



Rhizoremediation of Heavy Metal- and Xenobiotic-Contaminated Soil: An Eco-Friendly Approach

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Abstract

In the last few decades, staggeredly increasing human population, indiscriminate use of pesticides, polycyclic aromatic hydrocarbons, and discharge of effluents containing toxic heavy metals, dyes, etc. have negatively impacted the soil fertility and biodiversity to the extent of converting the agricultural land into barren land. These pollutants can be removed or altered by conventional and physical methods. However, these methods are neither cost-effective nor eco-friendly as they generate large amount of toxic intermediates. The most powerful eco-friendly approach is bioremediation. Microorganisms play a pivotal role in the maintenance and sustainability of any ecosystem. They are versatile and capable to adapt to any challenges posed by the environment. The biotic activities of a diverse group of bacterial populations residing in rhizosphere affect the dynamics of soil which in turn affect the crop productivity. They stimulate plant growth by making soil nutrients available to them, stimulating the production of various plant growth hormones, protecting the plants from pathogens by their antagonistic activity and remediation of the pollutants by sequestering toxic heavy metals, and degrading xenobiotic compounds such as pesticides, PAHs, etc. This chapter highlights the role of PGPR in remediation of heavy metal- and xenobiotic-contaminated soil for sustainable crop production.

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1 Introduction

The soil fertility and biodiversity have been adversely affected, in the last few decades, due to continuously increasing human population, indiscriminate use of pesticides, polycyclic aromatic hydrocarbons, and discharge of effluents containing toxic heavy metals, dyes, etc. Industries such as plating, mining, petroleum, paint, automotive, and battery manufacturing industries, printing circuits and chemical manufacturing industries, etc. are the major sources of pollutants in the environment. As per 2009 estimates, approximately 33,900 million liter per day (MLD) urban waste water and 23,500 MLD industrial waste water were produced in India which contaminated the water and soil resources (Saha et al. 2017). According to CEPI (Comprehensive Environmental Pollution Index) rating, 43 critically polluted industrial areas or clusters or zones have been declared in 16 states by CPCB (Central Pollution Control Board) that have more than 70 CEPI rating. Among the 43 zones, 21 sites have been reported in four states, i.e., Gujarat, Uttar Pradesh, Maharashtra, and Tamil Nadu (Saha et al. 2017).

These pollutants have adversely affected human health, plants, and associated soil microorganisms. These toxic compounds accumulate in the agricultural soils in which they are applied, threaten food security, and pose health risks to living organisms by their transfer within the food chain. These pollutants accumulate in different parts of the body such as the muscle, bones, brain, and kidney and cause several harmful diseases like headache, nausea, vomiting, gastrointestinal tract disorders, asthma, allergy, cancer, heart attack, renal failure, nervous disorder, liver toxicity, Alzheimer's diseases, and muscle weakness.

These toxic pollutants can be removed or altered by conventional and physical methods such as membrane separation, chemical precipitation, reverse osmosis, solvent extraction, filtration, flocculation, electro dialysis, electrochemical treatments, ion-exchange, advanced oxidation process, and adsorption by using nanocomposite materials (Saravanan et al. 2019). However, the conventional (physical and chemical) methods are complicated and expensive, require large energy consumption, and generate large amount of toxic intermediates responsible for secondary pollution. Moreover some techniques are not useful in removing the high concentration of contaminants. This has led to search for a cost-effective alternative method. Bioremediation is the most powerful eco-friendly approach. It is the biological process that detoxifies, removes, and immobilizes the hazardous pollutants from the environment by the use of naturally occurring bacteria, fungi, or plants. It is a very useful technique for restoration of contaminated soil. The concept of using plants for the treatment of pollutants has emerged a few years ago. When plants remove, destroy, and stabilize the contaminants from soil, it is referred to as

phytoremediation. This is a cost-effective and eco-friendly method, firstly investigated by Chaney (1983). This process removes the pollutants from soil by plants without affecting their biological activity, fertility, and structure of soil. It is also known as green remediation and agro-remediation (Pivetz 2001). The process of phytoremediation includes phytoextraction, phytoaccumulation, phytostabilization, phytostimulation/rhizostimulation, phytovolatilization, and rhizofiltration (Akpor and Muchie 2010).

