Chapter 13
Bioremediation of Heavy Metals: A New Approach to Sustainable Agriculture

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Abstract With the advancement in agricultural practices, use of various chemicals for better yield is posing huge threat to the society. These chemical containing variable amounts of heavy metals are the key players that have become threat to plants and human beings. The discharge of various harmful environmental pollutants from different industrial sectors has created a challenge for environmentalists and scientists concerning the sustainable development of mankind. Particularly in plants, heavy metals are essential for its growth and development, but when the concentration of each heavy metal crosses, its threshold concentration becomes harmful for plants itself. These heavy metals possess specific density of more than 5 g/cm^3 (Cr-7.2, Co-8.9, Ni-8.7, Cu-8.9, Zn-7.1, Mo-10.2, Cd-8.2 etc.). Various survey studies reveals intense exposure of heavy metals still continues in different parts of the world though its ill-effects are well documented. Some of the well-known heavy metals include arsenic, cadmium, copper, lead, nickel, zinc, etc., all of which cause risks for the environment and human health. Considering heavy metals as potential threat to different life forms, it has become an important and interesting issue since last few decades. This chapter attempts to review different strategies for remediating heavy metal contamination with the plants and microorganisms. An attempt has also been made to review and promote the sustainable development with the involvement of phytoremediation and micro-remediation technologies.

Keywords Phytoremediation · Micro-remediation · Heavy metals

1 Introduction

Heavy metals are natural constituents of the environment but with rapid industrialization and development; there has been a considerable increase in the discharge of pollutants in the environment (soil, air and water) (Nagajyoti et al. 2010). Unfortunately contamination of the environment with heavy metals has reached

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beyond the recommended limit (Chibuike and Obiora 2014). As compared to other pollutants, heavy metals are non-biodegradable, and so they persist for long time in the environment (Tak et al. 2013; Kumari et al. 2016; Yadav et al. 2016). Highly reactive heavy metals can enter soil and groundwater, bioaccumulate in food web and adversely affect biota. In the food chain, the non-biodegradable heavy metals get accumulated and cause damage to vital organs such as lungs, liver, kidney and nervous system (Kumar et al. 2015).

Thus, there is a need to remove these hazardous heavy metals from the environment. To seek solution to this problem, bioremediation is applied as a tool. The term bioremediation implies use of microorganisms and plants to degrade the environmental contaminants to less toxic forms (Mani and Kumar 2014; Upadhyay et al. 2016). The reason that bioremediation is used as a potential tool for this problem is because it helps to restore the natural state of the polluted environment. It has long-term environmental benefits and is cost-effective (Dixit et al. 2015).There are two strategies of bioremediation, viz. in situ and ex situ. In in situ bioremediation, the treatment of contaminated soil or water is done at the site in which it is found. It is more convenient and less expensive as compared to ex situ type. In ex situ bioremediation, the contaminated soil or water is excavated or pumped out of the location at which it is found. It is faster, easier to control and usually more able to treat a wide range of toxins from soils. Microorganisms have metabolic pathways which utilizes toxic heavy metals as a source of energy for growth and development. They possess characteristic enzymes for a particular contaminant which provide resistance against heavy metals. The microbes have cell wall which is anionic in nature and thus enables them to bind metal cations through electrostatic forces (Siddiquee et al. 2015). Not only microorganisms but plants also have the potential for remediation of environmental pollutants (Upadhyay et al. 2019). The various processes used by plants under phytoremediation are phytodegradation, phytovolatilization, phytostimulation and phytostabilization.

Bioremediation is less expensive as compared to other technologies. Blaylock et al. showed the cost-effectiveness of bioremediation. They made use of bioremediation for treatment of one acre of lead (Pb)-polluted soil and were able to save 50–60% of cost. The effectiveness of bioremediation depends on the wise selection of the microorganism, identification of the polluted environment and the technique chosen. The ability of the microorganism to degrade pollutants depends on the suitability of the environmental conditions such as temperature, pH and moisture (Verma and Jaiswal 2016). The objective of this chapter is to discuss the heavy metal pollution, its causes and effects along with the bioremedial potential to tackle this problem. A detailed account of bioremediation, various strategies employed, mechanisms, microorganisms used and merits and demerits associated with it has also been covered.

2 Heavy Metal Pollution

The heavy metals are defined as naturally occurring elements that have a high atomic weight and density five times higher than that of water and is toxic or poisonous even at very low concentration (Lenntech 2004). Due to rapid industrialization, the concentrations of heavy metals have reached beyond the threshold value (Dixit et al. 2015; Yadav et al. 2017). Some of the essential heavy metals exert biochemical and physiological functions in plants and animals. They make remarkable effects on plant physiology (Dixit et al. 2015). Pollution of heavy metals is a global concern. Many metallic elements are necessary for growth of plants and animals, but they are required in low concentration; if their amount in soil exceeds above the threshold value, it causes toxicity. Heavy metal toxicity in plants is a function of the bioavailability of these elements in the soil solution. According to Comprehensive Environmental Response Comprehension and Liability Act (CERCLA) USA, the permissible limit of some heavy metals Ar, Cd, Cr, Pb, Hg and Ag in water is 0.01, 0.05, 0.01, 0.015, 0.002 and 0.05 mg/l, respectively (Chaturvedi et al. 2015). According to Indian standards, the standard for soil heavy metal is 3–6, 135–270, 75–150, 250–500, 300–600 mg/kg for Cd, Cu, Ni, Pb and Zn (Dixit et al. 2015).

Heavy metals are naturally occurring element in soil. The naturally occurring heavy metals have a great adsorption capacity in soil, whereas heavy metals from anthropogenic sources are soluble and mobile and thus have a higher bioavailability as compared to naturally occurring heavy metals (Olaniran et al. 2013). Heavy metal accumulation in soil consequently in food items can pose health risks to the human beings. Many recent studies conducted at national and international levels reported heavy metal contamination in the soil and food crops. Agricultural soils have become a big reservoir of heavy metals due to extensive uses of different agrochemicals like fungicides, herbicides and phosphate fertilizers, organic manure and decaying plant and animal residues (Uwah et al. 2011). Table 13.1 below shows sources of some important heavy metals.

Table 13.1 Important heavy metals and their sources

Metal	Source	References
Arsenic	Mining, pesticides, smelting ores	Wahab et al. (2015) and Bissen and Frimmel (2003)
Cadmium	Fertilizer, pesticide, wielding, mining	Nagajyoti et al. (2010)
Chromium	Dyes and paints, steel fabrication	Barakat (2011) and Cervantes et al. (2001)
Copper	Copper polishing, mining, paint, plating, printing	Dixit et al. (2015), Nagajyoti et al. (2010) and Salem et al. (2000)
Mercury	Batteries, paint, paper industries, rock weathering, coal combustion	Fashola et al. (2016) and Ali et al. (2013)
Lead	Electroplating, batteries, coal combustion, mining, paint industries, water pipes	Fashola et al. (2016), Nagajyoti et al. (2010) and Ali et al. (2013)
Nickel	Electroplating, porcelain enamelling	Fashola et al. (2016)
Zinc	Brass manufacturing, mining, oil refining, plumbing	Gumpu et al. (2015)

3 Effects of Heavy Metals

Though some heavy metals have biological functions in living organisms, majority of them have no biological function and are extremely toxic even at very low concentration (Fashola et al. 2016). These heavy metals bind with protein sites by displacing original metals from their natural binding sites and thus causing toxicity. Research has indicated that deterioration of biological macromolecules is mainly due to binding of heavy metals to DNA and nuclear proteins (Flora et al. 2008).

