Recent Trends in Bioremediation of Heavy Metals: Challenges and Perspectives

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Abstract

Heavy metal pollution is a matter of serious concern worldwide. Movement of heavy metals starting from the extraction processes to their applications in a variety of industrial activities, results in their indiscriminate release in the environment. Prolonged exposure to these heavy metals can cause detrimental health effects in human as well as other living organisms. Heavy metals include a class of some highly toxic metals such as, Hg, Cd, Cr, Pb, Ni, Cu, and Zn. that are reported to have cytotoxic, carcinogenic, teratogenic, and mutagenic effects. Since, these heavy metals are nondegradable and have a tendency to accumulate in environment, their removal from aquatic and terrestrial system is required. Bioremediation is one of the promising techniques which can be used to remove these contaminants from water and soil using biological agents, including microorganisms (bacteria, fungi, and microalgae) and plants (phytoremediation). Microorganisms and plants are capable of taking up heavy metals from nature and use these toxic contaminants in their metabolic activities, or convert them to less/nontoxic forms. Thus, the microorganism- and plant-mediated treatment processes are widely accepted since these methods are based on natural mechanisms and also reduce the chances of generation of secondary pollutants as in the case of various conventional processes. This chapter thus studies the various bioremediation techniques for the removal of heavy metal from nature and will discuss the mechanisms of different biological agents used for the transformation of toxic heavy metals. Different methods for the assessment of heavy metals have been

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discussed for the effective monitoring of contaminants in nature. The review also presents the recent advances in the field of bioremediation in terms of use of plants and their metabolites, plant growth–promoting rhizobacteria and nanoparticles for efficient removal of heavy metals from contaminated sites.

Keywords

Heavy metals · Contamination · Toxicity · Bioremediation · Phytoremediation

5.1 Introduction

Rapidity of industrialization, rising demand of energy, mining, and careless exploitation of natural resources in the past many years are the key reasons for rise in environmental pollution which causes serious threats to biodiversity and ecosystem processes. Large amount of various toxic organic and inorganic pollutants causes soil and water pollution. Of these, one of the most toxic pollutants that have an adverse impact on environment is heavy metals (Gautam et al. 2016).

Heavy metals are naturally occurring elements with an atomic number greater than 20 and atomic density above 5 g cm^{-3} and exhibit the properties of metal such as luster, ductility, malleability, and high electric and thermal conductivity. These are one of the most challenging pollutants due to their highly toxic and nonbiodegradable nature. Also, heavy metals have high efficiency to get rapidly bioaccumulated in ecological systems since plants and animals absorb these from the contaminated environment in which they are residing.

Heavy metals can also biomagnify inside the human body due to consumption of bioaccumulated plants, animals, and contaminated water. They pose severe hazardous impacts even at very low concentrations. The natural sources of heavy metal discharge into the environment include geological weathering of bedrocks and volcanic eruptions. The anthropogenic sources include industries (dyeing, tannery, mining, electroplating, paints and pigments, fertilizer, etc.), sewage sludge, waste treatment plants and runoff from agricultural fields. The other sources include electronic waste, personal care products, cosmetics, and medicines. In addition to this, plant and animal waste matter decomposition, plant exudates, forest fire, wind erosion, oceanic spray, and airborne particles from volcanic activity also lead to the addition of heavy metals in environmental components. Emission of heavy metals into the environment also occurs in many other ways such as in air, at the time of combustion, due to extraction and processing of metal-containing ores, to surface waters by means of runoff and also due to releases from storage and transport. Roadways and vehicular emissions are also one of the major contributors of heavy metals in the environment (Jobby and Desai 2017). After their release from the source, these heavy metals tend to remain in the environment for longer time periods attributed to their nondegradable nature. They can also impose toxic and irreversible effects on the associated microorganisms, plants, animals, and human beings (Rahman and Singh 2019).

Currently the term "heavy metal" has been used to explain metallic chemical elements and metalloids which are harmful to the environment and human beings. Of the total 90 naturally occurring elements, 53 are considered as heavy metals. Some heavy metals more common in our everyday life are chromium (Cr), cadmium (Cd), copper (Cu), arsenic (As), lead (Pb), mercury (Hg), nickel (Ni), zinc (Zn), manganese (Mn), iron (Fe), cobalt (Co), silver (Ag), gold (Au), platinum (pt), molybdenum (Mo), tin (Sn), vanadium (V), and titanium (Ti) (Briffa et al. 2020). These metals and metalloids in less concentration play a vital role for tissues and cells of all living organisms because they serve as cofactors for proteins and enzymes. They are also responsible for maintaining the osmotic potential. On the other hand, these metals show highly hazardous effects in high concentration (Tahir et al. 2019). Among all heavy metals, chromium, lead, mercury, cadmium, and arsenic are widely present in the environment. These heavy metals cause various health impacts such as chromium (VI) and arsenic are carcinogenic in nature. High doses of cadmium can cause a degenerative bone disease. Lead and mercury in high concentration cause damages to the central nervous system in the human body (Fatima and Ahmed 2018).

5.2 Heavy Metal Pollution

Heavy metals are characterized into three forms which are of great concern, comprising (a) toxic metals (Cr, Hg, Zn, Pb, Ni, Cu, As, Cd, Sn, Co, etc.), (b) precious metals (Pd, Pt, Ru, Au, Ag, etc.), and (c) radionuclides (Th, U, Am, Ra, etc.). In addition of these, some metals are considered to be essential elements (Cu, Ni, Co, Zn, etc.), while others are considered as nonessential metals (Cd, Pb, As, Ag, Au, etc.). Soil contamination with heavy metals is of great concern because it causes adverse effects to humans and the ecosystem directly. Heavy metals from contaminated soil can easily enter the food chain through direct ingestion. Living organisms may also intake heavy metals by means of drinking contaminated groundwater. Metal toxicity in plants results in reduction in food quality and less use of land for agricultural production, ultimately leading to food insecurity and land tenure problems (Jobby and Desai 2017).

Heavy metals enter into the environment through various sources such as industrial wastewater and sewage discharge which are the significant sources of metal pollution in water life. Soils also get contaminated due to the accumulation of heavy metals and metalloids that are emitted from industrial areas, dumping wastes, from leaded gasoline and paints, mine tailings, agricultural activities such as use of fertilizers and pesticides, sewage sludge, irrigation with wastewater, residues of coal combustion, runoff from terrestrial systems, effluents from industrial and domestic sources, accidental leakages spillage, and atmospheric deposition (Table 5.1).

Various adverse impacts of heavy metals are well known. They are very lethal to living organisms even at very low concentrations (Table 5.2). They can cause potential health impacts that can be cytotoxic, carcinogenic, teratogenic, and mutagenic in nature (Fig. 5.1).

Table 5.1 Sources of some toxic heavy metals in the environment

Metal	Sources
Chromium (Cr)	Mining, road runoff, coolants from industries, leather tanning
Lead (Pb)	Lead acid batteries, mining, smelting, paints, e-waste, ceramics
Mercury (Hg)	Thermal power plants, fluorescents, dental amalgams, hospital wastes
Arsenic (As)	Smelting operations, thermal power plants, fuel burning, pesticides
Copper (Cu)	Mining, electroplating, smelting operations, road runoff
Nickel (Ni)	Combustion of fossil fuels, electroplating, battery industry, road runoff, thermal power plants
Cadmium (Cd)	Ni or Cd batteries, sludge from paint industry, fuel combustion, e-waste
Zinc (Zn)	Road runoff, electroplating, smelting

Table 5.2 Major heavy metal contaminants, prescribed standards (in drinking water) and their human health effects

Heavy metal	Maximum concentration levels (mcl) (mg/l) USEPA[a]	Tolerance limits (mg/l) IS:10500, 1992[b]	Potential health effects
Arsenic (As)	0.05	0.05	Skin lesions and carcinogenic effects
Cadmium (Cd)	0.005	0.01	Kidney problems
Chromium (Cr)	0.1	0.05	Allergic dermatitis
Copper (Cu)	1.3	0.05	Gastrointestinal distress (acute exposure) Liver and kidney damage (chronic exposure)
Mercury (Hg)	0.002	0.001	Kidney and spinal cord ailment
Lead (Pb)	0.015	0.05	Late physical and mental growth, central nervous system (CNS) complications, kidney and high blood pressure issues
Nickel (Ni)	0.1	0.05	Allergic skin diseases, carcinogenic effects, immunotoxic, neurotoxic, genotoxic

[a] United States Environmental Protection Agency Prescribed
[b] Indian Standard: 10500, 1992