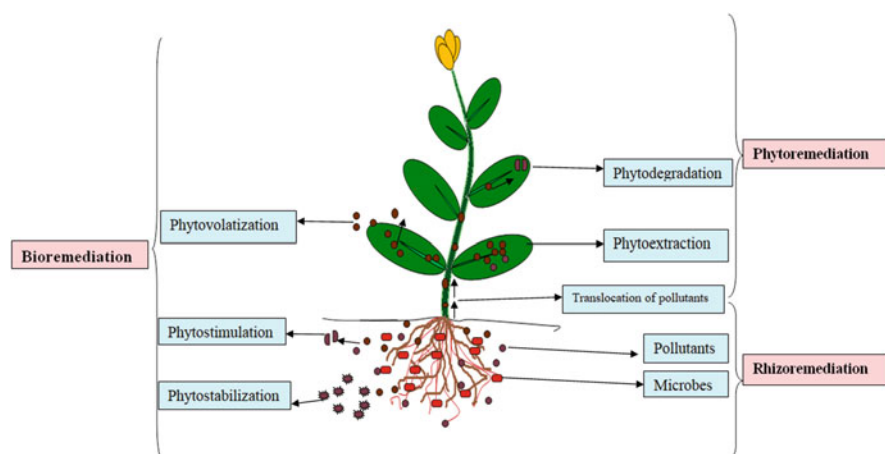
Though phytoremediation reduces the contamination of many hazardous pollutants, the large-scale field application requires long time period. It suffers serious limitations by pollutant-induced stress which affects seed germination, plant development, and plant biomass. The use of rhizospheric microbes is the alternative way for reducing deleterious effect of pollutants to plants (Burd et al. 2000). The remediation of pollutants by rhizospheric microbes is called rhizoremediation (Kuiper et al. 2004). The rhizospheric bacteria have the extraordinary ability to promote the growth of plant by various mechanisms such as nitrogen fixation, solubilization of minerals, production of plant growth regulators and siderophores, and transformation of nutrient elements. Generally, the plant is not directly involved in the bioremediation process. The rhizobacteria can directly carry out the remediation process. Both endophytic and rhizospheric microbes have the potential to carry out the remediation of pollutants. Thus, among the various methods for the removal of pollutants, rhizoremediation is the most potential eco-friendly approach in which pollutants are removed and degraded by the interaction of microbes and plant roots. Plants provide the essential nutrients to microbes, while microbes convert the toxic compound into nontoxic minerals and make them available for plants. So, microbes enhance the soil health by addition of nutrients. These rhizobacteria play an important role in biological restoration and reclamation of contaminated sites. This process completely destroys pollutants and converts hazardous compounds into harmless products, which also eliminates the possibility of future liability associated with the treatment and disposal of contaminated materials (Abtenh 2017). A consortium of microbes detoxifies the pollutants more efficiently rather than single strain or species. This chapter focuses upon the rhizoremediation of heavy metals and xenobiotics from environment.

2 Phytoremediation

It is a Latin word in which *phyto* means plant and the *remedium* means restoring balance which maintains a permissible concentration of toxic material in the environment. It is a promising and emerging area of interest because it is an environmentally safe method for restoration of barren land. 0.2% of angiosperms tolerate and accumulated high concentration of metals referred as hyperaccumulators (Baker and Brooks 1989). There are several examples of plants which act as hyperaccumulators like *Alyssum* species, *Arabidopsis halleri*, *Astragalus racemosus*, *Brassica juncea*, *Noccaea* sp., and *Viola calaminaria* (Ganesan 2012). Plants use various mechanisms to remove pollutants from soil (Table 1, Fig. 1).

Table 1 Mechanisms used for bioremediation of for heavy metals and xenobiotics

Mechanism	Pollutants	Plants	References
Phytovolatilization	Hg	<i>Chara canescens</i> (musk grass) and <i>Arabidopsis thaliana</i>	Ghosh and Singh (2005)
Phytoextraction	Pb As	<i>Glycine max</i> L. <i>Pteris vittata</i>	Aransiola et al. (2013), Xie et al. (2009)
Phytodegradation/ phytotransformation	Total Petroleum carbon (TPH)	<i>Scirpus grossus</i>	Al-Baldawi et al. (2015)
Phytostimulation/ rhizodegradation	Biodiesel Phenanthrene and pyrene	<i>Pisum sativum</i> Kandelia candel	Hawrot-Paw et al. (2019) Zhang et al. (2011)
Phytostabilization	As	<i>Piricum sativum</i>	Jonnalagadda and Nenzou (1997)
Rhizoremediation	Cr Lindane PHCs	<i>Helianthus annuus</i> <i>Withania somnifera</i> <i>Lolium perenne</i>	Gupta et al. (2018) Abhilash et al. (2011) Kukla et al. (2014)

**Fig. 1** Bioremediation of pollutants

These are the following. (1) Phytoremediation—It is the uptake of pollutants from soil by root system which are then converted into volatile form and released out in the atmosphere. (2) Phytoextraction—It is the translocation of pollutant by root from soil which gets accumulates in as hyperaccumulators, and once the pollutant has reached the saturation limit, then plants are cut and disposed of. (3) Phytostabilization—It is the immobilization of pollutant in soil by absorption and precipitation. (4) Phytodegradation—It is the degradation and mineralization of pollutants via the metabolic processes of plants after uptake from the soils.