In humans, heavy metals lower the energy levels and damage the functioning of vital organs such as the brain, heart, kidney, lungs and cause deterioration of physiological activities (Mupa 2013). They are also responsible for muscular and neurological degenerative processes that imitate diseases such as Alzheimer's disease and muscular dystrophy; long-term exposure can also lead to cancer. One such example of heavy metal toxicity was the "Minamata disease" caused by mercury poisoning in Japan. Lead is a heavy metal which can leach into drinking water and enter food items. Children are highly susceptible to lead and mercury, exposure of lead and mercury in children during their growing years leads to reduced intelligence and impaired development (Wuana and Okieimen 2011). Plants require some heavy metals for their growth and development, but their excess amount becomes toxic. Plants are capable of absorbing the heavy metals; they absorb toxicants either directly from the atmosphere through leaves or from soil and water through roots (Gaur et al. 2014). The excess amount of heavy metals in soil, water and air may lead to various direct or indirect effects on plants and human being. Direct toxic effects include inhibition of the cytoplasmic enzymes and damage to cell structure due to oxidative stress. Indirect toxic effects include replacement of essential nutrients at cation exchange sites of plants. Loss of fertility in plants, yield and food production. Destruction of chlorophyll pigments (Pichhode and Nikhil 2015). Some heavy metals have adverse effects on soil microorganisms. Heavy metals and microorganisms have a strong affinity; many of the heavy metals disrupt the normal metabolic functioning by competing with the essential elements due to their similar chemistry with the essential elements like similar size, charge and oxidation state. Secondly heavy metals pose a restriction on the biodegradation of majority of metallothionein which then accumulate and are harmful for the cells (Ahluwalia and Goyal 2007). Heavy metals have significant effect on soil environment also. It disturbs the buffering capacity of the soil. Heavy metal-contaminated soil limits plant habitat due to toxicity resulting in ecological, evolutionary and nutritional problems as well as severe selection pressure (Abdul-Wahab and Marikar 2012). Table 13.2 shows the hazardous effects of heavy metals on all life forms.

The entire bioremediation process can be studied under micro-remediation (remediation technique using microorganisms) and phytoremediation (remediation of soil and water by using plants) strategies.

Table 13.2 Effects of heavy metals on different life forms

Heavy metal	Human	Plants	Microorganisms	References
Arsenic	Brain damage, respiratory disorder, skin cancer	Cell membrane damage, inhibition of growth, interferes with critical metabolic processes, loss of fertility in plants and fruit yield, oxidative stress	Enzyme deactivation	Wahab et al. (2015) and Bissen and Frimmel (2003)
Cadmium	Bone disease, emphysema, kidney and lung disease, prostate cancer, testicular atrophy, anaemia	Chlorosis, plant nutrient content decrease, growth inhibition and reduced seed germination	Denaturation of proteins, nucleic acid damage, transcription inhibition and inhibition of carbon and nitrogen mineralization	Nagajyoti et al. (2010) and Fashola et al. (2016)
Chromium	Chronic bronchitis, emphysema, headache, skin itching liver and lung disease, renal failure, cancer and loss of reproductive ability	Delayed senescence Wilting, chlorosis, reduced growth, oxidation stress, biochemical lesions	Elongation of lag phase, i.e. slow growth, inhibition of growth and oxygen uptake	Barakat (2011) and Cervantes et al. (2001)
Copper	Abdominal pain, headache, vomiting, anaemia, liver and kidney damage, metabolic disorder	Oxidative stress and retarded growth	Cellular function disruption and inhibition of enzyme activities	Dixit et al. (2015), Nagajyoti et al. (2010), Fashola et al. (2016) and Salem et al. (2000)
Mercury	Blindness, deafness, dizziness, loss of memory, kidney problems and reduced immunity	Inhibition of photosynthesis, enhanced lipid peroxidation, inhibition of plant growth and yield	Denaturation of nucleic acids and proteins, inhibition of enzyme activities	Fashola et al. (2016), Ali et al. (2013) and Wang et al. (2012)
Lead	Neuronal damage, hyperactivity and high blood pressure, insomnia (lack of sleep), reduced fertility	Reduced photosynthesis and growth inhibition, inhibits enzyme activity and oxidative stress	Inhibition of enzyme activities and transcription	Nagajyoti et al. (2010), Fashola et al. (2016), Wuana and Okieimen (2011) and Mupa (2013)

(continued)

Table 13.2 (continued)

Heavy metal	Human	Plants	Microorganisms	References
Nickel	Cardiovascular diseases, kidney and lung diseases, chest pain and shortness of breath, nasal cancer	Decreased chlorophyll content, inhibition of enzymatic activities and reduced nutrient uptake	Cell membrane disruption and oxidative stress	Fashola et al. (2016) and Chibuike and Obiora (2014)
Zinc	Gastrointestinal irritation, kidney and liver failure, lethargy and metal fume fever, prostate cancer	Affects photosynthesis, inhibition of growth rate, chlorophyll reduction and reduced germination	Decrease in biomass and growth inhibition	Chibuike and Obiora (2014) and Gumpu et al. (2015)

4 Bioremediation by Microorganisms

Microorganisms are considered as most cosmopolitan organisms as they have the ability to thrive in wide range of environmental conditions due to their amazing metabolic ability. Further, they are highly versatile in their nutrition uptake, which that this property makes them very useful for decontaminating the immediate environment. Rock-bottom and economic growth requirements (such as carbon dioxide and sunlight) and the advantage of being utilized simultaneously in for multiple technologies (e.g. biofuel production, carbon mitigation and bioremediation) make microorganisms a perfect candidate for many environment-friendly technologies that may be useful for remediation of soil and water (Kumar et al. 2015). In due course of development, these microorganisms have developed substantial array of mechanisms (extracellular or intracellular) to survive the contaminations led by the heavy metals in soil and waterbodies (Kumar et al. 2015). The microbes that are responsible for acting as an agent for bioremediation are called as bioremediators. Bacteria, archaea and fungi are considered as the classic prime bioremediators (Strong and Burgess 2008). Classic bioremediators are those that can convert, modify and then utilize the converted product to obtain energy and biomass (Tang et al. 2007) and thereby cleaning up the environment and restoring the original natural conditions (Demnerova et al. 2005).

Bacteria, microalgae and fungi employ several methods to decontaminate the soil, and these modern techniques are considered to be more efficient than the conventional techniques. The older conventional techniques for removal of heavy metal toxicity includes hydroxide precipitation, carbonate precipitation and sulphide precipitation, chemical oxidation or reduction, lime coagulation, ion exchange (using resins, starch xanthate, etc.), reverse osmosis, solvent extraction, evaporation recovery, cementation, adsorption (involving use of activated carbon), electrodeposition, reverse osmosis and electrodialysis (Rich and Cherry 1987; Ahalya et al. 2003; Gray 1999; Ahluwalia and Goyal 2007). These conventional methods are also able to remove heavy metals but up to a limited extent. But once the heavy metal concentration reaches the range of 1–100 mg/l, these conventional processes become ineffective (Nourbakhsh et al. 1994). Furthermore, the conventional methods are less efficient and require high expenditure of energy and reagents (Ahalya et al. 2003), have low metal uptake selectivity, generate pernicious wastes or sludge (Ahalya et al. 2003; Ahluwalia and goyal 2007) and bear high investment and regeneration cost (Oboh et al. 2009). So, for more efficient removal of contaminants, introduction of new approaches and techniques that are sustainable becomes a must phenomenon of the era. The main reasons behind the need for enforcement of new technologies are to reduce the heavy metal contamination content below its permissible limit. According to Khan et al. (2008), the contamination beyond the permissible limit in aquatic environment can lead to direct toxicity to aquatic life forms and human beings too. Therefore, the need of the hour is to look for better technologies that are much efficient and capable of removing heavy metal toxicity to satisfy the requirements (Sheng et al. 2004). Moreover, the modern and new technologies to be introduced for removal of heavy metal contamination should be cost-effective and consistent and are able to reduce the contamination to such levels that are acceptable to natural field conditions (Kumar et al. 2015).