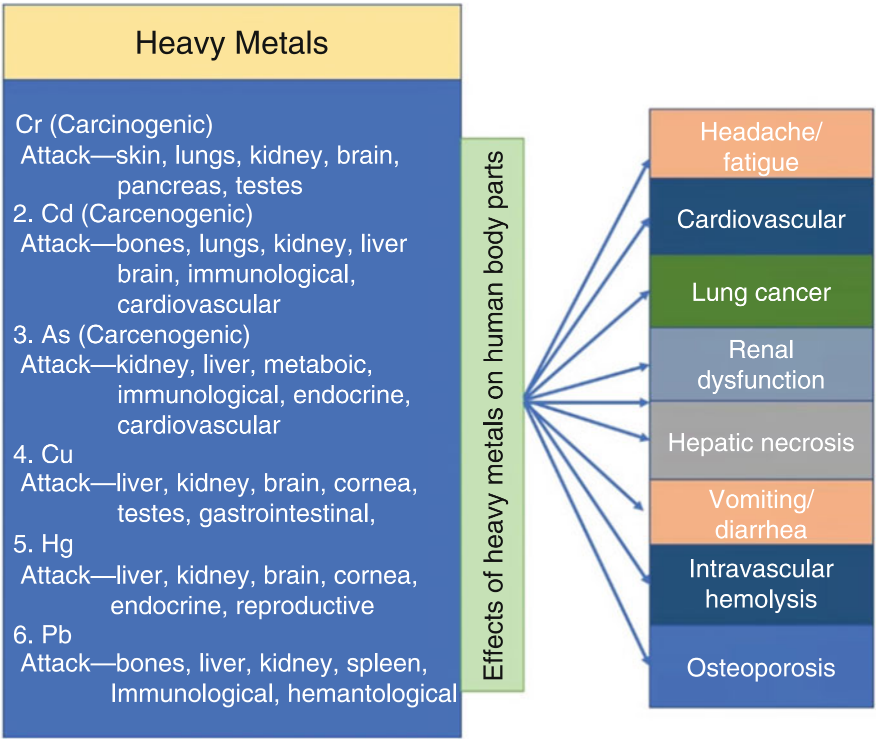


Fig. 5.1 Toxicity to humans due to heavy metals

5.3 Bioremediation of Heavy Metals: Principles, Mechanisms and Factors

Presently a number of methods such as ion exchange, adsorption, chemical precipitation, floatation, electro dialysis, solvent extraction, electrochemical deposition, and reverse osmosis are available in order to eliminate or remove these toxic heavy metals from the environmental components. But these techniques have many limitations such as high cost and very low efficiency. Further, these techniques have the potential to cause deleterious effects on soil and thereby changing its original composition. In order to overcome these drawbacks new eco-friendly methods are being invented and developed which have no such adverse effects. These methods use microorganisms such as bacteria, fungi, or plants to remove heavy metals either by absorbing or by changing the valency of metal element and make them less toxic (Pratush et al. 2018). These methods are collectively recognized as Bioremediation techniques. Eco-friendly and cost effectiveness are more advantageous features of bioremediation as compared to other chemical and physical methods of remediation (Azubuike et al. 2016).

Bioremediation is an ecologically sound and up-to-the-minute technique which by means of natural biological processes completely removes toxic contaminants from the environment. It can be any process which with the help of microorganisms (bacteria, fungi), green plants, or their enzymes brings the modified and contaminated environment into its natural original condition. The time period between the late 1980s and the early 1990s was the golden period for bioremediation (Mani and Kumar 2014).

5.3.1 Principles of Bioremediation

Principle of bioremediation includes the use of microorganisms to destroy the harmful contaminations or convert them into less toxic form. Three essential components of bioremediation are (a) microorganisms, (b) food, and (c) nutrients, which are together known to be the bioremediation triangle. The effective role is of microorganisms which metabolizes the chemical compounds to produce water, carbon dioxide (in aerobic conditions) or methane (in anaerobic conditions), microbial biomass and metabolites (Paul et al. 2021).

In bioremediation, the native microflora predominates in the contaminated site and suitable conditions such as more food for suitable growth is provided to them to make them grow to their full potential. This helps in the production of more enzymes as secondary metabolites which have potential to break down the complex contaminants into simpler constituents more efficiently. The process of bioremediation takes place through the breakage of chemical bonds and release of energy. This release energy is again used by the microorganisms for their metabolism and growth. The microbial species used for heavy metal transformation can either be isolated from aerobic or anaerobic or both of the environments. However, the microorganisms isolated form aerobic environments are mostly exploited for the process of bioremediation as compared to the ones isolated from anaerobic environment (Pratush et al. 2018). Complete breakdown of pollutant needs the action of several microbes. The process of biodegradation depends on suitable environmental conditions, type of the pollutant and its solubility, and the bioavailability of the pollutant to the microbial population in order to achieve fast and effective biodegradation. Environmental conditions are also manually controlled or manipulated to facilitate sufficient microbial growth (Tyagi and Kumar 2021).

5.3.2 Mechanisms of Bioremediation

Microorganisms are widespread and easily convert heavy metals from toxic form to nontoxic simpler forms. In the process of bioremediation, the organic pollutants or contaminants are converted into carbon dioxide (CO_2) and water (H_2O), and/or to other metabolic intermediates by microbial activity and the converted materials are utilized as primary substrates required for cell growth. Microbial communities are capable of two-way protection. Firstly, they produce degradative enzymes for the

Table 5.3 Microorganisms used for heavy metal removal from contaminated sites

Microorganisms	Heavy metals	Reference
Bacillus polymyxa *Pseudomonas aeruginosa*	Cu, Zn	Philip et al. (2000), Gunasekaran et al. (2003)
Saccharomyces cerevisiae	Pb, Hg, Ni	Chen and Wang (2007), Talos et al. (2009), Infante et al. (2014)
Pseudomonas aeruginosa, *Aeromonas* sp.	U, Cu, Ni, Cr	Sinha et al. (2011)
Geobacter spp.	Fe, U	Mirlahiji and Eisazadeh (2014)
Bacillus safensis (JX126862) strain (PB-5 and RSA-4)	Cd	Priyalaxmi et al. (2014)
Aerococcus sp., *Rhodopseudomonas palustris*	Pb, Cr, Cd	Sinha and Paul (2014), Sinha and Biswas (2014)
Pseudomonas fluorescens, *Pseudomonas aeruginosa*	Fe, Zn, Pb, Mn and Cu	Paranthaman and Karthikeyan (2015)
Lysinibacillus sphaericus CBAM5	Co, Cu, Cr, Pb	Peña-Montenegro et al. (2015)
Microbacterium profundi strain Shh49T	Fe	Wu et al. (2015)
Aspergillus versicolor, *A. fumigatus*, *Paecilomyces* sp., *Trichoderma* sp., *Microsporum* sp., *Cladosporium* sp.	Cd	Soleimani et al. (2015)

degradation of target pollutants and secondly, they become resistant to relevant heavy metals. Diverse types of mechanisms including bioaccumulation, biosorption, biomineralization, biotransformation, metal–microbe interactions, and bioleaching are utilized for bioremediation. Microorganisms use chemicals for their growth and development in order to remove the heavy metals from the contaminated site. The metals get dissolved, reduced, or oxidized through microbial activity. Microorganisms restore the contaminated environment into its original form by oxidation, immobilization, volatilization, transformation, and binding of heavy metals.

The success of bioremediation in a particular location can be ensured by thorough understanding of the operating mechanism, controlling activity, and growth of microorganisms, enhancing their response through metabolic capabilities to environmental changes. Organic contaminants tend to disrupt cell membranes. However, microbial cells have the potential to develop defense mechanisms which comprise the formation of outer cell-membrane-protective material, often hydrophobic or solvent efflux pumps. The prevalent example includes the formation of plasmid-encoded and energy-dependent metal efflux systems. These systems include ATPases and chemiosmotic ion/proton pumps and are reported for resistance against Cd, Cr, and As in many bacteria (Dixit et al. 2015). The selection or choice of microorganism, however, depends on the nature of contaminant or pollutant material to be degraded and environmental conditions. Certain microorganisms which have been implied for heavy metal removal are given in Table 5.3.

5.3.3 Factors Affecting Bioremediation

The efficiency and potential of bioremediation techniques depends on physicochemical characteristics of the environment, concentration and chemical nature of the pollutants, and their bioavailability to existing microorganisms (El Fantroussi and Agathos 2005). Important factors which are important for the growth of microorganisms in order to remove the heavy metals from the contaminated sites include the following.