(5) Phytostimulation—It is the degradation of contaminants by microbial activity. Various types of xenobiotics are degraded by the process of phytoremediation. Although phytoremediation is a promising approach, it has some limitations which include the following: (1) long time period is required for remediation; (2) rate of plant growth and accumulation should be fast for reduction of time period; and (3) efficiency of phytoextraction is dependent upon translocation and bioconcentration; some plants do not allow translocation; and (4) the accumulation of higher concentration of pollutants by plant affects the growth and development of plants. The transgenic plants have been used to improve remediation, but they inhibit the growth of native plants by absorbing excess nutrients from soil.

3 Rhizoremediation

The word, “rhizoremediation” is composed of two words, *rhizo* (a latin word which means roots) and remediation (which means the process of removal of pollutants). It is a natural process and cost-effective and highly efficient method in which pollutants of soil are remediated by rhizospheric bacteria (Kala 2014). Rhizoremediation is a mutual relation between plant roots and soil microbes in the rhizosphere in which plants provide essential nutrients in the form of root exudates to microbes, and in return plants get protection from pathogens and help in nitrogen fixation. The plant roots secrete some substances such as sugars, organic acids, sterols, and amino acids which are utilized by the microbes for degradation of pollutants (Kuiper et al. 2004; Saravanan et al. 2019). These root exudates make a nutrient-rich environment in the rhizosphere where microbial metabolic activity and number are too high. The root exudates play an important role in rhizoremediation because these root exudates attract a specific microbial group. Rhizoremediation is enhanced by expanded root system of plants which provide large surface area for microbes which help in effective uptake and removal of contaminants (Praveen et al. 2019). This process is the combination of bioaugmentation and phytoremediation, which solves the problems that arise during the use of individual methods (Bhatia and Malik 2011). Thus, this process benefits the plants to enhance plant growth by making soil nutrients available to them, stimulating the production of various plant growth hormones, and protecting the plants from pathogens by their antagonistic activity and remediation of the pollutants by sequestering toxic heavy metals and degrading xenobiotic compounds such as pesticides, PAHs, etc. In the process of rhizoremediation, the rhizospheric microbes change the bioavailability of metals by changing the pH of soil, secretion of chelating agents (organic acids, siderophores, etc.), and oxidation/reduction reactions (Rajkumar et al. 2012). Plant growth-promoting rhizobacteria (PGPR) along with mycorrhizal and arbuscular mycorrhizal fungi (AMF) carry out remediation. The fungi spread around the plant roots increase competition for uptake of pollutants and reduce the uptake of pollutants by plants (Praveen et al. 2019). The microbial diversity and activity in the rhizosphere of plants are much more than that in bulk soil. Microbes residing in the rhizosphere of plant are more potent to degrade or transform pollutants than the microbes of bulk

soil (Kamaludeen and Ramasamy 2008). The microbes of rhizosphere form a mutual association with root which has potential for detoxification of hazardous pollutants (De Souza et al. 1999).

Thus, there are many advantages of rhizoremediation like it is a cost-effective and eco-friendly approach, no need to add any chemical for degradation, and negligible side effects while chemical degradation adversely affect the environment. The efficient microbial strain and consortium has to be provided, and no investment is required (Saravanan et al. 2019). In addition to phytoremediation, rhizoremediation can be a better alternative for the removal of pollutants such as heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), etc.

3.1 Heavy Metals

Metals are the natural components of soil. Some of them are essential and required for various metabolic activities. Zn, Fe, Mg, Mn, Cu, Cr, Ni, Na, and Ca are essential micronutrients. Fe^{3+} is essential for all bacteria, while Fe^{2+} is required for only anaerobic bacteria and other metals such as Cd, Hg, Au, and Ag are nonessential and very toxic to soil microbes and other organisms. Heavy metals occur in soil as free cations in the form of complex with organic/inorganic ligands and soil colloids. Now pollution of heavy metals has become a severe problem for the environment. The increasing anthropogenic activities like industrial waste disposal, waste incineration, vehicle exhausts, energy and fuel production, mining and smelting of metals, long-term application of urban sewage *sludge*, and excessive use of agrochemicals and pesticides, etc. are the major source of heavy metal contamination which poses serious threat to soil ecosystem and human health (Khan 2005).

