Rhizoremediation of Environmental Contaminants Using Microbial Communities

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Abstract

Over last few decades, the contamination of water and soil has become a major threat to ecosystem and human health. Bioremediation is an attractive tool to overcome the challenges posed by the traditional methods such as incineration and excavation. Recently, phytoremediation has been widely used to remediate the pollutants (such as organic and inorganic) from the environment, but certain compounds and heavy metals tend to inhibit the growth of the plants. In this chapter, we have emphasized on most accepted bioremediation process known as rhizoremediation, which involves the mutualism between microorganisms and plants that degrades the recalcitrant compounds present in the soil and makes eco-friendly environment. Furthermore, we discussed the important factors such as temperature, pH, and organic matter present in the soil, which affects the growth and metabolism of not only the organism but also the plants, interaction between plant and microorganisms, and role of endophytic and rhizobacteria in bioremediation of heavy metals and organic pollutants.

Keywords

Phytoremediation • Rhizoremediation • Endophytic bacteria • Heavy metals • Organic pollutants

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17.1 Introduction

Over the past few centuries, dramatic rise in industrialization has been witnessed leading to enhanced release of anthropogenic compounds into the natural ecosystem. A xenobiotic compound includes petroleum hydrocarbons (PHC), polycyclic aromatic hydrocarbons (PAHs), solvents, metals, pesticides, and salts. These chemicals remain persistent in nature, creating negative effect on ecosystem and human health (Prabhu et al. 2014; Gerhardt et al. 2009; Meagher 2000). Remediation of soil-contaminated sites with the help of conventional techniques such as landfilling and incineration is expensive. Methods such as incineration cause air pollution, while landfilling generates leachates in the form of gases and liquids that can contaminate ground water, and the excavation of soil/land can lead to the generation of toxic air emissions (Kuiper et al. 2004). Hence, there is a need of an hour for alternative methods for restoring the polluted sites that is less expensive, less labor intensive, and eco-friendly. In last few years, bioremediation and phytoremediation have emerged as an alternative method to the previously existing conventional methods. It involves microbes and other biological components to degrade harmful pollutants from the environment (Caplan 1993; Dua et al. 2002). Bioremediation can be applied in situ without the removal and transportation of polluted soil and without causing any disturbance to the soil matrix. Another advantage is that the bacterial degradation of chemicals and pollutants usually results in complete breakdown and mineralization (Heitzer and Sayler 1993).

In situ bioremediation process such as biostimulation, monitored natural attenuation (MNA), bioaugmentation, and phytoremediation (including rhizoremediation) has been used to restore and rehabilitate the contaminated sites. However, one remediation technology is not enough to treat the on-site pollutant as it depends on the contaminant type and source of the contaminant/pollutant (Truu et al. 2015). In recent years, two different approaches for the bioremediation are extensively used to remediate polluted/contaminated soils: microbial-assisted plant remediation (rhizoremediation) and phytoremediation. Phytoremediation is a process which uses the plants to extract, sequestrate, or decontaminate terrestrial and/or aquatic environment, while rhizoremediation utilizes the exudates released by plants which will increase the rhizospheric microorganisms that will help plant growth and the degradation/breakdown of contaminants (Gerhardt et al. 2009). In the present chapter, we discuss about the challenges and potentials of rhizoremediation to remove the persistent chemical and metals from the environment (Fig. 17.1).

17.1.1 Phytoremediation

In phytoremediation process, plants are used to sequester, extract, or detoxify pollutants. This method is cost-effective and eco-friendly since the structural integrity of the soil will be maintained (Khan et al. 2000). In phytoremediation process, genetically engineered or special plants are targeted that have the potential to uptake the pollutants from the environment (Macek et al. 2000). This process is applicable

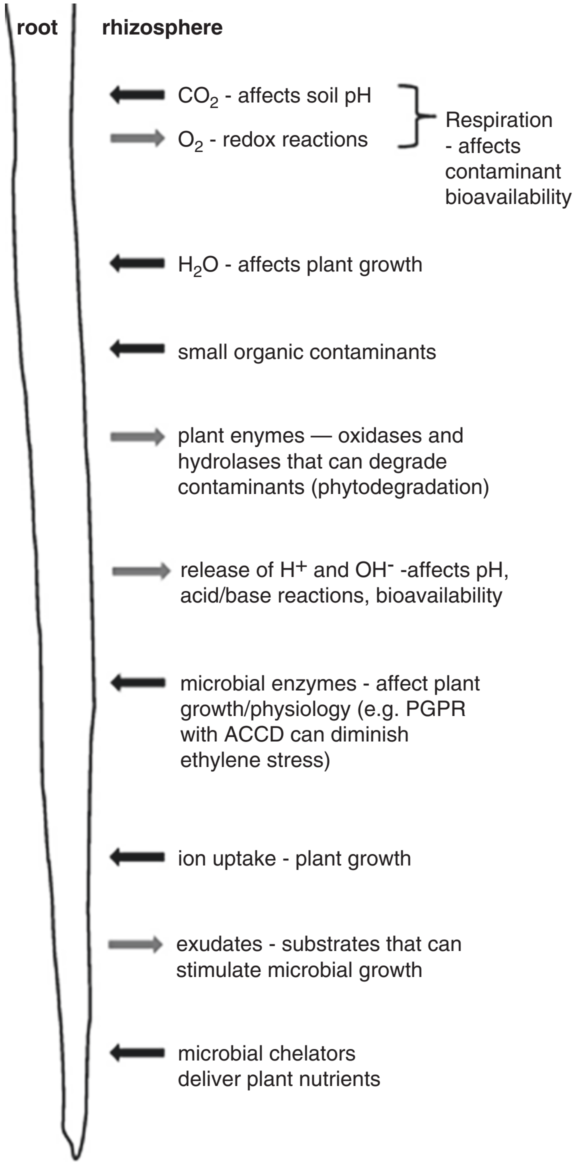

Fig. 17.1 General processes affecting rhizoremediation: plant roots support microbial growth at the root surface and in the rhizosphere. Roots create channels in soil that allow for movement of O_2 and H_2O and that are wide enough for “trapped” contaminants to become accessible to microbes. PGPR, plant growth-promotingrhizobacteria; ACCD, 1-aminocyclopropane-1-carboxylate deaminase (Adapted from Gerhardt et al. 2009)

