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Aravind Jeyaseelan
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Sustainable and Cleaner Technologies for Environmental Remediation

Avenues in Nano and Biotechnology

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Environmental Science and Engineering

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Chapter 2
Biological and Eco-Friendly Cost-Effective Measure for Remediation

Anil Kumar Moola, Selvam Sathish, S Mari Selvam, Balasubramanian Paramasivan, Sujatha Peela, Harish Kumar Seenivasan, and Dhandapani Gurusamy

Abstract The natural environment is being spoiled with harmful chemicals from tannery effluent, industrial effluent, and domestic wastewater by human uses. Therefore, the avoidance of pollution and the degradation of harmful chemical compounds are essential for the future mankind. Several microorganisms are involved in the biodegradation of harmful contaminants in the soil including bacteria, fungi, and algal species. Phytoremediation is the best choice for many developing and developed nations to as it is an eco-friendly process for the decontamination of several environmental pollutants. Green plants absorb the wastes materials in the soil through their roots. Nowadays, *in-vitro* studies on the phytoremediation of emerging pollutants are giving better results. This chapter focuses on the recent studies on phytoremediation through various roots, including hairy roots, adventitious roots, and normal roots. It also reports the mechanism of biodegradation and enzymatic reactions that

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trigger the phytoremediation process. This information is essential for assessing the feasibility of a plant for the phytoremediation process before its application in the target site.

Keywords Biodegradation · Harmful chemicals · Microorganisms · Plant · Phytoremediation · Soil

2.1 Introduction

Nowadays, the global nations were having a challenging problem with the need for groundwater. But, the groundwater is rapidly polluted by urbanization, use of pesticides, heavy metals (HMs), tannery effluents, phenols, organic matter, explosives, azo dyes, and industrial wastes (Carolin et al. 2017). From the industrial wastewater, large quantities of heavy metals are released, such as Hg (II), Pb (II), Cd (II), Ag (I), Ni (II), and Cr (VI). These are highly toxic, non-degradable, and have infinite lifetimes (Mokarram et al. 2020). The harmful chemical compounds are synthesized in two different ways, including natural elements and industrial wastewater. Natural compounds can easily be degraded by external factors than industrial wastewater. In this sense, many biological methods can remove these harmful substances from industrial wastewater and contaminated wastewater (Crini and Lichtfouse 2019). In removing toxic compounds from soil and water, various methods are followed like adsorption on activated carbon, chemical oxidation, incineration, and solvent extraction. These methods have several disadvantages, including low efficiency, high cost, or by-product generation. Therefore, phytoremediation is the most valuable method to remove harmful chemical compounds from soil and wastewater (González et al. 2006; Al-Baldawi et al. 2018). In this technology, the root system of the green plants absorbs the harmful chemicals, while emerging pollutants are also removed from the wastewater simultaneously. Phytoremediation is classified based on the mechanism of action employed by the plants as follows: phytoextraction (accumulation of toxic chemicals and elimination of toxicity), rhizofiltration (root system for removing toxic chemicals from polluted water), phytostabilization (removal of bioavailability of toxic chemicals in the soil), phytovolatilization (evaporation of several toxic chemical compounds) (Antoniadis et al. 2017). Various strategies and processes are involved in phytoremediation for improving the uptake of heavy metals, such as genetic strategies (genetic modification of plants) and chelate-assisted strategies (Sharma 2021). Hairy root culture is one of the most valuable phytoremediation processes to uptake the toxic compounds from soil and water. It can grow faster in a toxic environment than the regular root system (Gujarathi et al. 2005; Perotti et al. 2020). Microorganisms also help in the process of phytoremediation as bacterial and fungal pathogens in hyperaccumulator plants. Based on the 'literary reports, the review on comprehensive discussion on synergistic mechanism of plants, microbes and other additives has been rarely reported.

Hence in this chapter, features and the enzymatic reactions that take place in plants and microbes during the process of phytoremediation has been described. The key concepts of plant, microbe and biochar assisted remediation has been annotated. This chapter helps the researchers to understand the benefits of phytoremediation and its significance on synergistic relation with bacteria and other additives.