1. *Nutrients*: Availability of nutrients are less at the contaminated sites because of organic pollutants which are more at contaminated site and get depleted or degraded during microbial metabolism. So an additional supply of nutrients like nitrogen (N), phosphate (P), and potassium (K) is given from outside to the affected or contaminated site to stimulate the growth and cellular metabolic activities of microorganisms and thereby increasing the rate of bioremediation process. The higher efficiency of bioremediation can be achieved by improving the bacterial C:N:P ratio (Abatenh et al. 2017).
2. *Nature of Pollutants:* Bioremediation depends upon the type or state of pollutants such as solid, semisolid, liquid, and volatile in nature.
3. *Soil Structure*: Soil structure comprises different textures depending upon variable contents of silt, sand, and clay. Powdery, well-structured, or well-maintained soil helps in the effective supply of nutrients, water, and air to microbial consortia for in situ bioremediation.
4. *pH*: Optimum range of pH for the microbial growth and degradation of the contaminants is 5.5–8.0. Higher or lower pH values may slow down the removal process due to high susceptibility and sensitivity of metabolic processes to even minor changes in pH levels (Wang et al. 2011).
5. *Moisture content:* Water acts as a primary and important factor to determine the dielectric constant of soil and other such mediums. The moisture content of soil for efficient bioremediation should be in the range of 25–28%.
6. *Microbial Diversity*: The presence of various types of microorganisms at contaminated site such as *Flavobacteria, Pseudomonas, Chlorobacteria, Aeromonas, Corynebacteria, Mycobacteria, Streptomyces, Acinetobacter, Arthrobacter, Aeromonas, Bacilli,* and *Cyanobacteria* favors the remediation process.
7. *Macrobenthos Diversity*: A consortium of aquatic plants *E. crassipes*, *S. molesta*, *C. demersum* with aquatic animals *A. woodiana* and *L. hoffmeisteri* is very effective to degrade organic and metal load in domestic wastewater.
8. *Temperature:* The optimum temperature ranges from 15 to 45 °C for efficient bioremediation. Temperature influences the physiology of microorganisms resulting in speeding up or slowing down of the bioremediation process. The rate of microbial activities firstly increases with increase in temperature and achieves its maximum level at an optimum temperature. After then, the rate declines suddenly with any further increase in temperature and ultimately stops after specific temperature (Abatenh et al. 2017).

9. *Oxygen*: Oxygen is utilized for the initial stages of breakdown of the hydrocarbons present at the contaminated sites. The presence of oxygen determines whether the process of bioremediation will occur under aerobic or anaerobic conditions.

5.4 Techniques for Detection and Assessment of Heavy Metals in the Environment

Heavy metals are the contaminants of greatest concern due to their adverse effects on living organisms. Human and other living organisms can get exposed to the elevated concentration of heavy metals from contaminated soil, water, groundwater, and plants. Therefore, appropriate methods are essentially important for accurate detection and assessment of heavy metals in various environmental samples. Highly sensitive instrumental techniques such as flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorption spectroscopy (GFAAS), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma atomic emission spectrometry (ICP/AES) are widely used to assess the qualitative and quantitative analysis of heavy metals in various samples from the environment. However, rapid detection techniques, for example, X-ray fluorescence spectrometer (XRF), for assessing the heavy metals in variety of environmental samples are most preferred nowadays. Other techniques such as X-ray absorption spectroscopy (XAS) and X-ray diffraction (XRD) have been used to study the heavy metals and their interaction within biosystems such as soil and plants.

X-ray Fluorescence (XRF): It is a nondestructive method of analysis which involves the emission of X-ray photon followed by atom ionization through a primary X-ray beam. The primary X-ray beam upon hitting the sample interacts with the electron and removes this it from its inner shell forming. This process creates voids in the inner shell that exhibit an unstable state of the atom. The voids get rapidly filled by electron of the outer shell accompanied by emission of X-rays with a specific wavelength. This X-ray of specific wavelength is the measure of elemental composition of a sample (Meirer et al. 2010).

X-ray Absorption Spectroscopy (XAS): X-ray absorption spectroscopy (XAS) gives a researcher a detailed information on the environmental coordination of metals absorbed by plants. XAS is used to investigate the atomic geometry of heavy metals and their interactions within biosystems, that is, soil and plants (Gardea-Torresdey et al. 2005).

X-ray Diffraction (XRD): X-ray diffraction (XRD) is a fast technique used for the phase identification in crystal material in order to analyze the structure of the material (minerals, inorganic compounds, etc.). In the recent years, XRD is being widely used to heavy metal remediation studies. Shao et al. (2020) used XRD to confirmed the formation of a stable mineral pyromorphite ($Pb_5(PO_4)_3Cl$) in the soil containing Pb metal after treating with low-cost phosphorous-containing amendments.

5.5 Techniques of Bioremediation

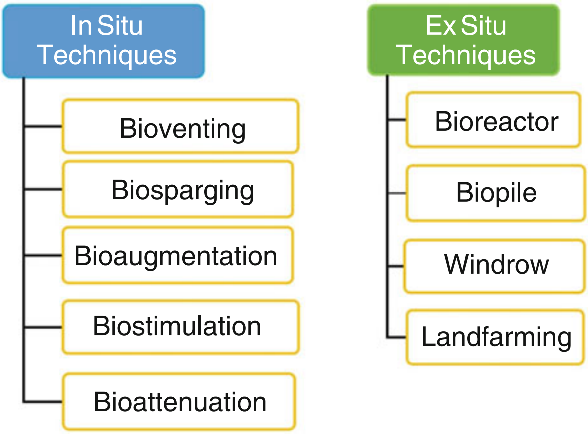
The process of bioremediation can be used for soil and water contaminated site through in situ and ex situ techniques (Kapahi and Sachdeva 2019). Both "in situ" and "ex situ" bioremediation approaches involve microbial metabolism. In situ methods are to restore the soil and water contaminated without excavating the sample from contaminated sites while ex situ methods are to degrade the chemical pollutants of excavated samples (Fig. 5.2). Ex situ techniques are more expensive as compared to in situ techniques.

5.5.1 In Situ Techniques

In situ bioremediation techniques involve biological degradation of contaminants at the site in natural conditions. In addition to the removal of heavy metals, "in situ" bioremediation is also used for the treatment of chlorinated solvents, dyes, and hydrocarbon polluted sites. To make "in situ" bioremediation more successful, several factors such as oxygen supply, moisture content, pH, temperature, and nutrient supply are needed to be made suitable for potential microbial growth. Of these factors, the availability of molecular oxygen is the one of the major problems which needs to be tackled. Various in situ techniques are discussed below.

1. *Bioventing*: It is a technology to stimulate on site natural degradation of organic compounds which gets adsorbed on soil particles in the unsaturated zone. The process is basically accomplished by inducing air or oxygen to existing and introduced microorganisms into the unsaturated zone of soil to favor their growth.
2. *Biostimulation*: In biostimulation, the indigenous microorganisms are provided with rate limiting nutrients such as nitrogen, phosphorus, oxygen, electron

Fig. 5.2 Types of bioremediation techniques for various contaminants



acceptors, and adequate amounts of water to accelerate their growth and bioremediation potential.

3. *Bioaugmentation:* Bioaugmentation is the introduction of specific indigenous or nonindigenous microorganisms that may be autochthonous, allochthonous wild type or genetically modified to the contaminated sited to remove the target compounds. This technique is aimed at increasing the gene pool and genetic diversity at the site to accelerate the rate of degradation of hazardous substances.
4. *Bioattenuation*: This technique involves the removal of heavy metals without human interference in passive mode suitable for both biodegradable and intractable contaminant. It includes aerobic and anaerobic types of degradation comprising physicochemical methods, namely dispersion, dilution, and ion exchange. The process involves the removal of chemicals by means of tiny bugs or microbes which eat and then digest the contaminants to convert them to water or less toxic forms.
5. *Biosparging*: In this technique, injection of air is given into saturated zone or subsurface soil in order to improve the rate of biodegradation of contaminants by naturally occurring bacteria. Nutrients may also be added to enhance the microbial growth. Pollutant biodegradability and soil permeability plays important role in the effectiveness of biosparging. In stressed conditions, metal-adsorbing materials are produced by bacteria. These materials chemically interact with contaminants and pollutants and cause their precipitation. During biosparging, the oxygen supply produces aerobic condition which are quite appropriate for the degradative action of native microbes.