for organic and inorganic contaminants, which are in solid and liquid form (Salt et al. 1998). Generally, phytoremediation of pollutants by a plant involves the following steps: uptake, translocation, transformation, compartmentalization, and sometimes mineralization (Schnoor et al. 1995). Several extensive research studies were performed in greenhouse laboratory level prior to the field trails. These experiments provided valuable information regarding particular type of phytoremediation mechanism of different organic contaminants. This mechanism includes transportation of some organic compounds through the plant membranes. Especially, the compounds with low molecular weight habitually removed from the soil and are released via evapotranspiration processes through leaves. This method is also known as phytovolatilization. Some of the nonvolatile compounds can be converted or degraded into nonhazardous entities by catalytic effect of enzymes and chemical sequestration in plants. This is referred as phytodegradation and phytoextraction, respectively. The highly stable compounds in the plants can be degraded along with the biomass during sequestration or incineration (Truu et al. 2015). The uptake of the organic compounds, distribution, and transformation depends not only on physical but also chemical property of the compound (molecular weight, water solubility) and environmental condition (temperature, pH, and soil moisture content) including the plant characteristics (root system and enzymes) (Suresh and Ravishankar 2004). The phytoremediation can be used to target two major kinds of pollutants: elemental pollutants and the organic pollutants (Meagher 2000).

17.1.1.1 Elemental Pollutants

This group of pollutants includes radionuclides and toxic heavy metals, which are very difficult to remediate and only few techniques are available for it. In recent years, plants have become an attractive tool to remediate heavy metals from soil (Clemens et al. 2002; Cobbett and Goldsbrough 2002; Khan et al. 2000). The process of heavy metal removal using plants includes (1) extraction of the contaminants from soil and translocation to aboveground tissues, (2) sequestering of the contaminants in the root system to prevent/stop further spreading and leaching into soil and/or groundwater, or (3) conversion into less harmful and toxic chemicals. For this purpose some of the plants such as sunflower, tobacco, mustard, maize, and sand rocket are used because of their capacity to absorb and hyperaccumulate the pollutant (Meagher 2000). Usually the plants growing in the region enriched with heavy metals have the ability to hyperaccumulate the heavy metals and were thought to have developed a defense mechanism against herbivores. However, plants with such capabilities are rarely available, and hence in modern era, scientists are exploring to develop plant with high metal absorptivity through genetic engineering (Kuiper et al. 2004).

17.1.1.2 Organic Pollutants

This class of pollutants includes organic compounds such as polycyclic aromatic compounds, polychlorinated biphenyls, nitro-aromatics, or linear halogenated hydrocarbons. Plants like willow, alfalfa, and other grasses have the ability to completely mineralize these kinds of compounds. However, the underlying mechanisms of mineralization of these compounds are not clearly understood. Nevertheless, plants have high potential of remediating organic compounds (Kuiper et al. 2004). In addition to several advantages of using phytoremediation, it also possesses some limitations, which includes slow growth rate of the plant, limitation of plant-root penetration in soil, time-consuming, sensitive for some pollutants, and the problem of being part of a food chain, and the process is completely dependent on the climatic changes (Khan et al. 2000).

17.1.2 Rhizoremediation

A combined action of plant and microbial remediation led to a more successful approach to bioremediation of pollutants that particularly belongs to organic compounds. This approach includes bioremediation methods such as phytoremediation and bioaugmentation to remediate the contaminants. Rhizoremediation refers to the use of microbes present in and around the rhizosphere of plants, which are utilized for phytoremediation purposes (Mosa et al. 2016). In recent years, it has popped out as the most effective method to remediate recalcitrant compounds. There will be an interaction between roots, root exudates, rhizosphere soil, and microbes resulting in breakdown of organics to nontoxic or less toxic minerals. The 40% of a plant's photosynthesis is deposited into the soil as organic acids, sugars, and larger organic compounds (Gerhardt et al. 2009). Soil microbes utilize these compounds as carbon, nitrogen, and energy source (Leigh et al. 2002).The rhizosphere of the soil consists of 10–100 times more microbes per gram of soil than un-vegetated soil. In soil containing large volumes of roots, microbial populations can reach titers of 10^{12} cells/g of soil. The plants can gain various benefits by these microbial consortia such as reducing stress hormones in plants, act as a chelators for delivering key plant nutrients, protect plants from pathogens, and reduce the negative effect of recalcitrant compounds on plants by converting/degrading (Hontzeas et al. 2004; Kuiper et al. 2004). The initial study of the rhizosphere is mainly focused on breakdown of herbicides and pesticides. These research studies suggest that the bacteria tend to degrade these compounds and protect plants from negative impact of these compounds (Hoagland et al. 1994; Jacobsen 1997). In the current scenario, many reports are available on breakdown of organic compounds such as TCE (Walton and Anderson 1990), PAHs (Radwan et al. 1995), and PCBs (Brazil et al. 1995). It was observed that grass varieties and leguminous plants, viz., alfalfa, are suitable for rhizoremediation, as these plants can harbor huge number of bacterial consortium on their root system (Kuiper et al. 2004).

