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Omics Insights in Environmental Bioremediation

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Contents

Plant–Microbe Associations in Remediation of Contaminants for Environmental Sustainability

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Ragavi Chidambaram, Ravina Devi Rajagopal, Ivo Romauld Sagayaraj, and Vivek Pazhamalai

Abstract

Pollutants are the substances that lead to undesired effects on the environment and pose a threat to all forms of life. The accumulation of these pollutants in the environment causes several diseases which affect both human and animal health. Several methods are implemented to degrade contaminants among which better results are obtained for the bioremediation technique. Plants and microbes are trappers of contaminants and they remove pollutants from the environment in an effective way. When both microorganisms and plants are combined, they showed an increase in their reduction activity compared to other remediation methods. Plant-associated microbes such as endophytes and rhizospheric microorganisms are utilised in the remediation of toxic compounds and are also used to enhance the treatment process. Thus, plant-associated microbes are considered as a promising approach in the remediation of contaminants. A broad knowledge about plant–microbe interactions and the challenges faced during remediation process is more important for the development of new technologies to remove various contaminants. This chapter highlights the need for plant microbes and how they play a vital role in the remediation of contaminants. More approaches should be implemented using plant microbes for the betterment of polluted environments.

Keywords

Plant–microbe interaction · Bioremediation · Contaminants · Signalling molecules

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4.1 Introduction

Environmental pollution is not a new occurrence, but still it continues to be the world's greatest problem. The most striking reason for environmental pollution is the eradication of the relationship between man and the environment due to the increasing rate of exploitation of natural resources, urban growth, and industrialisation. The source of environmental pollution is not only restricted to deforestation, landfills, dumping of waste into water bodies, population growth, mining but also triggered by the release of harmful substances such as toxic metals, sewage, industrial wastes, and gaseous pollutants into the environment (Kumar and Chandra 2020a; Kumar et al. 2020a, 2021a; Singh et al. 2021). Several physical and chemical techniques are available for reduction of pollutants, but they are expensive and lead to other environmental problems (Appannagari 2017). Thus, the most efficient, eco-friendly, and economical approaches should be considered for removing pollutants. Bioremediation is considered worldwide since it is eco-friendly and feasible (Kumar et al. 2018).

Phytoremediation and microbial bioremediation are the most common type and efficient technique of bioremediation (Chandra and Kumar 2018; Agrawal et al. 2021; Kumar et al. 2022). Phytoremediation is the technology that employs plants to eliminate contaminants from soil. Excessive use of pesticides and fertilisers contaminate soils by releasing heavy metals. This causes several human health problems. Plants can remediate these soils by recycling heavy metals. Phytoremediation inhibits the entry of contaminants into the environment, as they do not allow their entry into groundwater (Chandra et al. 2018). They have the potential to recycle a wide range of contaminants in the environment. The main disadvantage of phytoremediation is it is slower than other techniques and can affect the survival and growth of plants (Kumar 2021). Microbial techniques are also one of the cost-effective and eco-friendly methods of bioremediation. They employ various techniques such as bioreduction, biosorption, bioleaching, and biomineralisation for removal of pollutants. The main disadvantage of this type of bioremediation is it also is a slow process. Unlike phytoremediation, remediation by microbes cannot be monitored by the naked eye. In recent times, cost-effective technologies which employ the use of a combined system of plants and microbes for remediation of pollution has been researched (Rajkumar et al. 2006). The utilisation of combined systems of plants and microbes enhances the removal of contaminants from the environment. This review aims to provide a better understanding of plant–microbe interactions. Various methods using plants and microbes for remediation of polluted sites are discussed.

4.2 Plant–Microbe Interaction

Plant–microbe interaction is a complex and continuous process where the microbes have both positive and negative impacts on plants. Thus, understanding plant–microbe interactions would help in differentiating their positive and negative

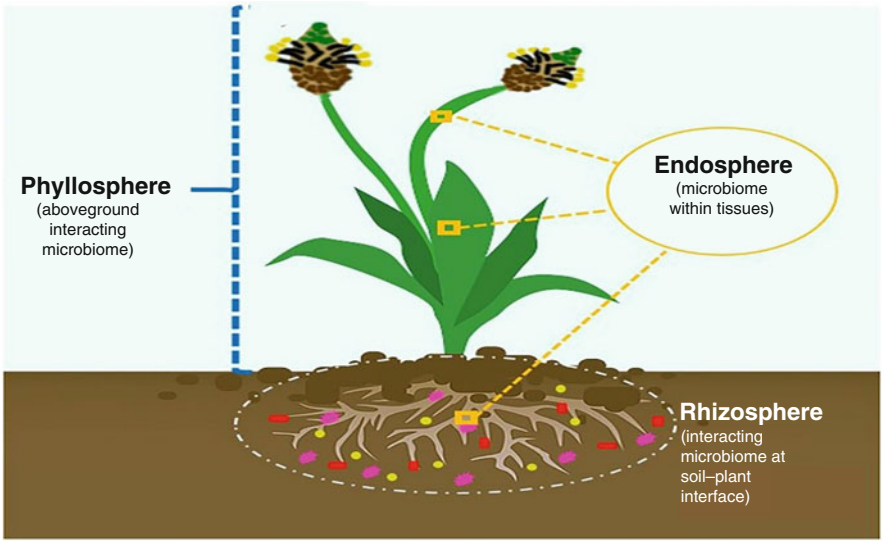

Fig. 4.1 Three main regions involved in plant–microbe interactions

impacts (Kumar and Chandra 2018a, b; Kumar et al. 2021b, c, d). It is a widely accepted fact that certain plant–microbe interactions help in enhancing the growth of plants, protecting from harmful pathogens, and maintaining soil fertility. The plant-associated microbes leading to positive impacts are grouped into three categories. They are phyllospheric, endophytic, and rhizospheric microorganisms (Fig. 4.1) (Kaul et al. 2021). Phyllosphere is the region that covers the aboveground plant parts. Phyllospheric microbes have the ability to withstand the abiotic stress of UV radiation and high temperatures of 30–35 °C than other plant microbes. They protect crops through various plant growth–promoting (PGP) mechanisms. Some examples of phyllospheric microbiome are *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Agrobacterium*, and *Xanthomonas*. Endophytic microbes inhabit the internal tissue of plants such as root, stem, flowers, fruits, and seeds. They protect the plants from harmful pathogens and increase stress tolerance. Some examples of endophytes are *Enterobacter*, *Achromobacter*, *Streptomyces*, *Pseudomonas*, *Microbiospora*, and *Nocardioides*. Rhizospheric microbes inhabit the area around roots where the microbes around the soil are attracted towards the plants due to the release of root exudates. Some examples of rhizospheric microbes are *Methylobacterium*, *Pseudomonas*, *Rhizobium*, *Arthrobacter*, *Acinetobacter*, *Azospirillum*, and *Bacillus* (Yadav 2021).

4.2.1 Endophytic Microbiome

Endophytes are microorganisms that live within the tissue of the plant without causing any diseases. They produce secondary metabolites and protect plants against

various pathogenic microorganisms. Bacterial endophytes enter the plant through roots and are divided into three types: passenger endophytes, opportunistic endophytes, and competent endophytes. Passenger endophytes and opportunistic endophytes have the capability to spread to the root cortex, whereas competent endophytes spread throughout the vascular tissues. Opportunistic endophytes also promote root proliferation. Bacterial endophytes enter into the vascular tissue, where they spread in and colonise the vegetative area of the plants. Microbes that colonise the rhizosphere enhance plant growth and also increase plants' ability to adapt to extreme environmental conditions. A recent study on rice seed colonised by bacterial endophytes has shown an increase in plant growth. Some endophytes have showed antifungal activities against various harmful pathogens present in plants. It has been reported that bacterial endophytes isolated from the wheat cultivar seeds have shown biocontrol activities towards *Fusarium graminearum.* A study conducted on endophytic bacteria isolated from halophytes has shown that they help in reducing plant stress by regulating plant hormones. It is also reported that these bacteria enhance nitrogen fixation and also assist in the uptake of nutritional compounds. Similar to bacterial endophytes, fungal endophytes have also been found in vegetative parts of plants and are categorised into two types: clavicipitaceous and non-clavicipitaceous endophytes. The non-clavicipitaceous endophytes have three subclasses: Class 2 endophytes, Class 3 endophytes, and Class 4 endophytes. Class 2 endophytes spread to rhizomes, roots, and shoots; Class 3 endophytes grow in shoots; and Class 4 endophytes grow in roots of plants. Fungal endophytes also produce secondary metabolites and volatile organic compounds to help plants withstand biotic and abiotic stresses. Fungal endophytes suppress plant pathogen growth by producing secondary metabolites. It has been demonstrated that secondary metabolites released by *Fusarium verticillioides* help reduce the growth of the plant pathogen *Ustilago maydis.* But physiological and environmental conditions are to be considered for the release of secondary metabolites by endophytic fungi. Some endophytic fungi help in plant growth by increasing the efficiency of glycolysis and tricarboxylic acid (TCA) cycle. For example, the endophytic fungus AL12 helps in improving plant growth by increasing the rate of these metabolic pathways in the plant *Atractylodes lancea* (De Mandal et al. 2021).

