Microorganisms for Sustainability 9
Series Editor: Naveen Kumar Arora

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Narendra Kumar *Editors*

Phyto and Rhizo Remediation

Springer

Microorganisms for Sustainability

Volume 9

Series Editor
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Microorganisms are performing diverse and huge number of tasks on our planet, most of which are making earth a habitable and sustainable ecosystem. Many of these acts of the microorganisms are being or can be utilized as low input biotechnology to solve various problems related to environment, particularly caused due to anthropogenic activities. Microbial technologies can be used for chemical free agriculture, replacing harmful pesticides as well as fertilizers for crop protection and enhancing the yields. Similarly, microbes can be used to provide green alternatives to the fossil fuels thus combating the problems related to pollution and global warming. Microorganisms can be the magic bullets for reclamation of wastelands/ stress affected regions, bioremediation of contaminated habitats and biodegradation purposes. The series proposed will cover all these wider aspects and explain how microbes can be used for sustaining our planet in a greener way. Each volume of the series will have a different Editor with his/ her own area of expertise.

The book series will focus on the role of microbes in sustaining the ecosystems. Naturally, microorganisms play so many important roles in sustaining plant and animal life on earth. Global issues such as remediation of polluted sites, degradation of pollutants, biofertilizers and biopesticides to replace chemicals in agriculture, reclamation of barren soils, combating climate change, green fuels are the major contemporary issues and focus of researchers.

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Preface

The exponentially increasing human population and the associated anthropogenic activities have negatively influenced the ecosystems and life on Earth. The continuous addition of agrochemicals, heavy metals, and industrial wastes/effluents in the ecosystems has caused great harm, including loss of productivity, biodiversity, climate change, and diseases in plants, animals, and humans. The toxification level of several ecosystems has already surpassed the permissible limits, and this has endangered sustainability of life on Earth. The man-made pollutants have dearly impacted soil and water ecosystems around the globe, reducing their productivity and resulting in several other issues, particularly related to human health and increase of marginal and barren lands. Hence, there is an urgent need to reverse the impact of dangerous pollutants. Conventional or non-biological methods such as physical and chemical, have serious issues and have not proven to be sustainable for remediating the polluted habitats and have concerns such as being costly, resulting in formation of toxic intermediates, and loss in biodiversity of local microbial flora. However, bioremediation has no such issues and not only is cost-effective but has long-term beneficial impact on the ecosystems. Bioremediation involves the utilization of biological systems, mainly plants (phytoremediation) or microorganisms or both in combination (rhizoremediation) for the removal or degradation of pollutants and to revive the habitats. Both phyto- and rhizoremediation are used for in situ remediation of the polluted habitats and thus are far more effective. Microorganisms in combination with their symbiotic partner plants can carry out remediation effectively by mechanisms such as biosequestration, biodegradation, bioextraction, or volatilization. Recently, there have been many success stories related to bioremediation involving plants or plant-microbe interactions. These success stories are related to the removal of heavy metals, pesticides, polyaromatic hydrocarbons, explosives, radionuclides or reduction of biological oxygen demand, total dissolved solids, total suspended solids, and oil spills in water bodies. Rhizoremediation has also been successfully used for reclamation of saline or marginal soils. Although phyto- and rhizoremediation have shown great potential, still these techniques have been underutilized. With the range of pollutants and the total area (on Earth) covered by these toxic chemicals, it is important that these eco-friendly technologies be utilized in a better way and there is great scope to enhance the compound annual growth rate (which is currently 8.3% for bioremediation) of bioremediation market to be several notches higher. For this to achieve, intensive research is required so as to improve the efficiency and replicability of this sustainable biotechnology.

The chapters presented in this volume throw light on the recent happenings, research, and success stories related to bioremediation of polluted habitats through phytoremediation or rhizoremediation. The book also highlights some of the significantly important plant and microbial species involved in remediation; the physiology, the biochemistry, and the mechanisms of remediation by various plants and microbes; and suggestions for future improvement of bioremediation technology. The tome provides inputs for treatment of water bodies through rhizofiltration using aquatic macrophytes as well. The work presented in the manuscript is contributed by the researchers from across the globe. There are detailed reviews on the use of endemic plant varieties, weeds, grasses, and wild flora, along with the microbial partners, in restoring the fertility and detoxifying the disturbed terrestrial and aquatic ecosystems. The authors also provide the use of lichens in reducing the airborne organic pollutants in some unique and fragile ecosystems of the Himalayas. The main aim of the book is to focus on the use of bioremediation in cleaning the polluted habitats off toxic pollutants and make the depleted or degraded fields/water bodies fertile and rejuvenated in order to maintain the sustainability.

Finally, we, the editors, would like to express our deepest gratitude and warmest appreciation to all those who have participated and helped in compiling this knowledgeable volume. We acknowledge all the authors who have given their valuable contributions and shared their knowledge with us to make the manuscript interesting. Thanks to Dr. Mamta Kapila and Ms. Aakanksha Tyagi from Springer (India) for her motivation and support. We would also like to thank Springer (India) for giving us the opportunity to accomplish the project and share the knowledge with the scientific and academic fraternity. Last but not the least, we would like to acknowledge the support of our colleagues, Dr. Vertika Shukla and Dr. Pankaj Arora, and research scholars, Dr. Dhananjay Kumar, Ms. Isha Mishra, Ms. Tahmish Fatima, Ms. Sushma Verma, Ms. Priya Mishra, Ms. Priyanka Verma, Ms. Ankita Bhattacharya, and Ms. Chanda Bharti, for their support during the phase of compilation and editing.

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About the Editors

Prof. (Dr.) Naveen Kumar Arora, PhD in Microbiology, Professor and Head in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India, is a renowned Researcher in the field of Environmental Microbiology and Biotechnology. His specific areas of research are rhizosphere biology and plant growth-promoting rhizobacteria (PGPR). He has more than 60 research papers published in premium international journals and several articles published in magazines and dailies. He is Editor of 15 books, published by Springer, Member of several national and international societies and of the Editorial Board of four journals, and Reviewer of several international journals. He is also the Editor in Chief of the journal *Environmental Sustainability* published by Springer Nature. He has delivered lectures in conferences and seminars around the globe. He has been Advisor to 118 postgraduate and 9 doctoral students. He has also received awards for excellence in research from the Honorable Governor of Uttar Pradesh, Asian PGPR Society, and Samagra Vikas Welfare Society. Although an Academician and Researcher by profession, he has a huge obsession for the wildlife and its conservation and has authored a book, titled *Splendid Wilds*. He is President of the Wildlife Conservation Society and is also Secretary of the Society for Environmental Sustainability (website: www.ses-india.org).

Narendra Kumar has obtained his MSc and PhD degrees in Environmental Science from the Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow, India. Dr. Kumar has also qualified the UGC-NET in the subject Environmental Science. He is an active Researcher with 17 years of postgraduate teaching and research experience. In 2002, he joined the Department of Environmental Science, Institute of Bioscience and Biotechnology, CSJM University, Kanpur, UP, India, as a Lecturer. He has been working as an Assistant Professor in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, since 2005. He has published more than 40 research papers and 15 book chapters with reputed national and international publishers. Five PhD degrees have been awarded under the supervision of Dr. Kumar, and currently two research scholars are working under his guidance for doctoral degree. In addition, he has guided more than 40 students for their MSc dissertation work. He has coedited three books, namely, *Environmental Concerns and Sustainable Development* in two volumes and *Plant Adaptation Strategies in Changing Environment* published by Springer Nature.

Chapter 1
Natural and Artificial Soil Amendments for the Efficient Phytoremediation of Contaminated Soil

Poonam and Narendra Kumar

Abstract Anthropogenic pollution caused by excessive use of chemicals, metals, radioactive substances, and organic pollutants has deteriorated quality of environmental assets, i.e., air, water, and soil. To restore the quality of all these essential systems, scientists and researchers are trying to stabilize contaminants in-situ rather than in in-vivo conditions. One of such effort is phytoremediation, which utilizes the application of green plants, herbs, and shrubs at contaminated sites to restrict the movement of pollutants and to decontaminate polluted sites. Application of various kinds of plants for bioremediation of polluted soils is an eco-friendly approach, with negligible effect over environment and also without disturbing the soil physico-chemical properties. This technology also offers an opportunity to rejuvenate precious metals and utilize left biomass for the production of bioenergy. The present book chapter deals with different aspects of phytoremediation processes for remediation and recovery of contaminated soil and improving its efficiency with augmentation of different organic and inorganic amendments. Soil amendments can lessen up the bioavailability of contaminants in soils and decrease the risk of food chain contamination. These amendments include the application of biochar, vermicomposting, slow-release fertilizers, and nanoparticles to the soil to enhance the phytoremediation process. Role of these amendments on bioavailability of contaminants, their uptake, translocation, bioaccumulation, and its effect on growth and developments of plants has been thoroughly addressed in the present chapter. Further, different constraints like slow growth rate and effect of seasonal variations on development of plants have also been discussed.

Keywords Phytoremediation · Soil pollution · Organic amendments · Inorganic amendments · In situ remediation · Organic pollutants

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1.1 Introduction

Soil is the basic support system for growth and development of living beings. For fulfillment of nutritional and developmental requirements, agricultural activities are being done since the dawn of civilization. Nowadays these practices are the main source of pollution of soil as in ancient times people were dependent normally on natural substances and did not use any kind of chemical amendments to enhance the production rate. But, as the time passed, the human population increased rapidly resulting in urbanization and industrialization. This increasing population required higher production of crops and cereals to fulfill their increasing nutritional demands. To cope up with such a condition, farmers started applying fertilizers, pesticides, herbicides, homicides, etc. to enhance the production rate and to protect the crop from pathogens. Thus, soil around the globe became contaminated with different organic and inorganic contaminants and lost its original constituents, texture, and fertility. Further, the industrial development and afforestation have also made the situation worst as different industrial effluents containing harmful contaminants like potentially toxic metals e.g. Cr, Ni, Pb, As, Cu, Zn etc., organic pollutants e.g. peroxyacetyl nitrates (PAN), volatile organic compounds (VOCs), persistent organic pollutants (POPs), pesticides, herbicides also polluted soil (Arora et al. 2018).

1.1.1 Soil Pollution

Soil pollution is defined as any undesirable change in the physicochemical and biological properties such as color, texture, nitrate, phosphate, potassium, organic carbon contents, bacterial and fungal population, which may be harmful to the health of plants as well as human beings. Soil is mainly polluted by chemicals such as pesticides and sodium and potassium fertilizers, which may get accumulated in the ecosystem. These pollutants have the potential to contaminate the food chain directly or indirectly and thus may cause harm to immune system by malfunctioning of vital organs in humans (Kamel 2013).