5.5.2 Ex Situ Techniques

Ex situ techniques involve the removal or transportation of contaminated environmental component or site to another site for remediation. The location and environmental conditions of the contaminated site, cost, type of the pollutant, and level of pollution are the main criteria for “ex situ” bioremediation techniques. The “ex situ” type bioremediation techniques are comparatively easier to regulate and control the processes. These are useful to treat a wider range of contaminated soils and toxins. In this mixing of material is done to have a good supply of air and nutrients so that degradation of contaminants is much faster as compared to “in situ” techniques. The various ex situ techniques are described below.

1. *Bioreactor*: in bioreactor technique, large vessels are used to remove the pollutants from wastewater by means of microbes. The different operating modes of the bioreactor are (a) batch, (b) fed-batch, (c) sequencing batch, (d) multistage, and (e) continuous. Temperature, pH, moisture, concentration of substrate, agitation rate, and aeration rate are the important parameters required for working of bioreactors. Due to certain limitations, bioreactor-based bioremediation is not suitable for removal of heavy metals. Firstly, it requires more man power. Also, there is requirement of high cost for the transfer of pollutants from

the contaminated site. Secondly, various bioprocess variables are involved in bioreactor technique. If any variable remains uncontrolled, it turns out to be a limiting factor and leads to reduction of microbial activities and hence makes the technique very less effective.

2. *Biopile*: Biopile-based bioremediation involves conversion of contaminated soils into piles. The pile formation is followed by application of nutrient and aeration to make bioremediation effective by increasing microbial activity. Different terms are used for of biopiling such as bioheaps, biocells, and biomounds for alleviating the problem of contamination from soils and sediments. Temperature, pH, moisture, and nutrients are important parameters to accelerate biodegradation in biopiles. Biopiles are useful to remove heavy metals from soil, and they are a better pollutant removal strategy as compared to other techniques such as land farming and composting which are based on bulk transfer of nutrients, water, and air.
3. *Windrow*: Windrow includes the turning of polluted soil for increasing and improving aeration along with the application of water, uniform distribution of nutrients, contaminants, and microbial degrading activities. The process occurs through assimilation, mineralization, and biotransformation. This treatment is not suitable for remediation of soils which are polluted with toxic volatile compounds since it involves periodic turning.
4. *Landfarming*: Less Equipment is required during landfarming technique operation. However, production of leachate take place during landfarming operation which should be taken care of to prevent the groundwater contamination. Tillage and irrigation with appropriate biological activity enhances the rate of bioremediation by enhancing heterotrophic bacterial counts. The enzyme microbial dehydrogenase, a good indicator of biostimulation, is used in landfarming. Landfarming is the simplest bioremediation practice. However, there are certain limitations to it such as it requires a large operating space, requirement of additional and high cost during excavation, reduction in microbial activity due to unfavorable environmental conditions and less effectivity in removal of inorganic pollutant. These limitations make this technique more time-consuming which in turn makes it less efficient.

5.6 Plant-Mediated Heavy Metal Removal

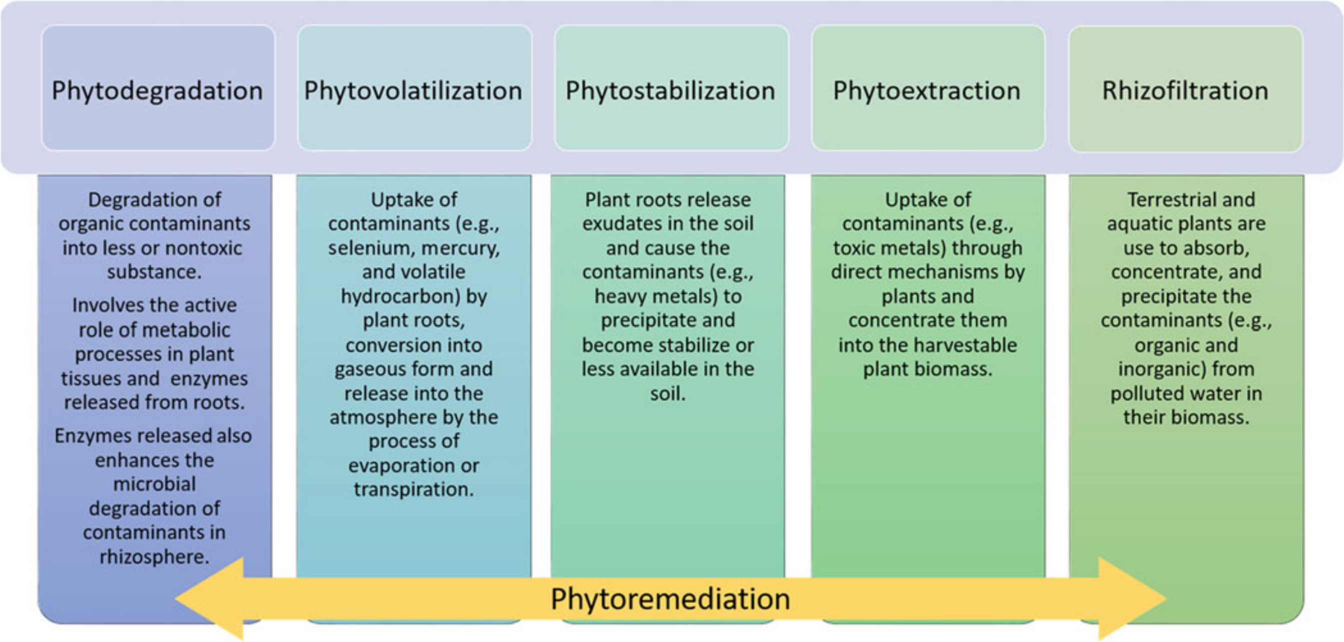
Plants are well known to remove metal contaminants from environment. Plant-based remediation technologies, also known as phytoremediation, have been widely studied for extracting and accumulating the heavy metals significantly. Phytoremediation is an ecological remediation technology where plants are used as an important source for the removal of contaminants whether, organic or inorganic. In the process of phytoremediation plants remove contaminants through different mechanisms such as extraction, sequestration, and detoxification. Plants use different approaches for the removal of heavy metals from the ecosystem, which include phytoextraction, rhizofiltration, phytovolatilization, and phytostabilization. Thus, the techniques of phytoremediation can be further classified as phytodegradation, rhizofiltration, phytostabilization, phytoextraction, and phytovolatilization (Fig. 5.3).

Heavy metals, for example, Cu, Zn, Fe, and Mo, make an integral part of many enzymes and participate in redox reactions, electron transfer, and also in nucleic acid metabolism, and thus are considered as essential for plants. These metals act as cofactors and activator of important enzymatic activities, thus playing an important role in the formation of enzyme–substrate metal complex (Nagajyoti et al. 2010). Zn, acting as cofactors for over 300 enzymes and 200 transcription factors involved in maintaining membrane integrity, auxin metabolism, and reproduction (Singh et al. 2016). Heavy metals at microlevels are important for plant growth, but at elevated concentrations can exert toxic effects in plants. The most common toxic effects of essential and nonessential heavy metals on plants include inhibition of growth and photosynthesis, chlorosis (loss of the green coloration of leaves), low biomass accumulation, altered nutrient assimilation and water balance, and senescence, which ultimately leads to plant death. There are four proposed mechanisms for the toxicity of heavy metals on plants (Singh et al. 2016).

1. Similarities to nutrient cations, lead competitive uptake on root surface; for example, As and Cd compete with phosphorus (P) and Zn, respectively, for their uptake.
2. The heavy metals directly interact with the sulfhydryl (-SH) group of functional proteins. This interaction disturbs their structure and function, thereby rendering them inactive.
3. Movement of essential cations from particular binding sites causes functional collapse.
4. Reactive oxygen species (ROS) generation as a result damages the macromolecules.

Plants have potential adaptive mechanisms for tolerance toward the high concentrations of heavy metals, their extraction, and accumulation in the above ground parts (Singh et al. 2010; Pal and Rai 2010). Plants having potential to uptake or tolerate a large amount of heavy metals known as hyperaccumulators make them unique to be used as a tool for the remediation of heavy metal. Such plants are also known as “metallophytes.” Over 400 plant species vary from annual to perennial herbs, shrubs, and trees belonging to over 45 families have been identified to accumulate significant amount of heavy metals (Giri et al. 2015). Baker (1981) recognized the types of plant–soil relationships, that is, accumulators, indicators, and excluders (Fig. 5.4) growing on metalliferous soils, as discussed below.