2.2 Bio-based Technologies

In recent days, the world faces various problems owing to pollution of oil, air, water, and land. These problems are temporarily solved with several bio-based technologies like bioaugmentation, biostimulation, composting, bioventing, and biosparging. Bioremediation is the process of removing contaminants from polluted soil or water with the help of several processes including bioaugmentation and biostimulation (Tyagi et al. 2011; Lellis et al. 2019). The types of bioremediation techniques has been illustrated in Fig. 2.1 (Sharma 2020). Biostimulation is one of the bio-based technologies to promote the degrading rate of hazardous chemical compounds from contaminated soil. In this technology, low-energy lasers are used to stimulate the rate of wound healing and degradation of contaminants (Wasewar 2022). Bioaugmentation is another most important method in bio-based technology for bioremediation of polluted environments and it is one of the best methods to degrade pollutants than other methods. Two different processes are involved in this method including the addition of a pre-adapted pure bacterial strain and the addition of a transformed gene vector into indigenous microorganisms (Li et al. 2018; Huang et al. 2020). Bioaugmentation is successful in agricultural land, forestry, and wastewater treatment with the help of indigenous microorganisms. Bioaugmentation has been reported significant achievement in anaerobic digestion of lignocellulosic residues (Tsapekos et al. 2017), waste activated sludge (Cayetano et al. 2021), food waste (Jiang et al. 2020) to enhance the process with the help of microbial enrichment culture. In bioaugmentation, microbial communities play a major role in remediation. Microbial strain selection and their subsequent survival and activity determine the success rate of bioaugmentation. Hence the basic or initial and most important step of bioaugmentation is strain selection due to the involvement of microorganisms in fulfilling the whole process. For instance, Yang et al. (2019) reported that both hydrogenotrophic and aceticlastic methanogenic pathway has to be accounted for bioaugmentation where the former and latter showed 71.1 and 59.7% higher efficiency than control. In addition to microbial strains, the efficiency of the plant in bioaugmentation is determined based on several factors including the chemical nature and concentration of pollutants, reactor setup and physicochemical characteristics of the environment (El Fantroussi and Agathos 2005; Yang et al. 2019). Several new approaches were developed in bioaugmentation including bioaugmentation with nanomaterials comprehensively defined as nanobiohybrids (Guo et al. 2020), cells encapsulated/immobilized performing more efficiently compared to free system, gene modified bioaugmentation (Nzila et al. 2016), rhizosphere and plant assisted augmentation