1.2 Soil Formation

This is a very complex process involving several physical, chemical, and biological transformations (Fig. 1.1). The processes involved are described below.

Addition Minerals are added by decomposition of vegetation and dead organisms or deposition of minerals by wind or water. This process makes the upper most soil which supports maximum bio-forms.

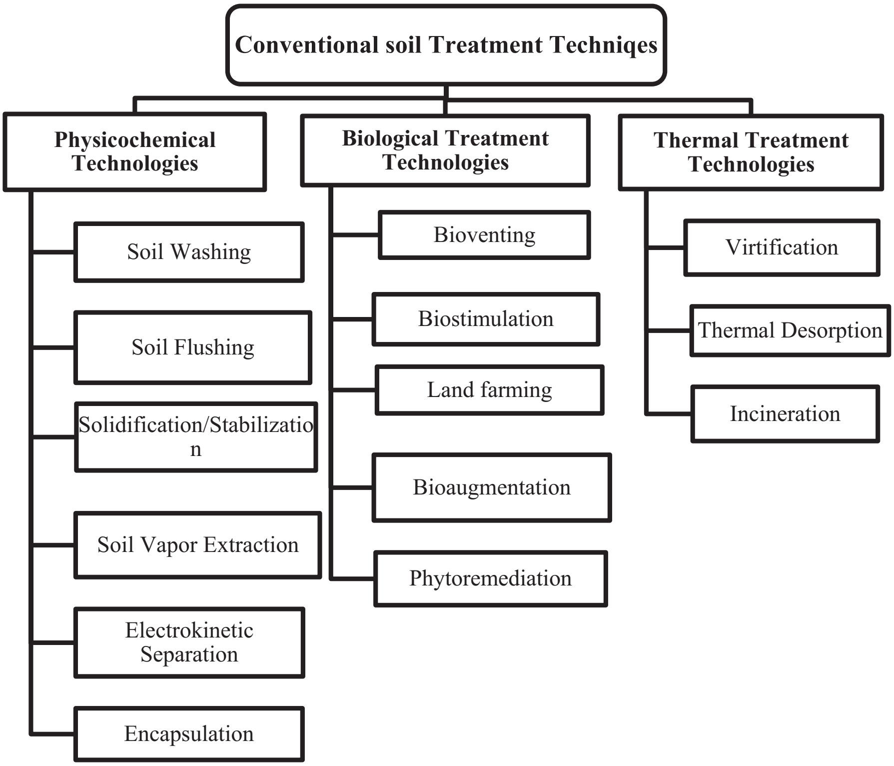

Fig. 1.1 Conventional soil treatment technologies

Losses By the movement of air and water streams or by human activities, constituents of soil (sand, gravel, silt, clay, and organic matters) can be leached or eroded or harvested from soil. This process may alter chemical and physical composition of soil.

Transformation This includes chemical weathering of soil particles, materialization of clay minerals, and conversion of coarse organic matter into resistant organic matter by decomposition, i.e., humus.

Translocation This process takes place by means of mechanical mixing, water transportation, and nutrient uptake by plants (organic and minerals) within the profile or horizon. This process can be visualized by alteration in color, texture, and structure of soil of a particular place in some time duration (Santra 2005).

1.3 Types of Soil Pollutants

Several activities contribute toward soil pollution. Some of these are described below.

Acidification The main reason of acidification of soil is acid rain, long-term leaching of base, microbial respiration, nitrification, excessive land use practices, use of inorganic nitrogen fertilizers and land drainage. All these processes lead to production of either H^+ ions or CO_2 which further get dissolved into soil water and forms carbonic acid.

Salinization and Sodification These are the two naturally occurring processes, but human activities like afforestation, poor irrigation practices, and improper maintenance of irrigation channels and canals lead to increase in soil salinity and sodicity. These have detrimental effect on both physicochemical aspects of soil quality as it disturbs pH of soil which in turn affects availability of nutrients to plants. Further, elevated salt and Na^+ ions concentration in soil may cause toxicity to plants by disturbing the nutrient balance.

Polluted Water Industrial effluents, sewage, and household wastewaters containing different kinds of contaminants, e.g., toxic metals, detergents, soaps, oils, and organic pollutants, may pour into the soil. These contaminants may result in physical and chemical degradation of soil. Physical degradation includes erosion, compaction, and structural damage, whereas chemical degradation includes changes in macro- and micronutrient composition of soil.

Agrochemical Pollutants A number of chemical fertilizers, pesticides, insecticides, and herbicides are used in agricultural practices to upsurge the production rate. These agrochemicals can influence the quality of soil by leaching through drainage, exchange in soil (fixation), gaseous and surface loss by runoff, volatilization and erosion.

Accidental Oil Spills These may take place during transportation and storage of chemicals at different oil stations. This may deteriorate the quality of soil and make it unsuitable for cultivation.

1.4 Causes of Soil Pollution

The main reason for soil pollution is manmade; naturally produced waste, e.g., dead plants, carcasses of animals, rotten fruits, and vegetable don’t pollute soil and they even increase the soil fertility. But, anthropogenic sources release different chemicals that are not naturally present and cause soil pollution. Some of the common sources of soil pollution, major pollutants, and their effect on soil and plants are given in Table 1.1.

Table 1.1 Sources and effect of pollutants on soil and plants

S. no.	Sources	Major pollutants	Effect on soil and plants
1	Industrial dumps, landfill sites	Heavy metals, radio-nuclides, persistent organic pollutants (POPs), etc.	These pollutants affect the soil fertility, growth, and development of plants.
2	Agricultural practices	Insecticides, herbicides, rodenticides, fungicides, different fertilizers, etc.	These pollutants have the tendency of bioaccumulation in plant tissues. This contaminates the food chain.
3	Sewage	Heavy metals, nitrates, phosphates, detergents	May affect growth and fruiting of plants.
4	Acid rain	Soot and dust, spray drift, pesticides, heavy metals, ozone, etc.	May alter the structure of soil, affect the fertility by harming beneficial microorganisms.

Source: Santra (2005)

1.5 Phytoremediation: An Overview

Phytoremediation is a universal term for utilization of green trees, herbs, and shrubs to absorb and store contaminants present in soil, sediments, water, or even the air, into their root, shoot, and leaves (Nichols et al. 2014). The word "phytoremediation" is composed of two words, ancient Greek "phyto," meaning plant, and "remedium" in Latin, meaning restoring balance or remediation (Hadi et al. 2015; da Gomes et al. 2016). The effectiveness of the process depends upon selective uptake abilities of plant root systems, and with bioaccumulation, translocation, and degradation abilities of selected plants (daGomes et al. 2016). It is used for cleaning up the medium (soil, water, and air) from different groups of contaminants like chlorinated solvents, petroleum, diesel, pesticides, explosive, potentially toxic metals, radionuclides, landfills and xenobiotics (Ali et al. 2013; Sharma et al. 2015).

Plants can successfully remediate contaminants by adjusting physicochemical properties of soil, discharge of root exudates, and improvement in aeration by freeing oxygen straight to the root zones, with increase in the porosity of different soil zones. Plants don't affect the top most soil during whole process and thus, conserve and improve the soil fertility by addition of organic matter (Ali et al. 2013). These processes intercept and retard the movement of chemicals by transformation of metabolic, enzymatic activities and decrease in relocation of pollutants to groundwater (Chang and Corapcioglu 1998). Phytoremediation can be categorized into direct and ex-planta phytoremediation or rhizoremediation.

When plants remediate contaminants by absorbing them through roots and translocating into upper aerial parts, it is called as *direct phytoremediation*, and when by accumulating and degrading contaminants into their root system, it is called as *ex planta phytoremediation/rhizoremediation* (Sharma et al. 2015).

1.6 Mechanisms of Phytoremediation

Different kinds of mechanisms are involved in processes of phytoremediation. Some of these include transformation of chemicals; some involve breakdown of pollutants to lesser toxic substances or stabilization of pollutants in the root system or hyperaccumulate in their aerial parts (Jagatheeswari et al. 2013; Coinchelin et al. 2012). A brief description of different modes of phytoremediation used for pollutants removal is given in Table 1.2. Mechanisms involved in different modes of phytoremediation are described below.

Phytoextraction Phytoextraction is also known as phytoaccumulation that involves removal of contaminants present in soil or water through absorption through root system and their translocation and accumulation in upper parts of plant system, i.e., shoots and leaves (Rafati et al. 2011). This biomass can be harvested by destruction of the phytoremediator plants, which can be done by incineration, anaerobic digestion, appropriate treatment, or recovery of energy, and possibly of some added-value chemicals and metals that is also possible by the process of phytomining (Schwitzguébel 2015; Ali et al. 2013). Hyperaccumulator plant species have high metal tolerance and high pollutant accumulation capacity which are used in this technique (Sharma et al. 2015). The extraction of contaminants depends upon a number of factors such as soil properties, metal bioavailability to plants, and concerned plant’s characteristics, i.e., plant growth rate, biomass, and hyperaccumulation power (Shabani and Sayadi 2012). The main objective of this is to decrease the concentration of contaminants to regulatory level within a reasonable time period. Plants extracting contaminants and accumulating none or partially degradable contaminants in their aerial parts are also known as “hyperaccumulators.” These can accumulate maximum amount of pollutants in comparison with other plant species which may feel stressed and cannot survive (Susarla et al. 2002).

Phytostabilization It involves immobilization of pollutants present in soil and sediments at particular place and minimization of its leaching to groundwater or food chain (Erakhrumen 2007). So, this process is also known as “phytoimmobilization.” This process takes place by means of sorption, precipitation, complexation, and alteration in oxidation states of metals from toxic to lesser toxic one (Wuana and Okieimen 2011). It takes advantage of capability of root system to alter soil properties like pH, moisture content, and root exudates that help in precipitation and complexation of contaminants (Susarla et al. 2002). In roots, vacuole impounding or cell wall attachments inhibit leaching of heavy metal ions and other pollutants into groundwater that prevents further translocation of contaminants into the shoot and upper parts of plants. Thus, some contaminants which are non-biodegradable and have the ability to persist in the soil for longer periods like organic pollutants (poly aromatic hydrocarbons (PAHs), POPs, pesticides) are immobilized, and their entry into the food chain can be restricted (Sharma et al. 2015).