Among all different kinds of microorganisms such as bacteria, fungi, algae or microalgae, it is the microalgae that possess immense capability to remediate the contaminated waterbodies. Moreover, these microalgae are thought to be more superior to the prevalent physicochemical processes used for eradication of heavy metal toxicity (Kumar et al. 2015). Microalgae are fresh and marine water dweller organisms that can photosynthesize in very similar way as land plants does. They are considered to be the world's largest group of organisms in terms of biomass that can photosynthesize and thus are responsible for at least 32% of global photosynthesis (Priyadarshani et al. 2011). They are well equipped with proper and systematic molecular mechanisms that have the ability to discriminate the essential heavy metals from non-essential ones (Perales-Vela et al. 2006), and as being the renewable natural biomass, they exhibit distinct affinities towards different kinds of heavy metals. This distinctive ability makes them eligible for acting as biosorbent materials (Doshi et al. 2006; Mallick 2002). According to Monteiro et al. (2012), living and non-living microalgal biomass have the ability to remove the heavy metal contamination present at very low concentration. These microalgae are also having the affinity for polyvalent metals and so can be efficiently employed for cleaning waste water containing dissolved metal ions (de Bashan and Bashan 2010). Apart from all these capabilities, they are very eco-friendly and user-friendly too and can be established easily in polluted area as well. Table 13.3 shows heavy metal removal efficiency of different microalgae (living and non-living) at different pH.

Table 13.3 Shows heavy metal removal efficiency of different microalgae (living and non-living) at different pH

Metal	Organism	pH	Type of biomass	References
Copper (Cu^{2+})	*Anabaena cylindrica*	4.0–5.0	Live	Tien et al. (2005)
Copper (Cu^{2+})	*Chlamydomonas reinhardtii*	5.5	Cells without cell wall	Macfie and Welbourn (2000)
Copper (Cu^{2+})	*Chlamydomonas reinhardtii*	5.5	Cells with cell wall	Macfie and Welbourn (2000)
Copper (Cu^{2+})	*Ceratium hirundinella*	4.0–5.0	Non-living	Tien et al. (2005)
Copper (Cu^{2+})	*Ceratium hirundinella*	4.0–5.0	Live	Tien et al. (2005)
Copper (Cu^{2+})	*Aulosira fertilissima*	5	Non-living	Singh et al. (2007)
Copper (Cu^{2+})	*Aulacoseira varians*	4.0–5.0	Non-living	Tien et al. (2005)
Copper (Cu^{2+})	*Aulacoseira varians*	4.0–5.0	Live	Tien et al. (2005)
Copper (Cu^{2+})	*Asterionella formosa*	4.0–5.0	Non-living	Tien et al. (2005)
Copper (Cu^{2+})	*Asterionella Formosa*	4.0–5.0	Live	Tien et al. (2005)
Copper (Cu^{2+})	*Anabaena spiroides*	4.0–5.0	Live	Tien et al. (2005)
Mercury (Hg^{2+})	*Chlorella vulgarisCCAP211/11B*	7	Non-living	Inthorm et al. (2002)
Mercury (Hg^{2+})	*Chlorella vulgaris BCC 15*	7	Non-living	Inthom et al. (2002)
Mercury (Hg^{2+})	*Chlamydomonas reinhardtii*	6	Non-living	Tüzün et al. (2005)
Iron (Fe^{3+})	*Chlorella vulgaris*	2	Non-living	Romera et al. (2006)
Nickel (Ni^{2+})	*Aulosira fertilissima*	5.0–5.5	Non-living	Ferreira et al. (2011)
Nickel (Ni^{2+})	*Arthrospira (Spirulina) platensis*	5	Non-living	Singh et al. (2007)
Nickel (Ni^{2+})	*Chlorella* spp.		Live	Doshi et al. (2006)
Nickel (Ni^{2+})	*Chlorella* spp.		Non-living	Doshi et al. (2008)
Lead (Pb^{2+})	*Microcystis novacekii*	5	Non-living	Ribeiro et al. (2010)
Lead (Pb^{2+})	*Oscillatoria laetevirens*	5	Live	Miranda et al. (2012)
Lead (Pb^{2+})	*Pseudochlorococcum typicum*	7	Live	Shanab et al. (2012)
Lead (Pb^{2+})	*Spirogyra hyaline*		Non-living	Kumar and Oommen (2012)
Zinc (Zn^{2+})	*Arthrospira (Spirulina) platensis*	5.0–5.5	Non-living	Ferreira et al. (2011)
Zinc (Zn^{2+})	*Planothidium lanceolatum*	7	Live	Sbihi et al. (2012)

(continued)

Table 13.3 (continued)

Metal	Organism	pH	Type of biomass	References
Zinc (Zn^{2+})	*Chlorella vulgaris*	5.0–5.5	Non-living	Ferreira et al. (2011)
Zinc (Zn^{2+})	*Desmodesmus pleiomorphus*	5	Non-living	Monteiro et al. (2009)

4.1 Mechanism of Uptake of Heavy Metals by Microalgae

The common pathway taken by microorganisms such as microalgae to remove heavy metals from solutions include (i) use of viable microorganisms in accumulation or precipitation of metals in extracellular space; (ii) cell-surface sorption which can be accomplished with both the community of microbes, i.e. living as well as dead microorganisms; and (iii) and the accumulation of heavy metals in intracellular spaces that requires microbial activity (Cossich et al. 2002). Here, both living and dead cells are more or less much efficient in metal accumulation, but the main difference lies in the mechanism that they involve. So, the mechanism of remediation with the help of microalgae could be mainly listed into two categories: (i) bioaccumulation by living cells and (ii) biosorption by non-living, nongrowing biomass. This first process (comprising bioaccumulative uptake) forms the principle involving the process for detoxification of waste materials (e.g. biological fluidized beds employing continually growing biofilms) (Kumar et al. 2015). On the other hand, the dead (heat-killed, dried, acid and/or otherwise chemically treated) cells can accumulate heavy metal in much a similar way, rather to greater extent as compared to the growing or resting cells (Aksu 1998).

Although both living and non-living biomass have the potential to accumulate the heavy metals in them, the living biomass have much interesting mechanism of the same due to different barriers provided by cell walls, plasma membrane, cell organelles, etc. (Kumar et al. 2015). Initial barrier provided by cell wall of microalgae stands to be less effective as the wall comprise mainly of polysaccharides, proteins and lipids, which offer several functional groups (e.g. carboxyl, –COOH; hydroxyl; –OH; phosphate; $–PO_3$; amino; $–NH_2$; and sulfhydryl–SH) that provides net negative charge to the wall. This galaxy of negative charges proves profitable for the positively charged cations such as cadmium, chromium, copper, etc. (Chojnacka et al. 2005). Much in the similar way, the plasma membrane also provides the second barrier to the heavy metals. In microalgae there exist two kinds of transport proteins, that is, Group A and Group B transporter proteins, where Group-A transporters {such as NRAMP (natural resistance-associated macrophage proteins), ZIP (Zrt-, Irt-like proteins), FTR (Fe transporter) and CTR (Cu transporter) families} help in moving metals inside the plasma membrane and Group-B transporters {such as CDF (cation diffusion facilitator), P1B-type ATPases, FPN (FerroPortiN) and Ccc1 (Ca (II)-sensitive cross-complementer 1)/VIT1 (vacuolar iron transporter 1) families} help in the exocytosis of excess metals (Blaby-Haas and Merchant 2012). Apart from these two ways of uptake of heavy metals by microalgae, some more ways are possible such as ion exchange concept (very similar to the concept of cell wall uptake), sequestration and compartmentalization in vacuoles (Monteiro et al. 2012) and sequestration to the chloroplast and mitochondria (Perales-vela et al. 2006; Shanab et al. 2012).