According to the report of Central Pollution Control Board (CPCB), in India, the states of Gujarat, Maharashtra, and Andhra Pradesh have 80% contribution in the generation of toxic pollutants including heavy metals. The report of Ministry of Mines, Government of India (2018), suggested that India is the major source of different types of metallic and nonmetallic minerals. Maximum numbers of mines are present in Tamil Nadu and Madhya Pradesh (Kumar et al. 2019). High concentration of heavy metal in soil are absorbed and accumulated by plant which ultimately reaches into the human body (Mishra et al. 2017) via food chain. The nonbiodegradable nature of these toxic heavy metals results in their persistence for long period in the environment. Even a very low concentration of these metals enters into the human body via contaminated food and contaminated drinking water and gets accumulated in animals and plants which can have lethal effects.

Various physical and chemical methods such as excavation and stabilization or solidification are used for removal of heavy metals, but these technologies have some demerits like less cost-effective, generation of hazardous products, difficult to operate, and cannot remove heavy metals permanently (Akshata et al. 2014). The remediation of heavy metals by phytoremediation takes a long time period while the addition of suitable microbes increases the rate of remediation. Microbes

reduce the toxicity of metals by converting the metals from toxic to nontoxic form, by stabilizing the metal in rhizosphere so that pollutants cannot be used by plants and enhance the degradation of metals by increasing bioavailability.

The rhizosphere region of plants which is growing in heavy metal-contaminated soil harbors a large population of microbes (Idris et al. 2004) which are able to tolerate high concentration of metals. They convert toxic metals into bioavailable form in soil by mechanisms like precipitation, chelation, acidification, redox reactions, and complexation (Ma et al. 2016). These microbes have developed various resistance mechanisms for survival in the presence of heavy metal-contaminated soil such as bioabsorption, bioaccumulation, bioleaching, biotransformation, biomineralization, and plant-microbe interaction. Besides this PGPRs also act as biofertilizer which enhance the yield of crops by efficient way of heavy metals remediation in soil (Ganesan 2012).

Madhaiyan et al. (2007) reported that *Methylobacterium oryzae* strain CBMB20 and *Burkholderia* sp. strain CBMB40 reduce the toxicity of nickel (Ni) and cadmium (Cd) in tomato by reducing uptake and translocation of heavy metals to shoot in plant and also promote the plant growth in contaminated soil. Fatnassi et al. (2015) reported that at high concentration of copper (Cu), the growth of plant *Vicia faba* (fava bean) was inhibited, but when plant was inoculated with *Rhizobium* and *Pseudomonas*, the inhibitory effect was decreased. Wu et al. (2006) observed that *Azotobacter chroococcum* HKN-5 promoted the growth of *Brassica juncea* by phytoextraction from the soil which was highly contaminated by lead (Pb). Dary et al. (2010) reported that the consortium of *Bradyrhizobium* sp.750, *Ochrobactrum cytisi*, and *Pseudomonas* sp. protected and promoted the growth of *Lupinus luteus* in multi-metal-contaminated soil Cu, Cd, and Pb by the process of phytostabilization and also helped in the reclamation of soil. The rhizobacteria *Pseudomonas aeruginosa* and *Burkholderia gladioli* were reported to facilitate the plant growth of *Lycopersicon esculentum* seedlings by reducing the accumulation of Cd metal due to immobilization of metal on the root that prevented the translocation of metal towards shoot and reduce the toxicity of Cd by decreasing bioavailability (Khanna et al. 2019). Wani et al. (2008) reported that *Bacillus* sp. PSB10 improved the growth, nodulation, chlorophyll seed yield, etc. of chickpea grown in chromium-contaminated soil by reducing the uptake and toxicity of contaminant from soil. Another study was performed by Gupta et al. (2018) on sunflower (*Helianthus annuus* L.) in which inoculation of strain *Pseudomonas* sp. CPSB21 reduced the Cr6+ toxicity and enhanced the plant growth and nutrient uptake. Some examples of microbes involved in rhizoremediation of heavy metals are listed in Table 2.