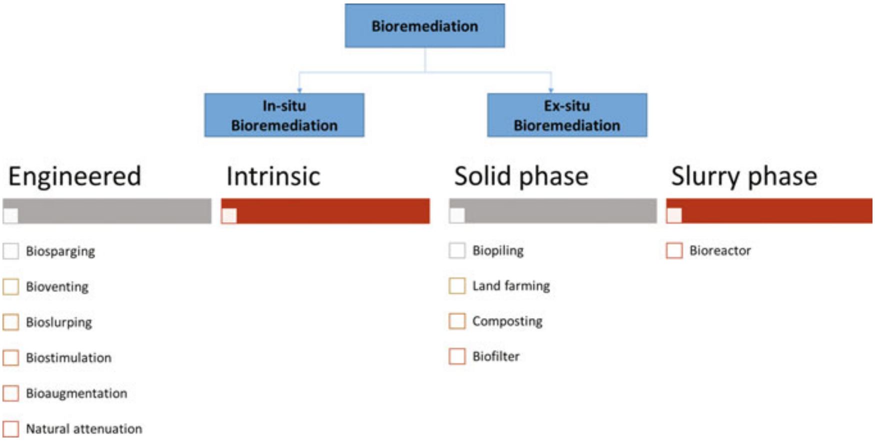

Fig. 2.1 Types of bioremediation techniques

(Itusha et al. 2019; Viji et al. 2022). Phytoremediation is one of the best techniques in bioaugmentation to remove pollutants from green plants with help of microorganisms. Microbial-enhanced sugarcane (*Saccharum officinarum*) was successfully eliminated the lindane from doped soil (Salam et al. 2017). Phytoremediation has various applications including degrading the pollutants, stabilizing the soil condition, increasing the biomass, decreasing the carbon dioxide level, and increasing the oxygen level. In the phytoremediation process, the plant roots thoroughly uptake the pollutants with the help of microorganisms in the rhizosphere. The pollutants then translocate into the upper regions of the plant for nutrient utilization and the remaining pollutants evaporate into the atmosphere through transpiration (Weyens et al. 2009; Ekta and Modi 2018).

2.3 Phytoremediation—An Overview

The term phytoremediation (Latin word) indicates the process of removal of the toxic contaminants by plant roots from the environment (Etim 2012). Generally, phytoremediation is defined as cleaning the polluted environment with natural plants. In phytoremediation, the green plants reduce the number of toxic compounds from the environment with root-associated soil microbes (Ali et al. 2013). Phytoremediation checks out the pollution of the contaminated environment through a series of processes namely phytofiltration, phytostabilization, phytoextraction, phytovolatilization, and phytotransformation, rhizosecretion. The detailed mechanism has been represented schematically in Fig. 2.2. Its mechanism includes various processes such as heavy metal uptake, metal translocation to shoots, heavy metal tolerance, exclusion of toxic compounds, vacuolar compartmentalization, production of phytochelatins, and metallothioneins (Sharma 2021). In phytoremediation,

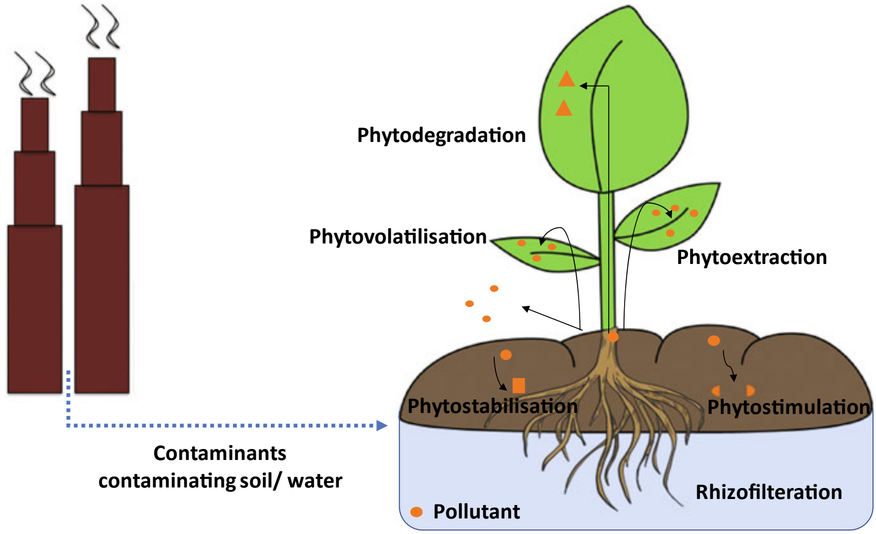

Fig. 2.2 General mechanism involved in phytoremediation by using plant roots

bioabsorption has several significant steps, including chemisorption, complexation, adsorption on surface and pore, ion exchange, micro precipitation, hydroxide condensation onto the surface (Shinomol & Bhanu 2021). Several factors including redox potential, pH, soil organic matter, clay content, nutrient balance, cation exchange capacity (CEC), concentrations of other trace elements in soil, soil moisture and temperature influences the uptake of metal from the contaminated soil (Neilson and Rajakaruna 2015). In translocating pollutants, the plant system has different tolerance processes including sequestration/ compartmentalization, binding/ chelation, excretion from aerial plant components, enzymatic and non-enzymatic antioxidants, and protection, stress recovery, and damaged protein repair (Antoniadis et al. 2017). Particularly, persistent organic pollutants have gradually become one of the most critical compound pollutants in the world due to their long-term bioavailability. Specific attention is paid to polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, organochlorine pesticides, and halo hydrocarbons.