4.2.2 Plant Growth–Promoting Rhizobacteria

Rhizobacteria are the bacterial group present in the rhizosphere which is the region present near the root system of plant. Plant growth–promoting rhizobacteria (PGPR) are bacterial groups which help in plant growth. The rhizobacteria not only stimulate plant growth but also act as an effective system for disease control by direct and indirect means. In direct method, PGPR stimulate plant growth by nitrogen fixation and enhance nutrient production. In indirect method, PGPR stimulate plant growth by exhibiting biocontrol activities towards harmful pathogens (Alotaibi et al. 2021). A study was conducted on PGPR isolated from maize plant to investigate the plant growth–promoting properties. Bacterial strains were isolated from *Zea mays* (maize)

and analysed for phytohormone production. Phytohormones are responsible for plant growth and development. Eighty bacterial strains were checked for production of indole-3-acetic acid (IAA), siderophore, and hydrogen cyanide (HCN) and phosphate solubilisation. The bacteria that show higher plant growth–promoting activities were selected for the study. The bacterial isolates showed higher production of IAA, a majorly abundant auxin phytohormone. Siderophore production was also high in the strains; siderophore increases the production of iron content in plants. Most of the phosphorus remains in insoluble form and solubilisation of phosphorus is essential for plant growth. The selected bacterial isolates showed higher phosphorus solubility. Using 16S rRNA gene sequencing, the bacterial isolates were identified as *Pseudomonas aeruginosa* AK20, AK31; *Pseudomonas fluorescens* AK18, AK45; and *Bacillus subtilis* AK38. Plant growth analysis was checked on *Oryza sativa* (rice plant). After 30 days, plants were harvested for analysing plant growth. All bacterial isolates showed increased growth in the roots and shoots of the plants. The study has provided evidence that PGPR help in plant growth and development, and further research is required for the process to be applied in field conditions (Karnwal 2017). Many studies reported that *Bacillus* sp. and *Pseudomonas* sp. present in tomato, chickpea, and wheat crops act against pathogenic microbes, showing their biocontrol ability towards the pathogens (De Mandal et al. 2021).

The interaction between plants and microbes occurs through various signalling molecules. The interaction starts by exchange of signals produced by the host or microbes, which in turn results in biochemical, physiological, and molecular responses. The signals are recognised by microbes and help form symbiotic relationships with plants through physical interactions using flagella, pili, and adhesion. Various mechanisms of plant–microbe interactions through signalling molecules are discussed here, and the current research focuses on a signal pathway that can recognise rhizospheric microbes. The interaction processes are controlled by several metabolites and exudates which lead to plant growth, protection against pathogens, availability of nutrients, and so on. Some of the plant–microbial interaction signals are illustrated in Fig. 4.2.

4.2.3 Plant-Released Signals

Root exudates are organic chemicals that are released through root system for inhibition of harmful microbes and promoting plant growth. Root exudates supply nutrients and other energy source for microbes. Thus, microbes trigger exudation of roots in plants. Root exudates help in phytoremediation process by stimulating the plant to adapt to any metal stress. Some of the studies suggested that in the presence of root exudates, bacterial strains are metabolically active and utilise the compounds present in the exudates. The interaction between plants and arbuscular mycorrhizal fungi (AMF) involves hyphae which promote root colonisation. Certain signals are assumed to be involved before colonisation starts. The AMF interaction is not species specific, so certain plant-released signals and compounds are involved to

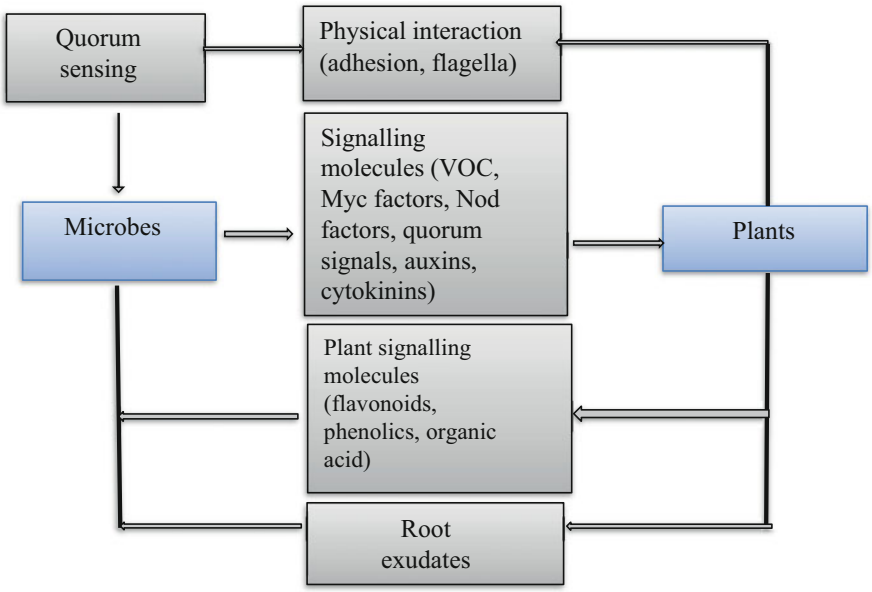

Fig. 4.2 Various signals involved in plant–microbe interactions

make it species specific. Some of the plant-released compounds are amino acids, sugars, and phenolic compounds which are potential signalling molecules in plant–microbe interaction. Flavonoids present in root exudates act as signalling molecules for plant–microbe interaction. They promote AMF spore germination and root colonisation in AMF. During root colonisation, flavonoids are released by the AMF fungus which promote hyphal growth. Flavonoids are species specific and during plant–AMF interaction their levels change. Flavonoid levels are moderate during root penetration and higher at later stages. After root colonisation the flavonoid levels get changed which play an important role in plant–AMF interaction (Ma et al. 2016). Flavonoids can also show negative impacts on other fungi as they are specific in nature, so their interaction with AMF is still unclear. Flavonoids also act as chemoattractants and help in rhizobia growth. They activate nod genes, that synthesise nod factors which stimulate nodule formulation in the host. Chemotaxis provides the organism with the ability to sense and respond to various signals induced by plants. Due to their specificity rhizobia can easily recognise and attach to the correct host plants. Ponce et al. conducted an experiment to determine the role of flavonoids in the plant–microbe relationship. The flavonoids were isolated from the plant *Trifolium repens* and molecular characterisation was carried out. The plants were allowed to grow in both under the presence and absence of AMF, *Glomus intraradices*. The flavonoid content in roots and shoots in the absence of AMF was analysed. But the role of flavonoids involved in the plant–microbe interaction is unknown. Only in the presence of AMF, the flavonoid are known to exist. Quercetin, a flavonoid, promotes AMF spore germination. Acacetin and rhamnetin showed

ability for inhibiting the eukaryotic topoisomerase. Ponkanetin promotes cytotoxicity and mutagenicity. The study suggested a clear interaction of AMF and flavonoid metabolism in plants. Similarly, plants secrete certain chemicals like phenolic compounds and organic compounds which help in the plant–microbe association. Plants produce phenolic compounds during their growth and development. Phenolic compounds are also synthesised in response to pathogenic infections and environmental stress factors. During a pathogenic infection, plants respond to infection through a series of defence mechanism which leads to the production of antimicrobial compounds. This mechanism is regulated by signalling pathways in which phenolic compounds play an important role. The phenolic compounds undergo changes in soil so that microbes can utilise it as a carbon source. During root and shoot development in *Arachis hypogaea*, the plant releases phenolic compounds, which the rhizobacteria present in the soil use as a carbon source and undergo changes. The responses also facilitate the formation of root nodules of plants. Plants associate with microbes by stimulating catabolic genes in microorganisms which are responsible for root nodulation. Plants and microbes share a mechanism through signals which makes bacteria to enter the root and shoot of plants. The root colonisation by bacteria is said to occur in a series of steps. The first step involves the movement of bacteria towards the root by flagellar activity, which is facilitated by the plant-released compounds. Attachment of the bacteria to the root occurs, after which the interaction between plant and bacteria takes place. In this step, root exudates are released and the bacteria enter the site of damage and promote plant growth. The exudates produced by the plant in response to tissue damage are utilised by microbes as a food source for colonisation. The association between plants and microbes is very specific and most of the studies suggested that the legume plant interacts well with rhizospheric bacteria (Ponce et al. 2004).

