

Chapter 6

Sustainable Approaches to Remove Heavy Metals from Water



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Abstract Water contamination by heavy metals is a worldwide issue undermining the whole biosphere and influencing the life of a huge number of individuals around the globe. Not exclusively is water contamination by metals one of the chief worldwide hazard factors for sickness, ailments and death, yet it likewise adds to the nonstop reduction of the accessible drinkable water around the world. These metals are discharged from an assortment of sources such as mining, urban sewage, smelters, tanneries, textile industry and chemical industry. Technologies utilized for their expulsion from aquatic bodies incorporate reverse-osmosis, ion-exchange, electro-dialysis, adsorption, etc. Most of these technologies are quite costly, energy intensive and metal specific. These conventional technologies for the expulsion of the dangerous heavy metals are most certainly not practical and further create colossal amount of harmful chemical sludge. Delivering valuable solutions, which are easy to implement and affordable, often remains a challenge. Bioremediation is considered as one of the safer, cleaner, cost effective and promising sustainable approach for heavy metal removal from waste water. The objective of this chapter is to conduct a comprehensive review on different sustainable tools for treating heavy metals present in the water.

Keywords Heavy metals · Water pollution · Bioremediation · Eco-sustainable approach · Biotechnological techniques · Genetic engineering

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6.1 Introduction

According to recent estimates, more than 1.2 billion people worldwide do not have access to the most fundamental component of life i.e. clean drinking water (GWI 2018). There has been an extensive increment in the release of waste into the environment, particularly in water bodies, with the development of industries, and this has prompted the gathering of heavy metals, particularly in urban regions (Dixit et al. 2015, Musilova et al. 2016, Masindi and Muedi 2018). The discharge of untreated industrial waste into the water has turned into a preeminent interest in the developing nations and is seen as one of the most significant ecological issues (Burri et al. 2019). The unpredictable discharge of heavy metals into the water is a noteworthy wellbeing concern around the world, as they cannot be degraded into harmless forms and accordingly have enduring consequences for the biological systems (Mishra et al. 2018). Plants and animals require metals for their biological systems, but at raised levels, they meddle with metabolic responses of living beings. The decline in the plant development is attributed to the presence of various lethal heavy metals as it brings down the photosynthetic rate by reducing the enzymatic activity and also causes deprivation of essential mineral nutrients (Nematian and Kazemeini 2013). Heavy metals are potential carcinogenic agents since they have the ability to cause cancer in humans even at low concentrations (Dixit et al. 2015). Consumption of contaminated food causes collection of heavy metals through food chains and becomes a well-being danger to living organisms (Tak et al. 2013). Heavy metals cause free radical production by the process of oxidative stress (Chandra et al. 2015a, b; Mani 2015). Oxidative stress aids in the production of reactive oxygen species (ROS) (Chibuike and Obiora 2014) and hydrogen peroxide (H_2O_2), which causes breakage of cellular DNA and eventually results in cell damage (Chandra et al. 2015a, b; Mani 2015; Kapoor et al. 2019). Antioxidant system which protects the cells from reactive oxygen species (ROS) is suppressed by heavy metal toxicity which causes overproduction of ROS. In the event that this condition proceeds, the normal functioning of the living being is influenced, and this may constantly prompt cell death (Ojuederie and Babalola 2017). Thus, it is very important to remove or reduce the heavy metal contamination in water so as to prevent or reduce the contamination of environment and the possibility of uptake in the food web.

Bioremediation is a process being acknowledged as the standard practice for the reclamation of heavy metal-polluted sites since it is a more eco-accommodating, advantageous, and sustainable technique than the traditional chemical and physical strategies, which are frequently extravagant and inadequate (Igiri et al. 2018). Optimum temperature, pH, and moisture are important environmental factors which govern the ability of microbes to degrade the pollutants (Massoud et al. 2019). Bioremediation can possibly reestablish heavy metal-contaminated sites (Dowarah et al. 2009). However, an absence of data related to the elements controlling the microbial development and metabolism (Li et al. 2013) in contaminated conditions frequently confines its execution. Bioinformatics, in light of proteomics and

genomics (Chauhan and Jain 2010; Poirier et al. 2013), offers momentous guarantee as tools for addressing long-standing inquiries with respect to the molecular components engaged in controlling mineralization pathways (Kim and Park 2013; Govarthanam et al. 2013; Achal et al. 2012). This chapter examines the sources of heavy metals in the aquatic environment and how they can be successfully remediated with the help of sustainable approaches, viz., bioremediation and phytoremediation as well as their mechanisms. The potential prospects and impediment of genetic engineering for bioremediation are also discussed.

6.2 Sources of Heavy Metals in the Aquatic Environment

Combustion of fossil fuels, forest fires, mining and smelting, weathering, municipal wastes, fertilizers, pesticides, and sewage are the basic sources of heavy metals in the environment (Rai 2009; Kabata-Pendias and Pendias 1989; Pillai 2010; Fig. 6.1). In developing countries like India, coal mining industries are also the major contributing source of heavy metal pollution (Sharma 2003; Rai 2012). Discharge of wastes containing heavy metals from different industries presents genuine dangers to water quality of rivers, lakes, and reservoirs and their biodiversity (Concas et al. 2006).