1. *Accumulators:* These plants concentrate the toxic metals in their aboveground parts.
2. *Indicators:* Uptake and transportation of metals to the shoots of plant is regulated in a manner where internal concentration reflects the external levels.



Degradation of organic contaminants into less or nontoxic substance.

Involves the active role of metabolic processes in plant tissues and enzymes released from roots.

Enzymes released also enhances the microbial degradation of contaminants in rhizosphere.

Uptake of contaminants (e.g., selenium, mercury, and volatile hydrocarbon) by plant roots, conversion into gaseous form and release into the atmosphere by the process of evaporation or transpiration.

Plant roots release exudates in the soil and cause the contaminants (e.g., heavy metals) to precipitate and become stabilize or less available in the soil.

Uptake of contaminants (e.g., toxic metals) through direct mechanisms by plants and concentrate them into the harvestable plant biomass.

Terrestrial and aquatic plants are use to absorb, concentrate, and precipitate the contaminants (e.g., organic and inorganic) from polluted water in their biomass.

Fig. 5.3 Different processes of phytoremediation

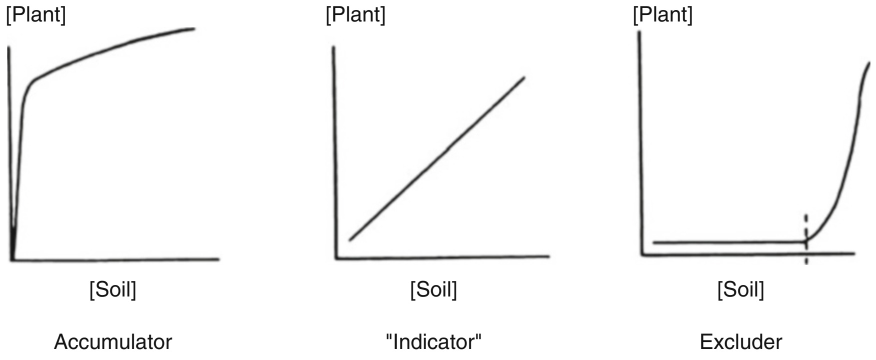


Fig. 5.4 Three ways in which the response of plants to increasing soil metal levels may be reflected by the metal concentrations in aerial plant parts. Reproduced with permission from Baker (1981). (Copyright 1981, Taylor & Francis Group)

3. *Excluders:* Plants maintain toxic metal concentrations in their parts as constant up to a critical soil value and above which unrestricted transportation of contaminants occur.

Plants use their root system to absorb the ionic compounds present in soil. Plants develop a rhizosphere ecosystem by extending their root system into the soil matrix. The extensive root system of plants helps them to accumulate heavy metals and regulate their bioavailability. In this way, plants not only reclaim the contaminated soil but also stabilize soil fertility (Jacob et al. 2018; DalCorso et al. 2019). Plant roots release some exudates into the soil and enhance the bioavailability of heavy metals by modifying soil pH. Root exudates are primary metabolites (sugar, amino acids, and organic acids) released from plants' root tip and play a crucial role in shaping the interaction between plants and soil, especially nutrient mobilization in rhizosphere soil (Canarini et al. 2019). Apart from root exudates, pH of rhizosphere also influences heavy metal uptake by plants/hyperaccumulators. It has been reported protons released in the rhizosphere by roots enhanced metal dissolution (Singh et al. 2016). Plant roots follow either of the two pathways for nutrient and heavy metal uptake, that is, apoplastic pathway and symplastic pathway. The apoplastic pathway is the passive diffusion of soluble metals through the space between cells, whereas the symplastic pathway is active transport of nutrients/ soluble heavy metals against electrochemical potential gradients and concentration across the plasma membrane (Peer et al. 2005).

For successful implementation of the process of phytoremediation plants must possess the heavy metal detoxification mechanism. Plants with constituent and adaptive mechanisms to extract, collect, and tolerate high concentrations of their rhizospheric contaminants are preferred for the application of phytoremediation procedures. Plants have developed a range of potential mechanisms for tolerating and avoiding the toxic effect of high concentrations of metals. By avoidance and tolerance strategies, plants are able to keep cellular concentration of heavy metals

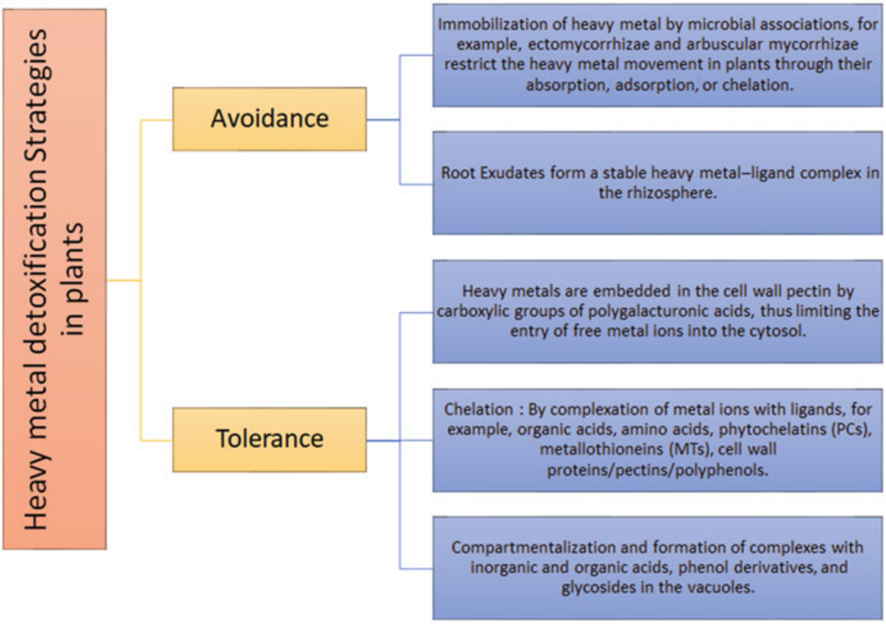


Fig. 5.5 Avoidance and tolerance strategies used by plants against heavy metal toxicity

below toxicity thresholds (Hall 2002). Avoidance is the first defense mechanism used by plants, whereby the entry of heavy metals into plant tissues is restricted by roots, whereas tolerance is the second- and intercellular-level approach adopted by plants to deal with the accumulated heavy metal ions inside plant cells (Dalvi and Bhalerao 2013). Various avoidance and tolerance approaches adopted by plants against heavy metal toxicity are depicted in Fig. 5.5.

***Plants tolerate the toxic metal concentration in the cytoplasm by complexation and chelation of metal ions with organic acids, thus reducing their bioavailability. Plants accumulates various metabolites in their cytoplasm in order to tolerate or detoxify the effects of high heavy metal concentration. Different metabolites and their roles are discussed in Table 5.4. Kramer et al. (2000) reported chelation of nickel (Ni) by citrate and accumulation in the vacuoles of leaves of the hyperaccumulator *Thlaspi goesingense*. Similarly, chelation and accumulation of Cd in the leaves of *Solanum nigrum* by acetic acid and citric acid was reported by Sun et al. (2006). Sun et al. (2011) observed a positive correlation between the Cd concentration and both tartaric and malic acids in the leaves of *Rorippa globosa* and in *Rissopsetia islandica* the rise in acetic acid levels was observed with Cd concentration, thus suggesting that the accumulation of Cd is associated with tartaric and malic acids in the leaves of *R. globosa* and acetic acid in *R. islandica* (Sun et al. 2011). Similarly, accumulation of amino acids such as proline is one of the strategies used by plants to avoid environmental stress (e.g., heavy metals, salt, water, UV radiation, and excess and deficiency of minerals). It has been observed that oxidative

Table 5.4 Metabolites and their role in plants to avoid toxic effects of heavy metals

Metabolites	Role in plants to avoid toxic effects of heavy metals
Organic acids (such as citric acid, oxalic acid, and malic acid)	Chelates metalloids inside the cells and reduce their toxic effect
Amino acids (proline, asparagine, cysteine, etc.)	Proline works as an osmolyte, radical scavenger, and macromolecule stabilizer Asparagine plays key role in metal–asparagine complex and reduce heavy metal stress Cysteine is a key metabolite in antioxidant defense and metal sequestration. Cysteine is also required in methionine and glutathione (phytochelatins) synthesis
Heat-shock proteins (HSPs)	HSPs are expressed or produced in response to stress like high temperature and heavy metals. Work to protect and repair the proteins under stress condition. HSPs also protect the membrane against metal damage
Betaines	Betaines (glycine betaines) observed to accumulate under water stress and in metal stress also
Metallothioneins (MTs)	Intracellular complexation. Metallothioneins are cysteine-rich metal-binding proteins/peptide ligands
Phytochelatins (PCs)	Intracellular complexation. Phytochelatins are also metal-binding polypeptides/peptide ligands, help to sequester and detoxify toxic metal ions

stress is one of the most common effects of heavy metal toxicity in plants; thus, enhanced antioxidant capabilities of hyperaccumulators make it possible to tolerate high heavy metal concentrations (Peer et al. 2005).