4.2.4 Microbial Signals

Microbes such as rhizobia and fungi have the ability to respond to plants through various signalling molecules. These signalling molecules can be volatile organic compounds (VOC), nod factors, myc factors, exopolysaccharides, and so on. Both plants and microbes secrete volatile organic compounds and communicate through it. Plants defend themselves from harmful organisms by releasing VOC. Similarly, microbes also emit VOC for communication and attacking. Microbial VOC modulate the activation of plant defence response and stimulate plant growth. Volatile organic compounds present in bacteria help in promoting plant growth and defence by nutrient production. It was first reported in *Arabidopsis thaliana*. After this discovery, a new line of research emerged in the plant–microbe association. Signalling molecules that are produced by myc factors and nod factors are utilised in the formation of new organs and root nodules (Ma et al. 2016). Certain flavonoids can also promote chemotaxis and bacterial growth. Some studies stated that chemotaxis plays an important role in plant root colonisation. De Weert et al. (2002) investigated root colonisation of tomato in which they used selected *P. fluorescens* strains and its

chemotactic mutants to investigate the chemotaxis towards root exudates of tomato. Movement is an important criterion for root colonisation. The movement towards root exudates is checked whether it is facilitated through chemotaxis. Chemotaxis of *P. fluorescens* occurs towards the root exudates of tomato which helps in root colonisation (de Weert et al. 2002). Rhamnolipids present in *P. aeruginosa* inhibit pathogenic fungi and give protection to grapevine. Varnier et al. (2009) conducted an experiment on rhamnolipids, biosurfactant molecules that protect grapevine against *Botrytis cinerea*, a necrotrophic fungus, and are also said to have a direct effect on spore germination and fungal growth. Rhamnolipids were isolated from *P. aeruginosa* and checked for their ability to activate the defence mechanism in grapevine. Different concentrations of rhamnolipids were used, and at higher concentrations, cell death resulted. The rhamnolipids were further tested for the defence mechanism along with chitosan and *B. cinerea*. Chitosan induces H_2O_2 production, which is enhanced by rhamnolipids. Oxidative burst was induced in the cells of grapevine by rhamnolipids. Rhamnolipids were tested for fungal activity against *B. cinerea* which results in inhibition of spore germination and growth of mycelium which also protects grapevine from the fungus (Varnier et al. 2009). Furthermore, various studies carried out on different plant species like *Solanum lycopersicum*, *Z. mays*, and *Lactuca sativa* showed response to VOC action. Another study reported that VOC present in *Bacillus subtilis* promoted increased growth in *Cucumis sativus*. VOC of fungal species also promote growth on different plant species. Various studies showed that Bacillus species have a high impact on lateral root development. Microbial VOC can also improve plant stress tolerance. For example, *Pseudomonas chlororaphis* increased the *A. thaliana* tolerance to drought by emitting 2R, 3R-butanediol (Ma et al. 2016). Recent research on microbial VOCs illustrates stimulation of plant growth and bacterial–plant interaction by VOC. However, the mechanism of bacterial VOC is still not clarified. Their signal perception, signal cascades, and cellular responses are still unknown. Thus, further studies are required for employing VOCs in various applications.

4.2.5 Quorum Sensing

Quorum sensing is a process which involves signalling for communication between bacterial cells. This bacterial cell–cell communication helps in monitoring the bacterial population density. Both gram-positive and gram-negative bacteria use different signalling molecules. Quorum sensing is a gene regulatory mechanism which involves N-acyl-L-homoserine lactones (AHL), a quorum sensing signal that is present in many gram-negative pathogenic bacteria. Plants can recognise bacterial AHLs, which in turn helps in promoting plant growth and defence mechanism (Ma et al. 2016). AHL signal is an auto-inducer signalling produced by *luxI* type gene. The signalling perception helps the bacterial cell to easily adapt to gene expression on environmental changes. The most recent study suggested that AHLs play an important role in abiotic stress of plant and help plants withstand the abiotic stress. Osmolytes and phytohormones play an important role in salt and drought

tolerance. The auxin indole-3-acetic acid is a key component in water stress tolerance. The bacteria associated with the root of plants secrete phytohormones which are utilised by the plants having low levels of phytohormones. This is successfully used to improve plant growth in salt-affected soils by combining bacterial phytohormones and seaweeds possessing a high level of osmolytes. IAA helps in increasing lateral root surface area, thus improving the facility to absorb more water and minerals. Auxin plays a key role in plant–microbe interaction by promoting plant growth development. IAA, a major naturally occurring auxin, acts as a signalling molecule in the plant–microbe interaction. Over 80% of rhizobacteria are reported to produce IAA. Tryptophan act as a precursor in the production of IAA, which indicates that higher concentration of tryptophan results in a higher production of IAA. Bacterial IAA sets the plants to adapt to metal stress by stimulating changes in cell metabolism (Hartmann et al. 2021). Phosphate-solubilising bacteria potentially reduce metal toxicity and also helps in plant growth. *P. fluorescens* is investigated for plant growth–promoting property and biocontrol activity against *Fusarium oxysporum.* Bacterial strains were isolated from the plant *Vigna mungo*. The metal resistance of the strains was checked against different metals and results revealed their high resistance to copper. The IAA production of bacteria was analysed by using high performance liquid chromatography (HPLC). The biocontrol activity of the strain was demonstrated and the strain showed inhibition against diverse groups of bacteria. Many soil bacteria stimulate plant growth by phosphate solubilisation, IAA production and exhibit certain antimicrobial activity. For example, IAA produced by both rhizobacteria and plants, transmitted as a signal, stimulates the production of antibiotics in *Streptomyces*. The signal perception also inhibits other competitive microbes (Upadhyay and Srivastava 2010). Transcriptional changes in legumes were observed when AHLs communicate with the roots of *Arabidopsis thaliana*. When treated with AHLs, there were changes observed in genes that are responsible for plant growth and genes that regulate growth hormones. The study illustrated that the interaction of N-hexanoyl-DL-homoserine-lactone (C6-HSL) and *A. thaliana* resulted in transcriptional changes in roots and shoots, and also induced plant growth (von Rad et al. 2008). Plant roots utilise water-soluble AHLs through an energy-dependent mechanism and this process occur in plants which have AHL-degrading enzymes such as *A. thaliana*, wheat, or barley. Thus, rhizobacteria provide support to the plant by promoting plant growth and development even under stress conditions. AHLs have provided a promising approach by increasing stress tolerance of plants against various environmental factors (Hartmann et al. 2021). Furanones are AHL mimic compounds which can antagonise AHL type behaviour. They can selectively bind to bacterial AHL receptors just like AHLs and affect the bacterial signalling. Root exudates which are responsible for root colonisation are also involved in quorum sensing. Studies found that some Bacillus species that have the capability to degrade AHLs exhibit biocontrol activity against plant diseases. AHL-degrading bacteria reduced the pathogenicity of plant pathogens. Bacteria were isolated from soils where tobacco grows and 54 bacterial strains were allowed to grow in enrichment media. Among them 25 bacterial isolates showed degradation of AHLs. The bacterial isolates W2

and W3 completely degraded the N-caproyl-L-homoserine-lactone (C6-HSL). W2 isolate showed a degradation efficiency of about 95%. The bacterial isolates belong to *Pseudomonas* sp., *Variovorax* sp., *Variovorax paradoxus*, *Comamonas* sp., *Comamonas testosteroni*, and *Rhodococcus erythropolis*. These bacterial groups were analysed for N-acyl homoserine lactone (N-AHSL)-degrading efficiency. *R. erythropolis* showed higher degradation properties and it is further allowed to interact with quorum signals from other bacteria. Bacteria such as *Chromobacterium violaceum*, *Agrobacterium tumefaciens*, and *Pectobacterium carotovorum* subsp. *carotovorum* were allowed to interfere with *R. erythropolis*. The interaction did not affect the growth of the bacteria. *R. erythropolis* interferes with the violacein production which is responsible for the virulence of bacteria. Violacein is produced by *Chromobacterium violaceum.* A decrease in violacein production was observed. The interaction between *Agrobacterium tumefaciens* and *R. erythropolis* was checked, and it showed reduction in Ti plasmid conjugation. The pathogenicity of *P. carotovorum* subsp. *carotovorum* was analysed in potato plant using *R. erythropolis.* The inoculation of both strains prevented breakdown of tissues of the plant. The study demonstrated that the targeting of quorum sensing (QS) signalling molecules interacts with the pathogenicity of microbes (Uroz et al. 2003). Thus, AHL signalling molecules serve as a promising approach for stimulation of plant growth and abiotic stress tolerance. Studies suggested that plants and microbes associate through various signalling molecules. Further research is essential to select highly specific plant and microbial signalling molecules to uncover novel strategies.