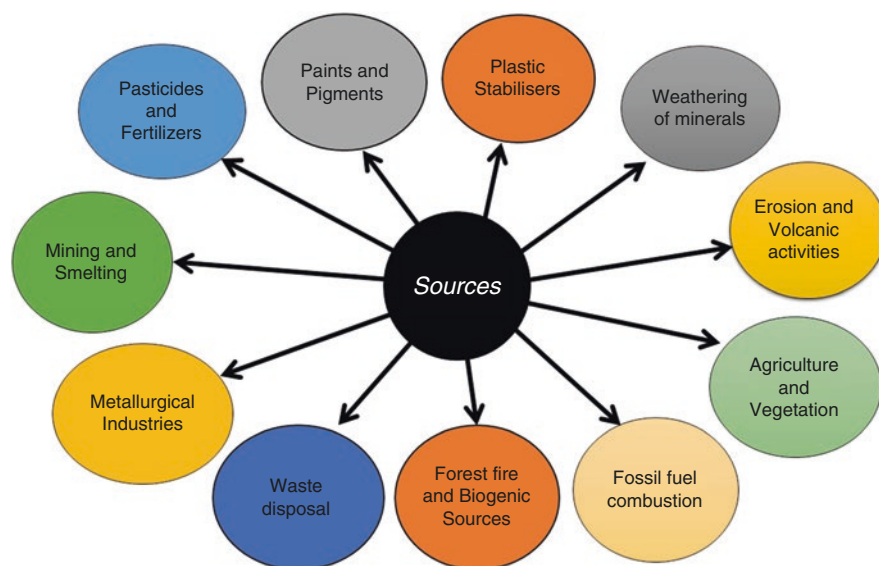


Fig. 6.1 Sources of heavy metals in the aquatic environment

6.3 Prospects of Sustainable Approaches in Heavy Metal Management

This chapter reviews the prospect of sustainable approaches in heavy metal eradication in comparison to traditional chemical technologies. Different traditional technologies such as chemical precipitation method, ion exchange, sedimentation, microfiltration method, and reverse osmosis are not only cost-effective but also eco-friendly, as they represent a genuine danger to life in aquatic system because of different side effects and associatively pollute the aquatic environment (Igiri et al. 2018).

Bioremediation is an eco-accommodating, sustainable, and effective strategy for recovering sites polluted with different pollutants by utilizing the intrinsic biological systems of microbes and plants to degrade toxic contaminants (Kannabiran 2017; Prasad and Aranda 2018). Bioremediation is very cost-effective as compared to chemical technologies because the biosystems utilized in this process are prepared from the naturally available or waste biomass of bacteria, fungi, or algae which are clearly extremely modest (Kratochvil and Volesky 1998; Ayangbenro and Babalola 2017). Different plant wastes such as rice husks, spent grain, sawdust, sugarcane bagasse, fruit wastes, and weeds were used as adsorbents for different heavy metals, viz., Cd, Cu, Pb, Zn, and Ni (Nghah and Hanafiah 2008; Acharya et al. 2018). Bioremediation should be possible on location, in this way diminishing presentation dangers for cleanup faculty, or conceivably more extensive exposure because of transportation mishaps. Other than the above focal points, bioremediation is more affordable, disposes of waste enduringly, takes out long-term liability, and can be combined with physical or chemical treatment technologies.

Moreover, it is a non-obtrusive method that can make the environment flawless (Vidali 2001). Nonetheless, it is difficult to anticipate the pace of cleanup for a bioremediation practice as a few ecological variables are engaged with choosing the destiny of bioremediation, and till date, researchers are looking for standards for foreseeing the pace of removal of contaminants from various parts of the environment (Machackova et al. 2012).

6.4 Bioremediation

Bioremediation is a remedial process that mainly involves application of microbes and/or their enzymes to detoxify environmental contaminants for restoring its original form (Ayangbenro and Babalola 2017). This is a naturally occurring process where microorganisms act as major players that clean up pollutants of soil, water, and other environmental sources. In this process, the growth of certain microorganisms can be stimulated as they utilize these pollutants as a source of nutrition and energy (Ostrem Loss and Yu 2018). Through the metabolic activities of microbes, a variety of contaminants, especially heavy metals, can be degraded. Therefore,

bioremediation can be very well used as a sustainable solution for heavy metal pollution which is constantly increasing due to industrialization and human activities (US EPA 2011; Masindi and Muedi 2018). Bioremediation process involves degradation, removal, alteration, immobilization, or detoxification of several heavy metals from the environment through the cellular processes of plants and microorganisms like bacteria and fungi (Artin 2010).

