residues of phytochelatins and its sequestration occurs inside the vacuole (Zhu et al 2004; Kumar et al. 2017). In hyperaccumulator plants, toxic effects of Ni were overcome by enhancing GSH-dependent antioxidant mechanism that protects the plant from

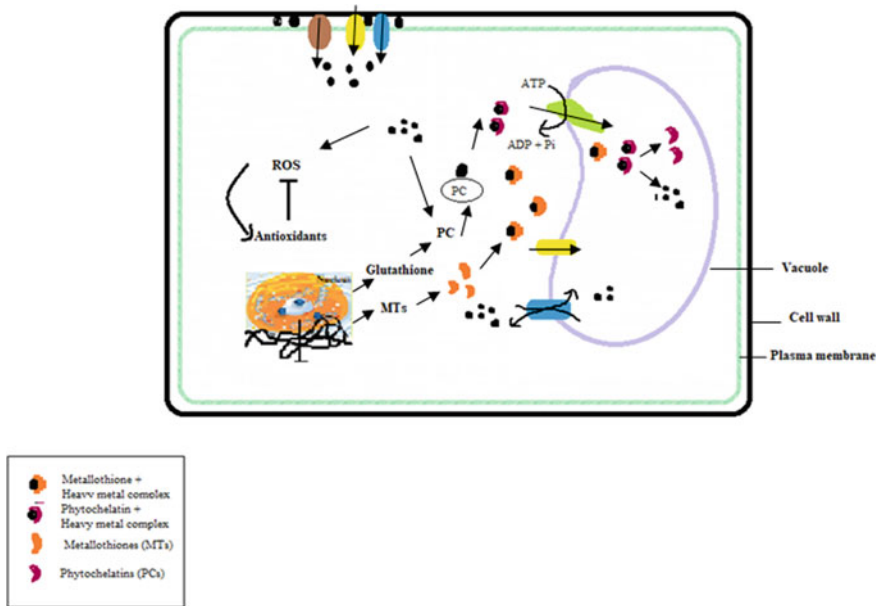


Fig. 4.4 Sequestration and detoxification of heavy metals (HM) in plant cell

oxidative damage (Freeman et al. 2005). Metallothioneins are metal-binding proteins that modulate the concentration of metals inside the cell by binding heavy metal ions to cysteine and thiol groups (Khan et al. 2004). Mn^{2+} metal detoxification involves uptake of ions from the plasma membrane, binding with malate and transportation through tonoplast to vacuole where Mn unbinds from malate and form complex with oxalate (Memon et al. 2001).

Heavy metal toxicity hindered the functional group of important molecules that disrupt the metabolic enzyme activity and consequently inhibit or suppress photosynthetic rate, respiration rate and all physiological and biochemical processes of plants (Gupta et al. 2015; Ali et al. 2013). Naturally, plants develop various defense mechanisms against heavy metal stress inside the plant body which include compartmentalization reduction, suppression of high-affinity phosphate transport system, sequestration and translocation (Zhao et al. 2009). When metal ions cross enter into plant tissues by crossing these barriers then various cellular defense mechanisms (as a second line of defense viz., ROS production, antioxidants) are initiated to detox the adverse effect of noxious heavy metals (Silva and Matos 2016).

4.7 Conclusion and Future Prospects

The pollution due to heavy metals is of great concern because of its potential impact on human and animal health. It is imperative to protect the natural resources and biodiversity, by using cheaper and effective technologies. In phytoremediation, the plants have to retain the pollutant in their root or other parts by producing large biomass and microbes converting toxic forms of heavy metals to non-toxic forms. But till now no plant is known to fulfil both these criteria. At the heavily contaminated sites with both organic and inorganic pollutants, there is a limitation of plant growth and microbial activity, thus having reduced plant assisted bioremediation efficiency. Recent progress in molecular, biochemical and plant physiology fields provides a strong scientific base for achieving this goal. During the last decade, substantial efforts have been made by the researchers to identify plant hyperaccumulators, bioremediators for heavy metals and their mechanism of uptake, translocation. There is a huge genetic variation in different plant species, even among the cultivars of the same species. So, research must be carried out to study the mechanism of metal uptake, accumulation, exclusion, translocation and compartmentation for each species as they play a specific role in phytoremediation. Further, research is needed to study metal uptake at the cellular level including influx and efflux of different metals by different cell organelles and membranes.

- There is a need of microbial profiling of rhizosphere under controlled and field conditions to examine the antagonistic and synergistic effects of different metal ions in soil and polluted waters.
- Selected essential rhizosphere microorganisms and microbial strains able to degrade toxic pollutants can be studied in the natural habitat. Molecular techniques will further help in elucidating the fate and effect of these selected strains in the soil environment.
- Standardization of the methods for heavy metal recovery from the hyperaccumulator plants will allow the detection of the new strains of the micro-organisms who can degrade or reduce the toxic metals to non-toxic metals as well as improve the fertility status of the soil.

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