4.3 Remediation of Contaminants by Plant–Microbe Combination

4.3.1 Removal of Pollutants from Aquatic Environments

Increase of pollutants in environment causes imbalance of ecosystem and serious health problems (Chandra and Kumar 2017a, b). Using plant–microbe interaction, several studies were done on treating pollutants. Here, some studies on cleaning up of pollutants from aquatic environments are discussed. Kristanti et al. investigated the effectiveness of four nitrophenol (NP)-degrading bacteria, namely *Pseudomonas* sp. (strain ONR1), *Cupriavidus* sp. (MFR2), *Rhodococcus* sp. (PKR1), and *Rhodococcus* sp. (DNR2) and the plant *Spirodela polyrhiza* in degrading the nitrophenol from aquatic system. Nitrophenols such as 1-NP, 2-NP, 3-NP, and 4-NP were used for degradation. The experiment was carried out with combinations of strains such as ONR1 with *Spirodela*; DNR2 with *Spirodela*; ONR1–DNR2 with *Spirodela*; and four strains, ONR1, DNR2, MFR2, PKR1, with *Spirodela*; and combinations without *Spirodela*. The degradation efficiency is higher in the presence of *Spirodela* compared to the test without *Spirodela*. Also, ONR1–DNR2 association with *Spirodela* showed greater degradation rate than that of ONR1 and DNR2 individually with *Spirodela*. Then the four strains with *Spirodela* were tested for

degrading the nitrophenol mixtures, which also showed promising results. ONR1 reduced the concentration of nitrophenol to about 92% (2, 4-dinitrophenol), 94% (2-NP), 3% (3-NP), 70% (4-NP), and the degradation efficiency of DNR2 is about 87% (2-NP), 32% (3-NP), and 100% (4-NP, 2,4-DNP). The inoculation of both strains degraded about 100% (2-NP, 4-NP, 2,4-DNP) and 30% (3-NP) over 4 days. In the absence of the bacterial strains, plants can degrade 17% (2-NP), 24% (3-NP), 3% (4-NP), and 9% (2,4-DNP) of pollutants. *Spirodela* without NP-degrading bacteria clean up a minimal quantity of nitrophenol which is stable on repeating cycles. But the degradation in the case of the bacteria along with *Spirodela* is not the same in repeated cycles. NPs often present in mixture, which causes a serious problem in treating them. Thus, studies on treating mixtures of NP are essential and would offer an attractive tool for degradation of NPs. A study analysed the efficiency of plant–microbe combinations in degrading pollutants. But selection of appropriate microbes and plants is essential for efficient degradation (Kristanti et al. 2014). Many industries release organic and inorganic compounds such as phenol and chromium, which cause pollution to the environment. Ontañon et al. conducted an experiment on rhizoremediation of phenol and chromium. Sediment samples were collected from a chemical and petrochemical industry and phenol-, chromium-resistant bacteria were isolated. On the basis of tolerance to the contaminant, a bacterial strain, *Pantoea sp*. FC 1, was selected and confirmed using 16S rRNA gene sequence amplification. The bacterial tolerance against phenol and chromium was investigated individually. FC 1 showed a higher degradation activity for chromium compared to phenol, since phenol affects bacterial growth at higher concentrations. As many authors suggested that hairy roots from different plant species have the capability to degrade phenolic compounds, the study employed association of FC 1 with hairy roots of *Brassica napus* in remediating the contaminants. For degradation process, removal of phenol exhibited with hairy roots alone and with a combination of hairy roots and FC 1. Hairy roots removed 60% of phenol and the efficiency increased on inoculation of FC 1. For chromium, the combined system removed 100% after 3–4 days. This study suggested that association of FC 1 and hairy roots of *Brassica napus* serves as an innovative system in degrading phenol and chromium pollutants (Ontañon et al. 2014). Hu and Li (2021) conducted a study on treating sewage using aquatic plants and microbes. The plants selected for the process were *Eichhornia crassipes*, *Cyperus alternifolius*, *Phragmites communis*, and *Scirpus validus Vahl* and grown in plastic buckets containing sewage. The phytoremediation test is conducted first for a duration of 15 days. The nitrogen and phosphorus contents were checked every 2 days. The nitrogen degradation effects in sewage for the four plants were different. Similarly, the phosphorus content also decreased in sewage treated with different plants. Microbial and plant combination tests were done with the same four plants for treating sewage. Similar to phytoremediation test, the nitrogen and phosphorus contents were analysed for 2 weeks. The nitrogen degradation effect in every test was different. The efficiency of degradation by microbes, plants, and plant–microbe systems were 64.85%, 40.61%, and 77.67%, respectively. Similarly, the phosphorus content degradation efficiency by microbes, plants, and plant–microbe combinations

was 61.56%, 39.23%, and 71.97%, respectively. When comparing all degradation tests, it is quite clear that plant–microbe systems showed better removal compared to plants and microbes alone. Microbial-assisted remediation showed higher efficiency compared to phytoremediation. From the above discussed studies, it is evident that a plant–microbe combined system can enhance the removal of contaminants in an aquatic system.

4.3.2 Removal of Pollutants from Terrestrial Environment

There are numerous studies that showed positive results in removal of contaminants from soil and/or sludge (Chandra and Kumar 2017b; Kumar et al. 2020b, 2021e; Kumar and Chandra 2020b). Microbes growing in metal-contaminated soils tend to have a high tolerance towards concentration of metals. Soil and plants are also benefited from these microbes. Among these microbes PGPR show high benefits by altering metal bioavailability. They do this by altering pH, producing phytohormones and thus improving the phytoremediation process. He et al. demonstrated a study in which 11 plant species showing metal tolerance and rhizosphere soil were collected from a copper mine wasteland. Thirteen copper-resistant bacterial strains were isolated and selected based on their different morphological appearance. The characterisation of bacterial strains was analysed by evaluating their production of IAA, acetyl-CoA carboxylase (ACC), and siderophore. The bacterial isolates were tested against different metals. The bacterial strains showed different degrees of resistance towards metals. The bacterial strains *Sphingomonas sp.* YJ3 and *Microbacterium lactium* YJ7 showed resistance towards Cu. The strain *Acinetobacter sp*. SWJ11, *Arthrobacter sp.* MT16, *Azotobacter vinelandii* GZC24, and *Arthrobacter* sp. YAH27 showed resistant towards Ni, Zn, Pb, and Cu when inoculated in *Brassica napus*. The bacterial strains also enhanced root elongation in the plant species (He et al. 2010). *Arabidopsis thaliana* and the bacterium *Sphingobium* are used to remove isoproturon from contaminated zones. In this study, phytoremediation and bioaugmentation processes were combined. The resulting intermediate of isoproturon is treated with *Sphingobium*, which can mineralise it. This resulted in enhanced and complete removal of pollutants. The increase in remediation is due to a synergistic relationship between the transgenic plant and *Sphingobium* (Yan et al. 2018). A study was conducted on *Cytisus striatus* to degrade hexachlorocyclohexane (HCH) with a combination of two endophyte microbial strains *R. erythropolis* ET54b and *Sphingomonas* sp. D4. The study was carried out on two different soils differing in their organic content. The results showed a higher efficiency in remediation of HCH compared to the treatment which does not involve the endophytes, and also enhanced plant growth. The efficiency of degradation in soil depends on the content of organic matter (Becerra-Castro et al. 2013). A 4-month study was conducted on three plant species, namely *Scorzonera mongolica Maxim*, *Atriplex centralasiatica*, and *Limonium bicolor*, in relation to their degradation of crude oil present in the contaminated soil at an oil refinery land farm. The rhizosphere soil adhered to the plant species

were analysed and the microbes present were investigated for metabolic activity. The utilisation of carbon sources by microbes was analysed. The most probable number (MPN) method was used for the identification of the number of microbes that have the capability of degrading petroleum hydrocarbons. The degradation efficiency of the three plant species showed no significant difference. The plant biomass was investigated and the results showed enhanced root system in *A. centralasiatica* compared to other plants. Microbial activity was higher for *A. centralasiatica.* The pH value of the soil of the three species decreased due to production of root exudates and microbial metabolites. The decrease in pH value is due to the increased utilisation of phosphorus, which promotes enhanced plant growth and breakdown of petroleum hydrocarbons. The results showed a decrease in the concentration of petroleum hydrocarbons using the plant–microbe remediation system. Both plants and microbes benefit each other and also enhanced the process of remediation (Ying et al. 2011).

4.3.3 Removal of Pollutants from Atmosphere

The major health risk for humans is caused by air pollution which involves ammonia, nitrogen oxides, particulate matter, sulphur dioxide, VOC, and so on. An efficient remediation method is essential to eradicate these pollutants. Selection of appropriate plant and microbial species for remediation is crucial in removing pollutants (Molina et al. 2021). Here, some of the studies on plant–microbe combination to clean up the pollutants are discussed. A study on endophytes confirmed that they have the ability to promote plant growth and also degrade polycyclic aromatic hydrocarbons (PAH). In this study, three willow endophytes, namely WW1, WW3, and WW11, and three poplar endophytes, namely SX61, PD1, and PTD3, were allowed to grow in a medium containing PAH and they showed high growth in the presence of naphthalene. Of all the endophytes, the strain PD1 showed highest growth with the inoculation of PAH. As a result, PD1 was chosen for further studies. PD1 was used to degrade phenanthrene and the results showed that about 60% of phenanthrene was degraded from the medium. Using 16S rDNA sequencing the endophytic bacteria were identified as *Pseudomonas putida*. This study illustrated that the endophyte-assisted phytoremediation showed higher efficiency in degrading PAH (Khan et al. 2014). Yutthammo et al. investigated the activities of the bacteria which have the capability to degrade PAH. The study focussed on the bacteria present in the phyllosphere of ornamental plants. The physical and chemical characteristics of leaves of the ten ornamental plants such as *Ixora* sp., *Murraya paniculata*, *Wrightia religiosa*, *Bougainvillea* sp., *Jasminum sambac*, *Codiaeum variegatum*, *Ficus* sp., *Streblus asper*, *Pseuderanthemum graciliflorum*, and *Hibiscus rosa-sinensis* were investigated. The plant species were compared for the number of phenanthrene-degrading bacteria. A large number of phenanthrene-degrading bacteria were found in *W. religiosa* and *H. rosa-sinensis*, which were further chosen for analysis to check the activities of the bacteria. The results showed higher reduction of phenanthrene level in *W. religiosa* leaves compared to *H. rosa-*