3.2 Pesticides

Pesticides are organic chemical which were introduced in agriculture for increasing agricultural yield and soil productivity, to protect agricultural products from crop pests, and to control insect vectors for prevention of outbreaks of human and animal epidemics. Pesticides are used for many purposes such as livestock farming,

Table 2 Remediation of heavy metals by rhizobacteria

Plant	Pollutant	Microbes	References
<i>Brassica juncea</i>	Pb	<i>Azotobacter chroococcum</i> HKN-5	Wu et al. (2006)
<i>Glycine max</i>	Mn	<i>Glomus etunicatum</i> or <i>G. macrocarpum</i>	Nogueira et al. (2007)
<i>Pteris vittata</i>	As	<i>Pseudomonas</i>	Huang et al. (2007)
<i>Zea mays</i>	Pb, Zn, Cu	<i>Brevibacterium haloterans</i>	Abou-Shanab et al. (2008)
<i>Brassica juncea</i>	Ni	<i>Pseudomonas</i> sp., <i>Bacillus megaterium</i>	Rajkumar and Freitas (2008)
<i>Brassica juncea</i>	Cu	<i>Achromobacter xylooxidans</i> strain Ax10	Ma et al. (2009)
<i>Cicer arietinum</i>	Cr	<i>Bacillus</i> sp. PSB10	Wani and Khan (2010)
<i>Helianthus annuus</i> , <i>Ricinus communis</i>	Ni	<i>Psychrobacter</i> sp. SRS8	Ma et al. (2010)
<i>Lupinus luteus</i>	Cu, Cd, Pb	<i>Bradyrhizobium</i> sp.750, <i>Pseudomonas</i> sp., and <i>Ochrobactrum cytisi</i>	Dary et al. (2010)
<i>Orychophragmus violaceus</i>	Zn	<i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Flavobacterium</i> sp., <i>Pseudomonas aeruginosa</i>	He et al. (2010)
<i>Crotalaria juncea</i>	Cd	<i>Achromobacter</i> sp. AO22	Stanbrough et al. (2013)
<i>Oryza sativa</i>	Ni	<i>Bacillus licheniformis</i> NCCP-59	Jamil et al. (2014)
<i>Triticum aestivum</i> (wheat)	Zn	<i>Pseudomonas fluorescens</i>	Sirohi et al. (2015)
<i>Vicia faba</i>	Cu	<i>Rhizobium</i> , <i>Enterobacter cloacae</i> , and <i>Pseudomonas</i> sp.	Fatnassi et al. (2015)
<i>Brassica nigra</i>	Cu	<i>Kocuria</i> sp. CRB15	Hansda et al. (2017)
<i>Leucaena leucocephala</i>	Zn, Cd	Rhizobia	Rangel et al. (2017)
<i>Suaeda nudiflora</i>	Zn, Pb	<i>Bacillus megaterium</i> , <i>Pseudomonas aeruginosa</i>	Jha et al. (2017)
<i>Helianthus annuus</i>	Cr	<i>Pseudomonas</i> sp. CPSB21	Gupta et al. (2018)

cropping, horticulture, forestry, home gardening, homes, hospitals, kitchens, roadsides, and industrial areas. Pesticides are a cost-effective method to control pest and increase agriculture productivity. But now the use of pesticides has become a serious threat as they cause many adverse effects upon environment and the human health. Residues of pesticides remain in the environment (air, soil, groundwater, and surface water) for long periods. Pesticides pollute the rivers, ponds, and lakes and also cause acid rain, which is hazardous to animals and especially amphibians

(Gilden et al. 2010). Every year around two million tonnes of pesticides are consumed worldwide, of which 24% is consumed by the United States, 45% in Europe, and the remaining 25% in the world (Abhilash and Singh 2009). Thus, three quarters of the total pesticide used worldwide (USEPA 2009) are consumed by the developed countries such as North America, Western Europe, and Japan (Yadav et al. 2015). In India, the use of pesticides started from 1948 where dichloro diphenyl trichloroethane (DDT) was used for the control of malaria and benzene hexachloride (BHC) for locusts. Now, India accounts for 3% of the world consumption of pesticide. After China, India is the second largest pesticide manufacturing country in Asia. The most common pesticides used in India are organophosphates, organochlorine, neonicotinoids, etc. The pesticides which are mostly used in India include monocrotophos, endosulfan, phorate, chlorpyrifos, methyl parathion, quinalphos, mancozeb, paraquat, butachlor, isoproturon, and phosphamidon (Bhushan et al. 2013). Ideally, according to definition a pesticide should be lethal only for targeted pests, but unfortunately, it has become lethal for nontargeted species including humans also. The persistent nature of pesticides is making the agriculture land barren.

Phytoremediation is a cost-effective technology that removes organic and inorganic contaminants used in polluted areas. But it is not an efficient technology without the contribution of rhizobacteria. Plant microbial interaction is very useful in remediation of contaminants. Romeh and Hendawi (2014) reported the degradation of organophosphorus insecticides, chlorpyrifos, chlorpyrifos-methyl, cyanophos, and malathion degraded by some phosphate-solubilizing bacteria like *Azospirillum lipoferum* and *Paenibacillus polymyxa* on mineral salt media as a carbon and phosphorus source. Various pesticides like acibenzolar-S-methyl, metribuzin, napropamide, propamocarb hydrochloride, and thiamethoxam have been reported to be degraded by *Bacillus subtilis* GB03, *Bacillus subtilis* FZB24, *Bacillus amyloliquefaciens* IN937a, and *Bacillus pumilus* SE34 in liquid culture and soil microcosm (Myresiotis et al. 2012). Some examples of microbes involved in rhizoremediation of pesticides are listed in Table 3.