Table 1.2 Different modes of phytoremediation

S. no.	Process	Description	Target pollutants	Plants	References
1	Phytoextraction/ phytoaccumulation/ phytosequestration/ phytoabsorption	Accumulation of pollutants in harvest table aerial parts	Heavy metals (Cd, Cr, Ni, Pb, Zn, etc.), radionuclides; organic pollutants (benzene, ethyl benzene, toluene and xylenes, penta-chloro-phenol, etc.)	Rapeseed plant, Indian mustard, maize, barley, castor hop, crucifers, serpentine plants, shrubs	Mukhopadhyay and Maiti (2010); Malik et al. (2010); Abreu et al. (2012)
2	Phytostabilization/ phytoimmobilization	Uses roots to restrict availability and movement of contaminants in soil	Heavy metals, phenols, tetra-chloro-methane and tri-chloro-methane, etc.	Mesophytic trees, trees with dense root system, poplar, mustard	Mukhopadhyay and Maiti (2010)
3	Phytovolatilization	Convert contaminants to gaseous form and subsequently release them in the atmosphere	Tetra-chloro-ethane, tri-chloro-methane, and tetra-chloro-methane, Hg, Se, etc.	Tobacco, canola, Chinese brake	Mukhopadhyay and Maiti (2010)
4	Phytodegradation/ phytotransformation	Utilizes plant enzymes to degrade organic xenobiotic compounds inside plant systems	Nitro-benzene, nitro-ethane, nitro-methane, nitro-toluene, picric acid, atrazine, chloroform, hexa-chloro-ethane, vinyl chloride, DDT PCBs, etc.	Willow, cotton wood, Bermuda, sohagram, clover, alfaalfa, horseradish, potato, white radish	Susarla et al. (2002)
5	Rhizodegradation/ phytostimulation	Degradation of organic xenobiotic compounds in the rhizosphere by microbial activities	Heavy metals, radionuclides, oil, and petroleum, PAHs, etc.	Mulberry, apple, orange,, rye fescue, Bermuda, wheat grass, aquatic plants	Mukhopadhyay and Maiti (2010), Schwitzguébel et al. (2011)
6	Phytofiltration/ rhizofilteration	Treatment of wastewaters by well-developed root systems	Heavy metals (Cr, Cd, As, Pb Fe etc.), organic solvents	Aquatic plants, pond weed, cattail, duckweed, Hydrilla, macrophytes with dense root system	Mesjasz-Przybylowicz et al. (2004), Sharma et al. (2015)
7	Phytodesalination	Application of halophytes for recovery of saline soils	Sodium, potassium salts, acids and metallic salts	Herbaceous seepweed, shoreline purslane, red mangrove, *Arthrocnemum indicum*, salt bush, etc.	Ali et al. (2013)

Phytovolatilization This is the process where plants convert contaminants into unstable forms by volatilization and transpire or evaporate these into the atmosphere (Ghosh and Singh 2005). This mode of remediation is utilized mainly for organic contaminants that have volatile nature. The extraction of contaminants is done by roots and xylem cells, which translocate these into shoot system. This process may be defined as biological transformation of toxicants into gases which are released into atmosphere by shoots and leaves (Sharma et al. 2015). However, the main constraint of phytovolatilization is that it could not remove contaminants entirely. The process involves transfer of pollutants from one aspect of environment, i.e., soil, to another, i.e., atmosphere, that have the possibility of recycling into the environment (Ali et al. 2013).

Phytodegradation This involves uptake, metabolization, and degradation of pollutants either inside the plants or in soil by enzymatic activities (of plants). It doesn't depend upon the action of microorganisms associated with the root system. This process is sometimes referred to as "phytotransformation." Plant enzymes and enzyme cofactor facilitate this process, e.g., dehydrogenase enzymes are involved in the transformation of chlorinated compounds, peroxidase for phenolic compounds, nitroreductase for explosives, and other nitrate compounds (Susarla et al. 2002; Schwitzguébel 2015).

Rhizodegradation It involves microbial breakdown of organic contaminants in the rhizospheric region of soil. It is also known as "phytostimulation." Plant and microorganisms often have a symbiotic relationship making rhizospheric area very active for microbial activities (Ali et al. 2013). Plants secrete root exudates that have the ability to encourage microbial commotion 10–100 times higher in the rhizospheric region. These exudates contain sugars (carbohydrates), proteins (amino acids), and pigments (flavonoids), which act as source of C and N to microbes and provide nutrients for enhancing microbial activities that help in degradation of contaminants. Further, some enzymes are also secreted by plants that also aid in degradation of organic toxicants (Yadav et al. 2010). Plant roots penetrate the soil and create zone of aeration and thus stimulate aerobic degradation of contaminants (Susarla et al. 2002).

Phytofilteration It involves purification of wastewater originated from industries, run off, or household utilities by the help of different plants (Mukhopadhyay and Maiti 2010). It prevents groundwater contamination as the contaminants are absorbed or adsorbed onto root surface or inside them (Ali et al. 2013). Phytofiltration may be of following types—rhizofiltration, blastofiltration, and caulofiltration, which involve roots, seedlings, and excised plant shoots for removal of pollutants, respectively (Mesjasz-Przybylowicz et al. 2004). In this process, firstly, plants are grown up into nutrient-rich water without using soil (hydroponically), and after the development of dense root system, plants are relocated to wastewater sites for treating it (Sharma et al. 2015). Cell membranes work as barriers to prevent the access of pollutants inside the root system (Yadav et al. 2011). Different factors like pH (high with higher pH) and temperature (increases with increasing temperature), ionic populations (cations cause competition for uptake of metals) affect the removal of toxicants (Xing et al. 2013).

Phytodesalination This process is recently reported for removal of salts from soil by the help of halophytic plants (Ali et al. 2013). The halophytic plants are used to be naturally adapted to saline soils, so these plants are used in phytodesalination of different contaminants including heavy metals (Manousaki and Kalogerakis 2011).

1.7 Advantages of Phytoremediation

1. Eco-friendly (Sharma et al. 2015)
2. Cost 10–20% of mechanical treatment and thus cost effective (Schwitzguébel 2015)
3. Fewer air and water emission
4. Recovery and reuse of metals (phytomining)
5. Easy for maintaining and monitoring the process (Aken 2009)
6. Aesthetical approach and pleasant (Ali et al. 2013)
7. Suitable for larger sites where other systems are not feasible (Garbisu and Alkorta 2003)
8. Improves eminence and consistency of soil at remediation sites (Gerhardt et al. 2009)

1.8 Limitations of Phytoremediation

1. Limited to depth and surface area occupied by roots (Pilon-Smits 2005).
2. Slow growth rate and lesser biomass makes it a time-consuming process, so the remediation rate is slower (EPA 2006).
3. Contaminants may leach out into groundwater.
4. Bioaccumulated toxic substances may contaminate the food chain (Susarla et al. 2002).
5. Influenced by soil and climatic conditions and different abiotic and biotic stressors.
6. Survival rate of plants depends upon toxicity and general condition of contaminated soil (Ali et al. 2013).

1.8.1 Conventional Soil Treatment Techniques

There are a number of conventional technologies used for in-situ remediation of contaminated sites, but, choice of the efficient technology should be based on types of pollutants, location physiognomies, governing necessities, budgets, and time restrictions (Khan et al. 2004). Different conventional soil treatment techniques are presented in Fig. 1.1 and their brief discussion is summarized in Tables 1.3 and 1.4.

1.9 Soil Amendments

Soil amendments are additional substances added (to soil) for improving its capacity to support the growth and development of plants. It enhances the nutrient contents, water retention, infiltrative aeration, porosity, texture, and pH of the soil. Thus amendments build a proper ecosystem to smoothen the optimum growth and development of plants. Some of the commonly used soil amendments are compost, peat moss, biochar, clay, gypsum, lime, and different agricultural wastes. In addition soil amendments improve soil texture and root proliferation, which prevent soil degradation and soil erosion. It is observed that any kind of soil can be made fertile by using different soil amendments at an optimum rate and proportion (West Coast Seeds 2011). Soil amendments are categorized into two major categories viz. organic and inorganic. Organic amendments are produced in the nature from living/dead biomass (peat moss, manure, vermicompost), by-product of plants or mills processing (sawdust, rice hulls, bagasse), or waste disposal (compost, biosolids, processed sewage sludge), whereas inorganic amendments are either man-made or mined (vermiculite, perlite, tire chunks, pea gravel and sand, salts, and nanoparticles) and need to be processed further. Various soil amendments and their role in phytoremediation have been presented in Fig. 1.2 and comparisons between organic and inorganic amendments have been listed in Table 1.3.

1.9.1 Natural Soil Amendments

All the organic/inorganic substances derived from living or nonliving biomass are termed as “natural amendments.” A detailed description of different natural amendments has been given in this section.

Agro-Industrial Wastes These include by-products or waste of different agricultural as well as industrial procedures, e.g., sawdust, wood chips, bark, straw, food wastes, poultry litter, cow slurry, molasses, municipal solid wastes/sludge, sugar beet residue, fly ash, lime stone, etc. (Shaheen and Rinklebe 2015; Galende et al. 2014). These substances are used as soil amendments due to their organic content and C/N ratio and recover soil texture by generation of huge amounts of humus. In

Table 1.3 Various conventional techniques for treatment of contaminated soil

S.no.	Technologies	Description	Advantages	Disadvantages	References
Physicochemical techniques					
1	Soil washing	The soil washing process separates soil on the basis of size and involves desorption of pollutants from binding site in solid matrix through action of simple water, organic solvents	Semiorganic compounds (SVOCs), petroleum, and fuel residuals, heavy metals, pesticides, poly aromatic hydrocarbons, etc. are the target toxicants Lager volumes of soils are cleaned up	The liquid phase containing the adsorbed toxicant needs secondary biochemical techniques for satisfactory decontamination It does not destroy or immobilize the contaminants Organics adsorbed onto clay particles are difficult to remove	Kuppusamy et al. (2016)
2	Soil flushing	It uses an injector filled with extraction fluid which is injected in the contaminated soils. The fluid helps in moving pollutants at sites where these are detoxificated	Helps in recovery of groundwater to meet regulatory guidelines for public usages Supports in-situ wastewater treatment Easy to handle, no need of excavation, and transference of toxicants	The quality of treated water may not be significant and require secondary and tertiary treatment	Son et al. (2003), Khan et al. (2004)
3	Solidification/ stabilization	It encapsulates waste to form a solid material and reduces the movement of lethal materials in the environment by different physicochemical processes, i.e., precipitation, complexation, and adsorption reactions	It converts contaminant into immovable and less toxic forms It helps in removal of toxic metals	The physical nature of wastes is not altered It is not or less effective against organic pollutants like pesticides	Khan et al. (2004), U.S. EPA (2006)

(continued)

Table 1.3 (continued)