sinensis. The bacteria along with *W. religiosa* was then experimented with different compounds of PAH such as acenaphthylene, acenaphthene, and fluorine. The unsterilised leaves showed higher efficiency in removal of PAH than sterilised leaves. The study suggested that the PAH-degrading bacteria are common in phyllosphere and also have the capability to increase the efficiency of the plant leaves, thus playing an important role in removal of urban air pollutants. Each plant in its phyllosphere has a unique bacterial group due to the differences in leaf morphology and chemical compounds present in the leaf. The study demonstrated that the leaves of ornamental plant contain various phenanthrene-degrading bacteria (Yutthammo et al. 2010). An investigation was made on chloromethane-degrading bacteria isolated from *Arabidopsis thaliana.* The study involved isolation of bacterial strains *Methylorubrum extorquens* CM4, *Hyphomicrobium chloromethanicum* CM2 from Russian petrochemical factory soil, and *Hyphomicrobium* sp. *strain* MC1 from industrial sewage. These three strains originated from the leaves of *Arabidopsis thaliana* and a gene, *cmuA*, responsible for dehalogenation of chloromethane was identified. The ability of the three strains to use chloromethane as a carbon source was compared with that of the reference strain. *Hyphomicrobium* sp. showed higher efficiency in degrading chloromethane compared to other strains, since it is well adapted to grow in the presence of chloromethane (Nadalig et al. 2011). The above studies prove that plant–microbe interaction-associated remediation not only degrades the contaminants but also benefits both plants and microbes by producing phytohormones. Even though the method is efficient, the mechanism of remediation by plants and microbes is still not clear. The above-discussed studies on degradation of pollutants by various plant–microbe interactions are illustrated in Table 4.1.

4.4 Examples of Bacterial-Assisted Phytoremediation

Phytoremediation is the process wherein plants are used to clean up pollutants, and this process involves various mechanisms such as phytodegradation, phytoextraction, phytostabilisation, and phytostabilisation, as shown in Fig. 4.3. Phytoremediation removes both organic and inorganic pollutants, but there are certain limitations that make the process less efficient. Plants suffer from stress caused by contaminants, which leads to reduction in plant growth, development, and seed germination. These limitations are said to be reduced by rhizobacteria. The microbes in the polluted site easily get adapted and use the polluted substance as a nutrient source. The mechanisms of phytoremediation, when assisted with bacteria, showed higher efficiency in degrading pollutants. Bacteria play an important role in the phytoremediation process by producing siderophores, IAA, ACC, and enhancing the bioavailability of heavy metals by mechanism of precipitation, redox reaction, chelation, and acidification (Gaur et al. 2021). Thus, intensive research on the phytoremediation mechanism helps in understanding the metabolic breakdown of pollutants by plants and microbes. The following are some of the research works carried out on the microbial-assisted phytoremediation process (Table 4.2).

Table 4.1 Enhanced remediation of pollutants by plant–microbe interactions

Pollutants	Microbes	Plants	Process	References
Nitrophenol	*Pseudomonas* sp., *Cupriavidus* sp., *Rhodococcus* sp., *Rhodococcus* sp.	*Spirodela polyrhiza*	Degradation of nitrophenol in five consecutive cycles	Kristanti et al. (2014)
Phenol, chromium	*Pantoea* sp.	*Brassica napus*	Hairy roots of *Brassica napus* and Pantoea sp. degrade 100% of pollutants	Ontañon et al. (2014)
Nickel (Ni) Lead (Pb) Zinc (Zn) Copper (Cu)	*Sphingomonas* sp. YJ3, *Microbacterium lactium* YJ7, *Acinetobacter* sp. SWJ11, *Arthrobacter* sp. MT16, *Azotobacter vinelandii* GZC24, *Arthrobacter* sp. YAH27	*Brassica napus*	Increased plant growth and root elongation; increased resistance towards heavy metals	He et al. (2010)
Isoproturon	*Sphingobium* bacterium	*Arabidopsis thaliana*	Combined process of phytoremediation and bioaugmentation; mineralisation of isoproturon	Yan et al. (2018)
Hexachlorocyclohexane (HCH)	*Rhodococcus erythropolis* ET54b *Sphingomonas* sp. D4	*Cytisus striatus*	Increased plant growth and degradation of pollutants based on organic matter content	Becerra-Castro et al. (2013)
Crude oil	Rhizospheric microbes	*Scorzonera mongolica maxim, Atriplex centralasiatica*, and *Limonium bicolor*	TPH-degrading bacteria	Ying et al. (2011)
Phenanthrene	Poplar endophytes PD1	*Pseudomonas putida*	60% degradation of phenanthrene and increased bacterial growth	Khan et al. (2014)
Polycyclic aromatic hydrocarbons	PAH-degrading bacteria	*Wrightia religiosa, Hibiscus rosa-sinensis*	Phenanthrene concentration decreases in leaves.	Yutthammo et al. (2010)
Chloromethane	*Methylorubrum extorquens*, *Hyphomicrobium chloromethanicum*, *Hyphomicrobium* sp. *strain* MC1	*Arabidopsis thaliana*	Higher expression level of gene *cmuA* which degrades chloromethane	Nadalig et al. (2011)

HCH Hexachlorocyclohexane TPH Total petroleum hydrocarbon; PAH Polyaromatic hydrocarbons

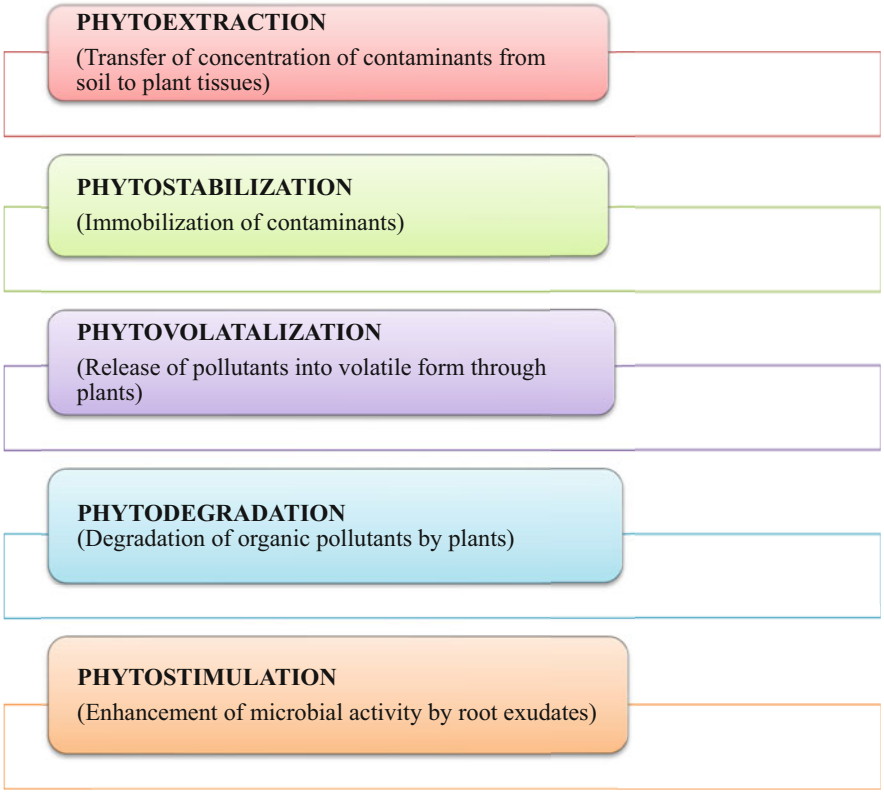

Fig. 4.3 Various phytoremediation strategies

4.5 Microbial-Assisted Phytoextraction

Phytoextraction is the process which involves the use of hyperaccumulating plants to remediate pollutants from contaminated environments. Hyperaccumulating plants are the plants that have the capability to remove metals from the soil. Phytoremediation is best known for its remediation of heavy metals. Various mechanism of phytoremediation involved in metal accumulation is illustrated in Fig. 4.4 (Gaur et al. 2021). Metal-accumulating capability and biomass production are the important factors in phytoextraction process. The optimisation of these factors enhances the phytoremediation strategy. The limitation of this process involves slow growth and their low tolerance to metal stress. Some of the studies illustrated that microbial-assisted phytoextraction has higher efficiency in removal of pollutants. Khonsue et al. investigated *Vetiveria nemoralis* and *Ocimum gratissimum* with cadmium-resistant bacteria. Two cadmium-resistant bacterial strains *Ralstonia* sp. TAK1 and *Arthrobacter* sp. TM6 were isolated from the