The phytostimulation is the process of promoting microbial biodegradation in the rhizosphere. Phytotransformation is one of the most important metal absorption processes in the degradation of harmful pollutants comprising the degradation, decolourization and transformation to less toxic compounds. The phytotransformation of methylene blue from water using *Azolla pinnata* has been found to show decolourization efficiency of 85% removal validating the fact of ability of plants to transfrom organic compounds (Al-Baldawi et al. 2018). The taller plants have two different strategies for adsorbing heavy metals including exclusion (uptake of pollutants from soil to shoot through root) and accumulation of toxic compounds (detoxification of toxic compounds) (Luo et al. 2016). The accumulators can accumulate the harmful chemical compounds in the aerial parts of plants and exclude the accumulation of toxic compounds in plants' biomass. Metal uptake and translocation of toxic compounds in the plant cell membrane also involves proton pumps (ATPases that consume energy and generate electrochemical gradients), co- and anti-transporters (proteins that use the electrochemical gradients generated by ATPases to drive the active uptake of ions), and channels (proteins that facilitate the active uptake of ions). Some organic acids also enhance the uptake of metal ions from contaminated soil. Heavy metal contaminated soil treated with citric acid assisted *Brassica napus* in hydroponic method reduced significant amount of cadmium, hydrogen peroxide (H_2O_2), electrolyte leakage, malondialdehyde (MDA) accumulated in plant roots (Ehsan et al. 2014).

Nowadays, air pollution is a primary challenge for all developed nations due to urbanization, vehicles, and industrialization. This makes the ozone in the surrounding environment depleted. Various methods were discovered to remove the toxic gases (including carbon monoxide, carbon dioxide, and greenhouse gases) from the natural air. Phytoremediation is one of the best methods to remove toxic gases from a contaminated environment. *Sida hermaphrodita* is one example of the phytoremediation of toxic gas through absorption from fossil-fuel combustion (Uchman et al. 2017).

2.3.1 Plant Roots in Phytoremediation

The harmful contaminants in the environment include heavy metals and complex chemicals that the plants directly uptake by their roots and few specific plants limit the uptake amount of heavy metals such as arsenic since its toxicity limit is 40 and 200 mg /kg in sandy and clay soils. The plants involved in phytoremediation adopt different mechanisms including passive uptake through the apoplast, direct transcellular transport from the environment to the plant vascular system, and active uptake through the symplast by plants during heavy metal uptake. The uptake of trace metal elements is controlled by root factors, including soil acidification by root exudates, translocator activity and selectivity, root membrane activity, avoidance strategy mechanisms, the release of redactors or oxidants, chelator root excretion, phytosiderophores, acids, and hydrogen ions (Vithanage et al. 2012).

Rhizosecretion is one of the essential roles of plant roots involved in phytoremediation. In this process, the valuable natural products, including enzymes, alcoholic compounds, and other substances, were secreted by plant roots (Takkar et al. 2022). The plant roots exudate compounds with specific biological activity in low quantity. Plant exudates are in multiple forms of sugar, alcohol, and acids. The exudates are annually produced ranging from 10−20% by photosynthetic plants. The exudates level is identified by the chemical or chromatographic method due to the low amount of secreted compound. The secretion of exudates is affected by various factors, including plant species, age of the plant, temperature, light, plant nutrition, soil microorganism, medium composition, soil moisture, and root damage. The primary source of exudates is the tip of the root (Antoniadis et al. 2017). Some of the essential enzymes including laccases, dehalogenase, nitroreductases, nitrilases, and peroxidases were secreted by the plant for degrading the organic chemical compounds. The xenobiotic compounds are also degraded by the exudates of plants (Hussain et al. 2018). The rhizospheric microorganisms help to improve the uptake of metal by their enzymatic action on these metals (Kumar et al. 2017). With root exudates, microbial biodegradation is stimulated to minimize the toxicity of the chemicals. The microbial interaction of plants was highly influenced in phytoremediation to remove the pollution from contaminated soil and water. The plant growth-promoting rhizobacteria promote nitrogen fixation, phytohormone production, specific enzymatic activity, and plant protection (Moola et al. 2021). The energy is more important for uptake of the base metals (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) through the active transport mechanism. However, organic chemicals can easily be diffused into the plant root cells by a passive transport mechanism. If the substrate concentration is low, the degradation can be explained by Michaelis−Menten kinetics. The first-order reaction is considered, while the higher substrate concentrations are limited. According to Monod kinetics, bacteria can be used as a substrate for development, and the growth of substrates is better at higher availability. However, it was noted that bacteria have limited degradation capability if the substrate's concentration is minimal, which can prohibit the biodegradation of contaminated sites. Combining with the phytoremediation potential of plants, bacteria can therefore be used to overcome problems with environmental pollutants. (Hussain et al. 2018). Examples of some plant species and their root system utilized for enhanced remediation of inorganic and organic pollutants are depicted in Table 2.1.