S.no.	Technologies	Description	Advantages	Disadvantages	References
4	Soil vapor extraction (SVE)	It is also known as *soil venting* or *vacuum extraction* Vacuum pressure is created to control subsurface air flow for removal of volatile and semivolatile organic compound from different zones of soil to surface for their treatment Adsorption and thermal oxidation processes are commonly used in this method	It is a cost-effective technique for remediation of volatile compounds present in the soil This technique works well in sand and gravel containing soils that have low organic carbon. It also helps in decontamination of groundwater	This type of system is not significant for combinations of various contaminants which are generally present in the environment Soils with high carbon content are hard to treat and require modifications	EPA (2006), Khan et al. (2004)
5	Electrokinetic separation	It works on the principle electric field gradients which are applied at two sides of soils Pollutants are used to brought to two poles treatment chambers for further treatments via electromigration, electroosmotic flow, or electrophoresis	This process is significant for low permeable soils The installation and operation of the process are easy and also cost efficient The process never harms the natural qualities of the soils	It cannot maintain pH of soil system efficiently and the treatment efficiency is low	Yao et al. (2012), Pazos et al. (2010)
6	Encapsulation	It involves physical isolation of the pollutants present in the soil by mixing it with lime, cement, and concrete and thus prevents dispersal, leaching, or migration of pollutants in the clean soils and groundwater	It helps in both soil and water treatment and remediation of the polluted sites	This method obstructs cultivation in polluted as well as adjacent soils The working is dependent upon lithology of the location and time The process is costly when concerned with deep soil remediation	Khan et al. (2004), Anderson and Mitchell (2003)
Biological techniques					

1	Bio venting	It comprises of injecting a gas into subsurface to provide aerobic or anaerobic conditions which increase biodegradation of a contaminant It also provides oxygen supply to contaminated soils to facilitate aerobic biodegradation by microbes It is of three types—aerobic, anaerobic, and catabolic bioventing	The installation and operation of the equipment are easy The time required is less, i.e., needs only 6 months to 2 years to complete whole process The process is compatible with other technologies	It's a successful phenomenon for treatment of soil contaminated with petroleum products It has lesser efficiency for soils with low permeability and rich in clay contents It cannot clean up minute amounts of pollutants	Khan et al. (2004)
2	Biostimulation	It involves introduction of additional nutrients into the contaminated sites to enhance the activities of native microbes to degrade contaminants It also involves optimized conditions such as aeration, addition of nutrients, pH, and temperature control to work effectively for removal of contaminants	Bioremediation takes place by already present native microorganisms that are well-suited to the subsurface environment	Bioavailability of nutrients depends upon lithology of the particular place Additional nutrients might promote growth of heterotrophic microorganisms which are not innate degrader and increase competition for nutrients	Kanissery and Sims (2011), Adams et al. (2015)
3	Land farming	Land farming generally uses tilling or ploughing to reduce contaminant levels biologically The degradation of pollutants involves transformation and immobilization by microbiological processes Microorganisms selected for the process are frequently added for successful breakdown of contaminants	Easy to operate The expenses involved in installation, operation and maintenance are comparatively low Can remove 95% of petroleum The optimum temperature range is 25–40 °C which is general	Requires larger areas of lands Volatile compounds must be pretreated or they may cause air pollution Possibility of movement of contaminants toward pollution-free sites	Hejazi (2002), Khan et al. (2004)

(continued)

Table 1.3 (continued)

S.no.	Technologies	Description	Advantages	Disadvantages	References
4	Bioaugmention	It involves applications of indigenous or genetically modified microbes to polluted sites	It relies on natural processes to dissipate contaminants through biological transformation	Process is dependent upon the survival rate of the strain of the microorganism used	Mrozik and Piotrowska-Seget (2010), Castiglione et al. (2016)
		The selected microorganisms used have high growth rate, easy to cultivate, potential to tolerate high concentrations of toxicants and worst environmental conditions	Effective removal of PAHs and petroleum components	Abiotic and biotic stresses also affect the process	
5	Phytoremediation	It is removal of contaminants from soil by using green plants by in situ treatment through phytoextraction, rhizodegradation, phytodegradation, phytovolatilization, and phytostabilization	Plants have the following potential: uptake, bioaccumulation, and degradation of contaminants present in soil Cost effective and eco-friendly Effective for heavy metals, PAHs, VOCs, and pesticides also Recovery and reuse of metals Chance of generation of secondary pollutants is minimum	Time-consuming process Depend upon the climatic conditions Contaminants can leach out in groundwater Can affect the food chain Require special disposal of the used plants	Schwitzguébel et al. (2015)

Thermal techniques					
1	Vitrification	It is heating the soil at temperature of 1400–2000 °C, in which process the organic matters volatilize or decompose For ex situ vitrification, energy may be provided by fossil fuel or electrode directly heating, plasma, and microwave transfer For in situ processes, the energy may be supplied through electrodes inserted into the contaminated soil	The vitreous is 10 times powerful than concrete Highly efficient for heavy metals removal The high temperatures destroy any organic constituents, resulting in few by-products Heavy metals and radionuclides are assimilated into assembly made up of glasses and are tougher, resilient, and impervious to leaching of contaminants	Requires more energy supply Costly and non-eco-friendly The depth of the contaminants may limit these processes Long-term monitoring is required	Acar and Alshawabkeh (1993), Dermatas and Meng (2003)
2	Thermal desorption	In this process contaminated soil is excavated, screened, and heated to release petroleum from the soil The mechanism comprises of heating of soils at 100–600 °C which causes vaporization of pollutants within whose boiling points occur within this range and become isolated from the soils. Vapors are compressed and treated further	Thermal treatments do not destroy pollutants completely but convert them into treatable forms The efficiency of desorption can be as high as 99% and may treat higher concentrations of pollutants present in the soil This technology is eco-friendly, simple, and easy to operate, and remediated soils may be reused for cultivation	The presence of water reduces its effectiveness since more than the contaminant water is being vaporized Operation cost is higher	Vidonish et al. (2016)
3	Incineration	It involves burning of hazardous wastes like halogenated solvents, explosives etc. at 870 to 1200 °C in the presence of oxygen	The demolition and elimination competence of properly operated incinerators surpasses 99.99% for hazardous wastes	Incinerators may release carcinogenic and poisonous substances from their stacks and produce products of incomplete combustion which is not eco-friendly	Chen et al. (2013a), Islam et al. (2012), Kuppusamy et al. (2016)

Table 1.4 Various characteristics of organic and inorganic amendments

S. no.	Organic amendments	Inorganic amendments
1	It is produced from dead or living biomass	It is extracted from mine or man-made
2	These are abundant in the nature and easily available for the processing	These are produced by different physicochemical methods so relatively hard to find
3	It may increase soil fertility by improving organic substances (humus) which works as nutrient for growth and development of plants	Nutrient content of the soil remains unchanged
4	It improves aeration, water retention, texture, and infiltration rate of soil	It also improves soil texture and decreases excess water holding capacity and increase or decrease biomass of plants
5	Before incorporating these substances into the soil, it has to be composted. Hence, time consuming	These substances are applied as such in the soil and don't need any kind of process before treating the soil. So, it is not time consuming
6	These amendments have higher C:N ratio. If C:N ratio remains low it may be toxic for the health of soil and related living beings as it will produce ammonia or related salts	If the process is not monitored timely, it may alter pH and chemical composition of soil which will be harmful for the health of the soil and related living beings
7	It is cost effective and eco-friendly technique to increase the fertility of the soil	It is expensive as it is either purchased or processing charges are high. Further, energy consumption is also high during industrial process
8	It facilitates phytoremediation processes by increasing the soil water relation, root growth, and fertility	It also increases phytoremediation by altering chemical composition of soil by influencing pH, ion mobility, etc.
9	Operation is simple and no skilled supervision is required	Heavy machinery and usage of chemicals requires skilled supervision

addition these amendments improve physicochemical properties of soil (pH, alkalinity, elctrical conductivity (EC), cation exchange), reduce bioavailability of different toxic pollutants, and supply nutrients to plant and microorganisms present in the soil (Galende et al. 2014). Ion exchange, precipitation and coprecipitation reactions, and adsorption on the surface of amendments are the main mechanisms involved in the process of immobilization of toxicants and their uptake by plants (Grobelak 2016). These amendments also contain calcium (Ca) and magnesium (Mg) in addition to bases (OH^-, CO_2^-) which neutralize soil acidity and influence pH (Mekki et al. 2013). Galende et al. (2014) have used sheep manure, cow, dung, poultry litter, slurry, and paper mill sludge mixed with poultry litter as soil amendments for *Festuca rubra* crop. They reported decrease in lead (Pb) and zinc (Zn) extractability in soil and stimulation of plant growth through increased soil microbial biomass and activity. Increased phytoremediation of Pb and cadmium (Cd) was observed by seedlings of red oak (*Quercus rubra* L.) and Austrian pine tree (*Pinus nigra* Arn.), while using paper pulp waste as soil amendment (Doichinova and Velizarova 2013). Shaheen and Rinklebe (2015) reported a decrease in the immobilization and uptake of Cd and Pb by rapeseed (*Brassica napus*) during the usage of chitosan, organo-

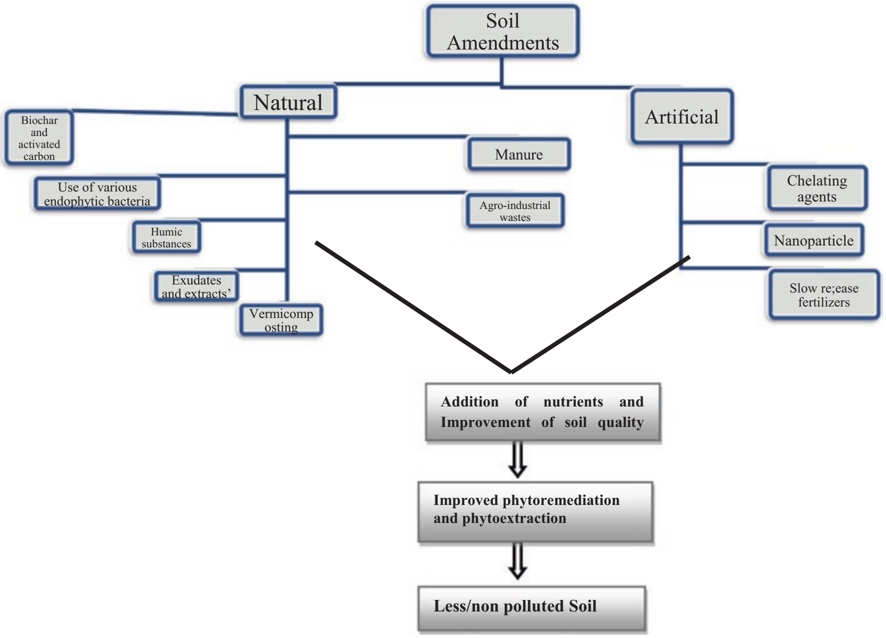

Fig. 1.2 Various soil amendments and their role in phytoremediation

clay, bentonite, cement kiln dust, fly ash, limestone, sugar beet factory lime, and zeolite in contaminated floodplain soil. They found that combination of these low-cost amendments may work more significantly in immobilizing the contaminants and their phytoavailability for energy plants. Similarly, compost, dehydrated manures, and digestate were used as soil amendments by Mekki et al. (2013) to observe the seed germination and the growth of tomato (*Lycopersicon esculentum*), alfalfa (*Medicago sativa*), wheat (*Triticum durum*), and sorghum (*Sorghum bicolor*). These amendments improved the physicochemical characteristics, i.e., pH, EC, water retention capacity, and soil organic matter of soils, which subsequently enhanced the microbial and respirometric soil activities to promote the seed germination and growth of these plants.