Microalgae being apt for the bioremediation are widely used in the environment, but the expertise exhibited by other microbes such as bacteria and fungi cannot be overlooked or underestimated. In environment, different types of contaminants are present in intermingled nature. So, the contaminants that exist in coordination with others are called as co-contaminants, for example, association of PAH (polycyclic aromatic hydrocarbons) with heavy metals (Liu et al. 2017). Different of microbes are thought to be used in treatment of these co-contaminants such as bacteria and fungi. Some commonly found bacteria that are used for PAHs and heavy metals bioremediation are *Bacillus*, *Escherichia* and *Mycobacterium.* They have the capability to breakdown the PAHs such as anthracene, naphthalene, phenanthrene, pyrene and benzopyrene in the presence of heavy metals and can diminish the repression brought about by some heavy metals such as Cd, Cu, Cr and Pb occurring together with PAHs (Table 13.4).

4.2 Factors Affecting Microbial Bioremediation

The efficiency of bioremediation depends on many factors including the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment and their availability to microorganisms (Fantroussi and Agathos 2005). The rate of degradation of contaminants by bacteria is more or less retarded due to less frequency of interaction between them. In addition to this, microbes and pollutants are not uniformly spread in the environment. The controlling and optimizing of bioremediation processes is a complex system due to many factors such as existence of microbial population capable of degrading the pollutants, the availability of pollutants, availability of contaminants to the microbial population and environmental factors such as the soil type and texture, temperature and pH, the presence of oxygen and other electron acceptors and nutrients (Abatenh et al. 2017).

4.3 Advantages and Disadvantages of Bioremediation by Microbes

Microorganisms are found naturally in the environment whether it is beneficial or non-beneficial. And the beneficial microorganisms are necessary for sustaining the natural connectivity of the food chain. In nature they exist as simple organisms, with less labour intensive to culture and are cheap due to their natural role in the environment (Abatenhet al. 2017). According to Dell anno et al. (2012), microorganisms used for bioremediation are environment-friendly and helps in maintaining

Table 13.4 Microorganisms, especially fungi and bacteria, capable of remediating heavy metals, oils, dyes, pesticides and many hydrocarbons

Microorganisms	Compounds	References
Penicillium chrysogenum	Monocyclic aromatic hydrocarbons, benzene, toluene, ethyl benzene and xylene, phenol compounds	Pedro et al. (2014) and Abdulsalam et al. (2013)
P. alcaligenes, *P. mendocina and P. putida P. veronii*, *Achromobacter*, *Flavobacterium*, *Acinetobacter*	Petrol and diesel polycyclic aromatic hydrocarbons toluene	Safiyanu et al. (2015) and Sani et al. (2015)
Pseudomonas putida	Monocyclic aromatic hydrocarbons, e.g. benzene and xylene	Safiyanu et al. (2015) and Sarang et al. (2013)
Phanerochaete chrysosporium	Biphenyl and triphenylmethane	Erika et al. (2013)
A. niger, *A. fumigatus*, *F. solani and P. funiculosum*	Hydrocarbon	AI-Jawhari (2014)
Coprinellus radians	PAHs, methylnaphthalenes and dibenzofurans	Aranda et al. (2010)
Alcaligenes odorans, *Bacillus subtilis*, *Corynebacterium propinquum*, *Pseudomonas aeruginosa*	Phenol	Singh et al. (2013)
Tyromyces palustris, *Gloeophyllum trabeum*, *Trametes versicolor*	Hydrocarbons	Karigar and Rao (2011)
Candida viswanathii	Phenanthrene, benzopyrene	Hesham et al. (2012)
Cyanobacteria, green algae and diatoms and Bacillus licheniformis	Naphthalene	Hesham et al. (2012) and Lin et al. (2010)
Acinetobacter sp., *Pseudomonas* sp., *Ralstonia* sp. and *Microbacterium* sp.	Aromatic hydrocarbons	Hesham et al. (2012)
Gloeophyllum striatum	Striatum pyrene, anthracene, 9-metilanthracene, dibenzothiophene lignin, peroxidase	Yadav et al. (2011)
Acinetobacter sp., *Pseudomonas* sp., *Ralstonia* sp. and *Microbacterium* sp.	Aromatic hydrocarbons	Hesham et al. (2012)
Gloeophyllum striatum	Striatum pyrene, anthracene, 9-metilanthracene, dibenzothiophene lignin, peroxidase	Yadav et al. (2011)
Acinetobacter sp., *Pseudomonas* sp., *Ralstonia* sp. and *Microbacterium* sp.	Aromatic hydrocarbons	Hesham et al. (2012)
Fusarium sp.	Oil	Hidayat A and Tachibana (2012)
Alcaligenes odorans, *Bacillus subtilis*, *Corynebacterium propinquum*, *Pseudomonas aeruginosa*	Oil	Singh et al. (2013)
Bacillus cereus A	Diesel oil	Maliji et al. (2013)

(continued)

Table 13.4 (continued)

Microorganisms	Compounds	References
Aspergillus niger, *Candida glabrata*, *Candida krusei* and *Saccharomyces cerevisiae*	Crude oil	Burghal et al. (2016)
B. brevis, *P. aeruginosa KH6*, *B. licheniformis* and *B. sphaericus*	Crude oil	El-Borai et al. (2016)
Pseudomonas aeruginosa, *P. putida*, *Arthrobacter* sp. *and Bacillus* sp.	Diesel oil	Sukumar and Nirmala (2016)
Citrobacter koseri and Serratia ficaria, *Pseudomonas cepacia*, *Bacillus cereus*, *Bacillus coagulans*	Diesel oil, crude oil	Kehinde and Isaac (2016)
B. subtilis strain NAP1, *NAP2*, *NAP4*	Oil-based based paints	Phulpoto et al. (2016)
Myrothecium roridum IM 6482	Industrial dyes	Jasin et al. (2012, 2013, 2015)
Pycnoporus sanguineus, *Phanerochaete chrysosporium* and *Trametes trogii*	Industrial dyes	Yan et al. (2014)
Penicillium ochrochloron	Industrial dyes	Shedbalkar and Jadhav (2011)
Micrococcus luteus, *Listeria denitrificans* and *Nocardia atlantica*, *Textile*	Azo dyes	Hassan et al. (2013)
Bacillus spp. *ETL-2012*, *Pseudomonas aeruginosa*, *Bacillus pumilusHKG212*	Textile dye (Remazol black B), sulfonated diazo dye, Reactive Red HE8B, RNB dye	Yogesh and Akshaya (2016) and Das et al. (2015)
Exiguobacterium indicum, *Exiguobacterium aurantiacums*, *Bacillus cereus and Acinetobacter baumannii*	Azo dyes effluents	Kumar et al. (2016)
Bacillus firmus, *Bacillus macerans*, *Staphylococcus aureus and Klebsiella oxytoca*	Vat dyes, textile effluents	Adebajo et al. (2017)
Cunninghamella elegans	Heavy metals	Bahobil et al. (2017)
Pseudomonas fluorescens and Pseudomonas aeruginosa	Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn2+ and Cu2	Paranthaman and Karthikeyan (2015)
Lysinibacillus sphaericus CBAM5	Cobalt, copper, chromium and lead	Peña-Montenegro et al. (2015)
Microbacterium profundi strain Shh49T	Fe	Wu et al. (2015)
Fumigatus, *Paecilomyces* sp., *Paecilomyces* sp., *Trichoderma* sp., *Aspergillus versicolor*, *A. Microsporum* sp., *Cladosporium* sp.	Cadmium	Soleimani et al. (2015)
Geobacter spp.	Fe (III), U (VI)	Mirlahiji and Eisazadeh (2014)