Table 4.2 Microbial-assisted phytoremediation of heavy metals and organic pollutants

Pollutants	Plants	Microbes	Process	References
Cadmium	*Vetiveria nemoralis*, *Ocimum gratissimum*	*Ralstonia sp.* TAK1, *Ocimum gratissimum*	Increased cadmium solubility, increased production of phytohormones (enhanced phytoextraction)	Khonsue et al. (2013)
Arsenic	*Betula celtiberica*	*Ensifer adhaerens* 91R, *Rhizobium herbae* 32E, *Variovorax paradoxus* 28EY, *Phyllobacterium myrsinacearum* 28EW	Increased production of indole-3-acetic acid (IAA), aminocyclopropane-1-carboxylic acid (ACC) deaminase, and siderophore, arsenic accumulation (enhanced phytoextraction)	Mesa et al. (2017)
9.Chromium	*Brassica juncea*	*Pseudomonas* sp. PsA4, Bacillus sp. Ba32	Increased plant growth and inhibitory effects against chromium (enhanced phytostabilisation)	Rajkumar et al. (2006)
Pesticides	*Cucurbita pepo* L., *Xanthium strumarium*	*Bacillus vallismortis*, *Bacillus aryabhattai*	Decrease in pollution stress and increased biomass production (enhanced phytostabilisation)	Nurzhanova et al. (2021)
Cd, Pb, Zn, Cu	*Hibiscus cannabinus*	*Enterobacter* sp. strain EG16	Increased phytohormone production, reduction in the concentration of metals and immobilisation of metals (enhanced phytostabilisation)	Chen et al. (2017)
Petroleum hydrocarbons	*Lolium multiflorum*	*Pseudomonas* sp. strain ITRI53 *Pantoea* sp. strain BTRH79	Increased alkane-degrading gene expression level, promotes plant growth (rhizoremediation)	Afzal et al. (2011)
Polychlorinated biphenyl (PCB)	*Morus alba*	*Rhodococcus* sp.	Increase the solubility of pollutants, reduction of inhibitory effects of plant growth (rhizoremediation)	Sandhu et al. (2020)
Diesel	*Scirpus grossus*	Rhizobacteria	Increase in the reduction of petroleum hydrocarbons (enhanced phytodegradation)	Al-Baldawi et al. (2015)

(continued)

Table 4.2 (continued)

Pollutants	Plants	Microbes	Process	References
Petroleum hydrocarbon	*Lotus corniculatus*, *Oenothera biennis*	Endophytic bacteria	Increased phytohormone production and degradation of hydrocarbons	Pawlik et al. (2017)
2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (dye)	*Gaillardia pulchella*	*Pseudomonas monteilii*	Decolourisation of dye is enhanced (enhanced phytodegradation)	Kabra et al. (2013)
Lead	*Lolium perenne*	*Trichoderma asperellum*	Increased plant growth and dry weight, increased metal extraction from soil (enhanced phytostimulation)	Sun et al. (2020)
Arsenic	*Pteris vittata*	*Agrobacterium radiobacter*	Increased phytohormone production and increased metal uptake (enhanced phytovolatilisation)	Wang et al. (2011)

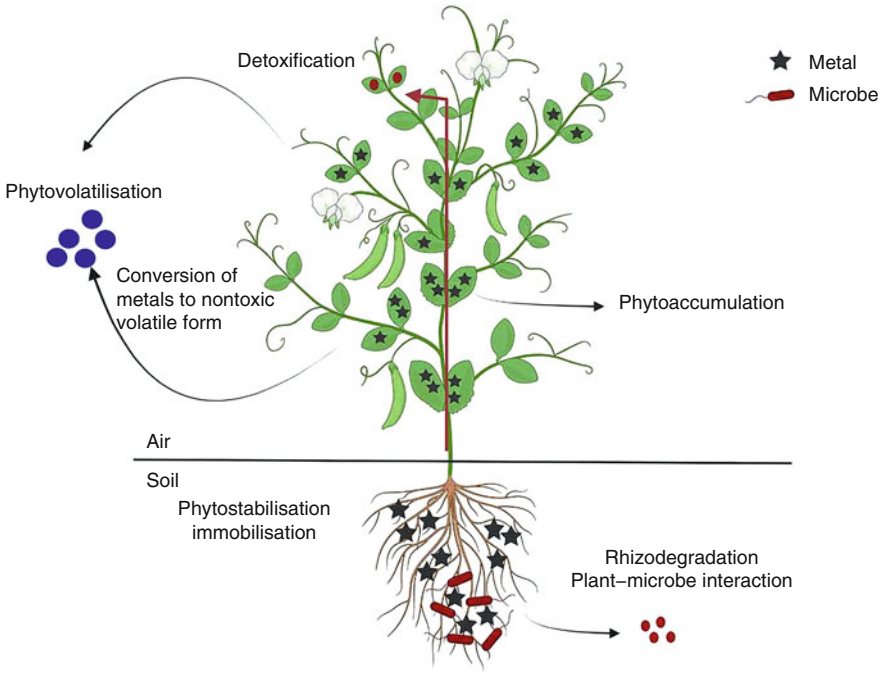

Fig. 4.4 Metal accumulation in plants and their remediation and detoxification mechanisms

cadmium-contaminated site. Two plants, *V. nemoralis* and *O. gratissimum*, were chosen and transplanted into the cadmium-contaminated soil. The experiment comprised four various treatments of plants: (1) without bacteria, (2) *Ralstonia sp*. TAK1, (3) *Arthrobacter sp.* TM6, and (4) EDTA. The plants were allowed to grow for 1 month. Plant growth and cadmium concentration were analysed. Plants inoculated with bacterial strains exhibited increased cadmium solubility of soil. Increase in solubility increases the mobilisation of heavy metals. An efficient phytoextraction method requires high solubility of heavy metals when it is in contact with plants. The bacterial strains were found to survive in the cadmium-contaminated soil. Cadmium inhibited the growth of root and shoot systems. The bacterial strains increased the production of phytohormones, which stimulated plant growth and development. It is observed that the accumulation of cadmium increases in the plants with bacterial strain inoculation. But cadmium accumulation is higher in root than in shoot, as mostly metal accumulates more in the root system. Cadmium concentration was analysed after 1 month of plant growth. The plant *V. nemoralis* treated without bacterial strains, with *Ralstonia* sp. TAK1, with *Arthrobacter* sp. TM6, and with EDTA showed decrease in cadmium concentration of 24.7%, 28.8%, 32.7%, and 32.2%, respectively. Similarly, in *O. gratissimum*, the decrease in cadmium concentration is 25.5% (Without inoculation), 23.2% (TAK1), 28.7% (TM6), and 32% (EDTA). The study reported that both bacterial strains stimulated the phytoextraction of cadmium. But *O. gratissimum* showed higher efficiency of

phytoextraction with *Arthrobacter sp*. Compared to *O. gratissimum*, the efficiency of accumulation of cadmium in *V. nemoralis* is higher. The findings in the study suggested that the synergistic relationship between microbes and plants in phytoextraction of cadmium-contaminated soils (Khonsue et al. 2013). Mesa et al. demonstrated the bacterial-assisted phytoextraction using endophytic and rhizosphere bacteria. Root samples were collected from *Betula celtiberica* grown on arsenic-contaminated soil. The first step involves isolation and characterisation of endophytic and rhizosphere bacterial communities. Totally, 68% of endophytic bacteria and 53% of rhizobacteria were able to produce IAA. Similarly, 5% of endophytic bacteria and 36% of rhizobacteria were able to produce acetyl-Co A carboxylase deaminase (ACCD). 32% of endophytic strain and 36% of rhizobacteria produce siderophore. A total of 54 rhizosphere bacteria and 41 endophytic bacteria were analysed for metal accumulation in plants and plant growth. Out of these, seven bacteria were considered for arsenic accumulation test. Based on IAA, ACC, and siderophore and arsenic resistance the bacterial strains were selected. The seven bacterial isolates were *Neorhizobium alkalisoli* ZY-4s, *Rhizobium herbae* CCBAU 83011, *Variovorax paradoxus* S110, *Phyllobacterium myrsinacearum* NBRC 100019, *Rhodococcus erythropolis* TS-TYKAKK-12, *Aminobacter aminovorans* LZ1304-3-1, and *Ensifer adhaerens* Sx1. These strains were inoculated into *Betula celtiberica* and allowed to grow for 30 days and analysed for metal accumulation. Out of these, four strains, *Ensifer adhaerens* 91R, *R. herbae* 32E, *V. paradoxus* 28EY, and *P. myrsinacearum* 28EW, were selected for field evaluation. *E. adhaerens* 91R promoted plant growth by increasing root and shoot length, whereas the other three strains were able to increase arsenic accumulation. The use of plants that grow naturally in a particular ecosystem provides an efficient way of phytoextraction by limiting the competition of neighbouring plants. It is noted that most of the endophytes have the ability to produce IAA, and siderophore production is more commonly reported in rhizospheric bacteria (Mesa et al. 2017).