6.4.1 Mechanism of Bioremediation

Bioremediation is the most economical way of heavy metal management. With the advent of technologies, it becomes possible to restore the heavy metal-polluted site by using different methodologies. These different techniques are employed based on various aspects such as aeration of area, characteristics of site, type of pollutant and its concentration, biosorption and bioavailability of pollutant, etc. (Smith et al. 2015; Azubuikwe et al. 2016; Ojuederie and Babalola 2017). However, no single technique can be helpful to achieve the complete restoration of heavy metal-contaminated environment. Therefore, different strategies are employed to clean up the environment (Verma and Jaiswal 2016). Broadly, bioremediation process can be carried out by two approaches as in situ and ex situ (Sharma 2012; Yuniati 2018).

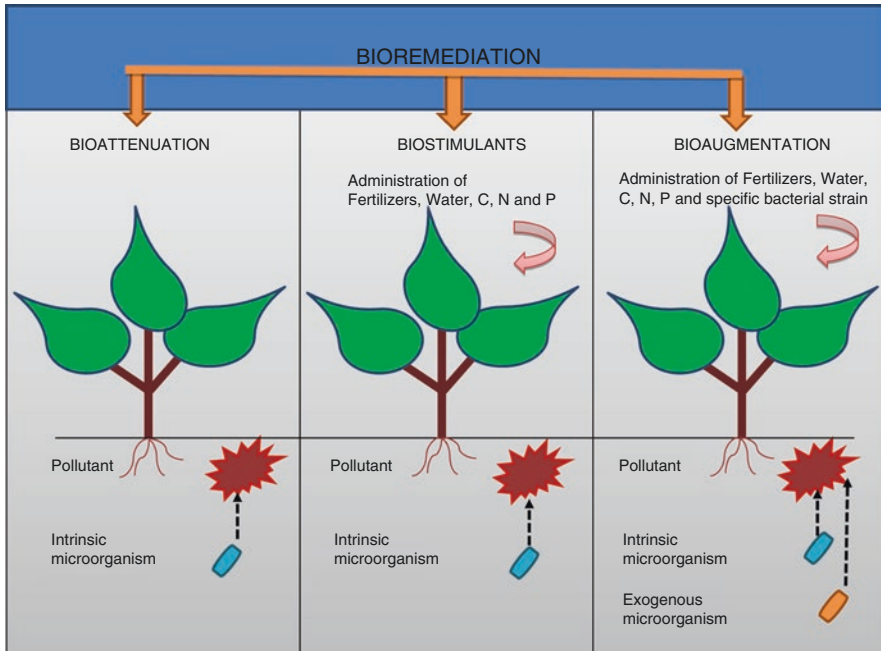


Fig. 6.2 Types of bioremediation

Bioremediation can be achieved through three different technological processes (Adams et al. 2015; Fig. 6.2) as follows:

6.4.1.1 Bioattenuation

Bioattenuation process is also known as natural attenuation as it relies on the natural way of heavy metal degradation which does not involve any intervention from humans (Mulligana and Yong 2004; Ying 2018). This process takes the advantage of metabolic diversity of intrinsic microorganisms present at the polluted site. Based on metabolic activities, these indigenous microbes can degrade, detoxify, neutralize, or transform the heavy metal (Abatenh et al. 2017). Bioattenuation comprises of various chemical, physical and biological processes in order to diminish the concentration and toxicity of recalcitrant. These processes encompass biodegradation by aerobic or anaerobic means, sorption, volatilization, stabilization, or transformation of pollutants (Mulligana and Yong 2004). The time required for natural attenuation of pollutants may vary from site to site depending upon the site conditions, type of contaminants, and degrading microbial flora of the site (Azubuiké et al. 2016). This process is applied to the site where concentration of pollutants is minimal and no other bioremedial technique can work. Bioattenuation process is applicable to control soil as well as water heavy metal pollution that mainly relies upon the appropriate degrading microorganisms (Yu et al. 2005).

6.4.1.2 Biostimulation

Biostimulation is the process that involves deliberate interventions of nutrients at a contaminated site to stimulate degradation of heavy metal contaminants by microorganisms. In other words, biodegradation process can be promoted by creating luxurious environment for degrading microorganisms present at the site (Kumar et al. 2011). Various physical and chemical properties of site affect the outcome of bioremediation process (Azubuiké et al. 2016; Abatenh et al. 2017). Generally, for biostimulation process, addition of macronutrients such as carbon, nitrogen, and phosphorus or micronutrients in proper ratio is needed to improve the degradation ability of indigenous or exogenous microorganisms (Wolicka et al. 2009; Ying 2018). These nutrients are otherwise available in low concentrations at the site, but nutrient addition can accelerate the process of bioremediation by increasing the population or activity of microorganisms naturally present at coordinated site (Perfumo et al. 2007). Biostimulation is a very promising technology of bioremediation as it uses the stable organic supplements which have high proportion of nutritious elements needed for growth promotion of varied microorganisms, thereby enhancing the biodegradation of pollutants in site (Tyagi et al. 2011).