(continued)

Table 13.4 (continued)

Microorganisms	Compounds	References
Bacillus safensis (JX126862) strain (PB-5 and RSA-4)	Cadmium	Priyalaxmi et al. (2014)
Pseudomonas aeruginosa, *Aeromonas* sp.	U, Cu, Ni, Cr	Sinha et al. (2011)
Aerococcus sp., *Rhodopseudomonas palustris*	Pb, Cr, Cd	Sinha and Paul (2014) and Sinha and Biswas (2014)
Saccharomyces cerevisiae	Heavy metals, lead, mercury and nickel	Chen and Wang (2007), Talos et al. (2009) and Infante et al. (2014)

sustainability in the environment by destroying the contaminants, thus maintain the cycle of nature. Moreover, the contaminants that are destroyed by these microorganisms are not simply transferred to different environmental media. Further, they are nonintrusive, potentially allowing for continued site use. According to Kumar et al. (2011), they are relatively easy to implement in ground-level experiments. Thus, the use of microorganisms stands to be the most effective way of remediating natural ecosystem from a number contaminates and acts as environment-friendly options (Singh et al. 2013). Although there are many advantages of using microorganisms for remediating the natural environment from various heavy metal contaminants, the benefits are limited to those compounds that are biodegradable in nature. In addition to this, not all compounds are susceptible to rapid and complete degradation (Abatenh et al. 2017). Also, there are some concerns that the products of biodegradation (in some cases) may be more persistent or toxic than the parent compound (Abatenh et al. 2017). In nature, biological processes are often highly specific and the site factors are quite important requisites. Important site factors required for success includes the presence of metabolically capable microbial populations, suitable environmental growth conditions and appropriate levels of nutrients and contaminants (Abatenh et al. 2017). A very high-profile research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants (solids, liquids or gases) that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids and gases.

5 Phytoremediation

An unequalled and rapid emerging branch of bioremediation that fits best as eco-friendly approach and employs natural properties of plants for removal of contaminants from soils is phytoremediation (Oh et al. 2014). This phytoremediation process has gained its importance due to its cost-effective, efficient and non-invasive way of decreasing the pollutants from water and soils (Mojiri 2012) without showing any negative effect on the environment. This technology is widely applicable in remediating inorganic contaminants such as heavy metals and radionuclide, as well as organic contaminants such as polyaromatic hydrocarbons, chlorinated solvents, etc. (Wang et al. 2003; Oh et al. 2013a, b). The urge for the enforcement of this process is due to continuous contamination of heavy metals beyond its threshold limit which is harmful to all forms of life (Gaur et al. 2014; Dixit et al. 2015; Tak et al. 2013). Earlier it was natural sources which were dominating over anthropogenic sources for heavy metal pollution, but nowadays due to rapid urbanization and industrialization, the anthropogenic sources of pollution left the natural sources way beyond the expectations. Industries that are energy intensive has been established for power an electricity production such as thermal power plants, coal mines, etc. pose to be major sources of anthropogenic pollution (Rai et al. 2007). Many large agrochemical industries such as chlor-alkali industries release large amount of range of heavy metals into the lakes and reservoirs thereby deteriorating the water quality (Rai et al. 2007). Different standards have been set for different heavy metals in water as well as soil to regulate its concentration. According to Comprehensive Environmental Response Compensation and Liability Act (CERCLA), USA, the maximum permissible concentration of heavy metals in water was given as 0.01 for Ar, 0.01 for Cr, 0.02 for Hg, 0.05 for Cd, 0.05 for Ag and 0.15 for lead (mg/litre) (Chaturvedi et al. 2015). Similarly, according to Indian Standards, the maximum concentration should be 3–6 for cadmium, 75–150 for nickel, 135–270 for copper, 250–500 for lead and 300–600 for zinc (in mg/kg) (Nagajyoti et al. 2010). Phytoremediation proves to be very modern and cost-effective technology as compared to old conventional techniques, viz. vapour extraction, soil washing, thermal desorption, etc., that leads to other problems such as air and groundwater pollution (Oh et al. 2013a, b). Among the conventional techniques, onsite management or excavation and then dumping of the same waste containing heavy metals pose to be a great threat as it just changes the site of contamination and are often act as a reason for hazard associated with transportation throughout the path of travel to dump area (Tangahu et al. 2011). There are chemical technologies and physical methods too that help in remediating the heavy metal contamination, but they are technically difficult to use and are too expensive and generate large volumetric sludge thereby contributing pollution to the environment again (Rakhshaee et al. 2009). On the other hand, phytoremediation stands to be very useful as it uses sunlight as its energy source and natural green plants for remediation of soil contaminants which can be done in situ. Moreover, this process has least or no secondary contaminants as it immobilizes them, thereby preventing their entry into the groundwater thus protecting the soil profile and enhancing the quality of soil and prevents the soil resources (Oh et al. 2013a, b). According to some workers, phytoremediating plants could metabolize large and highly toxic substances into small and non-toxic ones, but this capability greatly varies from species to species (Oh et al. 2013a, b). Phytoremediating plants have variable capabilities due to difference in their growth rates, their biomass, depth of root zone and their potential to transpire groundwater into the atmosphere (Oh et al. 2013a, b).

Besides all the advantages, phytoremediation has some shortcoming as it takes a long time to remediate the soil contamination and is limited by the climatic, geological conditions and the type of soil of that area.

Worldwide, dedicated research is going on to find different ways for remediating heavy metals from soil and water, and till date the phytoremediation process proved to be the best technique and only sustainable alternative to all kinds of remediation. Different plants species have been used to evaluate the phytoremediation capability of plants by varying the type of plant species, properties of medium (pH adjustment, fertilizer) (Prasad and Freitas 2003) and addition of chelating agent such as EDTA (Ginneken et al. 2007), etc. Tables 13.5 and 13.6 enlist some phytoremediating plants that can be used for phytoremediation of heavy metals (Table 13.5) and hydrocarbons (Table 13.6).

6 Different Processes of Uptake of Heavy Metals by Plants in Contaminated Soils

Plants are the amazing creation of nature being always help in bringing up the environment to stabilize itself by various means. Much on the same way, various plant parts absorb heavy metal contaminants present in soil and water leaving the environment pollution-free. Root, shoot and leaves accumulate the metals inside their tissues by many different processes leading to decontamination of important abiotic resources such as soil and water. The urgency of the process is due to the nasty property of heavy metals off being long time persistent and its non-biodegradable nature which increases the threat to human beings and other animal's health (Gisbert et al. 2003). Figure 13.1 depicts various areas of plants for uptake, absorption and evaporation of contaminants. Various processes are involved in the process of phytoremediation which are discussed below.