According to Fenoll et al. (2011), application of organic wastes, i.e., composted sheep manure and spent coffee grounds, often upsurged the adsorption of pesticides and decreased their mobility in the soil due to addition of humus and restricts their leaching in the soil and groundwater. They studied the removal of different pesticides (azoxystrobin, cyprodinil, fludioxonil, hexaconazole, kresoxim-methyl), triadimenol (fungicides), pirimicarb (insecticide), and propyzamide (herbicide) and reported drastic reduction in the movement of tested pesticides, except for pyrimethanil and pirimicarb for spent coffee grounds, in the leachates. Lamichhane et al. (2012) used molasses, a by-product of refinery of sugarcane or sugar beets, as soil amendment to phytostabilize the energetic (royal demolition explosive (RDX) and high-melting explosive (HMX) by guinea grass (*Panicum maximum*). Positive results were found for degradation of RDX in the root zone of plants, whereas for HMX these amendments did not work.

Biochar and Activated Carbon *Biochar* is a form of carbon-enriched pyrolyzed biomass obtained from agricultural and forestry wastes (wood, leaves, peels, manure, etc.). It is highly porous in nature and quite effective in surface adsorption of the soil contaminants (Jiang et al. 2012; Egamberdieva et al. 2018). *Activated carbon* is also a form of carbon-rich material produced from different carbonaceous materials, e.g., coal, coconut shell, peat wood, etc. These substances are transformed into activated carbon by different physicochemical modifications and thermal decomposition under controlled atmosphere and temperature. It has large surface area with porous structure that helps in the surface adsorption of soil contaminants. Both activated carbon and biochar are involved in the surface adsorption of contaminants with similarity in the process of thermal decomposition of parent material and porous structure.

Activated carbon also works as soil amendment because of its large surface area which attracts the pollutant and adsorbs them onto the surface. These are effective in adsorbing contaminants of diverse nature, i.e., organic or inorganic, polar or nonpolar, and neutral or ionized. Biochar immobilizes soil contaminants by altering the pH of the soil (below 4 or above 12) and EC and thus lowers bioavailability of contaminants and risk of food chain contamination (Lehmann et al. 2011). Success of the biochar as a soil amendment also depends upon its own properties like pH, surface area, cation exchange capacity, temperature of pyrolysis, degree of oxidation, and the properties of the contaminants to be encountered with. Biochar derived from bamboo and rice straw increased soil pH and immobilized heavy metals (Cd, Cu, Pb, and Zn) in *Sedum plumbizincicola*. Efficiency of removal was found to be dependent upon size of particles; smaller particles were more efficient in removal of Zn (Lu et al. 2014), although the optimum biochar dosage for effective phytoremediation as soil amendment ranges from 1% to 5% depending upon soil type, texture, moisture content, and kind of crop (Matovic 2011). Further, amendment of biochar improves physicochemical and biological characteristics of soil and makes it fertile by retaining water and water-soluble nutrients helping in the promotion of plant growth and development and reduction in the use of chemical fertilizers for the same processes (Liu et al. 2013; Houben et al. 2013). Sometimes, it is used in combination with various chemical and organic fertilizers, activated carbon, compost, and manure to enhance the process (Bielská et al. 2017; Liang et al. 2017). *Zea maize* showed increased phytoremediation of PAHs and potentially toxic elements (PTEs) after amending the soil with maize and pine-derived biochar and commercial-grade activated carbon (Brennan et al. 2014). Efficacy of both of the amendments lasted longer than any other form of the organic amendments (manure and compost). Biochar produced by different feedstock, i.e., pruning remainders, fir tree tablets, and manure pellets, was reported to phytostabilize the mine tailing by changing pH, EC, and cation exchange capacity of the soil. The plants used in the study were *Anthyllis vulneraria* subsp. *polyphylla* (Dc.) *Nyman*, *Noccaea rotundifolium* (L.) *Moench* subsp. *cepaeifolium*, and *Poa alpina* L. subsp. *alpina* (Fellet et al. 2014).

Houben et al. (2013) used biochar as soil amendment for removal of Cd, Zn, and Pb using rapeseed (*B. napus* L.). It was observed that bioavailability of the metals increased with increase in dosage of the biochar. The reduction rate was 71%, 87%, and 92% for Cd, Zn, and Pb, respectively, with 10% biochar. It was found to be efficient in improving soil fertility, rapeseed production, as well as reduction of metal concentration in shoot. Chen et al. (2013b) have reported increase in bacterial gene abundance in slightly acidic soil amended with biochar produced by rice paddy crop. Bian et al. (2013) also reported reduction in Cd content and reduce rice grain by biochar amendment. Both of these results were obtained due to increased soil organic carbon, nitrogen content, pH, and associated microbial biomass. Likewise, Cui et al. (2011) have also reported decreased Cd content in rice field due to increased pH by rice crop biochar amendment.

Exudates and Extracts Plants secrete a variety of organic compounds from their roots into the rhizosphere which help them to counteract with different infections caused by different pathogens; these chemicals are called as *root exudates*. These possess different metabolites of phenolic groups, sugars, amino acids, terpenoids, and aliphatic carboxylic acids (organic acids) having antimicrobial properties and work as chelators for different metals for efficient phytoremediation (Baetz and Martinoia 2014; Wiszniewska et al. 2013). It is the exudation of ions, free oxygen, enzymes, mucilage, and various collections of carbon containing primary and secondary metabolites (Zhu et al. 2009). Root exudates improve physicochemical and biological characteristics of soil, mobility, and bioavailability of soil contaminants, particularly in rhizospheric area, so they can be used as soil amendments which promote phytoremediation (Zhu et al. 2009; Mimmo et al. 2011).

Phillips et al. (2012) reported that root exudates affect the abundance and activeness of microorganisms to degrade hydrocarbons present in the rhizosphere. They examined the influence of root exudates secreted by *Elymus angustus* (wild rye) and *Medicago sativa* (alfalfa) on degradation ability of microbial communities in a weathered hydrocarbon contaminated soil. The root exudates from both of the plant's suppressed hydrocarbon mineralization with exudates from hydrocarbon stressed wild rye having the least suppressive impression. Likewise, Balseiro-Romero et al. (2014) examined the influence of root exudates (individually and in mixtures) produced from *Holcus lanatus* and *Cytisus striatus,* on mobility of fuel-derived compounds in contaminated soils. Contaminants, i.e., oxygenates {methyl tert-butyl ether (MTBE) and ethyl tert-butyl ether (ETBE)} and monoaromatic compounds (benzene, toluene, ethylbenzene, and xylene), were mixed in the substrate humic acid, montmorillonite, and A horizon from an alumi-umbric Cambisol. Results indicated upsurge in movement of the pollutants by root exudates, more effectively by *H. lanatus*, while, in A horizon and montmorillonite, natural or synthetic exudates reduced pollutant kinesis.

Biodegradation of pyrene and benzo[a]pyrene in *Phragmites australis* rhizosphere sediment by interaction of bacteria and root exudates was reported by Toyama et al. (2011). The root exudates of *P. australis* had increased phenolic compounds during exposure to pyrene, which evidently supported cell growth of pyrene-degrading *Mycobacterium* strain tested and stimulated benzo[a]pyrene degradation by the strain. Stingu et al. (2012) used extracts from different vegetal biomass resources (*Picea abies* bark, *Castanea sativa* chestnuts shell, and *Asclepias syriaca* plant) for Cd bioaccumulation in oat plants (*Avena sativa*). Positive results were reported for *P. abies* bark and *A. syriaca* plant extracts which encouraged translocation of Cd to aerial parts of plant. But, chestnut shell extract decreased the mobility of Cd. So, root exudates and extracts can be used as soil amendment to increase the phytoremediation of different pollutants, but its effectiveness depends upon the composition of the exudates, its interaction with rhizospheric microbes, physicochemical properties of soil, and levels of contamination.

Compost and Vermicompost Compost is produced from aerobic biological decomposition of organic matter, whereas vermicompost is produced from breakdown of organic matters by different worms, generally red wigglers, white worms, and earthworms. Organic matter may be of decaying vegetables or food wastes, animal wastes like cattle dung, animal droppings and municipal solid wastes, produced from different agricultural and household processes (Sinha et al. 2009). Both of these products improve physicochemical and biological characteristics of soil, enhance nutrient contents and organic substances, promote root and shoot growth and, thus, catalyze the phytoremediation process (Gopinath et al. 2010). In addition, these also add useful microorganisms as well as plant growth-promoting hormones to the soil and, thus, enhance their biotic potential and capability of self-renewal of soil fertility (Ouédraogo et al. 2001). The vermicomposting owns a greater capacity for cation exchange and a larger surface area which supports the phytoremediation efficiency of the plants in comparison with raw manure or composts (Yin et al. 2016). Comparatively, vermicompost binds with macro- and micronutrients, including NPK for longer duration as compared to other composts. Further, production of compost is a time-consuming process, whereas vermicompost takes only half time in comparison with compost to decompose organic substances. Compost and vermicompost have very high porosity, water retention tendency, aeration, and vast surface area which absorb and hold nutrients for extended periods.

It has been reported in many works that vermicompost is a better choice to assist phytoremediation of pollutants than compost because of its high nutrient contents, viz., nitrogen, phosphorus, potassium, sulfur, magnesium, humic acids, etc. (Atiyeh et al. 2000). Branzini and Zubillaga (2012) studied the immobilization of different heavy metals (Cu, Zn, and Cr) while using biosolid compost and phosphate fertilizer. They reported reduction in solubility and mobility, in combination of both the amendments, of Cu and Zn, whereas increased stabilization of Zn was noticed when compost with or without phosphate fertilizer was applied. Hartley et al. (2009) examined the mobility of arsenic in soil amended with green waste compost and biochar established with *Miscanthus*. Compost caused increase in growth and yield of plants in comparison to biochar but insignificant increase in arsenic uptake was noticed. The explanation of this behavior was given as increased availability of phosphorus limited arsenic uptake by the plant as behavior of phosphate (PO_4^{3-}) and arsenic is the same which makes them compete with each other for uptake sites. Zhou et al. (2017) reported that compost in combination with red mud reduced bioavailability of heavy metals (Cu, Zn, Cd, and Pb). Thus, it can be summarized that compost and vermicompost work more efficiently when combined with other potential organic or inorganic amendments.