6.4.1.3 Bioaugmentation

Bioaugmentation is one of the approaches of bioremediation that encompasses the induction of specific microbes with proficiency in degradation of heavy metal pollutants from contaminated site in order to improve the removal of contaminants through biodegradation (Goswami et al. 2018). Recently, bioaugmentation methods are gaining significant attention as a strategy of bioremediation. Basically, bioaugmentation is a one of the efficient ways of bioremediation where exogenous microorganisms with potential degradation ability are added to the site of contamination to speed up detoxification and decomposition of heavy metal pollutants. These altered microorganisms are either single strain of bacteria or consortia of microorganisms (Niu et al. 2009). These microorganisms can be isolated from natural environmental sources or genetically modified in the laboratory (Kulshreshtha 2013). The competency of bioaugmentation process relies upon many biotic and abiotic factors (Simon et al. 2004). The abiotic factors that affect bioaugmentation process include physiological and chemical properties of contaminated site, chemical structure, bioavailability of contaminants, and their concentration (Goswami et al. 2018). Biotic factors involve the selection of appropriate microorganisms that will have the ability to degrade heavy metal pollutants as well as to compete effectively with intrinsic microorganisms of the site (Abatenh et al. 2017).

Various approaches have been employed to make bioaugmentation as an efficacious remedial technique to recover contaminated site without destroying intrinsic microorganisms. Bioaugmentation process uses exogenous microorganisms which can be genetically modified in order to inherit desired catalytic capabilities among them. Therefore, genetically modified microorganisms exhibit enhanced decomposition ability covering numerous aromatic components (Abatenh et al. 2017). Moreover, bioaugmentation process can also be improvised by inoculating appropriate microorganism which is encapsulated using a variety of carriers like alginate (Mrozik and Piotrowska-Seget 2010). Certain newer approaches of bioaugmentation include gene augmentation technique where remediation gene is transferred to indigenous microorganisms. Rhizosphere bioaugmentation is another approach which includes introduction of microorganisms to the site along with plant to encourage microbial growth and degradation of pollutant (Kumar and Fulekar 2018). Phytoaugmentation approach does not involve the introduction of microbial inoculant; instead, it uses plants that are genetically engineered by transferring remediation genes (Gentry et al. 2004). It is believed that when bioattenuation and biostimulation process fail to work out, bioaugmentation technique should be employed (Mrozik and Piotrowska-Seget 2010).

Bioaugmentation approaches had been practiced to clean up undesirable compounds from a site that mainly include heavy metals. The selection of proper microorganism is very important for efficient bioaugmentation of the defected site. Several types of microbial species are used for bioaugmentation process. Many experiments exploit the efficiency of bacteria belonging to the genera *Pseudomonas*, *Bacillus*, *Sphingobium*, etc. The fungi belonging to the genera *Verticillium*, *Penicillium*, and *Aspergillus* had been experimented to remove undesirable heavy metals from polluted sites, especially to treat wastewater (Bahobil et al. 2017).

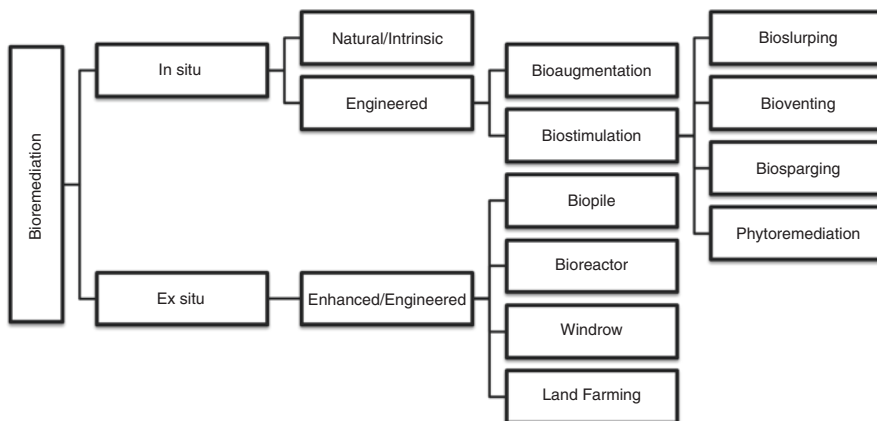


Fig. 6.3 Strategies of bioremediation process

Bioremediation process is used to eliminate various contaminants like pesticides, heavy metals, hydrocarbons and chlorinated compounds, dyes, plastic waste, greenhouse gases, sewage, oil spills, and nuclear waste (Azubuike et al. 2016). Implementation of in situ or ex situ bioremediation strategy is decided according to the site of application (Igiri et al. 2018). Several in situ (on site) and ex situ (off site) approaches (Tomei and Daugulis 2013; Fig. 6.3) are useful in controlling environmental pollution.