6.1 Phytoextraction

Phytoextraction is the process of absorption of soil contaminants by plants where it stores or concentrates them in the shoots and harvestable parts of the root. Nickel, copper and zinc are the best members to be absorbed by plants, and over 400 plants can absorb them easily, and they can be "removed permanently" from the soil and water (Etim 2012; Upadhyay et al. 2019). The plants that are selected for this process exhibit excellent property to produce high biomass. But, according to Evangelou et al. (2007), most of the metal accumulating plants are generally found to be slow growing and are having very low capacity to produce considerable amount of biomass. So, the plants with these properties are supposed to discourage the process of phytoextraction as it wholly and solely depend on tissue metal concentration and

Table 13.5 Table showing phytoremediating plants capable of heavy metal uptake

Name of plant	Metal	Process	References
Cerastium arvense (field chickweed)	Cadmium	Uptake/accumulation	Institute for environmental research and education (2003)
Claytonia perfoliata (miner's lettuce)	Cadmium	Uptake/accumulation	Institute for environmental research and education (2003)
Lupinus albus (white lupin)	Arsenic	Rhizoaccumulation	Esteban et al. (2003)
Vicia spp. (vetch)	Nutrients/metals	Uptake	McCutcheon and schnoor (2003)
Thlaspi caerulescens (alpine pennycress)	Cadmium, zinc, nickel	Hyperaccumulation	McCutcheon and schnoor (2003)
Solidago hispida (hairy goldenrod)	Metals	Hyperaccumulation	McCutcheon and schnoor (2003)
Gleditsia triacanthos (honey locust)	Lead	Phytoextraction	García et al. (2003)
Populus tremula (Aspen)	Lead	Extraction	McCutcheon and schnoor (2003)
Viola spp. (violets)	Metals	Phytoextraction/hyperaccumulation	Institute for environmental research and education (2003)
Water bloom/algal bloom (*Microcystis* sp.)	Metals	Uptake	Rai et al. (2007)
Reed (*Phragmites australis*; *Phragmites karka*)	Metals	Uptake	Bragato et al. (2006) and Vymazal (2007)
Water fern, water velvet (*Azolla caroliniana*, *Azolla pinnata*)	Metals	Uptake	Rai et al. (2007)
Bulrush/cattail (*Typha latifolia*, *Typha angustata*, *Typha domingensis*)	Metals	Uptake	Manios et al. (2003) and Hadad et al. (2006)
Poplar trees (*Populus deltoids*)	Metals	Uptake	Robinson et al. (2000)
Pond weed/curly leaf pond weed (*Potamogeton natans*; *Potamogeton crispus*)	Metals	Uptake	Fritioff and Greger (2006)
Parrot's feather (*Myriophyllum spicatum*)	Metals	Uptake	Lesage et al. (2007)
Umbrella plant (*Cyperus alternifolius*)	Metals	Uptake	Qian et al. (1999)
Duckweed (*Lemna minor*)	Metals	Uptake	DeBusk et al. (1996)

biomass production (Chaney et al. 2007). Different species of plants differ in their capability of concentrating metals in them, and the species that can accumulate 100 mg kg^{-1}f cadmium (Cd), 1000 mg kg−1 of arsenic (As), cobalt (Co), copper (Cu), lead (Pb) or nickel (Ni) or >10,000 mg kg^{-1} of manganese (Mn) and zinc (Zn) are considered as hyperaccumulator plants. There are several steps that are

Table 13.6 Table showing some phytoremediating plants capable of hydrocarbon accumulation

Canadian wild rye (*Elymus canadensis*)	Hydrocarbons	Rhizodegradation/ accumulation	McCutcheon and Schnoor (2003)
(Red fescue *Festuca rubra*)	Hydrocarbons	Rhizodegradation	McCutcheon and Schnoor (2003)
(Tall fescue) (*Festuca arundinacea*)	Pyrene, PAHs and NPK	Rhizodegradation/ phytoextraction	Christensen-Kirsh (1996) and McCutcheon and Schnoor (2003)
English ryegrass (*Lolium perenne*)	Hydrocarbons/ nutrients	Rhizodegradation/ uptake	McCutcheon and Schnoor (2003)
(Yellow sweet clover) *Melilotus officinalis*	Hydrocarbons and NPK	Rhizodegradation	Christensen-Kirsh (1996) and McCutcheon and Schnoor (2003)
Switchgrass (*Panicum virgatum*)	Hydrocarbons	Rhizodegradation	McCutcheon and Schnoor (2003)
Mulberry (*Morus rubra*)	PAHs and PCBs	Rhizodegradation	McCutcheon and Schnoor (2003)

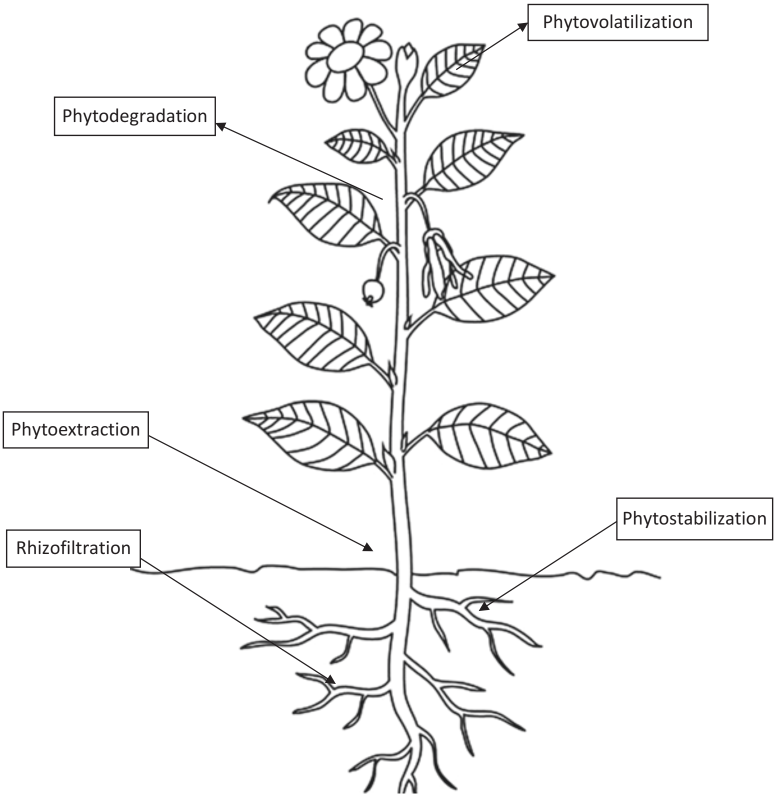

Fig. 13.1 Different methods of remediation of heavy metals contamination in soil by plants. (Modified from Oh et al. 2014)

necessary for hyperaccumulation of heavy metals by plants which includes absorption and transportation of metals across the membranes of root cells followed by uploading of metals into xylem for its transportation and then the translocation of these metals to the shoots and thus sequestration and detoxification of metals within plant tissues (Yang et al. 2005). According to Rascio and Navari-Izzo 2011, the plant epidermis, its trichomes and cuticle are the favourable lodging sites for detoxification of metals, and in several instances, the subsidiary and stomatal cells are protected against metal toxicity.

Since this process generally indulge in accumulation of metals in lower concentration due to inefficiency of plants to produce larger biomass, therefore these hyperaccumulator plants discourages their adoption in larger scale or for commercial purpose. But few plants with metal tolerable capacity can be thought to be effectively used for commercial scale (Saifullah et al. 2009). However, these species have an inherently low ability to absorb metals but can accumulate higher concentrations of metals if grown in the soils treated with chemical amendments to increase metal phytoavailability and plant uptake (Meers et al. 2005).

6.2 Phytostabilization

Sites with high concentration of contamination of heavy metals are difficult to remediate. So, this level of contamination is so much so that the phytoextraction of metals from such soils would take a considerably longer period of time which is neither economical nor suitable. In such cases if remediation technology is not applied quickly and effectively then, these could be a major source of metal dispersion into the environment. The risk posed by such soils can be decreased by using plants to stabilize or immobilized the metals in the soil (Marques et al. 2009). Such process of immobilization of soil contaminants by accumulating or precipitating it with the help of root and its exudates within rhizospheric region, to limit its spread to the food chain is called as phytostabilization. In the process of phytostabilization, plants readily immobilizes the metals present in the rhizospheric zone thereby leaving them less bioavailable and less toxic to plants, animals and humans or retain the metals in the roots by restricting their translocation to above-ground parts (Mendez and Maier 2008; Wong 2003). This technology is quite useful in treatment of lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn) (Etim, 2012).