Humic Substances These are dark brown organic materials, naturally found to be present in soil, sediments, and water basins produced by microbial decomposition of plant and animal residues (MacCarthy 2001). These are chemically composed of carbon (about 50%) and oxygen (40%) with small fractions of hydrogen (5%), nitrogen (about 3%), phosphorus, and sulfur (<1%). Humus comprises of mainly three fractions, viz., humic acid, fulvic acid, and humane. Among these three, humic and fulvic acids are extracted from soil with the help of strong alkaline solution, while humines cannot be extracted by any strong alkaline solution or strong acid (Bandiera et al. 2009). Both of them actively help the plants to take up nutrient ions that are often locked up in the soil. These substances have the power to work by chelating positively charged multivalent ions in the contaminated soil (Figueroa et al. 2008). Chelation facilitates the uptake of contaminants, prevents their precipitation and bioavailability, and results in detoxification of contaminants. Humic substances help in effective phytoremediation by motivating efficient breakdown of organic matter, escalate moisture holding capacity by chelation, stimulate root and shoot growth, and also increase soil fertility.

Hattab et al. (2014) reported that humic acids and fulvic acids, derived from composted sewage sludge, were the main organic fraction associated with Pb, copper (Cu), and molybdenum (Mo) and contributed toward their immobilization in soil. This amendment increased total organic carbon and nitrogen content by higher decomposition of soil organic matter which helped in immobilizing toxic metals.

Bandiera et al. (2009) studied aspects of humic and fulvic acid for phytoremediation of different toxic metals (As, Co, Cu, Pb, Zn, Cd, Cr, Mn, Ni) by *Raphanus sativus* L. *var. oleiformis* cultivated in pyrite cinders. Addition of these amendments increased the bioavailability of all of the metals and their translocation to shoots. The higher dosage of humic acids was found to be toxic, generally because of higher concentrations of toxic metals in upper plant parts. But foliar spraying of humic acid was able to minimize the phytotoxicity caused by higher levels of humic acids, by increased rate of leaf expansion. Humic constituents steered decrease in root diameter and enhancement in specific root length, facilitating translocation of heavy metals in shoot, thus encouraging efficient phytoremediation. Maji et al. (2016) also reported increase in the plant growth parameters by amendment of humic acid-rich vermicompost as compared to chemical fertilizers for *Pisum sativum*. Humic acid improved root nodulation and soil microbial colonization which in term increased soil fertility and plant growth.

Manure It is organic matter derived from animal (chicken, cow, horse, sheep, goat, pig, etc.) feces and is an outstanding fertilizer comprising of NPK and other nutrients. The nutritional value depends upon source, moisture content, storage, handling methods, species of animal, feeding pattern, amount of litter, bedding, and urine concentrated with it. Manure recovers soil structure, aeration, soil moisture-holding capacity, and water infiltration.

Pillai et al. (2013) studied the impact of organic manure, produced by cow dung, on phytoremediation potential of *Vetiveria zizanioides* for chromium (Cr) contaminated soil. They reported increase in growth, shoot, and biomass production of the plant, ensuring enhanced phytoremediation of Cr. It was also found that concentration of the metal was higher in root tissue, thus ensuring a successful rhizofilteration of Cr by *V. zizanioides* in soil amended with manure. Likewise, Efe and Okpali (2012) reported effective phytoremediation of oil-contaminated soil by *Cyperus* sp. and *Axonopus* sp. with the help of organic and inorganic manure amendments. According to the experiments performed by Marques et al. (2008), Zn contamination in the soil was reduced by about 80% with the help of manure compared to compost (40%) and arbuscular mycorrhizal fungi by *Solanum nigrum*. They found increase in biomass of root, shoot, and leaves when amended with manure due to an increased nutrient pool with lowest Zn accumulations in the different plant parts. Further, use of *S. nigrum* in the Zn-contaminated soil combined with manure led to a 70–80% reduction in the amount of Zn leached through the soil. Elouear et al. (2016) reported increased phytostabilization by alfalfa (*Medicago sativa* L.) of Pb, Cd, and Zn in the soil contaminated by mining processes by applying sheep manure amendment. Sheep manure amendment reduced metal toxicity and metal uptake by plants with a low risk of leaching into groundwater.

Microbial Agents Application of different microbial species for enhancing phytoremediation of polluted soil comes under this category. These microbes utilize enormous arrays of inorganic and organic substances for their metabolism to survive in contaminated environment. These microbes include endophytic and exophytic bacteria, plant growth promoting rhizobacteria (PGPR), fungi, root-colonizing bacteria, etc. Some of the endophytic bacteria are known to be diazotrophic and may fix nitrogen to the host plant (Doty 2008). Microbes degrade contaminants following various processes including chemical transformation, chelation, protonation precipitation, acidification, and sorption. Further, microbes produce chelating agents, sugars, and organic acids, viz., citric, oxalic, or acetic acids, which work as energy source for microbes (Li et al. 2009; Gerhardt et al. 2009). These microbes enhance phytoremediation by stimulating plant growth and biomass as they secrete various phytohormones (eg. indole acetic acid (IAA)), suppress stress by ethylene production, and improve plant nutritive status due to the presence of N_2 fixers, PO_4 solubilizers, or siderophore producers which intensify population and activity of native microbes to degrade contaminants by horizontal gene transfer (Sessitsch et al. 2013; Afzal et al. 2014). Plant growth and biomass enhancement have been observed by the amendment of different endophytic, rhizospheric and plant growth-promoting bacteria (Cabello-Conejo et al. 2011; Ghosh et al. 2011; Li et al. 2007).

Dell'Amico et al. (2008) observed that IAA, ACC deaminase, and siderophore producing rhizospheric bacteria promoted growth of *B. napus* in Cd-contaminated soil. Braud et al. (2009) have reported increase in bioavailability of chromium (Cr) and lead (Pb) by rhizospheric bacteria *Pseudomonas aeruginosa* for improved phytoremediation by maize plants. Contrastingly, some studies have also reported opposite trends by different microbes, e.g., Sinha and Mukherjee (2008) observed reduction in Cd uptake in roots and shoots of *Cucurbita pepo* and *Brassica juncea* by *P. aeruginosa* strain KUCd1. These may be because of variation in plant type, metal bioavailability, ability of plants for contaminants' uptake and its translocation (Rajkumar et al. 2012). Further, Hou et al. (2015) phytoremediated petroleum-contaminated soil by amending it with PGPR. They used tall fescue (*Testuca arundinacea* L.) for purpose and reported increased biomass by PGPR with higher removal of high molecular weight fractions (C21–C34) of aliphatic hydrocarbons and PAHs.

1.9.2 Artificial/Inorganic Substances

These substances are produced by manmade processes such as physicochemical processes, genetically modified organisms, nano-techniques, etc. for amending the soil to enhance phytoremediation. Some of these are discussed in this section.

Chelating Agents The word *chelation* means "to grab" or "to bind" and chelation process is the binding of ions and molecules to metal ions. When chelating substances are added to the contaminated medium, it "grabs" metals like mercury, copper, iron, arsenic, aluminum, nickel (Ni), etc. Chelation involves formation of two or more coordinate bonds between a central atom and multiple bonded ligands to form a ring structure. These ligands are organic compounds and are commonly known as *chelating agents,* and these may be synthetic or natural. Some of the common chelators are ethylenediaminetetraacetic acid (EDTA), N-(−2-hydroxyl)-ethylenediaminetetraacetic acid (HEDTA), [S,S]-stereo-isomer of ethylenediaminedisuccinic acid citrate (EDDS), nitrilotriacetate (NTA), and mugenic and avenic acids. These chelators increase the solubility of metal, prevent precipitation and sorption of metals and, thus, enhance their bioavailability to the plants to enable phytoextraction/phytoaccumulation in upper parts (Prieto et al. 2013).

Tariq and Ashraf (2016) studied effect of EDTA amendment on phytoextraction probability of *Helianthus annuus*, *Z. maize*, *Brassica campestris,* and *Pisum sativum* for removal of heavy metals (Cd, Cu, Co, Ni, Cr, and Pb). They reported increased phytoremediation of all of the metals with different plant species after addition of EDTA. *Z. maize* was found to be hyperaccumulator for Co and Cr after EDTA application, while *H. annuus* for Cd under similar conditions.

Further, EDTA is persistent in nature and may remain absorbed in soil even after soil remediation process and may adversely affect plant growth, development, and soil-associated microorganisms (Bandiera et al. 2009). Chen and Cutright (2001) reported an increased phytoremediation of Ni and Cd with effect of EDTA and HDTA amendments by *H. annuus*. Other chelating agents like EDDS, NTA, etc. are easily biodegradable and less toxic in comparison with EDTA, so these can be used as replacement of EDTA for better remediation of heavy metals from soil (Wenger et al. 2003; Meers et al. 2005). Duquène et al. (2009) reported increased phytoremediation of uranium, Cd, Cr, Cu, Pb, and Zn by Indian mustard (*B. juncea*) and ryegrass (*Lolium perenne*) after amendment of chelates of citric acid, oxalic acid, etc. Quartacci et al. (2006) have also reported similar, i.e., two to threefold increase of Cd, Cu, Pb, and Zn concentrations in shoots of Indian mustard after application of 5 mmol kg^{-1} NTA. But, comparative studies have shown that despite its toxicity to plants and persistent nature, EDTA was still more efficient than other chelating agents for phytoextraction as well as for solubilizing different metals from the soil (Luo et al. 2005). Saifullah et al. (2010) reported that EDTA was more efficient than citric acid (CA) or elemental sulfur (S) in solubilizing lead (Pb) from the soil with wheat (*Triticum aestivum* L.) crop.

Nanoparticles These are microscopic particles that have dimensions between 1 and 100 nm with surrounding interfacial layer (Thomé et al. 2015).Nanoparticles are synthesized from different nanomaterials (NMs) which may be either natural or anthropogenic in origin. Naturally present NMs include clay, organic matter, and iron oxide; and anthropogenic include NMs from air emissions, solid or liquid wastes, agricultural operations, weathering, etc. (Thomé et al. 2015). In labs these are produced by two methodologies; *top to bottom* where larger particles are broken down into smaller ones and *bottom to top*, where smaller particles build up bigger particles (Niemeyer 2001). There are different kinds of nanoparticles depending upon their composition. These are organic and inorganic, e.g., carbon nanotubes, quantum dots, metals, and oxides of metals like Zn, iron, manganese (Mn), tin, Cu, titanium, aluminum nanoparticles, etc. (Thomé et al. 2015). On the basis of smaller particle size with very large surface areas and higher reactivity, these are used as soil amendment for better phytoremediation of different pollutants (Klaine et al. 2008). Nanoparticles affect growth and development from seed germination to crop production by adsorbing nutrient and metals onto the surface and translocating them into different tissues. But at higher concentrations, these may also harm the health of the soil, plants, and microorganisms as these can cause toxicity and improve generation of reactive oxygen species that result in interruption of cellular metabolism (Siddiqi and Husen 2017). The bioavailability and ecotoxicity of nanoparticles depend upon different phases, i.e., dissolved, colloidal, or particulate phase, size, shape, and surface area (Pal et al. 2007; Madden et al. 2006; Siddiqi and Husen 2016).