Bioremediation techniques have been demonstrated as proficient and sustainable approaches in restoring polluted site containing a wide variety of pollutants (Gao et al. 2018). Microorganisms are the key players in bioremediation process; hence, it is important to consider variety in microbial species, microbial population, and their complexity present at contaminated environments for their appropriate exploitation and to decide the outcome of any bioremediation strategy. Additionally, environmental aspects that can affect microbial activities have to be maintained at the optimal level. Advanced microbial molecular detection methods such as genomics, proteomics (Singh 2006), metabolomics and transcriptomics (Chauhan and Jain 2010) have revolutionized the understanding of identification of microorganisms, their functions and metabolic pathways (Azubuike et al. 2016), which is required for developing microbial culture and its application. Less population of desired microorganisms, limitation of nutrients, low degradation capabilities, and bioavailability of pollutants are major governing factors that may affect the process of bioremediation. Therefore, optimization of such important parameters may determine the success of any bioremediation technique.

In a nutshell, bioremediation technology has been proven as an efficient, economical, and eco-friendly or sustainable approach for the restoration of contaminated site including soil and groundwater. Moreover, much attention should be paid to research specifically focusing toward development of more effective treatment design and performance. In addition to it, research should be also directed to develop

newer strategies and approach for enhancement of bioavailability and mass transformation of contaminants, bioprocess optimization, and multidisciplinary integrative approach to reduce environmental pollution.

6.5 Phytoremediation

In the twenty-first century, the major challenge all over the world is rapid increase of industrialization and urbanization that has led to environmental pollution with several toxic and hazardous materials. Heavy metal contamination is the most distinguished concern since it directly affects the efficiency, growth rate, developmental stages, and productivity of plants (White et al. 2006). Several approaches (physiochemical and biological) have been used or established to restore heavy metal-polluted waters/soils including the landfill/dumping locations (Das 2016; Ayangbenro and Babalola 2017). Thus, remediation approach is very critical to eliminate heavy metals from the water. These remediation techniques comprise several treatment methods for pollutant degradation, removal/separation (through accumulation or dissipation), or immobilization (Malik et al. 2017; Padmavathiamma and Li 2007). Phytoremediation approach takes account of soil microorganisms symbiotically associated with green plants to eliminate harmful contaminants from polluted soil and waters/wastewaters through degradation and detoxification mechanisms (Ali et al. 2013; Bharagava et al. 2017; Saxena et al. 2019). It can be efficient for the eco-restoration of locations mostly polluted with heavy metals, radioactive compounds, and several organic contaminants (Ali et al. 2013; Mahar et al. 2016). It is an environmentally friendly, non-invasive, and aesthetically attractive remediation technology that eliminates heavy metal contaminants from the polluted locations (Saxena et al. 2019). It consists of diverse phytoremediation techniques for the deterioration of numerous contaminants using altered mechanisms contingent on their applications. Based on the toxic contaminant source, field environments, required level of environmental clean-up, and plant nature, there are different phytoremediation techniques that can be used. These techniques include phytoextraction/phytovolatilization, phytodegradation, phytostabilization/phytoimmobilization, rhizodegradation, and rhizofiltration (Thangavel and Subbhuraam 2004; Saxena et al. 2019). Phytoremediation techniques consist of diverse plant-based technologies. The definition, mechanism, application, benefits, and restricted access of common and long-established phytoremediation practices are shown in Table 6.1.

Selected commonly recycled phytoremediation methodologies are as follows (Parmar and Singh 2015):

- (1) Phytostabilization relies on either precipitation or immobilization of pollutants from groundwater and soil using plants, therefore reducing accessibility.
- (2) Phytofiltration process uses roots and parts of plants to absorb pollutants from the water bodies.

Table 6.1 Description of phytoremediation mechanisms and applications

Phytoremediation processes	Definition	Mechanism	Pollutants	Applicability
Phytoextraction or phytoaccumulation or phytosequestration or phytoabsorption	Plants remove metal pollutants from contaminated sites via plant's root absorption and sequester/concentrate in aboveground harvestable plant parts	Hyperaccumulation	Pb, Cd, Zn, Ni, Cu, Pb, radionuclides, pentachlorophenol, aliphatic compounds (short chained)	Contaminated soil/sites, water, wastewaters
Phytofiltration or rhizofiltration	Plants concentrate and precipitate metal pollutants in low concentration from the aquatic environment in their roots	Rhizosphere accumulation	Pb, Cd, Zn, Ni, Cu, radionuclides (Cs, Sr, U), hydrophobic organics	Contaminated water and wastewaters
Phytostabilization or phytoimmobilization or phytotransformation	Plants immobilize or inactivate metal pollutants at their place involving absorption by roots, adsorption onto roots, and precipitation, complexation, and metal valence reduction in rhizosphere, e.g., reduction of Cr^{6+} to Cr^{3+}	Precipitation, complexation, and metal valence reduction	Pb, Cd, Zn, As, Cu, Cr, Se, U, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs), dioxins, furans, pentachlorophenol, DDT, dieldrin	Contaminated soil/sediments and sludge
Phytovolatilization or phytoevaporation	Plants take up metal pollutants through roots in low concentration, modify/transform them into less toxic form, and subsequently transpire/volatilize into the atmosphere through stomata	Volatilization or evaporation by leaves	Chlorinated solvents like carbon tetrachloride, trichloroethylene, methylene chloride, tetrachloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, Hg (mercuric ion), Se	Contaminated wastewaters, soil, sediments, and sludges
Phytodegradation	Plants break down/convert highly toxic organic pollutants into less toxic forms through the action of enzymes secreted within plant tissues and released in the rhizosphere	Degradation in plant tissues	DDT, PAHs, bisphenol A, organophosphorus compounds	Contaminated soil, sediments, sludges, groundwater, surface water, and wastewaters
Rhizodegradation or rhizoremediation or phytostimulation	Plants break down/convert highly toxic organic pollutants into less toxic forms through enzymatic activity of rhizospheric microorganisms	Degradation in rhizosphere	Atrazine, ammunition wastes, petroleum hydrocarbon, PCBs, PAHs, TCE, diesel fuel	Contaminated soil, sediments, sludges, groundwater, and wastewaters