The bacterial strain *Achromobacter xylosoxidans* JCp4 and *Ochrobactrum* sp. FCp1 was found to increase crop productivity by degrading chlorpyrifos from soil and promote the plant growth of *Vigna unguiculata* (Akbar and Sultan 2016). Fulekar (2014) studied the effect of consortium *Stenotrophomonas maltophilia* MHF ENV20, *Stenotrophomonas maltophilia* MHF ENV22, and *Sphingobacterium thalpophilum* MHF ENV23 on plant growth of *Pennisetum pedicellatum* and *Cenchrus setigerus*. The consortium was found to degrade pesticides chlorpyrifos, cypermethrin, and fenvalerate in soil. Jacobsen (1997) suggested that colonization of the plant roots by the herbicide-degrading *Burkholderia cepacia* DBO1 (pRO101) can protect the plant *Hordeum vulgare* by degradation of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the rhizosphere soil. Abhilash et al. (2011) reported that the integrated use of pesticide-tolerant plant *Withania somnifera* and rhizospheric microbe *Staphylococcus cohnii* decreases the concentration of lindane from soil and has practical application for in situ remediation of contaminated soils.

Table 3 Remediation of pesticides by rhizobacteria

Plant	Pollutant	Microbes	References
<i>Zinnia angustifolia</i>	Mefenoxam	<i>Pseudomonas fluorescens</i> and <i>Chryseobacterium indologenes</i>	Pai et al. (2001)
Greengram (<i>Vigna radiata</i>)	Fipronil and pyriproxyfen	<i>Pseudomonas aeruginosa</i> PS1	Ahemad and Khan (2011)
<i>Withania somnifera</i>	Lindane	<i>Staphylococcus cohnii</i>	Abhilash et al. (2011)
Grapevines (<i>Vitis vinifera</i>)	Profenofos	<i>Bacillus subtilis</i>	Salunkhe et al. (2013)
Cauliflower (<i>Brassica oleracea</i>)	Cypermethrin	<i>Serratia nematodiphila</i>	Tyagi and Prashar (2015)
Maize (<i>Zea mays</i>)	Thiamethoxam	<i>Bacillus subtilis</i> GB03) and <i>B. subtilis</i> FZB24	Myresiotis et al. (2015)
<i>Vigna unguiculata</i>	Chlorpyrifos	<i>Achromobacter xylosoxidans</i> JCp4, <i>Ochrobactrum</i> sp. FCp1	Akbar and Sultan (2016)

3.3 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated from both natural and anthropogenic processes and pose a serious concern to the health of aquatic life and humans. They are released naturally in the environment due to incomplete combustion of organic material. Due to their toxic, carcinogenic, and mutagenic effects on human health, 16 PAHs have been identified as priority pollutants by USEPA (United States Environmental Protection Agency) and monitored regularly in industrial wastes (Bisht et al. 2015; Gupte et al. 2016). Out of 16, seven priority pollutants are considered as probable human carcinogens by USEPA and IARC (International Agency for Research on Cancer) (Wang et al. 2018). There are various types of chemical methods used for removal of PAHs; however as mentioned earlier, due to their various demerits, phytoremediation and rhizoremediation are the most preferred method. Bisht et al. (2010, 2014) reported the rhizoremediation of PAHs by rhizospheric and endophytic bacteria in *Populus* sp. Some examples of microbes involved in PAH rhizoremediation are listed in Table 4.

Golubev et al. (2009) reported that *Sinorhizobium meliloti* was quite effective in degradation of PAHs besides its ability to produce surfactant and indole acetic acid (IAA). Kukla et al. (2014) reported that *Rhodococcus fascians* strain (L11) was able to degrade petroleum hydrocarbon and promoted the growth of ryegrass (*Lolium perenne* L.). Khan et al. (2014) reported that inoculation of *Pseudomonas putida* PD 1 to willow plant and ryegrass protected the plant from toxic effect by degrading phenanthrene and promoted plant growth. *Burkholderia fungorum* DBT1 along with hybrid poplar plant was reported to degrade PAHs from contaminated soil by the process of phytoextraction or rhizodegradation (Andreolli et al. 2013).