The effectiveness of the rhizoremediation depends on the microbes to efficaciously colonize on the growing root. A colonizing process involves multitude of genes from the microbial consortia (Capdevila et al. 2004; Lugtenberg et al. 2001; Silby and Levy 2004; de Weert et al. 2002). These genes include production of biotin and thiamine, synthesis of amino acid synthesis, O-antigen of lipopolysaccharide, and an efflux pump induced by isoflavonoids. Although the chemotactic response can be evoked by different compounds depending on the colonizing species, the key factor for successful root colonization is the chemotaxis, which is specific toward root exudate compounds (Capdevila et al. 2004; Kuiper et al. 2004; de Weert et al. 2002). Among the compounds that influence the colonization complex includes aromatic compounds such as coumarins and flavonoids which plays a key role. The accumulations of these compounds are very low as these compounds are degraded by microbial consortia and used as the carbon and nitrogen sources, respectively (Leigh et al. 2002). It is fortuitous that these aromatic compounds are similar to many organic contaminants structurally, viz., polychlorinated biphenyls (PCBs), PHC, and PAHs, thereby providing means to exploit natural processes in the rhizosphere for the bioremediation of contaminants (Jacobsen 1997).

17.1.3 Microbe-Plant Interactions in Phytoremediation

The investigation of plant-microbe interactions has been under investigation for over 50 years, but these studies were mainly focused on plant-pathogen interactions. Over the decades, the ecology of microbes in the rhizosphere was focused toward many kinds of decontamination processes. The group of organisms acquainted in the rhizosphere is associated with plants and aids in its metabolism. They were found to be in synergism with plant roots and are known as rhizosphere microorganisms. In the early twentieth century, Hiltner defined the term rhizosphere, as the volume/amount of the soil that is influenced by the roots of plants (Kavamura and Esposito 2010).

In general, the microbial consortia of rhizosphere are stimulated by the plant roots while providing proper aeration, releasing of exoenzymes, and excreting a root exudate compounds which not only provide nutrients but also provide surface for colonization, niches to protect bacteria against desiccation, and other biotic and abiotic stresses (Kuiper et al. 2004). In return, the rhizospheric microorganism boosts plant growth by nutrient mobilization, nitrogen fixation, decreasing the level of plant stress hormone, production of plant growth regulators, and degradation of pollutants before they negatively impact the plant (Fig. 17.2) (Chaudhry et al. 2005; Segura and Ramos 2013). This mutualism between plant and microbes known as rhizosphere effect results in increased number, diversity, and degradative capability of the microbes (Kent and Triplett 2002; Ramos et al. 2000). In most of the cases, the microbial consortia are responsible for biodegradation process. In rhizoremediation, the amount and composition of root exudates will be plant specific. These exudates are majorly composed of organic acids (lactate, oxalate, acetate, malate, succinate, fumarate, and citrate), amino acids, and sugars along with some secondary metabolites (viz., isoprenoids, alkaloids, and flavonoids). These are released into the soil as the rhizo-deposits; among them majority of organic acid secreted exudates are dissociated anions (carboxylates) (Jones 1998; Martin et al. 2014; Singer et al. 2003; Singh et al. 2004). Rhizo-deposition results over 10–44% of the fixed carbon (Bais et al. 2006). The exudates of the roots can be utilized by the microbial consortia as the carbon source (Singer et al. 2003). Many secondary metabolites possess a similar structure as that of contaminants thus inducing the expression of specific catabolic genes of microbial consortia, which are necessary for the degradation of the contaminant. Some of the secondary metabolites like salicylate induce the microbial degradation of PAHs (naphthalene, fluoranthene, pyrene, chrysene) and PCB (Chen and Aitken 1999; Master and Mohn 2001; Singer et al. 2000), while terpenes aid in breakdown of toluene, phenol, and TCE (Truu et al. 2015). In some cases, the metabolites cannot be used as sole carbon sources. Hence, the microbes utilize easily degradable root-exuded compounds which serve as co-metabolites (i.e., aerobic biodegradation of trichloroethylene). The interaction between rhizospheric bacteria and plant roots excretes some biosurfactants that enhance the bioavailability and uptake of pollutants (Schwitzguébel et al. 2002; Wenzel 2009). In aged soil, this process may be beneficial as they contain low contaminant (Dams et al. 2007; Gunderson et al. 2007).