Adapted from Yadav (2010), Ali et al. (2013), Jaishankar et al. (2014), Dixit et al. (2015), Sarwar et al. (2017), Saxena et al. (2019)

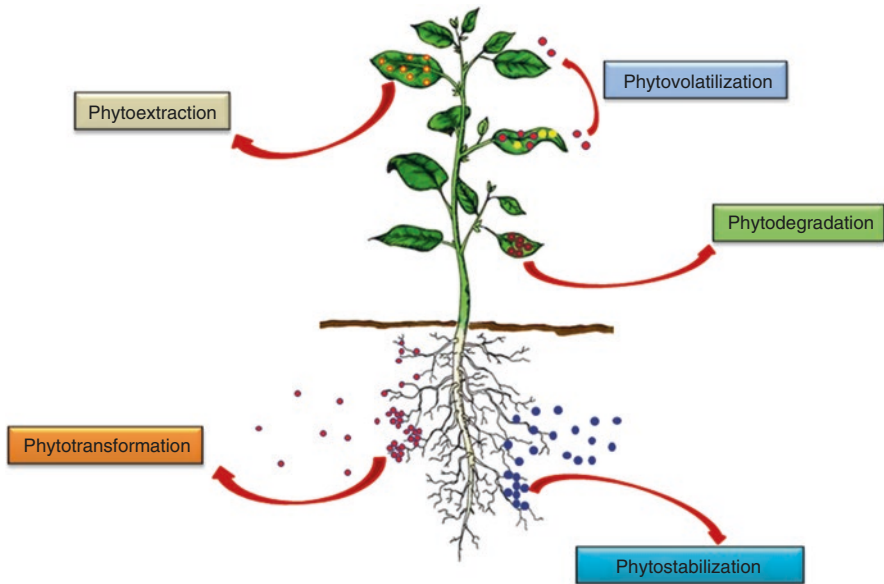


Fig. 6.4 Schematic representation of phytoremediation techniques

(3) Phytovolatilization can clean up groundwater and soil by means of plants that can evapotranspire pollutants like mercury (Hg), selenium (Se), and volatile hydrocarbons.

(4) Phytoextraction process involves uptake and absorption of metal pollutants from water and soil via plant tissues and consequent elimination of metal.

(5) Phytodegradation process utilizes plants or microbes for degradation of metals (or other contaminants) in groundwater as well as rhizospheric soil through metabolic activities.

(6) Phytotransformation implicates the uptake of organic pollutants from a polluted site and their transformation by plants into nontoxic or lesser toxic components.

(7) Evapotranspiration uses vegetative plants to prevent the leaching of pollutants.

Figure 6.4 shows the schematic presentation of the different phytoremediation approaches.

Uptake of metals is influenced by chemical speciation of metal and habitat characteristics of plants (aquatic, terrestrial, etc.). Thus, selection of plant is crucial for remediation of polluted sites. There are many plants exhibiting remediation characteristics that belong to diverse families, for example, Brassicaceae, Cyperaceae, Fabaceae, Poaceae, Lamiaceae, Caryophyllaceae, Euphorbiaceae, etc. (Sarma 2011). There are several plant species which are described as hyperaccumulators on the basis of their capability to sustain concentrations of toxic metal pollutants, as outlined in Table 6.2.

Of all the phytoremediation mechanisms, phytoextraction is the prominent technique to eliminate heavy metals from polluted locations. In phytoextraction

Table 6.2 Some metal hyperaccumulator plants (with metal accumulation ability) used for phytoremediation