2.3.2 Adventitious Roots in Phytoremediation

The adventitious roots develop from various tissues, cells, and neighboring cells of vascular tissues. Besides, they develop in the postembryonic stage of plant growth (Geiss et al. 2018). Plants generally adapt to their native natural environment, including water availability and micro-and macronutrient availability, concentration, and localization. Hence, the plants change their organ and tissues depending upon the environmental conditions. The adventitious roots are also a modification of the shoot by the plant's adaptation mechanism (Bellini et al. 2014). The growth of adventitious roots is inhibited when an excess amount of heavy metal is accumulated in the surrounding soil environment. The inhibition is high when the solute potential of the external medium is high that may deny the plant from nutrient uptake. Direct inhibition of enzymes that are of physiological importance, and inhibition of mitotic division of the meristematic cells may also occur (Odjegba and Fasidi 2004). *ThMT3* gene expression in the transgenic *Salix matsudana* increases the tolerance of copper stress and nitric oxide (NO) production (Yang et al. 2015). A high concentration of copper (5−500 mM) causes leaf toxicity, strongly affects adventitious root formation and produces ethylene at higher levels in *Populus alba*'s shoot culture (Franchin et al. 2007). The aquatic adventitious roots have the photosynthetic ability

Table 2.1 Examples of the plant species and their root system utilized for enhanced remediation of inorganic and organic pollutants

S.no	Plant species	Type of root system	Uptake elements	References
1.	*Armoracia rusticana*	Hairy root system	Uranium	Soudek et al. (2011)
2.	*Pteris vittata*	Hairy root system	Arsenic and chromium	Kalve et al. (2011)
3.	*Eleocharis acicularis*	Rhizome	Cu, Zn, As, and Cd	Sakakibara et al. (2011)
4.	*Brassica juncea* L	Hairy root system	Zinc and nickel	Ismail et al. (2012)
5.	*Nicotiana tabacum*	Hairy root system	Arsenic	Talano et al. (2014)
6.	*Brassica napus HR and Pantoea sp. FC 1*	Hairy root system	Chromium	Ontanon et al. (2014)
7.	*Sorghum bicolor* × *sudanense*	Adventitious roots	As, Cu, Pb and Zn	Babu et al. (2014)
8.	*Ipomoea aquatica*	Adventitious roots	Copper	Chanu and Gupta (2014)
9.	*Nicotiana tabacum*	Hairy root system	2,4-Dichlorophenol	Angelini et al. (2014)
10.	*Nicotiana tabacum*	Hairy root system	Copper	Perez-Palacios et al. (2015)
11.	*Solanum nigrum L*	Tap-root	Cadmium	Ji et al. (2015)
12.	*Alyssum bertolonii*	Hairy root system	Nickel	Ibañez et al. (2016)
13.	*Hyptis capitate*	Hairy root system	Copper	Malik et al. (2016)
14.	*Adenophora lobophylla A. potaninii*	Hairy root system	Cadmium	Malik et al. (2016)
15.	*Moringa oleifera Typha latifolia Cymbopogon proxmus*	Hairy root system	Cadmium	Ghada et al. (2017)
16.	*V. zizanioides*	Hairy root system	Arsenic	Moogouei (2018)
17.	*Vetiveria zizanioides*	Hairy root system	Nitrate	Moogouei (2018)
18.	*Amaranthus chlorostachys*	Hairy root system	Metformin and cesium	Moogouei (2018)
19.	*Elodea canadensis*	Adventitious roots	Cobalt	Mosoarca et al. (2018)
21.	*Lemna minuta Kunth*	Hairy root system	Cr(VI)	Paisio et al. (2018)
22.	*Brassica napus*	Hairy root system	Chromium	Perotti et al. (2020)