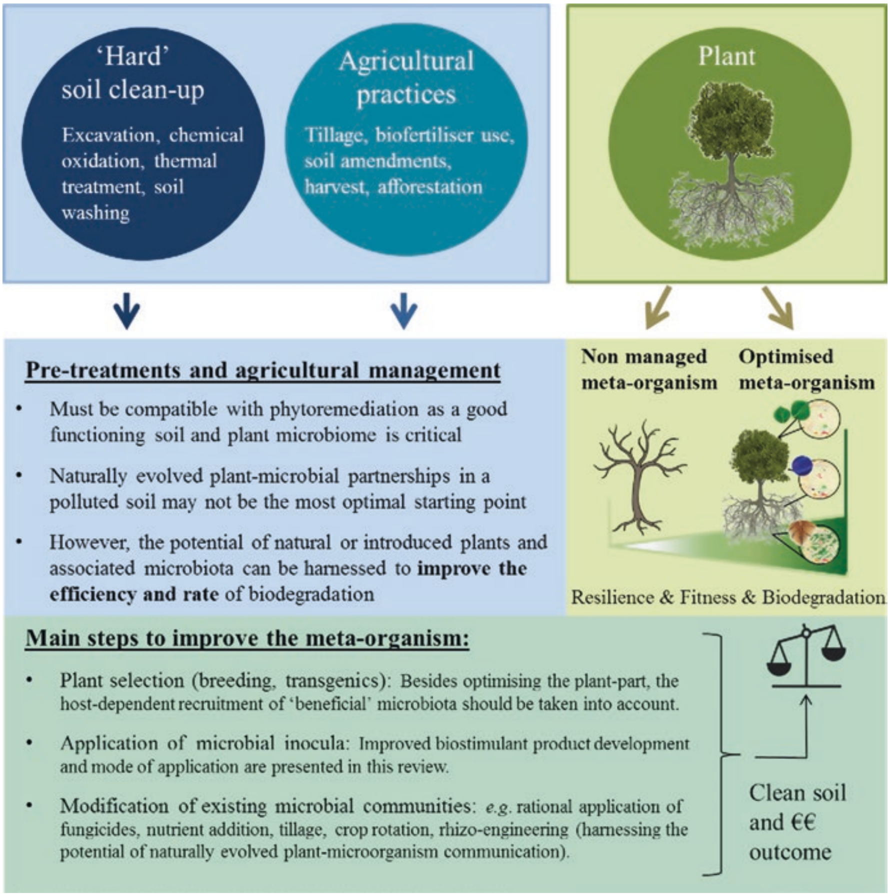

Fig. 17.2 Schematic diagram showing the integration of phytoremediation in soil cleanup treatment strains and optimization of the plant microbiome. Identification of the limiting factors to natural attenuation and overview of different approaches (e.g., rational plant selection and microbiome engineering) to turn the plant from a potential low-productivity state to a high-productivity, diverse, and resilient state with high phytoremediation activity (Thijs et al. 2017)

This microbial-assisted phytoremediation was investigated with both indigenous microbes and intentionally stimulated microbes through seed inoculation in the laboratory, greenhouse, and field. A wide range of enzymes has been found in plants, root-colonizing bacteria, endophytic bacteria, and fungi that can effectively degrade the contaminants. These include dehalogenases, dioxygenases, laccases, phosphatases, P450 monooxygenases, nitrilases, peroxidases, and nitro-reductases (Table 17.1).

Table 17.1 Plant and microbial enzymes with a role in degradation of organic compounds

Enzyme family	Catalytic action	Examples of known sources
Various plant enzymes for uptake, transport, sequestration, and degradation	General uptake and degradation	All plants
Dehalogenase	Hydrolyzes chlorine and fluorine from halogenated, aliphatic hydrocarbons (e.g., trichloroethylene), and aromatic hydrocarbons (e.g., PCBs, DDT)	*Xanthobacter autotrophicus* (B), Hybrid poplar (*Populus* spp.), *Sphingobium chlorophenolicum* (B)
Laccase	Degradation of various aromatic compounds	Alfalfa (*Medicago sativa*), *Trametes versicolor* (F), *Coriolopsis polyzona* (F)
Dioxygenase	Degradation of various aromatic compounds	*Pseudomonas* sp. (B), *Mycobacterium* sp. (B)
Peroxidase	Degradation of various aromatic compounds; reductive, dehalogenation of aliphatic hydrocarbons	Horseradish (*Armoracia rusticana*), *Phanerochaete chrysosporidium* (F), *Phanerochaetelaevis* (F), Alfalfa (*Medicago sativa*)
Nitrilase	Cleaves cyanide groups from aromatic and aliphatic nitriles	Willow (*Salix* spp.), *Aspergillus niger* (F)
Nitroreductase	Reduces nitro groups on nitro-aromatic compounds (e.g., 2,4,6-trinitrotoluene); removes N from ring structures	*Comamonas* sp. (B), *Pseudomonas putida* (B), Hybrid poplar (*Populus* spp.)
Phosphatase	Cleaves phosphate groups from organophosphates (e.g., pesticides)	Giant duckweed (*Spirodela polyrhiza*)
Cytochrome P450 monooxygenase	Hydroxylation of aromatic and aliphatic hydrocarbons	Most aerobic bacteria, all fungi, and all plants

Microbial sources are designated (B) for bacterium or (F) for fungus. All fungi except for *Aspergillus* are white-rot fungi (Gerhardt et al. 2009)

17.2 Factors Affecting Rhizoremediation

Rhizoremediation is mainly affected by various physical, chemical, and biological properties/compositions of the root-associated soil. Many studies were carried out to interpret the effects of soil moisture, pH, temperature, aeration, and organic matter composition on the breakdown of pesticides (Charnay et al. 2005; Rasmussen and Olsen 2004). Factors such as accessibility of mineral nutrients, the age of plants, and presence of contaminants affect the quantity and quality of exudates secreted. Since the rhizoremediation is majorly dependent on the nature and quality of the root exudates. The root exudates mediate the acquirement of minerals by plants, thus stimulating the microbial growth and activities in the rhizosphere, in addition to changing of some physicochemical conditions. Under stress condition, plants respond by varying the composition of root exudates, in turn controlling the metabolic profile and activities of rhizosphere microorganisms (Chaudhry et al. 2005).