Plant species	Family	Polluted medium	Metal	Metal accumulation capacity (mg kg ⁻¹ DW)	Phytoremediation mechanism and metal accumulation compartment	Reference
<i>Tagetes minuta</i>	Asteraceae	Water	As	380.5	Phytoextraction (shoots)	Salazar and Pignata (2014)
<i>Nocca caerulea</i>	Brassicaceae	Water	Pb	1700–2300	Rhizofiltration (aerial parts/root)	Dinh et al. (2018)
<i>Pteris vittata</i>	Pteridaceae	Water	As	20,707	Phytoextraction (shoots)	Kalve et al. (2011) and Oliveira et al. (2014)
			Cr	35,303	Phytoextraction (shoots)	Kalve et al. (2011)
			As	20,707	Phytoextraction (shoots)	Sakakibara et al. (2011)
<i>Eleocharis acicularis</i>	Cyperaceae	Water	Zn	11,200	Phytoextraction (shoots)	Sakakibara et al. (2011)
			Cu	20,200	Phytoextraction (shoots)	Sakakibara et al. (2011)
			Cd	239	Phytoextraction (shoots)	Sakakibara et al. (2011)
			As	1470	Phytoextraction (shoots)	Sakakibara et al. (2011)
<i>Azolla pinnata</i>	Salviniaceae	Water	Cd	740	Rhizofiltration (bioaccumulation)	Rai (2008)
<i>Lonicera japonica</i>	Caprifoliaceae	Water	Cd	–	Phytoextraction (shoots)	Liu et al. (2011a, b)

technique, green plants are used to eliminate contaminants from polluted locations through root absorption and their sequestration (Saxena et al. 2019). This technique can have commercial applications since it is economically feasible to remove metals from polluted locations using plant biomass extracted and employed as “bio-metal” to get valuable, useful, and efficient metals; the procedure can be referred to as phytomining (Ali and Singh 2018). Therefore, it can create income and offer further employment opportunity for the people. Phytoextraction proficiency of green plants mainly depends on two factors, viz., bioconcentration factor (BCF) and translocation factor (TF). BCF characterizes concentration of metals in root/soil, representing metal accumulation, while TF describes concentration of metals in shoot/root that signifies metal translocation (Ali et al. 2013; Antoniadis et al. 2017).

Currently, there is an increasing curiosity for the exploitation of metal-accumulating roots and rhizomes of aquatic and semi-aquatic vascular plants for the elimination of heavy metals from polluted water bodies (Mémon et al. 2001). For instance, *Hydrocotyle umbellata*, *Eichhornia crassipes*, *Tagetes minuta*, *Lemna minor*, *Pteris vittata*, *Lonicera japonica*, *Eleocharis acicularis*, *Noccea caerulescens*, and *Azolla pinnata* absorbed Cu, Cr, Hg, Pb, As, Cd, Fe, and Zn from polluted water bodies (Gallardo et al. 1999; Gustin et al. 2009; Oliveira et al. 2014). Furthermore, elimination of a widespread series of metal ions present in contaminated solutions using cell suspension cultures of *Datura innoxia* has been demonstrated (Wao et al. 2017). Maximum eliminated metals were strongly chelated by unrevealed constituents of cell walls in a manner which did not involve metabolic activity. The hyperaccumulation of metals in several plant species has been broadly studied, and till date, extensive improvement has been accomplished. It has been clearly investigated that diverse mechanisms of metal accumulation, elimination, and compartmentation occur in many plant varieties. Now, the ever-increasing knowledge of biochemical pathways and metabolic processes of plants in relation to uptake/absorption of heavy metal, its accumulation, transport, and resistance will persuade enhancements of phytoremediation via recent genetic techniques. Therefore, phytoremediation proficiency of plants can be significantly enhanced by using these genetic engineering techniques.

6.6 Role of Advanced Biotechnological Techniques and Genetic Engineering in Bioremediation Process

Microbes are used in bioremediation in light of their capacity to deteriorate ecological pollutants because of their metabolism by means of biochemical pathways identified with the life form movement and development. Microbes have the capacity to degrade the harmful substances of polluted environment into harmless end products by the process of co-metabolism (Ojuederie and Babalola 2017). Degradation of hazardous products into harmless products utilizing inbred microbes did not yield

much positive outcomes. Bioremediation of Hg from the polluted environment by indigenous bacteria has not been reported.

Nonetheless, recombinant DNA technology (RDT) has a noteworthy task to carry out bioremediation of heavy metals since it upgrades the remedial procedure (Kang et al. 2016). Introduction of genetic engineering techniques in bioremediation is intended to alter genotype of plants and microorganisms, thereby changing their functional proteins like enzymes, in order to use them as potential agents for deterioration of hazardous compounds (Wolejko et al. 2016). Azad et al. (2014) reported the use of genetically engineered bacteria for degradation of different heavy metals. Genetically engineered microorganisms (GEM) have been utilized to acquire skillful strains for bioremediation of polluted environment by having improved capacity to degrade an assortment of pollutants. Several studies have demonstrated the removal of Hg from polluted environments by using genetically modified *Escherichia coli* strain M109 and *Pseudomonas putida* with *merA* gene (Ojuederie and Babalola 2017). Different genes such as *merA* gene, *pheA*, *pheB*, *pheC*, *pheD*, and *pheR* genes (phenol catabolic genes), and *ArsM* gene have been extensively used for the removal of Hg, phenol, and As, respectively, with the help of genetic engineering (Liu et al. 2011a, b). Addition of *mer* genes into *Deinococcus geothermalis* bacterium from *Escherichia coli*, which are responsible for the degradation of Hg, enabled the bacterium for removal of Hg from polluted environments (Dixit et al. 2015). Sone et al. (2013) reported the addition of novel genes utilizing pMR68 plasmid for the synthesis of Hg-resistant strains of *Pseudomonas*. Thus, GEM can be used to assist remediation process to defeat the pollutants from the environment. It is also important to maintain the stability of these genetically modified microorganisms before applying them to the field, since the catabolic action of GEM is mainly related to the presence of stable recombinant plasmid in them (Ghosal et al. 2016).