through the uptake of oxygen microelectrode and CO_2. The adventitious root system of *Meionectes brownie* has the maximum rate of photosynthesis (0.38 μmol O_2 $m^{-2}s^{-1}$) (Rich et al. 2011). The function of the adventitious root system is based on the location of adventitious roots in plants. If the adventitious root generates from the stem, they help in tolerating the biotic stresses (Steffens and Rasmussen 2016). When there is flooding, it affects the plant that produces the aquatic adventitious roots due to the absorption of air (Ayi et al. 2016).

2.3.3 Hairy Roots in Phytoremediation

The hairy root (HR) system of the plants plays a major role in phytoremediation during the metal uptake. Generally, HRs have fast growth and development than normal root systems in natural plants. It have potential energy in the phytoremediation process (Majumder and Jha 2012). The root system has several processes to remove the pollutants, including adsorption, transport, translocation, hyperaccumulation or transformation, and mineralization. The carrot's hairy root culture was eliminating more than 90% of exogenous phenolic compounds from contaminated culture medium within 120 h (Zhou et al. 2013). The hairy root culture of *Brassica napus* removed 97–98% of 2, 4-dichlorophenol within 1hr treatment (Agostini et al. 2003). The root system of *Vetiveria zizanioides* has reached a high amount of Pb (2280 mg kg^{-1} DW) in 5000 mg Pb kg^{-1} of contaminated soil (Chen et al. 2004). *Agrobacterium rhizogenes* strain ATCC 15,834 infected *Helianthus annuus* has fast-growing hairy root culture and that catalyzes the disappearances of antibiotics including tetracycline (TC) and oxytetracycline (OTC) from the aqueous medium (Gujarathi et al. 2005). The hairy root culture induced by *A. rhizogenes* (NCIM 5140) in explants of *Sesuvium portulacastrum,* removed the harmful chemicals (Reactive green 19A HE4BD) from textile effluents within 5 days incubation periods (Lokhande et al. 2015). The normal root system of *Canna indica Linn* removed 41–55% of triazophos (O, O-diethyl-O-(1-phenyl-1, 2, 4-triazole-3-base) sulfur phosphate, TAP) in a hydroponic system (Cheng et al. 2007). *Vetiveria zizanoides* removed the phenolic content*s* through hydroponics technology (Singh et al. 2008).

2.3.4 Remediation with Microorganisms

Few microorganisms could act as biofertilizers in polluted soil and water. Naturally, plants interact with microorganisms for improved nitrogen fixation. Recent developments in bioremediation approaches have been made during the last 20 years with the intention of restoring damaged areas rapidly and inexpensively (Balloi et al. 2010; Leong and Chang 2020). Examples of some microbes involved in heavy metal bioremediation has been summarized in Table 2.2. Remediation through bioaugmentation with *Bacillus* sp., *Lysinibacillus* sp., or *Rhodococcus* sp. diminished