Table 4 Remediation of PAHs by rhizobacteria

Plant	Pollutant	Microbes	References
Grasses (<i>Lolium multiflorum</i>)	Naphthalene	<i>Pseudomonas putida</i> PCL1444	Kuiper et al. (2001)
<i>Festuca arundinacea</i>	PAHs	<i>Pseudomonas fluorescens</i>	Ho et al. (2007)
<i>Zea mays</i>	Phenanthrene	<i>Pseudomonas putida</i> MUB1	Chouychai et al. (2009)
<i>Populus deltoides</i>	Naphthalene and anthracene	<i>Kurthia</i> sp. SBA4, <i>Micrococcus varians</i> SBA8, <i>Bacillus circulans</i> SBA12	Bisht et al. (2010)
<i>Populus deltoides</i>	PAHs	<i>Bacillus</i> sp. SBER3	Bisht et al. (2014)
<i>Oryza sativa</i>	Phenanthrene	<i>Phomopsis liquidambaris</i>	Fu et al. (2020)

3.4 Factors Affecting Rhizoremediation

The physicochemical properties of soil play an important role in successful rhizoremediation. The microbial activity as well as diffusion of the chemicals in the soil is affected by moisture, redox conditions, temperature, pH, organic matter, nutrients and nature, and amount of clay. The effect of soil moisture on microbial mineralization of three aerobically degradable pesticides (benzolin-ethyl, isoproturon, and glyphosate) was quantified by Schroll et al. (2006). They observed a linear correlation ($p < 0.0001$). The increasing soil moisture (within a soil water potential range of -20 and -0.015 MPa) was found to increase the mineralization of pesticides.

3.4.1 Soil pH

pH of soil is an important factor that control the microbial growth and activity. Most of the microbes have optimum pH at 6.5–7.5. At particular pH, microbes secrete enzymes and organic acids that enhance the removal of pollutants. The catalytic activity of enzymes is dependent upon pH. The degradation process takes more time at acidic pH rather than alkaline and neutral pH. Singh et al. (2006) reported the biodegradation of organophosphate pesticide was found to be slow at low pH soil as compared to neutral and high pH soils.

3.4.2 Temperature

The removal and degradation rate of pollutants are affected by soil as the metabolic activity of microbes depends on temperature. Bioavailability of pollutants is increased with increasing temperature of soil. However the native microbes of low temperature soil like Arctic and Antarctic soils will degrade pollutants at low temperature. *Rhodococcus* isolated from an Antarctic soil was found to degrade various alkanes at -2 °C, while its activity was inhibited at high temperature (Oberai and Khanna 2018).

3.4.3 Soil Organic Matter

The soil organic matter is a rich source of nutrients which enhance microbial growth and thus affect the degradation of pollutants. The soil organic matters control the adsorption and desorption process. Sewage sludge which is a source of organic matter increases the biodegradation of isoproturon herbicide (Perrin-Ganier et al. 2001).

3.4.4 Plant Root Exudates

Rhizoremediation is mainly dependent on the quality of root exudates. The age of plants and presence of pollutants affect the quality and nature of root exudates which determine the microflora of the rhizosphere. It is a mixture of soluble organic compounds including sugars, amino acids, organic acids, and enzymes that influence the colonization of specific microbial community which promote the plant growth and protect the plant from soil pollutants, and microbes use root exudates for their growth and development.

3.4.5 Microbial Population

The composition of microbial community of rhizosphere can affect the rate and efficiency of rhizoremediation. There are many microbes like *Azotobacter*, *Bacillus* sp., *Pseudomonas* sp., etc. which are active colonizers of rhizosphere and have been reported to be efficient degraders of pollutants.

3.4.6 Pollutants

The nature of pollutants in soil can affect the composition of microbial population. The hydrophobic pollutants are degraded in aqueous medium such as the biodegradation of phenanthrene (Bouchez et al. 1995). The type and concentration of pollutants affect the process of rhizoremediation.

3.5 Methods to Improve Rhizoremediation

There are several limiting factors such as pH, temperature, organic matter, pollutant type, soil properties, source of energy, and microbes which slow the process of rhizoremediation. This process also depends on the capacity of plants to tolerate the maximum limit of pollutants which can be accumulated in the plants without any adverse effects to them. The efficiency of rhizoremediation can be enhanced by several techniques.