Some modern techniques such as site-directed mutagenesis and rational designing have been used to engineer the microbes for the degradation of heavy metal contaminants (Kumar et al. 2013). Microbial biosensors are presently being utilized to set up the measure of heavy metal contaminants rapidly and accurately and are created utilizing genetic engineering. Dixit et al. (2015) detailed the utilization of biosensors to assess the degrees of different heavy metals in polluted environments. Usage of genetic engineering guarantees more prominent open doors for acquiring powerful pollutant-degrading microbes as they could have higher capability of ecological cleanup than the inbred microorganisms.

Transgenic plants can be obtained with insertion of specific genes in the genome of plants with enhanced phytoremediation capability using genetic engineering. Genetically engineered endophytes and plant growth-promoting microbes (PGPM) can adequately degrade the heavy metals in contaminated environment (Dixit et al. 2015; Ojuederie and Babalola 2017). Mani and Kumar (2014) reported that the expression of *merA* genes in transgenic rice and tobacco makes them ten times more resistant to Hg than those that do not express *merA* genes. Chen and Wilson (1997) observed that the different transgenic plants, such as *Arabidopsis thaliana*, *Nicotiana*

tabacum, *Brassica juncea*, *Brassica oleracea* var. *botrytis*, and *Lycopersicon esculentum*, have been used for the degradation of heavy metals. Innovative research on the rapidly developing plants having capabilities of metal aggregation should be advanced. Additionally, microorganisms from different genera have to be explored for improving plants and rhizospheric microorganisms at genetic level, which can be eventually used for phytoremediation. Besides the *merA* genes, various genes ought to be investigated for their conceivable use in defeating a wide variety of heavy metals. Recombinant DNA technology is fundamental for the bioremediation procedure as it empowers analysts to examine, screen, and evaluate the execution of the procedure (Ojuederie and Babalola 2017). It ought to be utilized with alert and as per biosafety guidelines.

6.7 Conclusions

This chapter featured the heavy metal contamination sources and different mechanisms utilized by plants and microorganisms including their enzymes for the effective remediation of polluted environment, especially aquatic system. It uncovered the advantages of bioremediation as a superior alternate as well as sustainable approach in the expulsion of pollutants like heavy metals from the environment as compared to other existing physical and chemical strategies that are less effective and costly because of the measure of energy consumed. Microbes and plants have natural biological systems that empower them to make do under heavy metal pressure and expel the metals from the environment. Different processes such as precipitation, biosorption, enzymatic transformation of metals, and complexation are used during the bioremediation of heavy metals by the microbes for the removal of heavy metals from the polluted environments. Plants use phytoremediation techniques of which phytoextraction and phytostabilization have been very efficient. Environmental variables assume a noteworthy job in the achievement of bioremediation as the microorganisms utilized will be hampered if suitable ecological conditions are not accessible. Transgenic microorganisms and plants could successfully remediate polluted destinations of heavy metal and organic contaminations; however, its utilization ought to be liable to stringent biosafety techniques to guarantee that there is no well-being or ecological dangers.

Application of metagenomic approaches must be taken into consideration to understand the community structure of microorganisms present at the treatment site to explore metal-resistant genes for cleaning up various heavy metals by improving the degrading microbial strains.

Public impression of the utilization of different modern sustainable technologies for bioremediation will likewise have to change for its compelling usage; this determines necessity of collaboration among scientists and environmentalists.

6.8 Future Prospects

Rapid industrial development and innovation advancement pose negative effects on environment like water pollution where quality of water gets deteriorated. Because of the multifaceted nature engaged with the traditional approaches for biological treatment of water especially contaminated with heavy metals as a pollutant, the utilization of microorganisms has emerged as a help for bioremediation. Be that as it may, bioremediation innovation has impediments; few microorganisms cannot break down lethal metals into innocuous metabolites, and these affect microbial movement. Changes in the external layer proteins of microorganisms with potential bioremediation properties for improving metal restricting capacities are the possible method to upgrade their biotransformation ability of dangerous metals. Further investigations should concentrate on the variables engaged with improving in situ bioremediation methodologies utilizing GEMs and furthermore the applicability and flexibility of these GEMs in all the conceivable unfavorable/stressed conditions and environments polluted with different heavy metals. The hesitance among people in general to acknowledge GEM for bioremediation likewise needs to be taken into consideration as future investigations, and their non-harmfulness to the environment should be demonstrated.

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