17.2.1 Soil Conditions

The physicochemical nature/composition of the soil plays a crucial role in the success of bioremediation. The microbial metabolic activity and chemical diffusion in soil depends on factors, viz., moisture, redox conditions, temperature, pH, organic matter, nutrients and nature, and amount of clay. The aerobic microbial mineralization/degradation of selected pesticides (benzolin-ethyl, isoproturon, and glyphosphate) in different types of soil at different moisture content was evaluated by Schroll et al. (2006). They found a linear correlation ($p < 0.0001$) while increasing soil moisture content (within a soil water potential range of −20 and −0.015 MPa), which increases the relative pesticide mineralization/degradation.

17.2.2 Temperature

Temperature plays a vital role in biodegradation of recalcitrant chemical compounds by microbial consortia since majority of the biochemical reactions and metabolic activity of microbes depends on thermal thermodynamics. The cell membrane permeability and cell physiology-altering proteins are majorly impacted by temperature (Alberty 2006; Mastronicolis et al. 1998).

17.2.3 pH

Most of the putrefaction of compounds are due to the enzymes secreted by the plant-microbe interactions. The catalytic activities of these enzymes are pH dependent; the optimal bacterial growth was observed at the optimal pH 6.5 and 7.5 for most of the organisms. Siddique et al. (2002) noticed that the *Pandoraea sp.* isolated from an enrichment culture degrade the HCH isomer in the pH range of 4–9. They also observed that the growth and biodegradation of α- and γ-isomers of HCH seem to be optimal when pH of the soil slurry is 9. Similar observation was made by Singh et al. (2004) while studying the putrefaction of organophosphate pesticides in the soil. They understood that the degradation was slow at acidic pH compared to that of neutral or alkaline pH.

17.2.4 Soil Organic Matter

The organic matter in soil affects the adsorption/desorption process of pesticides in the soil including the nutrients for cell growth. Perrin-Ganier et al. (2001) monitored putrefaction of isoproturon (herbicide) by introducing phosphorus (P), nitrogen (N), and sewage sludge separately, thus observed that P and N had the greatest effect on the process of isoproturon degradation.

17.3 Role of Endophytes in Rhizoremediation

In recent few years, much is focused on the utilization of endophytic microbes/bacteria in phytoremediation to degrade xenobiotic compounds from the environment. These bacteria are nonpathogenic and find its existence in most if not all higher plant species. Some of these species such as *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Azospirillum*are found most abundantly in soil (Lodewyckx et al. 2002; Moore et al. 2006). The endophytes possess plant growth-promoting ability and also pathogen controlling capability (Berg et al. 2005; Ryan et al. 2008). The major advantage of employing endophytes over other rhizospheric bacteria in phytoremediation is that, in rhizospheric bacteria, there will be huge competition among the strains. This reduces the number of desired strains, and it is very difficult to control these organisms. Conversely, endophytic bacteria are acquainted in the internal membranes/tissues of plants thus reducing the problem of competition between bacterial strains (Doty 2008; McGuinness and Dowling 2009).

Genetic modification strategies of these endophytes have gained more attention in phytoremediation process. Barac et al. (2004) reported that introduction of toluene degradation plasmid (pTOM) from *B. cepacia* G4 into a natural endophyte such as yellow lupine is capable of degrading toluene up to 50–70%. While Germaine et al. (2006) reported that interaction of natural endophytes with a genetically modified endophyte possessed the capability of degrading 2,4-dichlorophenoxyacetic acid. The same group has also reported 40% higher degradation of 2,4-dichlorophenoxyacetic acid by using *Pseudomonas putida* VM1441(pNAH7). Weyens et al. (2009a, b) showed the co-culture of genetically modified TCE-degrading strain (i.e., *P. putida* W619-TCE) along with natural TCE strain of tree growing on TCE-contaminated soil showed 90% reduction of TCE evapotranspiration under the field conditions.

The genetic engineered endophytes were used to improvise the phytoremediation of organic/inorganicpollutants and toxic metals. The incorporation of modified yellow lupine which was inoculated with pTOM-Bu61 plasmid (encoding for trichloroethylene degradation constitutively) and ncc-nre (Ni resistance/sequestration in *B. cepacia* VM1468), along with the natural yellow lupine showed significant reduction in TCE and Ni phytotoxicity. This also promoted 30% enhancement in root biomass and 50% decrease in the enzyme activities involved in antioxidative defense in the roots. In addition, to the decreasing trend in TCE evapotranspiration, it showed about a fivefold higher Ni uptake after inoculation of two types of yellow lupine plants together (Weyens et al. 2010). The bioaugmentation of two grass species (*FestucaarundinaceaSchreb*. and *FestucapratensisHuds*) along with the endophytic fungi (*Neotyphodiumcoenophialum* and *Neotyphodiumuncinatum*) showed 80–84% and 64–72% of PAH and TPH reduction compared to that of control plants, which showed only 30% removal(Soleimani et al. 2010). Apart from the rhizosphereendophytes, the culturable endophytes in aquatic plants showed enhancement in phytoremediation(Chen et al. 2012). It was shown that genetically engineered endophyticbacteria possess much easier in application than genetic plants because it has the ability to colonize multiple plants, and it also benefits plants by reducing stress hormones, nitrogen fixation, and phosphate solubilization (Dimkpa et al. 2009; Doty et al. 2009; Gai et al. 2009).