Chelation of toxic metal ions followed the compartmentalization of the heavy metals in the vacuoles in order to reduce their toxic effects on other cell functions (Sheoran et al. 2010). Various studies mentioned certain secondary metabolites and high molecular weight compounds released from root which influence the root–microbe relation (Ahmed et al. 2018). Thus, heavy metal uptake by plants involves a series of processes which starts with heavy metal mobilization followed by root uptake, xylem loading, transportation from root to shoot, cellular compartmentation, sequestration, and volatilization (Peer et al. 2005).

5.7 Role of Microbes in Heavy Metal Removal

Microorganisms such as bacteria, fungi, yeasts, and microalgae possess great potential for the remediation of heavy metal–contaminated sites. Microorganisms possess certain resistance mechanisms against the metal toxicity which allow microbes to survive in heavy metal–contaminated environment (Fig. 5.6). The metal resistance mechanisms include (a) exclusion by permeability barrier, (b) intracellular sequestration by protein binding (cysteine-rich metal-binding protein, e.g., metallothionein), (c) extracellular sequestration, (d) active transport/efflux system, (e) enzymatic reduction to less toxic forms, and (f) reduction in the sensitivity of

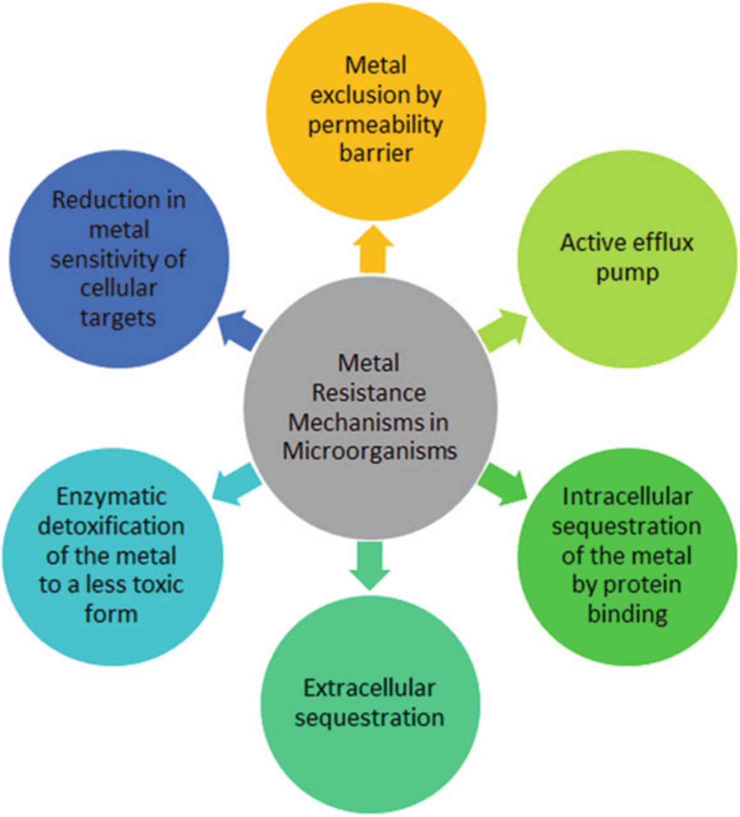


Fig. 5.6 Mechanisms of metal resistance in microorganisms

cellular targets to the metal ions (Ji and Silver 1995; Bruins et al. 2000; Ramasamy et al. 2007). Microbes play a vital role in modifying the bioavailability of heavy metals simply by solubilizing and/or immobilizing them, hence can be exploited for the treatment of heavy metal–contaminated sites (Ramasamy et al. 2007). Microbes interact with heavy metals through different mechanisms, namely biosorption and bioaccumulation, biomineralization, bioleaching, and bioimmobilization (enzyme-catalyzed transformations), which can be used to remediate the heavy metal–contaminated sites.

Biosorption is a passive mechanism of heavy metal sequestration, which uses living or dead cell biomass. In the process of biosorption the metal ions are stick through surface complexation onto the cell surface. Heavy metals interact with different functional groups available on microbial cell surface. Bacterial cell surface possesses variety of anionic ligands such as carboxyl, amine, hydroxyl, phosphate, and sulfhydryl groups are known to bind heavy metals. Living microbial cells are preferred by many workers for the biosorption of heavy metal due to their continuous metal uptake and self-replenishment characteristics (Hajdu et al. 2010; Shamim

2018). Microalgae can also biologically sequester heavy metals in aquatic environment. Microalgae possess great potential to bind metals on their cell surface and also to intracellular ligands. The availability of large surface-to-volume ratios, the presence of high-affinity groups and metal-binding groups (amino, sulfate, and carboxyl groups) are important features that enable microalgae to sequester metals. The distribution and abundance of cell wall components may vary across different groups of algae; as a result, the types of functional groups also vary.

Bioaccumulation is an active process in which microorganisms built up the heavy metals metabolically into the cellular interior. Heavy metals transport through microbial cell wall into the cytoplasm and becomes immobilized in the cell (Ramasamy et al. 2007). Biomineralization is the process of mineral formation associated with microbial transformation of metal ions into amorphous or crystalline precipitates. Dhami et al. (2017) studied two isolates of ureolytic fungi, namely *Aspergillus* sp. UF3 and *Fusarium oxysporum* UF8 for their biomineralization and metal recovery potential. The two isolates showed significant production of calcite and a coprecipitation of Pb and radionuclide strontium (Sr) in the form of carbonates (Dhami et al. 2017). Microbes by the processes of leaching, chelation, and redox transformation mobilize heavy metals from the contaminated site. Bioleaching is the process microbial extraction/leaching of metals from their ores. Many microorganisms through the enzymatic and nonenzymatic process reduces the heavy metals and other trace elements. The enzymatic reduction uses the metals as electron acceptors. The oxidized metals are highly soluble and have a potential to contaminate the water, while reduced metal forms are insoluble. A wide range of metal reducing bacteria can reduce the chromate ions (soluble) to Cr(III), which precipitate as $Cr(OH)_3$ (Ramasamy et al. 2007).

Microorganisms in the rhizosphere also play an important role in phytoremediation of heavy metals. These microbial communities are classified into two groups, namely, mycorrhizal fungi and plant growth–promoting rhizobacteria (PGPR). Mycorrhizal fungi such as arbuscular mycorrhizal fungi (AMF) exhibit mutualistic association with most plants and benefits them (Marques et al. 2009). Plant growth–promoting rhizobacteria can be classified into two major groups: (a) symbiotic and (b) free living rhizobacteria. PGPR enhance the tolerance in plants against the various stress, such as flood, water deprivation, and salt stress. According to the relationship of PGPR with plants, PGPR can be broadly classified into two major groups, namely (a) symbiotic rhizobacteria, also known as intracellular PGPR (e.g., nodule bacteria), and (b) free-living rhizobacteria, also known as extracellular PGPR (e.g., *Bacillus, Burkholderia, Azotobacter*, and *Pseudomonas*). The symbiotic PGPR invade the interior cells of the plants and survive there, while the free-living ones exist outside the plant cells. Nutrients (for example amino acids, organic acids, and sugar) exuded from the plants' roots influence the healthy concentration of rhizospheric bacteria. Plant growth-promoting rhizobacteria produces different growth-regulating compounds. The low molecular weight (400–1000 K Dalton) organic compounds produced by PGPR are known as siderophores that helps to solubilize or chelate unavailable forms of heavy metals by complexation reaction and make them available for microbial and plant cells (Pal and Rai 2010). Various

Table 5.5 PGPR and their associated growth-regulating compounds

Plant growth-promoting rhizobacteria (PGPR)	Plant growth-regulating compounds
Pseudomonas fluorescence	Siderophores
Bacillus, Azotobacter, Pseudomonas, and Azospirillum	Indole acetic acid (IAA), and phosphate (P)-solubilization
Micrococcus luteus	IAA and P-solubilization
Variovorax paradoxus, *Flavobacterium*, and *Rhodococcus* sp.	IAA and siderophores
Bacillus subtilis	IAA and P-solubilization
Brevibacterium sp.	Siderophore
Brevibacillus brevis	IAA
Pseudomonas and bacillus	Siderophores, IAA, and P-solubilization
Bacillus spp.	IAA, siderophores, P-solubilization, HCN, and ammonia
Azotobacter, Pseudomonas fluorescens, and Bacillus sp.	IAA, siderophore, ammonia, HCN, and P-solubilization
Pseudomonas spp., and *Bacillus megaterium*	IAA, siderophore, and P-solubilization
Pseudomonas chlororaphis and *Arthrobacter pascens*	P-solubilization
Achromobacter xylosooxidans	IAA, P-solubilization
Pseudomonas sp.	IAA, P-solubilization, and siderophores

PGPR and their associated compounds that promotes the growth of plants are discussed in Table 5.5.