4.6 Microbial-Assisted Phytostabilisation

Phytostabilisation is the process that involves immobilisation of the contaminants present in soil or groundwater by chemical compounds produced by plants. The immobilisation of contaminants occurs by absorption by roots and precipitation around the roots, which reduces the mobility of the pollutants. However, it has disadvantages such as existence of residues of pollutants in the soil, overuse of fertilisation, and decrease in plant growth due to exposure to metal stress. The following studies were investigated for bacterial-assisted phytostabilisation to check their efficiency in overcoming the disadvantages. Rajkumar et al. conducted a study on *Brassica juncea* inoculated with two chromium-resistant plant growth–promoting bacteria (PGPB), *Pseudomonas* sp. PsA4 and *Bacillus* sp. Ba32. The soil samples were collected from the metal-contaminated site and chromium-resistant PGPB are isolated. *B. juncea* plants are inoculated with PGPB and allowed to grow for 20 days. The growth parameters of the plants such as shoot length, root length,

and dry weight were measured and accumulation of chromium in roots and shoots was analysed. Sixteen PGPB strains were isolated and the strains *Pseudomonas* sp. PsA4 and *Bacillus* sp. Ba32 were selected as they use acetyl-CoA carboxylase (ACC) as a source of nitrogen. The strain using ACC as a source of nitrogen enhances plant growth. Inoculation of the strain PsA4 in the plants increases 59% root length, 55% shoot length, and 85% vigour index. Similarly, inoculation of Ba32 strain increases 55% root length, 29% shoot length, and 58% vigour index. The bacterial-inoculated and non-inoculated plants were observed. The bacterial-inoculated plant showed increased plant growth in the absence of chromium. PsA4 enhanced plant root length by 73% and shoot length by 28%. Ba32 improved plant root length by 53% and shoot length by 18%. The non-inoculated plants were subjected to different concentrations of chromium, which inhibits plant growth. Plant inoculated with the strains in the presence of chromium showed a moderate activity of increase of 62% root length, 31% shoot length in PsA4. At all concentrations of chromium, PsA4 exhibited higher efficiency in plant growth compared to Ba32. Several strategies were developed for plants to adapt to the polluted environment. But high concentrations of heavy metals restrict the uptake of Fe and P required for plant development. Microbes help by inhibiting the effects of metals and producing more phytohormones, which enhances the uptake of minerals and promotes plant growth. This study illustrated that the inoculation of PGPB bacteria in plants contaminated by chromium showed inhibitory effects against chromium (Rajkumar et al. 2006). Chen et al. carried out a study on the metal-tolerant bacterium *Enterobacter* sp. strain EG16 using *Hibiscus cannabinus* growing in metal-contaminated soil. The *Enterobacter* sp. that is cadmium resistant is isolated from *H. cannabinus* in a metal-polluted site. The bacterial growth is enhanced by the supply of Fe. The effect of cadmium on bacteria was analysed by checking for production of IAA, siderophore, Fe uptake, and the results showed decreased production. The inoculation of bacteria into plants, enhance the root and shoot length. The bacterial strain also stimulates increase of Fe supply in less Fe accumulation plant. The concentration of cadmium also reduces drastically in the plant. Since the contaminated soil was taken from site containing multiple metals, metals such as Pb, Zn, and Cu were also found. EG16 not only reduces the concentration of Cd but also significantly reduces the concentration of Pb and Zn. The Cu concentration remains unaffected by the bacterial strain. Bioavailability and metal mobilisation play an important role in toxicity of plants. The study also illustrated the reduction in bioavailability of Pb, Zn, Cd, and immobilisation of Cu in *H. cannabinus* (Chen et al. 2017). Nurzhanova et al. selected *Cucurbita pepo* L. and *Xanthium strumarium* that are tolerant to dichlorodiphenyltrichloroethane (DDT) and isolated bacterial strains from DDT-contaminated soil. The experiment was carried out in two types of soil. One is naturally obtained from the contaminated site and the other is artificially prepared in laboratory by inoculating DDT. The two soil groups tested here mostly consist of *Pseudomonas* and *Bacillus* dominantly, the next available genera are *Mycobacterium, Arthrobacter*, *Streptomyces*, and the least distributed group is *Micromonospora*. Seeds of the two plant species were transplanted into the two soil types and growth is monitored for 7 months. The

soil adhered to the roots of the plants are taken for isolation of bacterial strains. 580 microbial strains were isolated from the soil and two bacterial strains were selected for further experiments due to their capability to utilise dichlorodiphenyldichloroethylene (DDE). Based on 16S rDNA sequence analysis the bacterial strains were identified as *Bacillus vallismortis* and *B. aryabhattai.* After an incubation of 14 days, it is observed that *Bacillus aryabhattai* consumed around 89.3% of the pesticide and after 21 days, the consumption is around 93.4%. The inoculation of selected microbes and plants resulted in a decrease in pollutant stress and an increase in plant biomass. The bacterial-assisted treatment enhances the phytostabilisation strategy. The rhizosphere contains diverse groups of microbes and in this study the dominant group found in the polluted soil was *Rhodococcus*, which mostly executes dehalogenations, dehydrogenations, oxidation, and so on. Though *Rhodococcus* strains were present abundantly in the contaminated soil, bacterial strains of bacterial genera like *Bacillus* and *Pseudomonas* were present more in both soil types. Thus, two *Bacillus* strains were chosen for further processing in the studies. It is also reported that Bacillus spp. when mixed with fungi accelerate the degradation of DDT, since fungi break down DDT into DDD. The microbes and plants in the contaminated soil were able enhance the degradation process and also reduce plant stress (Nurzhanova et al. 2021).

4.7 Microbial-Assisted Phytovolatilisation

Phytovolatilisation is the process wherein plants take up the contaminants and release it to the air through their leaves as volatile compounds. This process helps the contaminants to reduce its toxicity. The disadvantage of this process may be the precipitation of contaminants into lakes or oceans. The phytovolatilisation process is said to be enhanced when it is microbial assisted. Some of the studies suggested the efficiency of microbial-assisted phytovolatilisation is approachable. Wang et al. conducted a study on arsenic (As) degradation by PGPR and *Populus deltoides*, a tree species. *Pteris vittata* plants were collected from arsenic-contaminated region and bacteria from soil adhered to the roots were isolated. Twenty-two As-resistant bacteria were isolated and checked for efficiency of production of IAA and siderophore. The strain D14 showed high resistance towards arsenic and using 16S rRNA gene sequencing, the strain is identified as one of *Agrobacterium radiobacter.* The strain also showed higher production of IAA and siderophore. *P. deltoides* was allowed to grow in the presence and absence of the bacterial strain for 5 months. Without bacterial inoculation, the plants showed a high efficiency in arsenic removal. However, with bacterial inoculation the arsenic removal was enhanced. As the bacterial strain was inoculated, the inhibitory effects of arsenic were reduced. The strain increased plant growth and uptake of the metal. The study evidently showed the capability of plants in metal uptake and the enhancement of the degradation process with microbes (Wang et al. 2011).

4.8 Rhizoremediation

Rhizoremediation is the elimination of contaminants around the soil employing microbes that are present in the surrounding soil. Using plant–microbe pairs, the rhizoremediation process is very efficient. The roots produce compounds which are used by microbes as a source of nitrogen, carbon, and phosphorus when attracted to root exudates. The utilisation of these compounds helps in resisting the toxicity of pollutants (Molina et al. 2021). The following are some of the examples of rhizoremediation strategies using plant–microbe pairs. Afzal et al. investigated on the plant–microbe association to clean up the soil contaminated with petroleum hydrocarbons and the activity of plant and microbes. Two bacterial strains, *Pseudomonas* sp. strain ITRI53 and *Pantoea* sp. strain BTRH79, were isolated. Seeds of *Lolium multiflorum* were harvested in three types of soil such as sandy soil, loamy sandy soil, and loamy soil. BTRH79 colonised better in the rhizosphere of the plant than ITRI53, which showed better colonisation in the shoot of plant. The bacterial strain expresses the gene which is responsible for the degradation of pollutants. This gene expression level is found higher in loamy soil and also plant growth is efficient. In sandy soil, the survival of the bacterial strain is low, the gene expression level is found only in the shoot region, which is also lower. From the study, it is evident that the type of soil determines bacterial abundance and expression of alkane-degrading genes. The hydrocarbon degradation is evaluated in the presence and absence of bacterial strain. The degradation level is low in plants not inoculated with the strains, and the degradation efficiency is just 12–20% even after 8 weeks; the ones inoculated showed higher degradation of hydrocarbons, and their efficiency is different in different soil types. Hydrocarbons reduce plant growth by hydrophobicity, which limits the plants in taking up nutrients and water. In the bacterial-inoculated plants, the growth of shoot region increased by 8–41%. Loamy soil resulted in higher degradation rate of hydrocarbons of 63% and showed higher plant growth compared to other soil types. The above experiment illustrated that selection of a suitable bacterial strain is essential for improved phytoremediation and in plant growth. It is also evident that the soil type influences plant growth and microbial colonisation (Afzal et al. 2011). Wu et al. did an investigation on sunflower plant and *P. putida* for rhizoremediation of heavy metals. A study was conducted on bacteria that have polychlorinated biphenyl (PCB)-degrading ability and the plant *Morus alba*. The soil sample was collected from the depth of the plant. The bacterial strain was isolated and characterised for biphenyl utilisation. The soil is enriched with four bacterial strains. The bacterial strain MAPN1 showed prominent results and was selected for further studies. Using 16S rDNA gene sequencing, the bacterial strain MAPN1 was identified as one of *Rhodococcus* sp. The bacterial strain is tested for PCB-degrading activity. The strain was allowed to react with naphthalene, anthracene, benzoic acid, salicylic acid, and dibenzofuran. The result was the MAPN1 strain grew on all the tested aromatic substances. The highest growth was observed for the compound anthracene. The strain showed prominent growth using biphenyl as the carbon source and increased the efficiency of phytoremediation strategy. MAPN1 strain has the capability to produce glycolipid