17.4 Polycyclic Aromatic Hydrocarbons (PAHs)

Rhizoremediation is a process which uses effect of both microbial degradation and plant growth for the breakdown of toxic compounds to less toxic/volatile compounds (Song et al. 2004; Tang et al. 2010). Tang et al. (2010) conducted the pilot plant experiments to analyze the outcome of bioaugmentation and environmental factors for rhizoremediation of petroleum-contaminated soils using different plant species. Among the tested sources, ryegrass resulted in 5% total petroleum hydrocarbons (TPH) degradation in soil. They observed that with different microbial species and plant growth-promoting rhizobacteria (PGPR), the TPH degradation increased in the following order: cotton +PGPR > cotton + EMA > cotton + PGPR > cotton > control. They suggested that rhizoremediation can be increased with proper optimization of the factors like plant growth and EMA microbial community in soil (Tang et al. 2010; Tyagi et al. 2011). Huang et al. (2005) developed a technique known as multiprocess phytoremediation system (MPPS) which consists of contaminant-degrading bacteria, land farming (aeration and light exposure), plant growth-promoting rhizobacteria (PGPR), and growth of the contaminant-tolerant plant, i.e., tall fescue (*Festucaarundinacea*). Using the MPPS, they were able to remove 90% of all fractions of TPHs from soil. Figure 17.2 clearly shows the combined strategies for phytoremediation.

17.5 Petroleum Hydrocarbons (PHCs)

Petroleum hydrocarbons (PHCs) are organic compounds comprised of carbon and hydrogen atoms arranged in varying structural configurations. They are classified in two main categories, namely, diesel range organics (DROs) and gasoline range organics (GROs). GROs include mono-aromatic hydrocarbons such as toluene, benzene, xylenes (BTEX), ethylbenzene, and short-chain alkanes (C6–C10) with low boiling points (60–170 °C) such as 2,3-dimethyl butane, isopentane, n-butane, and pentane. DROs consist of long-chain alkanes (C10–C40) and hydrophobic chemicals like polycyclic aromatic hydrocarbons (PAH) (Gkorezis et al. 2016; Kamath et al. 2004). Petroleum hydrocarbons (PHCs) are biodegradable and bio- and phytoremediable (Gkorezis et al. 2016). The plant-associated bacteria include phyllospheric, endophytic, and rhizospheric bacteria. The mutualism between these host plants and the bacteria allows for greater survivability and treatment of polluted soils by mutual benefitting both the organisms (Weyens et al. 2009b, 2015). Possible

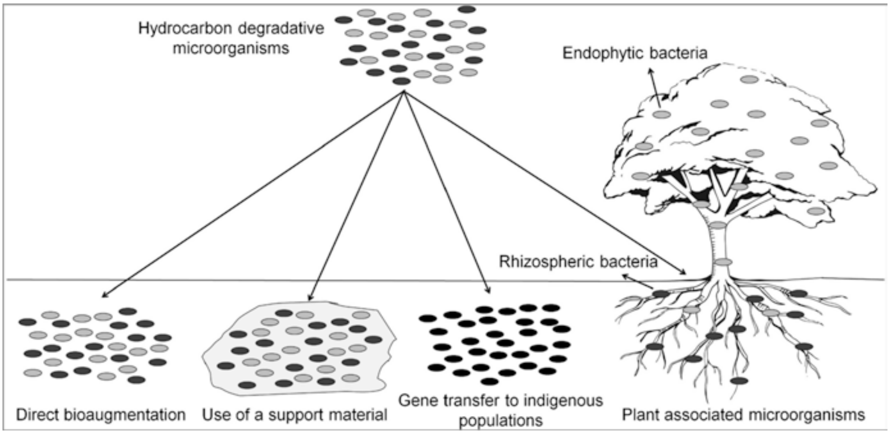

Fig. 17.3 Possible strategies for the bioremediation of PHC-contaminated sites (Gkorezis et al. 2016)

mechanism for the bioremediation/rhizoremediation of PHC-contaminated sites is shown in Fig. 17.3.

The capability of the microbes to breakdown PHCs is greatly contributed to the presence of catabolic genes and enzymes that helps them to use PHCs as energy source (Das and Chandran 2010). Table 17.2 shows the interaction of microbes and plant species for breakdown of PHC component. The advantages and disadvantages of phytoremediation over traditional technologies are shown in Table 17.3.

17.6 Rhizoremediation of Heavy Metals

Rhizoremediation, a special case of phytoremediation, is a process, which exploits the microbial species present in the rhizosphere of plants. These microbes share a symbiotic/mutualistic relationship with the roots of plants and aid in retrieving soils polluted with heavy metals (Fig. 17.4). These heavy metals not only possess a serious threat to the surrounding ecosystem but also are more probable to get absorbed by plants through roots and enter the food chain. Subsequently, it reaches the animal kingdom from Kingdom Plantae (Ganesan 2012). Heavy metals are classified from their traditional analogs in the sense that these metals have density greater than 5 g/cm^3 (Kareem et al. 2016). It is renowned that heavy metals are present ubiquitously in soil in trace amounts. However, from the advent of industrialization and urbanization over the past few centuries, it has been a customary habit for humans to release heavy metals and other harmful pollutants into the environment. Apart from natural occurrences, the main sources of heavy metals include industrial wastes, fertilizers, and petroleum byproducts. These heavy metals act as genotoxic substances and interfere with protein synthesis, respiration, and carbohydrate metabolism (Khan et al. 2009). Consequently, they result in poor growth and low

Table 17.2 Selected paradigms of successful rhizodegradation of PHCs (Gkorezis et al. 2016)