3.5.1 Biostimulation

It is a remediation method that refers to the addition of rate-limiting nutrients such as phosphorous, nitrogen, oxygen, carbon, and electron donors in the polluted soils to stimulate existing bacteria for degradation of pollutants (Tribedi et al. 2018). The activity of rhizobacteria is enhanced by the addition of fertilizers or minerals (N, P, and K) and compost in soil as they improve the root exudates. Biosurfactants improve the physicochemical property of soil and increase the bioavailability of

pollutants in soil. The use of additive such as biochar, cattle manure, and fly ash enhances the soil fertility. Biochar is known as a universal sorbent due to its strong ability to bind pollutants and has long been used as a sorbent for both organic and inorganic pollutants (Ahmad et al. 2014). Biochar increases soil fertility moisture content and nutrient retention and remediate heavy metals from soil by surface adsorption, precipitation, partitioning, and sequestration (Tang et al. 2013; Saravanan et al. 2019). Recently, biochar has been implemented to enhance phytoremediation efficiency by improving plant growth, water holding capacity, and soil structure. Qin et al. (2013) observed the total petroleum hydrocarbon (TPH) removal after applying biochar as a soil amendment.

3.5.2 Bioaugmentation

Bioaugmentation is the addition of bacterial culture to increase the rate of degradation of pollutants. The native microbes of contaminated sites are able to detoxify the contaminants. The process of rhizoremediation is also enhanced by introducing specific pollutant-degrading microbes or consortium in the soil by various methods such as seed coating, root dipping, and soil drenching. The ability of microbe to remove contaminants is limited because the catabolic pathway for some pollutants like polychlorinated biphenyls (PCBs) has not evolved in microbes. To combat this problem, genetically modified microbes are used to increase efficiency of rhizoremediation by modification of a gene (Lorito et al. 1998). The genes of siderophore receptor introduced into *Pseudomonas* sp. made it more useful in rhizosphere region (Praveen et al. 2019). The selection of suitable plant-microbe combination for efficient rhizoremediation is also quite important (Kuiper et al. 2004).

3.5.3 Transgenic Plants

For efficient rhizoremediation, transgenic plants are now used. Transgenic plants have increased ability to uptake, transport, and degrade pollutants. The plant is improved by either gene transfer or breeding. Janssen et al. (2015) have improved *Salix* sp. (Willow tree) into *S. viminalis* and *S. alba* by metal extraction. Both species have improved ability to accumulate Cd and Zn. Plants are genetically modified to improve stabilization and accumulation of the pollutants. Doty et al. (2000) reported enhanced degradation of the halogenated hydrocarbons by the introduction of cytochrome P450 2E1 gene into plants.

3.5.4 Rhizoengineering

The use of transgenic plants increases the root exudates that improve the rhizoremediation, but they are not effective for soil which is contaminated by multiple contaminants. Wu et al. (2006) utilized the advantages of plant-microbe symbiosis within the plant rhizosphere for effective cleanup technology. They demonstrated the expression of a metal-binding peptide (EC20) in *Pseudomonas putida* 06909 that not only improved cadmium binding but also decreased the cellular toxicity of cadmium and also improved the plant growth. Thus rhizoengineering becomes very important to reclaim the soil.

3.5.5 Nanotechnology

Nanotechnology is characterized by the use of very small manufactured particles (<100 nm) called as nanoparticles. The use of nanoparticles for the removal of pollutants for environment cleanup is an emerging technique that has gained attention recently. Bacteria have the ability to mobilize and immobilize the metals, and they can reduce the metals by precipitation. The removal of environmental pollutants such as heavy metals, organic and inorganic, from polluted sites by using nanoparticles/nanomaterial produced by plants, fungi, and bacteria is called nanobioremediation which has been reported to enhance the efficiency of phytoremediation or rhizoremediation (Yadav et al. 2017). Recently some studies have reported the degradation of organic contaminants such as atrazine, molinate, and chlorpyrifos by nanosized zerovalent ions (Zhang 2003; Ghormade et al. 2011). Liu and Zhao (2007) used iron phosphate (vivianite) nanoparticles for in situ immobilization of Pb^{+2} in soils and reported the reduction of bioavailability and mobility of Pb^{+2} from soil effectively. Some organic pollutants like hydrocarbons and organochlorines cannot be degraded by plant as well as microbes, but the combined use of nanotechnology and biotechnology solved this problem (Yadav et al. 2017).

4 Conclusions

The rhizoremediation is a cost-effective and eco-friendly approach that is the emerging technique for remediation of pollutants. It is the mutual interaction of microbes and plant roots that enhances the degradation of contaminants. The versatility and adaptability of microbes to any challenges posed by the environment make them perfect for remediation of environmental pollutants. In rhizoremediation the degradation of pollutant is higher than that of phytoremediation in plant and microbe alone. So rhizoremediation is the best research topic for understanding the interaction of soil-root-microbe for bioremediation of pollutants. Plants and microbes can be genetically modified for improvement of degradation ability. Thus, rhizoremediation can be an environment-friendly cleaning technique.

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