The main mechanism of phytostabilization is the precipitation and adsorption of heavy metals near the rhizospheric zone where these metals are converted into less soluble forms like carbonates and sulphides of metals, metal complexes with organic compounds, metal adsorption on root surfaces and metal accumulation in root tissues (Mendez and Maier 2008; Wong 2003). The presence of plants in metal-contaminated soils promotes heterotrophic microbial communities which may, in turn, promote plant growth and participate in metal stabilization. Metal-tolerant plants with the capacity to keep the metals out of metabolic sites (shoots) are the best candidates for phytostabilization. Although such plants have developed mechanisms to restrict the metals in the rhizosphere or roots, even then concentration of metals in shoots must be monitored (Mendez and Maier 2008). Among many phytoextracting plant species the *Cynodon dactylon* was found to be the best accumulator of As in roots and thus a promising candidate for phytostabilization and have wide adaptations in Pb- and Zn-contaminated soils also (Leung et al. 2007). Moreover, the mycorrhizae (interaction of fungi with the roots of higher plants) play an important role in stabilization by binding the metals with hyphae, and some mycorrhizae like ericoid and ectomycorrhizal fungi colonizing in *Cynodon dactylon* can modify the rhizosphere by excreting organic acids and thus stabilizing metals in the rhizosphere (Meharg 2003). *Vetiveria zizanioides*, *Sesbania rostrata*, herb legume and *Leucaena leucocephala* have been successfully grown in metal-contaminated soils for metal stabilization (Shu et al. 2002; Zhang et al. 2001). This phytostabilization technique is effective only when the phytoextraction method is not efficient (Sabir et al. 2014), and the efficiency of this process can be enhanced by performing and applying soil amendments like zeolites, beringite, steel shot and hydroxyapatite (Lothenbach et al. 1998).

6.3 Phytodegradation

Breakdown of organic contaminants by plants either internally through its metabolic pathway with the help of secreted enzymes or externally by root exudates and incorporation of these contaminants into plant tissues (Trap et al. 2005). This process is mainly used to degrade complex organic molecules and convert it into simpler forms in soils, groundwater medium and sledges. Some complex organic compounds that are reported to be degraded by this process are tetra-chloroethane by poplar species, 2, 4-dichlorophenol by *Brassica*, benzotriazoles by *Helianthus*, trifluralin and lindane by rye, gasoline by pothos, diesel and heavy oil by grasses native to California (Newman and Reynolds 2004).

6.4 Rhizodegradation (Phytostimulation)

Disintegration of contaminants in the soil through the activity of microorganisms, enhanced by the presence of root zone, is known as rhizodegradation (Tangahu et al. 2011). According to USEPA, 2000, the rhizospheric region contains at least 100 times more number of microbes as compared to non-rhizospheric region. This process mainly helps in remediating organic hydrocarbons such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), chlorinated solvents, pesticides, polychlorinated biphenyls (PCBs), benzene, toluene, ethylbenzene and xylenes (EPA 2000).

6.5 Phytovolatilization

In phytovolatilization contaminants such as selenium, mercury, arsenic, etc. that are absorb by the root of the plants are converted to more simple forms and are then volatilized through stomata of the leaves to the atmosphere. In phytostabilization metals are assimilated into organic compounds which are volatile in nature and ultimately released into atmosphere as biomolecules (Marques et al. 2009).This process is primarily used for removal of mercury (Hg) contamination from the soil along with other metals such as Se and As. During the course of development, molecular technology reveals the presence of a gene that is responsible for reducing mercuric ion into elemental mercury through enzyme mercury reductase (Rugh et al. 1996). The gene, merApe9, has been introduced into *Arabidopsis thaliana* which ultimately volatilizes large amounts of Hg into the atmosphere (Rugh et al. 1996). Although the advantage of this process is that the mercury ion can be easily transformed to the less toxic elemental form, unfortunately the disadvantage associated with it is much greater as there is huge probability of recycling of mercury by precipitation and thus its accumulation in lakes and oceans with pose a great threat to aquatic life forms (USEPA 2000). A very similar phenomenon is observed in case of selenium. *Brassica juncea* has been shown to volatilize Se into the atmosphere through assimilation of Se from the soil into organic seleno-amino acids, selenocysteine and seleno-methionine which later can be biomethylated to form the volatile compound dimethylselenide (Banuelos et al. 1993; Terry et al. 2000). These processes have not gained much importance as the probability of recycling of volatile metallic compound is very high.

Thus, different plant species employing different processes of uptake of heavy metals from metal-contaminated soils can be depicted in Table 13.7 which shows plant species remediating different heavy metals by different mechanisms.

7 Advantages and Limitation of Phytoremediation

Phytoremediation have some significant advantages in terms that they help in reducing the heavy metal ion concentration either by changing its form or by reducing them to low levels with the help of inexpensive biosorbent materials (Rakhshaee et al. 2009). According to Rodrigues (2005), there are various methods that are used for phytoremediation which lead to degradation of heavy metal contents in soil. Moreover, phytoremediation shows its transparency towards lowest remediating capacity, that is, a cost-effective method accompanying least expensive approach for remediation of the environmental media, mainly appropriate for large sites containing relatively low levels of contamination (Ginneken et al. 2007). Furthermore, the phytoremediation technology can also be used for remediating wide range of toxic metals and radionuclides and are equally useful for detoxifying organic as well as inorganic contaminants to a level that are acceptable to the society and

Table 13.7 Plants species with different processes for heavy metal remediation from contaminated soil

Plant	Phytoremediation type	Metal	Mechanism	References
Sedum alfredii H	Phytostabilization	Pb and Cd	Induction of glutathione biosynthesis that bind metals in roots	Anjum et al. (2012), Gupta et al. (2010) and Sun et al. (2007)
Athyrium wardii	Phytostabilization	Pb and Cd	Root retention of metals	Zou et al. (2011)
Ceratophyllum demersum	Phytoextraction	Cd	Production of phytochelatin for metal binding in shoots	Mishra et al. (2009)
			Activation of cysteine synthase, glutathione-S-transferase, glutathione	
Pteris vittata	Phytoextraction	As	Increased colonization	Leung et al. (2007)
			Exploring more soil	
Sedum alfredii	Phytoextraction	Zn	Metals loaded into leaf sections and protoplast	Yang et al. (2005)
Imperata cylindrical, *Miscanthus floridulus*	Phytostabilization	Cd, Zn, Cu, Pb	Fibrous root system retaining the metals	Peng et al. (2006)
Cynodon dactylon	Phytostabilization	As, Zn, Pb	Binding with hyphae of mycorrhizae	Leung et al. (2007)
			Release of organic acids	

environment (Liu et al. 2017; Mwegoha 2008). Vivid researches on phytoremediation technique also help in improving the soils that are enriched with high aluminium and soil level (US Department of energy 1994). Although phytoremediation is good, reliable and cost-effective technique, it can be very a time-consuming process, i.e. it may take several growing season for completion of the process (Mwegoha 2008). It may take weeks to months for excavation and disposal of wastes, or it may extend up to several years to accomplish process exquisitely (Tangahu et al. 2011). Once the process is done, the by-products or the intermediate formed during the remediation process, which is either organic or inorganic in nature, may be cytotoxic to plants itself (Mwegoha 2008).