Plant species	Microorganisms	PHC component	References
Zea mays	*Pseudomonas* sp. strain UG14Lr, *Pseudomonas putida* strain MUB1	Phenanthrene/ pyrene	Chouychai et al. (2009, 2012)
Lolium perenne	*Pantoea* sp. strain BTRH79	Diesel oil	Afzal et al. (2012)
Lotus corniculatus	*Pantoea* sp. strain BTRH79	Diesel oil	Yousaf et al. (2010)
Medicago sativa	*Rhizobium meliloti* strain ACCC17519	Various PAHs	Teng et al. (2015)
Zea mays	*Gordonia* sp. strain S2RP-17	Diesel oil	Hong et al. (2011)
Lolium multiflorum	*Acinetobacter* sp.	Various PAHs	Yu et al. (2011)
Secalecereale, *Medicago sativa*	*Azospirillum brasilense* strain SR80	Crude oil	Muratova et al. (2010)
Lolium multiflorum	*Rhodococcus* sp. strain ITRH43	Diesel oil	Andria et al. (2009)
Sorghum bicolor	*Sinorhizobium meliloti* strain P221	Phenanthrene	Muratova et al. (2009)
Hordeum vulgare	*Mycobacterium* sp. Strain KMS	Pyrene	Child et al. (2007a, b)
Triticum aestivum	*Pseudomonas* sp. strain GF3	Phenanthrene	Sheng and Gong (2006)
Trifolium repens	*Rhizobiumleguminosarum*	Chrysene	Johnson et al. (2004)
Hordeum vulgare	*Pseudomonasfluorescens*, *Pseudomonas aureofaciens*	Phenanthrene	Anokhina et al. (2004)
Lolium multiflorum	*Pseudomonas putida* strain PCL1444	Various PAHs	Kuiper et al. (2001)
Hordeum vulgare	*Pseudomonas putida* strain KT2440	Various PAHs	Child et al. (2007a, b)

Table 17.3 Advantages and disadvantages of phytoremediation over traditional technologies (Das and Chandran 2010; Stępniewska and Kuźniar 2013)

Advantages	Disadvantages
Relatively low cost	Longer remediation times
Easily implemented and maintained	Climate dependent
Several mechanisms for removal	Effects to food web might be unknown
Environmentally friendly	Ultimate contaminant fates might be unknown
Aesthetically pleasing	Results are variable
Reduces landfilled wastes	
Harvestable plant material	

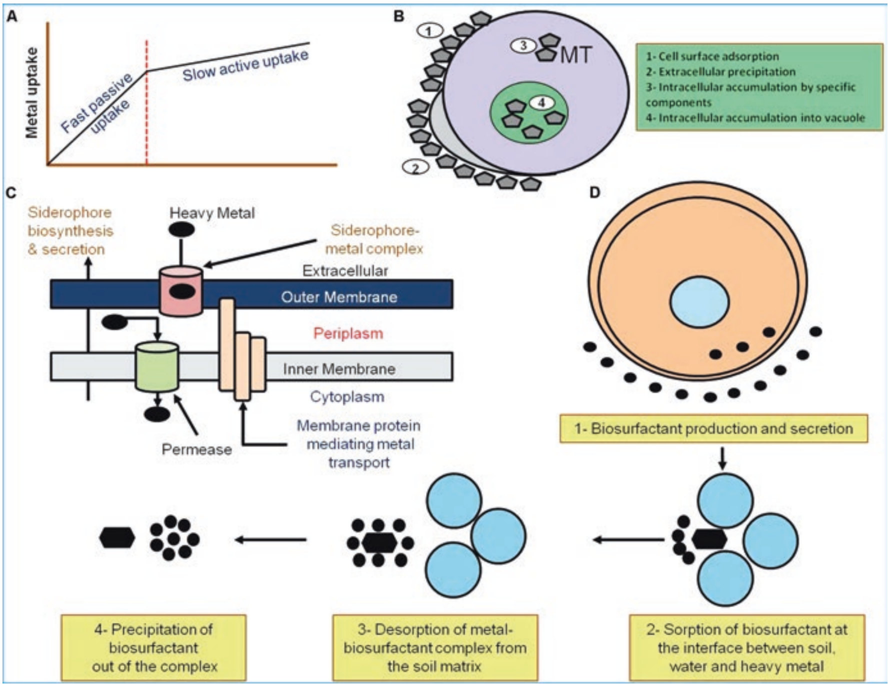

Fig. 17.4 Mechanism of microbial remediation. (**a**) Passive and active heavy metal uptake by biological materials. (**b**) Mechanisms of heavy metal biosorption by bacterial cells. Bacterial biosorption of heavy metals through (1) cell surface adsorption, (2) extracellular precipitation, (3) intracellular accumulation through special components, such as metallothioneins (MT), or (4) intracellular accumulation into vacuoles. (**c**) Heavy metal remediation via siderophore formation. (**d**) Mechanism of bacterial heavy metal remediation through biosurfactant production (Adapted from Kareem et al. 2016)

yield of crops. Different origins of heavy metals, their density, and toxicity to living beings are shown in Table 17.4 (adapted from Seshadri et al. 2015).