biosurfactants which have the ability to solubilise the pollutants. Plant growth was observed for different concentrations of biphenyl and with inoculation of bacteria. The biphenyl showed an inhibitory effect on plants, in the absence of the bacterial strain. When the bacterial strain is inoculated, the inhibitory effect was reduced and plant growth was enhanced. For 15 days, plant growth was slow. After that plant growth at all different concentrations of biphenyl gradually increased. However, there is no clear study of *Rhodococcus sp*. on plant growth properties, so a better understanding is required to achieve successful enhanced phytoremediation (Sandhu et al. 2020).

4.9 Phytostimulation

Phytostimulation is the process which involves promotion of rhizosphere microorganisms by utilising the signalling molecules released by plant roots as a nutrient source. Sun et al. investigated on reduction in lead toxicity on *Lolium perenne* (perennial ryegrass) by *Trichoderma asperellum.* The fungus *T. asperellum* was isolated from the lead-contaminated region. The fungal isolates were cultured in media for 14 days. The experiment was done in four ways: plants were set up in soil which was not contaminated by lead (CK), lead-contaminated soil (T1), lead-contaminated soil with saw dust (T2), and inoculation of fungal isolate with saw dust in contaminated soil (T3). Then the plants were allowed to grow for 28 days. Plant growth was reduced in T1 condition which was exposed to lead; plant height was increased in T2 condition, but dry weight was reduced; T3 condition showed enhanced plant growth and dry weight. A higher concentration of lead was observed in roots than in shoots. The lead-resistant microbes enhanced the extraction of lead from the soil, thus improving remediation of the lead-contaminated soil (Sun et al. 2020). The modulation of signalling compounds in association with plants and microbes was investigated. Seeds of *Withania somnifera* are planted and allowed to grow for 14 days. Four endophytic strains were isolated from the leaves of the plant and tested for the production of IAA, ACC, and ammonia. The fungal isolate *Aspergillus fumigatus* was able to produce IAA, ACC, and ammonia, based on which the strain was chosen for further studies. The endophytic strains, when inoculated into the plants, were able to promote plant growth and colonised effectively. To check the ability of IAA in enhanced plant growth, it is inhibited and plant growth was analysed. The growth of roots was reduced to 66% and with IAA, the growth of maize root was 90%. Thus, it is evident that IAA plays a key role in phytostimulation of plants (Mehmood et al. 2018).

4.10 Microbial-Assisted Phytodegradation

Phytodegradation is the process which involves breakdown of organic pollutants either by metabolic activities occurring within the tissue or through enzymatic release from roots. *Scirpus grossus* is used for phytodegradation of pollutants in

contaminated water. The plant was allowed to grow in diesel-contaminated water and analysed. The polluted water, soil and plant samples were taken on 14, 28, 42, and 72nd days to check the effect of the phytoremediation process. The number of rhizobacteria present in the roots of *S. grossus* was estimated by the serial dilution method. The potential rhizobacteria were isolated for running the biodegradation test, which was used to evaluate the plant–microbe interaction. After 72nd day of phytoremediation, the concentration of total petroleum hydrocarbons (TPH) was evaluated. Three plants were used in the experiment and the efficiency of degrading petroleum hydrocarbons was 81.5%, 71.4%, and 66.6%. The degradation test showed the effect of *S. grossus* and rhizobacteria in diesel-contaminated water. There was a difference in the removal of concentration of pollutants on from 14th day to 72nd day. It is due to interaction of plant and microbe. In the presence of a higher population of rhizobacteria, the removal efficiency is higher. The study demonstrated the ability of *Scirpus grossus* to withstand the petroleum hydrocarbons in the concentration of 0.1%, 0.175%, and 0.25%. It is also evident that the interaction between rhizobacteria and *S. grossus* enhanced the removal of diesel (Al-Baldawi et al. 2015). A study on *Lotus corniculatus* and *Oenothera biennis* has shown that hydrocarbon-degrading endophytic bacteria stimulate improved phytodegradation of pollutants. The plants were collected from the hydrocarbon-polluted site and divided into shoots, roots, and leaves. The soil adhering to the roots were taken and endophytic bacteria were isolated from the soil. The production of IAA, ACC and solubility of inorganic phosphate of bacterial isolates were estimated. About 58.33% of bacterial isolates from *L. corniculatus* and 28.7% of isolates from *O. biennis* showed the ability to solubilise phosphate. The capability of bacterial isolates to utilise hydrocarbon as a carbon source was checked. The bacterial isolates were analysed for identification of hydrocarbon-degrading genes. Five bacterial strains, namely *Pseudomonas mandelii* and four strains of *Rhodococcus* sp., showed positive results. All the bacterial isolates are screened for their capability to promote plant growth. All bacterial strains produce IAA and the highest production were observed on *Delftia lacustris* 5FXS, *Delftia lacustris* 6.1XS, and *Rhizobium* sp. 1XS. The highest production of siderophore was observed in *L. corniculatus.* The ability of the bacterial strain in plant colonisation was analysed by checking the cellulase activity. The cellulase production of *O. biennis* and *L. corniculatus* efficiency is 64.29% and 47.67%, respectively. The isolated bacterial strains showed a clear potential to degrade hydrocarbons by emulsification property. Bacterial strains of species such as *Serratia, Delftia, Rhodococcus, Rhizobium, and Pseudomonas, and Rhodococcus sp. 4WK* have the ability to produce biosurfactants which promote emulsification of hydrocarbons, resulting in degradation (Pawlik et al. 2017). Kabra et al. (2013) worked on the treatment of textile effluents by plant–microbe interactions. The soil adhered to the roots of the plant *Gaillardia pulchella* was cleaned and dye was added to it. The dye used in the experiment is 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). The plant inoculated with the dye decolourised it within 72 h. Using 16S rRNA gene sequencing the bacteria present in the roots was found to be *Pseudomonas monteilii*. Seeds of the *G. pulchella* were planted and allowed to grow for 4 weeks. The plant decolourised the dye with an

efficiency of 97% within 72 h, the efficiency of decolourisation by bacteria was 85% within 72 h, and the efficiency of combined system was about 100% within 48 h. The study shows the efficiency of plant–microbe combined system in decolourising the dye is higher than usage of plant and microbes separately (Kabra et al. 2013). From the studies illustrated in the review, it is evident that microbes when interacting with plants help in depleting pollutants and also help plants to withstand the stress caused by pollutants. Microbes provide plants with growth-promoting phytohormones and plants in turn provide carbon, nitrogen, and phosphorus sources to the microbes.

4.11 Challenges Faced During Remediation by Plant–Microbe Associations

Even though plant–microbe remediation has a potential ability to degrade pollutants, there are some disadvantages for this combined system, which make it challenging, and only few studies have been conducted on them to improve the efficiency of remediation. Differences in the efficiency of degrading pollutants have been observed, when plants with its associated microbes are used in different combinations. This observation suggests that selection of appropriate microbes and plants is required for remediation of specific contaminants. There is not much research on suitable selection of plant–microbe systems for remediating polluted environments. Some negative impacts on food chain also occur due to the problems faced in disposal of pollutants. Only few details about the composition of the microbes present in contaminated soils or regions are known. In degradation of pesticides, the parent biocompounds used also have toxic effects, which results in harmful impacts on humans. There is no clear understanding of the mechanism of degradation of contaminants, role of rhizospheric microbes, and the interaction of plants and microbes (Kuiper et al. 2004).

4.12 Conclusion

From this review, it is evident that plant–microbe interaction-associated remediation is more beneficial compared to other conventional methods. Various regulatory networks in plant–microbe interactions should be investigated. The synergistic relation between plants and microbes, and their role in metal mobilisation and degradation should be analysed clearly. Development of transgenic and recombinant microbes along with plants may also increase the efficiency of the treatment. Plant–microbe interaction-associated remediation will be a promising approach if the limitations are clearly acknowledged and resolved. For utilisation of microbe–plant combined systems to degrade contamination at industrial level, it is pretty much essential to better understand plant–microbe interactions. There is an urgent need in developing novel strategies for pollutant degradation so that we can create a better and safe environment.

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