8 Mechanism of Uptake of Heavy Metals in Plants

Accumulation of heavy metals in small quantities is essential for plant growth and metabolism; however, at higher concentration they stand to be potentially toxic to plants and thus the soil ecosystem (Nagajyoti et al. 2010). Living organism absorbs heavy metals directly or indirectly, and the over-accumulation of metals ultimately leads to the production of reactive oxygen species (ROS) followed by apoptosis (Shi et al. 2004). These heavy metals are initially encountered by the macromolecules such as proteins. So, to understand the real mechanism or the pathway taken by the heavy metals to get absorbed inside the tissues, we need to search for the proteins that bind these metals or metal ions to it. In fact, these heavy metal-binding proteins are encoded by specific genes. If these genes are properly searched out and thorough analysis is done, then it would be the answer to the mechanism of action of the heavy metals.

According to Trivedi and Ansari (2015), the expressed sequence tags (ESTs) analysis is the best technique that would help to elucidate the sites of accumulation or hyper-accumulation of the heavy metals. With the help of biotechnological techniques and immobilized metal ion affinity chromatography or IMAC, the metal ions are finally immobilized (Trivedi et al. 2003). Following the immobilization of the heavy metals, it initially binds with the cell walls or the membrane, which acts as an ion exchange agent of comparatively low selectivity. Further, the transport systems, with activation and deactivation of intracellular high-affinity binding sites, help in the uptake of these metals across the plasma membrane through secondary transporters such as channel proteins and/or H^+ -coupled carrier proteins (Chaney et al. 2007). These transporters act through a series of signalling events like phosphorylation cascades, hormones, mitogen-activated protein kinases and calcium-calmodulin systems (Shi et al. 2004).

In the last few years, extensive studies have been done on membrane transporter genes, and few membrane transporter gene families have been identified. After their identification they are characterized by heterologous complementation screens and sequencing of ESTs and plant genome studies. Many cation transporters have been identified in recent years, most of which are Zn-regulated transporter (ZRT), Fe-regulated transporter (IRT), natural resistance-associated macrophage proteins (NRAMP), Al-activated malate transporter (ALMT), cation diffusion facilitator (CDF), P-type ATPase (heavy metal associated), yellow stripe-like (YSL), copper transporter and nicotianamine synthase (NAS) (Guerinot 2000; Williams et al. 2000; Talke et al. 2006; Memon and Schroder 2009; Maestri et al. 2010). Once these heavy metals enter the plant tissues, the subsequent movement of metal takes place through the plant sap with the help of root pressure and by the process called transpirational pull (Robinson et al. 2003). Further the responsibility of transporting these heavy metals to the shoot parts are completed by the xylem cells. Since heavy metals at its higher concentration inside the cell become very much toxic to plants, they start an enzymatic process catalysing oxidation reduction reactions and thereby alter their chemistry from toxic to non-toxic forms. Two such examples are reduction of Cr^{6+} to Cr^{3+} in *Eichhornia crassipes* (Lytle et al. 1998) and reduction of As^{5+} to As^{3+} in *B. juncea* (Pickering et al. 2000). Besides this, some of the intracellular metals are detoxified by some different mechanism such as they either binds to low molecular mass organic compounds or they may get localized in the vacuoles as a metal-organic acid complex, or they may bind to histidine itself (Persans et al. 1999; Kramer et al. 2000). Heavy metal concentration in the cytoplasm can be regulated in many ways, and among many metals, Zn shows most diversified ways in regulating its concentration, which involves sequestration in a subcellular organelle to low molecular mass organic ligands, low uptake across the plasma membrane and precipitation as insoluble salts and active extrusion across the plasma membrane into the apoplast (Brune et al. 1994). There have been many investigations done in different disciplines of science to understand the mechanism of accumulation and tolerance of heavy metals. Finally, the molecular and genetic engineering technologies led to the well understanding of mechanisms of heavy metals in plants. Furthermore the information, of mechanism of remediation, the rate-limiting steps for uptake, translocation and detoxification of metals in hyperaccumulating plants can be thought to be used in the development of many transgenic plants with increased resistance and uptake of heavy metals and thus improving the applicability of the phytoremediation technology (Yang et al. 2005).

9 Remedial Technologies for Metal-Contaminated Soil

In today's era of scarcity of land under farming and the invariable increase in population size, there is always an urge of fertile land for cultivation and clean water for irrigation. Thus it has become very important for remediating the contaminants from soil and water by generating certain remediation technologies.

Remediation technologies can be classified according to (1) the nature of action that is applied on the metals immobilization or extraction, (2) the location where the process is applied in situ or ex situ and (3) technology type, i.e. containment/disposal methods, or chemical, physical, thermal and biological treatments or monitored

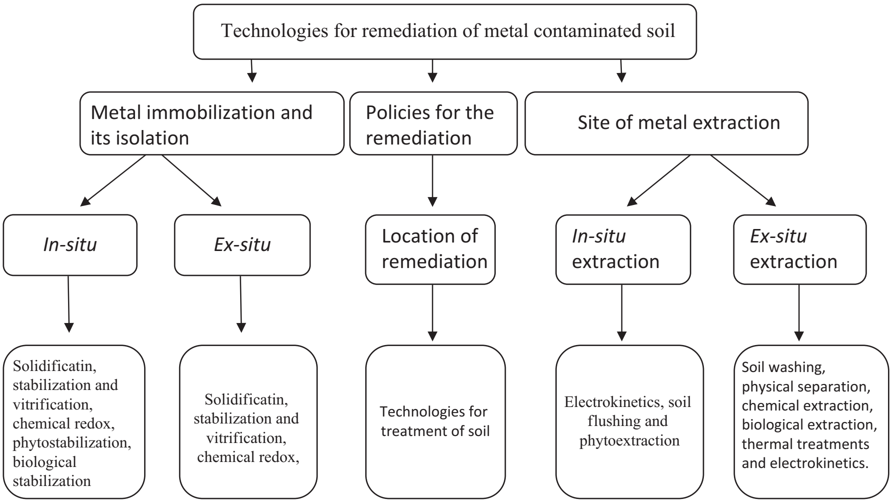

Fig. 13.2 Shows certain remedial technologies which can be useful in removal of metals from the metal-contaminated soil. (Taken from: Dermont et al. 2008)

natural attenuation (Dermont et al. 2008). Figure 13.2 shows certain remedial technologies which can be useful in removal of metals from the metal-contaminated soil.

10 Conclusions

Bioremediation is a powerful tool available to clean up contaminated sites. However, other applications are relatively new, and many other applications are emerging or being developed. Bioremediation occurs when the microorganisms can biodegrade the given contaminant and the necessary nutrients such as nitrogen, phosphorus, electron acceptors and trace elements. This process can be aerobic or anaerobic depending on the microorganisms and the electron acceptors available. This process may be natural (intrinsic bioremediation), or it may be enhanced by man (engineered bioremediation). Regardless of which aspect of bioremediation that is used; this technology offers an efficient and cost-effective way to treat contaminated groundwater and soil. But the effects for increasing the scope and efficiency of phytoremediation and for developing phytoremediation systems for sites contaminated with multi-contaminants are urgently necessary. Although some companies have started their business in phytoremediation, phytoremediation has not been fully commercialized. Further research is still needed, and the priorities on phytoremediation for the future should focus on establishing stable and efficient phytoremediation systems through finding more efficient remediating plants and microbes, monitoring current field trials to obtain thorough understanding, developing microbe-plant combination systems and using genetic engineering technology. Phytoremediation are expected to be used as a vital tool in sustainable management of contaminated soils. Contaminated site managers should consider phytoremediation when evaluating remedial alternatives.

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