Table 2.2 Example of microbes are mostly involved in degrading pollutants

S.no	Microbes	Pollutant	References
1.	*Kocuria flava*	Copper	Achal et al. (2012)
2.	*Stenotrophomonas sp.*	Copper	Zaki and Farag (2010)
3.	*Enterobacter sp.*	Copper	Bestawy et al. (2013)
4.	*Desulfovibrio desulfuricans*	Nickel	Ockjoo et al. (2015)
5.	*Enterobacter cloacae*	Nickel	Banerjee et al. (2015)
6.	*Acinetobacter sp.*	Zinc	Tabaraki et al. (2013)
7.	*Desulfovibrio desulfuricans*	Cadmium	Ockjoo et al. (2015)
8.	*Enterobacter cloacae*	Lead	Banerjee et al. (2015)
9.	*Pseudomonas aeruginosa*	Lead	Ahmady-Asbchin et al. (2015)
10.	*Termitomyces clypeatus*	Chromium	Fathima et al. (2015)
11.	*Trametes versicolor*	Zinc	Sahan et al. (2015)
12.	*Pleurotus mutilus*	Uranium	Mezaguer et al. (2013)
13.	*Pleurotus ostreatus*	Lead	Zhang et al. (2016)
14.	*Aspergillus lentulus*	Lead	Mishra and Malik (2012)
15.	*Candida parapsilosis*	Mercury	Muneer et al. (2013)
16.	*Bacillus cereus*	Cadmium	Jan et al. (2015)
17.	*Cupriavidus metallidurans CH34*	Chromium	Alviz-Gazitua et al. (2019)
18.	*Pseudomonas aeruginosa and Bacillus cereus*	Cadmium	Nath et al. (2018)
19.	*Pseudomonas aeruginosa*	Cadmium	Chellaiah (2018)
20.	*Bacillus megaterium*	Boron, lead, and cadmium	Esringu et al. (2014)
21.	*Bacillus licheniformis*	Mercury	Muneer et al. (2013)
22.	*Enterobacter cloacae*	Mercury	Al-Garni et al. (2010)
23.	*Enterobacter, Klebsiella, Leifsonia, Bacillus*	Zinc	Jain et al. (2020)
24.	*Rhizophagus clarus*	Mercury	Gontia-Mishra et al. (2016)
25.	*Trichoderma virens*	Cadmium	Khanna et al. (2019)

the metal contents of the HM-induced contaminated soils, according to Fauziah et al. (2017). The soil microbes have the efficiency of remediation to degrade the harmful chemical compounds. *Burkholderia fungorum* DBT1 is a soil microorganism that transforms dibenzothiophene, phenanthrene, naphthalene, and fluorine (Andreolli et al. 2013). *Lolium multiflorum* plant system degrades the petroleum hydrocarbons (Arabian medium crude oil) with the help of petroleum-degrading microorganisms including *Glomus intraradices* (AMF), *Sphingomonas paucimobilis* (bacterium), and *Cunninghamella echinulata* (filamentous fungi). Phytoremediation of metals in maize plants is successful with the addition of *Bacillus licheniformis* and *Rhodotorula dairenensis* (García et al. 2013). The plant's endophytic microorganisms produce economically valuable substances and plant growth-promoting substances such as phytohormones, plant growth regulators, and host protecting molecules. Various endophytic genera were found in plants, including *Serratia, Enterobacter, Acinetobacter, Agrobacterium, Bacillus, Herbaspirillum,* and *Klebsiella* strains. The endophytic microorganisms can improve plant growth by enhancing nutrient acquisition, water uptake, and decreased oxidative stress enzyme activities in host plants grown in contaminated soils (Feng et al. 2017). The bacteria's resistance to heavy metals like zinc and copper will be evaluated based on biochemical investigations of the bacteria. Some biodegradative bacteria were degrading the toxic chemical substances like herbicides, pesticides, refrigerants, solvents, and other organic compounds to beneficial plant growth substances (Zhang et al. 2020). The soil microbes participate in various remediation processes, including soil formation, energy transfer, and nutrient cycling. Through these processes, the microorganisms can promote revegetation and increase the stability of polluted ecosystems. But a high concentration of pollutants reduces biomass, population number, and diversity (Li et al. 2012). Soil microorganisms and plant root-associated bacteria were enhancing the production of plant root exudates, including organic molecules. Heavy metal phytotoxicity can be decreased by altering the bioavailability of metals in soil and preventing metal translocation through phytoremediation (Ma et al. 2016). The plant-associated microorganisms have the capacity of absorbing pollutants and promote plant growth through several direct and indirect mechanisms. The plant-associated microorganisms can produce plant growth regulators, including auxins, cytokinins, and gibberellins (Weyens et al. 2015). Bacterial and fugal strains degrade petroleum hydrocarbons from contaminated soil. From this study, the maximum phytoremediation rate was reported as 0.51 mg of TPH g^{-1} of dry plant d^{-1} at 60 days of culture and 90% of hydrocarbon was removed from the contaminated soil (Escalante-Espinosa et al. 2005). *Lolium perenne* plant was inoculated with microbial strains of *Bacillus subtilis, Sphingobacterium multivolume, Acinetobacter radioresistance, Rhodococcus erythropolis,* and *Pseudomonas fluorescens* for remediation of petroleum-contaminated soil. From this study, the maximum degradation rate was achieved up to 58% after 162 days from inoculation (Tang et al. 2010).