Michálkov et al. (2014) studied the stabilization of three oxides of Fe and Mn nanoparticles for elimination of Cd, Cu, and Pb from soil. Mn nanooxides were reported to be the most efficient amendment for declining mobility of these metals in comparison with nanomaghemite and nanomagnetite and also have higher potential for adsorptive removal of these metals.

Mohsenzadeh and Rad (2012) reported higher accumulation of all toxic metals (Cu, Zn, Pb, and Ni) in *Gundelia tournefortii, Centaurea virgata, Reseda lutea, Scariola orientalis, Eleagnum angustifolia*, and *Noaea mucronata* after addition of nanoparticles prepared from *Noaea mucronata.* Li et al. (2014) studied potential of two different particle sizes, i.e., nano- and micrometer hydroxyapatite amendments, for enhancing phytoremediation of heavy metal (Pb, Zn, Cu, and Cr)-contaminated soil. Nano- as well as microsized hydroxyapatite amendments showed significant immobilization of metals, but micrometer-sized hydroxyapatite amendments were more significant than nanosized hydroxyapatite. Further, Shaheen and Rinklebe (2015) compared different low-cost (activated carbon, fly ash, lime stone, and zeolite, etc.) and emerging (nanohydroxyapatite, biochar) soil amendments for Cd and Pb (im)mobilization and its uptake by rapeseed (*Brassica napus*). Among all of these amendments, nanohydroxyapatite showed highest potential for Pb immobilization in soil and increased phytostabilization.

Slow Release Fertilizers (SRF) SRFs are granulated fertilizers with slowly soluble nutrients in the soil. As the name indicates, these make nutrients available to plants in small amounts, gradually, over an extended period. These are used as soil amendments to upsurge the plant growth and development which helps in the effective phytoremediation of contaminants present in the soil. Examples include osmocote- and sulfur-coated urea, black rock phosphate, colloidal rock phosphate, methylene urea, etc. These are water insoluble and are released in the soil with the help of either microbial activity or by hydrolysis. Most of the times these are used for enhancing production rate but sometimes, these amendments may also be used to promote bioremediation/phytoremediation of contaminants. Kim et al. (2017) studied the combined effect of biochar and SRF on rice productivity, methane emission, and microbial abundances in rice paddy. They reported reduced methane emission after combining biochar and SRF which increased rice yield. SRF also diminished plant biomass, which may decrease methane transport and carbon substrate from plant debris and root exudates to reduce methanogenesis.

1.10 Conclusion

As industrial development had taken place at rapid pace, contamination of soil by organic and inorganic contaminants has become a serious issue all around the world. These vigorously contaminated soils have poor physicochemical and biological conditions which do not support the full growth and development of the plants and associated rhizospheric bacteria. Various conventional techniques, which have been discussed earlier in the chapter, are available to remediate soil from these contaminants, but, have their own limitations. Conventional techniques involve different processes (physiochemical and biological) to remove or degrade the contaminants to less toxic substances without removing the bulk soil. Further, chemical and physical means of remediation irreversibly affect soil quality and abolish biodiversity.

Hence, there is a need of suitable cost-effective and biological techniques for remediation of soil without affecting soil fertility and maintaining the sustainability. These limiting factors have forced scientists and researchers to use plants for decontaminating soil without harming the environment. For occurrence of successful phytoremediation, several amendments (organic as well as inorganic) have been used to remove contaminants to maximum levels. However, these amendments also suffer with different constrains in the way of implementation on large scale. One of the main limitations is the unavailability of single technique/amendment for remediation of all kinds of pollutants and diversities in physicochemical properties of soil to be remediated. Several times, more than one amendment is required to address different contaminants and combining two or more methods may inhibit the removal efficiencies. Further, adaptability and sustainability of the plants to be used for phytoremediation also vary from species to species. There is also a chance of generation of harmful secondary pollutants, which may remain bioavailable in the environment after completion of phytoremediation of contaminated soil. There is very limited commercialization of these amendments, so the knowledge regarding these are restricted to the labs and pilot field studies. Hence, these constraints need to be solved for increasing the confidence in this green technology and for the sake of pollutant-free, clean, and healthy environment/soil.

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Chapter 2
Rhizoremediation: A Sustainable Approach to Improve the Quality and Productivity of Polluted Soils

Isha Mishra and Naveen Kumar Arora

Abstract Soil contamination is a widespread problem which has been causing deleterious changes in the biology, structure, and also its productivity. Various recalcitrant and xenobiotic compounds, due to rapid pace of anthropogenic activities, have accumulated in soil resulting in its degradation and infertility. In addition to this, persistent nature of these pollutants allows them to enter into the food chain posing serious threats to living beings. Therefore, a holistic and sustainable approach which is ecofriendly, cost-effective, and organic in nature, is the need of the hour. Rhizoremediation is one such method which could obviate the problem of such hazardous compounds from soils. The co-evolutionary relationship between plant and their associated microbiota is being successfully used to reclaim and restore degraded soils without causing any harmful by-products unlike conventional methods. Not only this, application of rhizoremediation technique is also reported to improve soil organic matter (SOM), nutrient cycling, bioavailability of insoluble compounds, which in turn enhances biomass production rendering the soil fertile and productive for better agronomic purposes. Recent advances in genetic engineering and "omics" techniques have further strengthened our knowledge in this area which when exploited in future could be used to alleviate the problem of soil contamination with precision and in shortest possible time. In this way, this green technology along with amalgamation of biotechnological tools could be envisaged as an excellent substitute to chemical and physical methods to remediate contaminated soil making it fertile and productive.

Keywords Soil contamination · Anthropogenic activities · Rhizoremediation · Plants · Microbes · Productivity · Green technology

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2.1 Introduction

Soil, derived from a Latin word "solum" means floor, is the principal foundation to our agricultural resources, global economy, sustainability, and survival (Ramesh and Chhonkar 2001; Oh et al. 2013). Soil is crucially important in maintaining the quality of environment and most importantly linked to food production, land use and human health (Gomeiro 2016). However, elevation in urbanization, industrialization, and intensive agricultural practices has led to the degradation of soil quality and productivity around the globe (Paz-Gonzalez et al. 2000; Li et al. 2008). There are many land sites which are tainted with contamination of several xenobiotic compounds like polyaromatic hydrocarbons (PAH), heavy metals, pesticides, and some other pollutants (Marchand 2017). Soils themselves do not have the ability to filter these contaminants and cannot transform them into non-toxic forms. This has resulted in unhealthy soils and limiting crop production around the globe.

According to FAO (2017), about one-third of the world's soils are facing the problem of soil pollution due to unsustainable practices. It was estimated that amount of chemicals produced by European chemical industry was up to 319 million tons in 2015 out of which 117 were found hazardous in nature (FAO 2018). The Chinese Environmental Protection Ministry reported that 16% of the total soils in China and 19% of its agricultural soils come under polluted category (CCICED 2015). Furthermore, as per studies of European Environment Agency (EEA 2014), around 3 billion tons of sites with potential contamination have been estimated in European Economic area and approximately 80,000 contaminated sites across Australia (Department of Environment and Conservation, Australia DECA 2010). On the other hand, according to the reports from India, due to adverse effects of soil pollution, crop productivity has reduced to 15–25% over the years (Dakiky et al. 2002; Nath et al. 2005; Saha et al. 2017). The major concentrators and emitters of such compounds are agrochemicals like fertilizers and pesticides (herbicides, weedicides, fungicides, and other pest control products) (Merry et al. 1983; Mani et al. 2005; Chiaia-Hernandez et al. 2017) and industrial wastes like those generated from pulp, pesticides, petroleum, pharmaceuticals, engineering industries, and oil refineries (Rohrbacher and St-Arnaud 2016). These myriad of hazardous pollutants continue to exist in the environment for longer period of time and, therefore, create nuisance in the ecosystem by accumulating in the food chain (Mrozik and Piotrowska-Seget 2010; Ling et al. 2015). The contamination through these sources takes place in various ways. It can be the result of improper chemical production (such as from drilling of oil spills, manufacturing of explosives), transport (such as oil spills from tankers or pipelines), storage (such as leakage of chemicals from storage tanks), usage (such as pesticides, fertilizers from agriculture), or disposal processes (industrial wastes) (Truu et al. 2015). These contaminants interact with soil in three different ways: (i) complexion, (ii) precipitation (in case of inorganic contaminants, e.g., heavy metals), and (iii) sorption (in case of organic compounds, e.g., PAHs) (Tan 2009; Marchand 2017). These interactions change the soil fertility by changing its ion exchange capacity, pH, and bioavailability of elements, thus making it unproductive. Measures have been taken in the last few decades for restoration of such lands, and new environmental guidelines have also been developed for remediation of such sites (Marchand 2017). Several ex situ and in situ technologies for treatment of polluted soil have already been introduced. However, these methods are limited only to definite area or sites. There are also chemical methods for the treatment of such soils which carry out extraction of hazardous wastes such as through photochemical degradation (Clark et al. 2007; De Bruyn et al. 2012), chemical oxidation (Sirguey et al. 2008), and thermal desorption (Wang et al. 2012). On the other hand, physical treatments remove wastes through soil washing, incineration, solidification, and vapor extraction (Sessitsch et al. 2013). However, these traditionally used technologies have several side effects on quality of soil and are reported to lower its productivity. Furthermore, these are costlier to carry out particularly in developing countries, thus limiting their applicability (Oh et al. 2013).

Therefore, there is an urgent need for a biological remediation technology which is sustainable, environmentally non-evasive and is efficient to carry out (Al Ameeri and Al Sarawi 2017). One such technique which is gaining the attention of several scientists over the years is the use of plants and microorganisms in conjunction to degrade the toxic compounds present in soil. The term used for this biological method is known as "rhizoremediation." It has emerged as a potential approach for the reclamation of contaminated soils and stimulates pollutant degradation by the help of plants along with the associated communities of soil microbes (Kamaludeen and Ramasamy 2008).