PGPR influence the growth of plants and their efficiency to accumulate heavy metals in various ways, as discussed in Fig. 5.7. PGPR like *Pseudomonad* and *Acinetobacter* have been reported to increase the phytoremediation efficiency of nonhyperaccumulating maize (*Zea mays* L.) plants by improving the growth and biomass of plants (Lippmann et al. 1995). Different microorganisms use different mechanisms for plant growth and tolerance of high heavy metal concentration. Thus, it may be advantageous to design phytoremediation processes in conjunction with appropriate microbial consortia.

5.8 Recent Advancement in Heavy Metal Removal Techniques

In the last few years, research on bioremediation of heavy metal has gained much attention to understand the pathways (molecular and biochemical) of heavy metal movement (i.e., uptake, transport, and storage) in plants (Giri et al. 2015). In the recent years, work has been extensively done to improve the process of bioremediation by implementing the genetic engineering tools to the agents (plants and microorganisms) used for removal of heavy metal. Thus, with genetic engineering methods appropriate genes or hyperaccumulation traits can be transferred to the plants. Heavy metal detoxification system has also been explored at molecular levels in microorganisms such as yeast and bacteria. Transfer or overexpression of genes

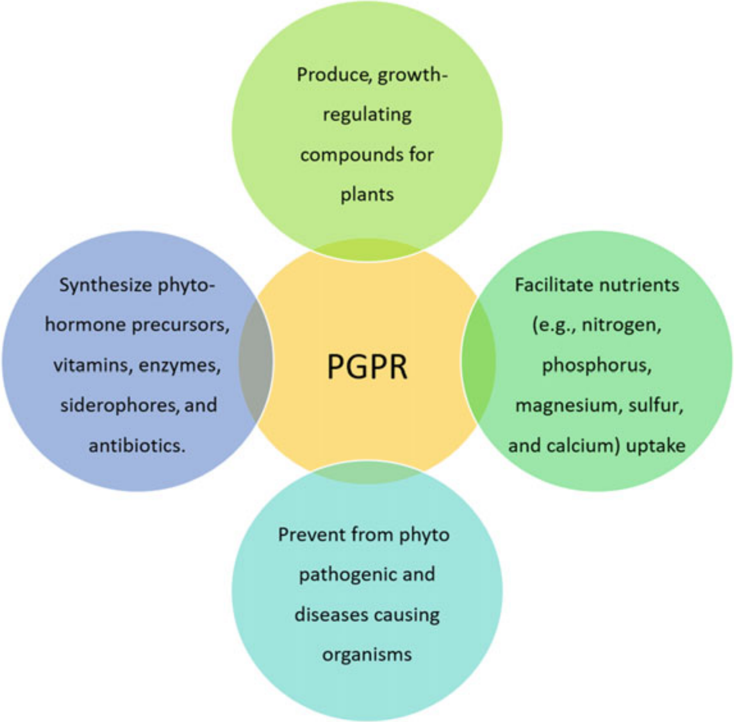


Fig. 5.7 Different advantages of PGPR for promoting plant growth

from microorganisms into plants is being done to improve the remediation potential of plants. Such genetic manipulations in plants have already yielded promising results. Some of the genetic modifications for enhanced metal tolerance include modifications in oxidative stress–related enzymes, overexpression of glutathione-S-transferase, peroxidase, and aminocyclopropane-1-carboxylic acid (ACC) deaminase (Eapen and D'Souza 2005).

The plants with high biomass production have been proven a good candidature for successful hyperaccumulation of heavy metals and genetic manipulations. Some of the high biomass producing plants are Indian mustard (*Brassica juncea*), tomato (*Lycopersicon esculentum*), sunflower (*Helianthus annuus*), and yellow poplar (*Liriodendron tulipifera*) (Eapen and D'Souza 2005). Chemically treated stems of *H. annus* have been used to optimize the adsorption of Cd (II) ions from water and statistical results confirmed 99.8% removal efficiency under optimized conditions (Jain et al. 2021a). Plants like *B. juncea*, *Nicotiana tabacum*, and *Populus angustifolia* have been extensively studied for genetic modification to enhance the heavy metal accumulation compared to their wild type. Van Huysen et al. (2004) reported the enhanced affinity for selenium (Se) uptake in transgenic *B. juncea* overexpressing ATP sulfurylase (APS transgenics) and cystathionine-gamma-synthase (CGS) than the wild variety. The two Indian mustard plants, with

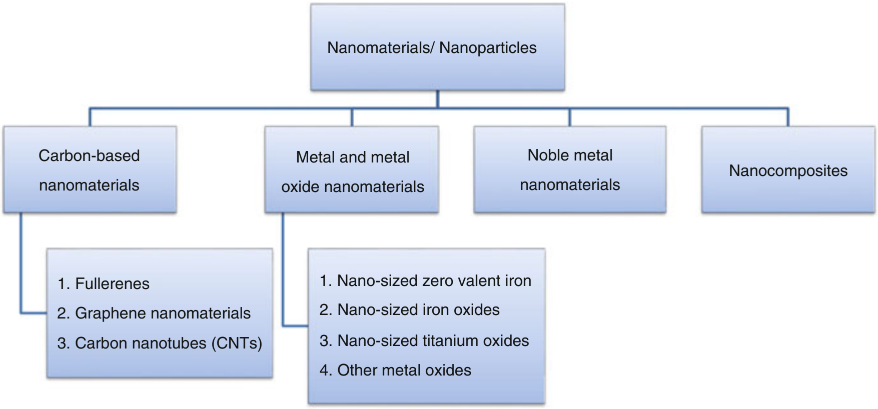


Fig. 5.8 Classification of nanoparticles

overexpressed genes encoding selenocysteine lyase (cpSL) and selenocysteine methyltransferase (SMT) enzymes were observed to possess great potential for the accumulation of Se from the contaminated soil (Bañuelos et al. 2007). Transgenic plants have proved to be a promising biotechnological approach for the bioremediation of heavy metal–contaminated soil.

In recent years, nanotechnology has also received considerable attention for its application in heavy metal remediation technologies. Nanomaterials are unique in their characteristics, that include nano size (≈1–100 nm size), high mobility in solution, high surface area-to-volume ratio, and high adsorption capacity and reactivity that make them suitable for use in remediation technologies (Yu et al. 2021). Nanomaterials can be of a variety of shapes, sizes (on nanoscale), and functions. Compared to conventional treatment processes, application of nanomaterials possesses various advantages over the conventional treatment practices, that includes enhanced reactivity, unique surface chemistry (i.e., target specific functional groups on surface), and physical properties of nanoparticles. Various nanomaterials can be grouped into carbon based, metal oxide based, noble metal nanomaterials, and nanocomposites (Fig. 5.8).

Graphene oxide is a carbon-based nanomaterial comprises a variety of functional groups (hydroxyl, carboxyl, carbonyl, and epoxy group) for the adsorption of metal contaminants (Lü et al. 2012). Many workers reported graphene oxide for its heavy metal adsorption potential. Ding et al. (2014) studied the adsorption capacity of graphene oxide layered fixed bed sand column for the removal of heavy metals (Cu (II) and Pb(II)) from aqueous solution. Nano-sized metal oxides have also been reported for their remarkable affinity toward the heavy metals such as Pb(II), Cu(II), Ni(II), Mn(II), Ni(II), Cd (II), and Cr(VI) (Engates and Shipley 2011). Jain et al. (2021b) studied the efficient removal of divalent nickel ions from aqueous media through adsorption by copper oxide nanoparticles and inferred that the technique can be utilized for effective sequestration of Ni (II) ions from wastewater. Nanoparticles can be used in a variety of approaches to remediate contaminated environment.