2.4 Biochar Mediated Remediation

Biochar is gaining remarkable attention in bioremediation techniques over years owing to its significant physicochemical characteristics. The porous surface, functional groups, surface area and charge properties of biochar enhances it use as a stand-alone bioadsorbent for various pollutants viz organic, petroleum, dyes, heavy metals, etc. (Cheng et al. 2021). Being produced from biomass like agricultural residues, biosolids, municipal solid wastes, it is carbon rich product possessing the heterogeneous characteristics mainly depending on feedstock and reaction conditions (Selvam and Paramasivan 2022). As concerned to the scope of this chapter, literary reports on biochar for bioremediation validate it to be cost-effective and sustainable bioadsorbent compared to commercial adsorbents. The effectiveness of its adsorption has been proved for antibiotics, hydrocarbons, pesticides and metal contaminants. The proposed mechanism for the biosorption is primarily upsurging the microbial community to degrade the contaminants in amended soils. In addition to that, biochar efficiently adsorbs contaminants like heavy metals and decreases its bioavailability and toxicity (Karppinen et al. 2017). Song et al. (2021) reported that bacteria immobilized on biochar in polycyclic aromatic hydrocarbon contaminated soil increased the removal rate of the contaminant compared with the individual applications. Bioremediation efficiency is improved significantly by the biochemical material especially the biochar being immbolized with plant growth promoting bacteria drives soil micro-ecology and enhances the remediation pathway with this accumulator system (Wu et al. 2019). The studies on utilizing biochar with phytoremediation report that amendment of biochar reduces heavy metal and metalloid bioavailability. However, few plants need elevated doses to accumulate the bioavailable metals and metalloids (Narayanan and Ma 2022). Summarizing these entries, advantages of biochar for bioremediation involve improved physicochemical properties of contaminated soil, enhanced microbial activities and population, and improved agricultural productivity.

2.5 Conclusion and Future Perspective

Using phytoremediation technology, heavy metal uptake by roots seems to be a prosperous way to remediate the polluted environment. Microbial remediation and phytoremediation are reliable, cost-effective, efficient, eco-feasible alternatives. When compared with other conventional methods, these methods are better biological tools to remediate polluted conditions. However, several factors must be considered to accomplish better remediation results. The future perspectives to enhance the research includes the following.

- The technologies providing low-cost solution and eco-friendly to remediate contaminates has to be explored further.
- Also, focus on developing agricultural techniques to enhance remediation efficiency to reduce the time and cost utilized in the removal of heavy metals from soil to a considerable level for the well-being of mankind and nature has to be enhanced.
- Exploring the plants that has higher biomass, environmental affordability and high heavy metal accumulation potential for overcoming the associated limitations of phytoremediation.
- Eco-friendly additives with phytoremediation for enhancing the process efficiency and simultaneous enhancement of soil structure has to be explored more.

- Most of studies so far reported is based on laboratory scale. Translative research on field demonstration and its execution has to be implemented.

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Conflicts of Interest The authors declare that they have no conflict of interest.

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