Many biological agents have entangled themselves in removing these hazardous entities and preventing the plants from getting being damaged. The microbial population in the rhizosphere tends to act alone or as a part of community in eliminating these metals. Plants and their mutually associated microbial allies are tabulated in Table 17.5 (adapted from Kamaludeen and Ramasamy 2008). This bacterium present around the roots tends to reduce/increase the absorption of metals by plants, stabilizes the metals by forming organo-complexes, and diminishes the heavy metal accumulation/aggregation in the rhizosphere. *Pseudomonas putida*, a gram-negative bacterium, was found to show high tolerance against heavy metals such as cobalt, zinc, cadmium, copper, nickel, and lead (Uslu and Tanyol 2006). *P. putida* TPHK-1 was found to be a highly efficient and unique strain especially in breaking down the diesel oil in the presence of heavy metals. Tolerance toward heavy metals tied together with celerity in deprivation of hydrocarbons from soil, even at high

Table 17.4 Sources of heavy metals in soils and their expected ionic species in soil solution

Metal	Density (g/cm^3)	Ionic species in soil solution	Contaminant sources	Toxicity[a]
Arsenic (As)	5.73	As(III): $As(OH)_3$, AsO_3^{3-}, As(V): $H_2As_4^-$, $HAsO_4^{2-}$	Timber treatment, paints, pesticides, geothermal	Toxic to plants, humans, and animals
Cadmium(Cd)	8.64	Cd^{2+}, $CdOH^+$, $CdCl^-$, $CdHCO_3^+$	Electroplating, batteries, fertilizers	Toxic to plants, humans, and animals
Chromium(Cr)	7.81	Cr(III): Cr^{3+}, CrO_2^-, $CrOH^{2+}$, $Cr(OH)_4^-$, Cr(VI): $Cr_2O_7^{2-}$	Timber treatment, leather tanning, pesticides, dyes	Cr(VI) toxic to plants, humans, and animals[b]
Copper (Cu)	8.96	Cu^{2+}(II), Cu^{2+}(III)	Fungicides, electrical, paints, pigments, timber treatment, fertilizers, mine tailings	Toxic to plants, humans, and animals
Lead(Pb)	11.35	Pb^{2+}, $PbOH^+$, $PbCl^-$, $PbHCO_3^-$, $PbSO_4$	Batteries, metal products, preservatives, petrol additives	Toxic to plants, humans, and animals
Manganese (Mn)	7.21	Mn^{2+}, $MnOH^+$, $MnCl^-$, $MnCO_3$	Fertilizer	Toxic to plants
Mercury (Hg)	13.55	Hg^{2+}, $HgOH^+$, $HgCl_2$, CH_3Hg^+, $Hg(OH)_2$	Instruments, fumigants, geothermal	Toxic to humans and animals
Molybdenum (Mo)	10.2	MoO_4^{2-}, $HMoO_4^-$, H_2MoO_4	Fertilizer	Toxic to animals
Nickel (Ni)	8.9	Ni^{2+}, $NiSO_4$, $NiHCO_3^+$, $NiCO_3$	Alloys, batteries, mine tailings	Toxic to plants and animals
Zinc (Zn)	7.13	Zn^{2+}, $ZnSO_4$, $ZnCl^+$, $ZnHCO_3^+$, $ZnCO_3$	Galvanizing, dyes, paints, timber treatment, fertilizers, mine tailings	Toxic to plants

Adapted from Seshadri et al. (2015)

[a] Most likely to observe at elevated concentrations in soils and water

[b] While Cr(VI) is very mobile and highly toxic, Cr(III) is essential in animal and human nutrition and generally immobile in the environment

concentrations, indicates that *P. putida* TPHK-1 is a promising strain in remediating both hydrocarbons and heavy metals simultaneously (Ramadass et al. 2016). Siderophores were found to be iron-chelating agents present in microbes such as *Pseudomonas fluorescens-putida* group and increased the yield of crops up to 144% (Joseph et al. 1980).

Plant growth-promoting rhizobacteria (PGPR) is the title given to the group of bacteria, which helps in growth of plants by remediating the soil. However, different routes are exploited by different bacteria in remediating the soil, which are contaminated with heavy metals as depicted in B, C, and D in Fig. 17.1 (Adapted from Kareem et al. 2016). The rate at which the metal is taken up can either be passive (fast) or active (slow). Similarly, other mechanisms like direct biosorption,

Table 17.5 Microbes and their communities associated with plants in metal rich soils

Plants	Microbe/Microbial communities and their characteristics	Soil nature
Thlaspi goesingense	*Holophaga/Acidobacterium division* and *α- proteobacteria*, *Methylobacteriummesophilicum*, *Sphingomonas*	Ni-rich serpentine soils
T. caerulescens	Ni-resistant bacteria predominant in rhizosphere than bulk soils	
Alyssum murale	Ni-resistant, siderophore, and acid producing bacteria more in rhizosphere than bulk soils	
	Sphingomonas macrogoltabidus, *Microbacterium liquefaciens*, *M. arabinogalactanolyticum*	
A. bertolonii	Gram-positive α-proteobacteria	
Rinorea bengalensis, *Dichapelatum gelonioidesssp. andamanicum*	*Pseudomonas*, *Bacillus*, *Cupriavidus sp.*	
Agrostis tenuis	*Arthrobacter*, *Ochrobactrum*, *Bacillus*, *Serratia* sp., and AM fungi – *Acaulospora*, *Gigaspora*	
Pteris vittata	*Pseudomonas*	As-contaminated cattle dip sites
Phragmites sp.	Cu-tolerant, exopolymer producing bacterial communities, predominantly, *Bacillus*	As-contaminated soils

Adapted from Kamaludeen and Ramasamy (2008)

siderophore formation, and remediation through biosurfactants are most common among microbes.

17.7 Conclusion

From the existing literature, it is imminent that phytoremediation is an attractive and potent tool for remediating the toxic pollutants present in the environment. Rhizoremediation, a special phytoremediation technique that involves both plants and microbes, elucidates their usage in removing hazardous components. However, with the exponential increase of population and ever-increasing pollution, the progress made in remediating is gloomy. On the other hand, it is promising to note that the allocation of assets and awareness in the society toward such eminent concerns is augmenting day by day. In conclusion, the near future holds more hope on a larger scale toward such promising maneuvers than the contemporaneous.

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