Different approaches used for the treatment of inorganic and organic contaminants include adsorption, separation, catalysis, photocatalysis, and disinfection, as discussed below.

1. *Adsorption:* Adsorption is a surface phenomenon of the adsorbent. Nanomaterials have unique features such as high adsorptive capacity, specific affinity toward the contaminants, and large surface area for adsorption, which make them a good candidate to be applied in treatment plants. The adsorptive capacity of nanomaterials can be enhanced by some structural improvements.
2. *Separation:* This includes processes filtration, size exclusion, and reverse osmosis. Nanofiltration membranes are especially designed to remove inorganic and organic contaminants from wastewater. Properties of nanofiltration membrane include high flux, high retention of anionic salts, and low maintenance and operational cost.
3. *Catalytic and photocatalytic activity:* Nanocatalysts and photocatalysts improve the chemical reactivity by enhancing the production of oxidative species at the material surface. TiO_2 is the most extensively studied nanophotocatalyst.
4. *Disinfection:* Nanoparticles can possess the properties of pollutant remediation as well as disinfectants. The carbon-nanofiber composite TiO_2/ZnO has been observed to treat toxic chemical dye and microbial contamination such as *Escherichia coli* (*E. coli*). The nanoparticles showed excellent antimicrobial activity along with fast adsorption and methylene blue degradation ability (Pant et al. 2013).

Various nanoparticles utilized for wastewater remediation are carbon–TiO_2 nanotubes, carbon–ZnO, graphdiyne–ZnO, graphene–SiO_2/Cu_2O, graphene–SiO_2 nanoplatelets, multiwalled carbon nanotube–metal-doped ZnO nanohybrid, carbon nanoparticles–gold, platinum nanoparticle, carbon aerogel–TiO_2, carbon nanotube-Ag_3PO_4 in Pickering emulsions, multiwall carbon nanotube–TiO_2–SiO_2, carbon–nitrogen-doped TiO_2–SiO_2, carbon nanofibers–Ag–TiO_2, carbon–Ag–TiO_2, silver nanoparticles, and so on. Development of new nanomaterials has advanced the present treatment techniques, but more research is still needed to make the process sustainable.

5.9 Advantages and Limitations

Bioremediation techniques are more economical than conventional methods because low installation and maintenance cost. The most of the pollutants get treated on the site of contamination which reduced the exposure risk to other biotic and nonbiotic components of the environment. The technique is more publicly accepted since it is based on natural attenuation. Further, bioremediation has the potential to eliminate or degrade a wide variety of pollutants completely and permanently. It can be operated on larger scale and can easily be coupled with other physical and chemical methods of remediation. It also does not let the transfer of contaminants from one environmental medium to other. Therefore, bioremediation offers a less energy-intensive, cost-effective, and yet efficient option to clean the environment. Advantages of phytoremediation includes recovery of precious metals, improvement in soil fertility, and decline in metal leaching and erosion.

Along with large number of benefits, some drawbacks are also associated with bioremediation. The process can be effective under certain environmental conditions which are required to be manipulated for enhanced microbial growth and faster degradation rate. There are also some compounds which are resistant to microbial attack such as chlorinated organic pollutants, high aromatic hydrocarbons, and radionuclides. The time scale of the process is relatively long, and also, the appropriate residual levels of contaminants may not always be achieved. The implementation of technique requires huge experience and expertise. Sometimes small-scale laboratory studies are required to be done before actual implementation in the field. The limitations associated with phytoremediation includes longer time scales, concentration of pollutants or contaminants and their bioavailability to plants, toxic effect of pollutants on plants and inability to degrade organic contaminants due to lack of specific degradative enzymes.

5.10 Application and Future Prospects of Bioremediation

Bioremediation technologies are more appropriate and offer many advantages compared to traditional treatment methods, such as cost-effectiveness, high efficiency, and reduced secondary waste production. These techniques also provide flexibility to work continuously, regeneration of biomass and metals recovery. Bioremediation with the recent advancement is becoming a widely acceptable and economically viable technology. Over the last decade the scientific community gathered information on potential modification of remediation processes for heavy metal removal on large scale. These processes include identification of low-cost and commercially applicable biosorbent, and development of transgenic plants and nanomaterials for remediation of heavy metals. Biosorption has been proven as low-cost technology to remediate the heavy metal–contaminated effluents and has received a great attention. Inexpensive biosorbents have been used to detoxify effluents from the metal plating, extraction, and ore-mining operations, as many research works have demonstrated the biosorption as an advantageous alternative to traditional treatments methods (Vijayaraghavan and Yun 2008). However, optimization of the process is required in order to understand the metal–microbe interaction, and regeneration of the material (biosorbent) for effective removal of the contaminants. The nanotechnological approach has contributed an extraordinary adsorption capacity and reactivity to the adsorbent that promotes heavy metal removal. Microbes are pervasive and grow rapidly, becoming habituated to varying concentrations of different toxic metal ions. Genetically engineered microbes (GEMs) have made the microbial remediation more effective, but their applications on the ground have their own concerns such as legality, ethics, and biosafety. Efforts are under way to achieve a better molecular understanding of mobilization, absorption, translocation, and accumulation of metals in plants.

For efficient phytoremediation of soils contaminated by heavy metals, the activity of plant symbionts in rhizosphere is necessary. Application of mycorrhizal fungi and plant growth-promoting (PGP) bacteria would benefit plant growth and facilitate the mobilization and bioavailability of heavy metals. Pramanik et al. (2017) reported the *Klebsiella pneumonia* K5, a PGPR strain highly resistant to cadmium, possessed several PGP characters, such as nitrogen fixation, phosphate solubilization, and indole acetic acid (IAA) production and also confirmed multiple resistance to heavy metals such as lead and arsenite. Mitra et al. (2018) also characterized a highly Cd-resistance strain *Klebsiella michiganensis* MCC3089 that exhibited many PGP traits such as IAA production, phosphate solubilization, nitrogen fixation, and reduction of oxidative stress. Nitrogen fixation is a common mechanism in the genus because *Klebsiella* species are well-known free-living nitrogen fixers. In a recent work, the phytoremediation potential of *Zea mays* inoculated with AMF *Claroideoglomus etunicatum*, bacterial diversity (*Microbacterium, Agrococcus, Lysobacter, Planomicrobium, Streptomyces, Saccharothrix*), and various unclassified bacteria and fungi was assessed by Hao et al. 2021. The results showed that arbuscular mycorrhizal fungi (AMF) facilitate the revegetation of heavy metal–contaminated soils through interacting with the rhizosphere microbiome (Hao et al. 2021). Thus, rhizosphere microbes are the important partner for stress tolerance in plants and bioaugmentation with AMF, and growth-promoting bacteria can be applied as a beneficial strategy for reclaiming the soil contaminated with toxic metals.

There is no question that molecular knowledge and nanotechnology have helped to explore new avenues to remediate heavy metal–contaminated sites. But more research is still needed to identify new strategies of heavy metal remediation concerning the issues relating biosafety, emerging pollutants, and efficiency of genetically engineered microbes, and transgenic plants. Future research is required aiming at the experimental approach for data collection from multidiscipline and mathematical modeling to achieve better prediction. And for better environmental application, the generated experimental data need to be integrated into different approaches to test the bioremediation effectiveness.

5.11 Conclusions

Bioremediation proves to be a fruitful and attractive approach to clean, manage, remediate, and recover the contaminated sites through indigenous or extraneous microbial activity. In recent era, where other physical, chemical, or mechanical methods are very costly and tedious to be put into implementation, bioremediation offers a low-cost and efficient approach toward a cleaner and greener environment. The effectiveness of the technique however depends on thorough understanding of microbial communities, their response to natural and contaminated environment, knowledge of genetic capabilities of microbes to degrade toxic pollutants. Also, the success requires frequent cost-effective field trails on sites specifically dedicated for research purpose. The speed of the process both in situ and ex situ is determined by competition within biological agents, adequate supply of essential nutrients, other environmental or abiotic factors such as oxygen supply, temperature, pH, moisture, and bioavailability of the contaminants. Therefore, to be more successful, bioremediation is carried out in manipulated environments rather than natural environments. Further, this review provides an insight in to the recent technologies such as use of nanoparticles for heavy metal removal from environmental components. More research is needed in the areas of commercially acceptable biosorbents, development of transgenic plants and advancements in nanotechnology to efficiently remediate the heavy metal–contaminated effluents. Regardless of certain limitations, the more advantages of bioremediation technology make it an acceptable, efficient, cost-effective, and green approach toward a clean environment.

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