The plant root system and the area which encircles it is known as rhizosphere, and it is characterized by microbial biomass which is 100 times richer and metabolically more active than bulk soil (known as rhizosphere effect) (Campbell and Greaves 1990; Erickson et al. 1995; Gunther et al. 1996). The symbiotic relationship between plants and microbes and co-metabolism in the rhizosphere releases nutrients, enzymes and biosurfactants which help in degradation of toxic compounds present in the contaminated soil (Alkorta and Garbisu 2001; Morikawa and Erkin 2003; Barea et al. 2005; Wenzel 2009). Rhizospheric bacteria obtain nutrients from roots of soil and in return make these xenobiotic compounds bioavailable to plants by a series of mechanisms described below (Kamaludeen and Ramasamy 2008; Oberai and Khanna 2018). There are two ways in which rhizoremediation technique is used, i.e., by naturally occurring microbial communities or by inoculating the affected soil with microorganisms capable of degrading contaminants (Gerhardt et al. 2009; Asemoloye et al. 2019). Several microbial species of bacteria, and fungi can be introduced externally in rhizosphere to stimulate the degradation process, and an efficient rate of remediation could be achieved (Blackburn and Hafker 1993). In this way, rhizoremediation has several advantages over other technologies. It is easy to implement, requires no maintenance and fuel cost and above all, unlike the conventional methods this green technology conserves the fertility of soil (Salam and Idris 2019). Therefore, microbe-assisted catabolic reactions for the remediation of such polluted soils are the most economic and environment-friendly procedures. In this chapter, we focus on the different types of pollutants associated with the contaminated soils and their remediation using this green technology.

2.2 Rhizoremediation of Contaminated Soils

Rhizoremediation is also known as rhizodegradation, microbe-assisted phytoremediation, or rhizosphere degradation (Truu et al. 2015) and is a specific subset of phytoremediation (Cook and Hesterberg 2013). The first-ever study for the degradation of a compound using this method was conducted on pesticides (Hoagland et al. 1994; Zablotowicz 1994; Jacobsen 1997). Important parameters for rhizoremediation are (i) nature of the pollutants, (ii) the soil structure and hydrogeology (movement of pollutants through soil and groundwater), and (iii) nutritional state and microbial composition of the site (Blackburn and Hafker 1993). It can also be optimized using compatible plant-microbe pairs. These can be combination of plants and PGPR or may be plant- and contaminant-degrading microbes. There are many plant species which have proven suitable for the process of rhizoremediation, like grasses (e.g., prairie grass), because of their extensive root system (Aprill and Sims 1990), leguminous plants (such as alfalfa) because of their ability to fix nitrogen (Kuiper et al. 2001), and trees like *Salix* sp. and *Populus* sp. due to their perennial growth, high absorption surface areas, and resistance to contaminants (Newman and Reynolds 2004; Barac et al. 2009). *Morus rubra* and *Betula pendula* are also efficient candidates because of their ability to produce high biomass and to colonize nutrient-depleted soils efficiently (Rezek et al. 2008). In addition, plant roots help in spreading microbes to impermeable layers of soil matrix (Kuiper et al. 2004). The microbes which take part in the degradation process in the rhizosphere belong to bacteria, actinomycetes, and fungi including arbuscular mycorrhizal fungi (AMF). Root exudates are the key players for the successful colonization of rhizospheric bacteria in the root vicinity and which would result in successful degradation of soil pollutants. The root exudates consist of organic acids, amino acids, sugars, proteins, alcohols, nucleotides, flavanones, enzymes and phenolic compounds (Hiltner 1904; Vancura and Hovadik 1965; Al Ameeri and Al Sarawi 2017). For example, linoleic acid secreted by plant roots acts as surfactant to increase the bioavailability of PAH (pyrene) by forming a layer on soil particles. This results in enhanced attachment of bacteria on the pollutant (Yi and Crowley 2007). The bacteria present in the rhizosphere of plants in such contaminated sites use these exudates as their carbon and energy source (Neumann 2006; Biswas et al. 2015) and synthesize various metabolites like siderophores (iron scavanging compounds), exopolysaccharides (metal chelator and biosorption), biosurfactants (solubilization of metals and hydrophobic compounds), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (lowers ethylene, a stress hormone), organic acids (mineralize metals and solubilize organic compounds), and many other enzymes, e.g., oxidoreductases (for oxidation of hydrocarbons), which bring about the degradation of contaminants (Mishra et al. 2017). The process of rhizoremediation of contaminated soils is shown in Fig. 2.1. The rhizoremediation technique has proved better over bioremediation (bioaugmentation and biostimulation) and phytoremediation processes because of the fact that it uses indigenous rhizospheric microbial communities which are greater by two to four orders of magnitude compared to bulk

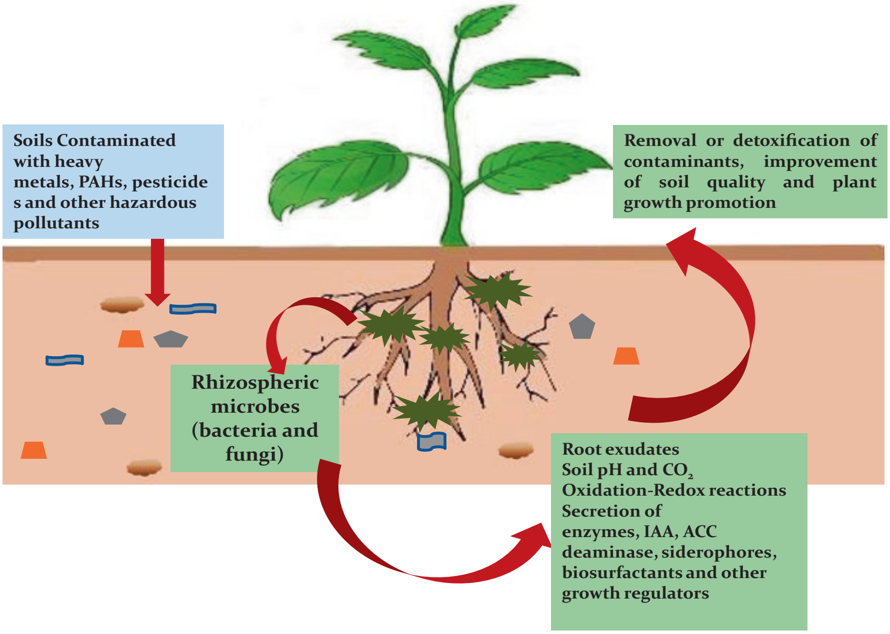

Fig. 2.1 Process of rhizoremediation in contaminated soils

soil (Salt et al. 1998; Marschner et al. 2001). The degradation rate of up to 65% to 90% has been observed for pollutants like total petroleum hydrocarbons (TPH) using ryegrass and PGPR as compared to bioremediation and phytoremediation methods (Huang et al. 2005). Another study has shown that mitigation of pyrene was reported more in rhizospheric soil than in bulk soil, and it was typically due to root exudates secreted by plant in the rhizosphere (Reilley et al. 1996). This proves that the key factor on which the process of rhizoremediation relies is the co-evolutionary process between plants and rhizospheric microbes and hence with the use of suitable plant cultivar along with suitable kind of rhizobacteria, enhanced reclamation process could be achieved (Kamaludeen and Ramasamy 2008). The degradation process also depends on various soil characteristics like soil pH, temperature, light, humidity and carbon content (Schroll et al. 2006; Biswas et al. 2015). It has been observed that degradation rate of contaminants by bacteria was slower in acidic soils as compared to alkaline or neutral soils (Singh et al. 2006). Likewise, some rhizobacteria such as *Sphingomonas* UT26, in absence of moisture content in soil, were unable to degrade lindane, a highly persistent pesticide in spite of being an excellent degrader (of this pesticide), proving importance of soil moisture (Segura et al. 2009). In this way, plant-microbe relationship helps to naturally attenuate, immobilize, or remove these compounds from soil and restores its quality and productivity. In the next section, major contaminants causing soil pollution and how these could be mitigated through the sustainable approach using rhizoremediation have been discussed in detail.

2.2.1 Heavy Metal Contamination

Pollution of hazardous heavy metals in soils is of great concern and poses serious threat to various environmental niches including soil ecosystems (Wuana and Okieimen 2011). Long-term residence of heavy metals in soil, non-biodegradable nature, and accumulation at different trophic levels of the food web makes heavy metal contamination a global concern (Bowen 1979; Clemens 2006; Khan et al. 2009). Presence of heavy metals in soil has been a natural phenomenon, but due to rapid industrialization, agronomic practices, and anthropogenic activities, their concentration has reached to an alarming level that is harmful to plants and human health (Chibuike and Obiora 2014). The major sources of heavy metal contamination are smelting, mining, use of pesticides and fertilizers, medical wastes, combustion of coal, leaded petrol, sewage sludge, and municipal wastes (Zhang et al. 2010; Rodrigues et al. 2012; Taiwo et al. 2016). Aluminum (Al), barium (Ba), arsenic (As), cadmium (Cd), chromium (Cr), cadmium (Cd), copper (Cu), cobalt (Co), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), lithium (Li), and zinc (Zn) are some common heavy metals found in contaminated soils (Adriano et al. 2005; Sparks 2005; Karthik et al. 2017). In the recent past, use of rhizospheric microbes in the context of cleaning up the heavy metal-contaminated soil has emerged as a suitable alternative, and the input provided by these rhizobial microbes is non-toxic (Kuiper et al. 2004; Ullah et al. 2015). The rhizomicrobial population is predominantly composed of *Actinobacteria*, *Proteobacteria*, and *Firmicutes* which help in decontamination of heavy metals from soil (Pires et al. 2017). Fungi belonging to *Ascomycotina* and *Basidiomycotina* and a large number of AMF have also been reported to reside in such contaminated sites (Khan et al. 2000). Although there are certain plants which are hyper-accumulators of some heavy metals and, therefore, help in remediation process (phytoremediation), the presence of rhizospheric microbes aids in the above process and enhances their growth in stressed conditions (Wenzel and Jockwer 1999; Glick 2012). Cr (VI) uptake in metal-contaminated soil was reduced by 37% in shoots and 56% in roots of green chili plant by reducing toxic Cr (VI) to non-toxic Cr (III) upon inoculation with *Cellulosimicrobium cellulans* (Chatterjee et al. 2009). Rhizobacterial strains *Microbacterium* sp. CE3R2, *Microbacterium* sp. NE1R5, *Curtobacterium* sp. NM1R1, and *Microbacterium* sp. 166 NM3E9 are reported to be the potent candidates in remediation of As(V), Pb(II), Cu(II), and Zn(II) in the agroecosystems (Romano et al. 2017). These microbes are tolerant to the benign effects of heavy metal toxicity and increase the bioavailability and solubility of heavy metals. There are various mechanisms by which cleanup process is carried out by rhizospheric microbes, i.e., through chelation, acidification, redox reactions, precipitation, and complexation (Mishra et al. 2017). According to Rajkumar et al. (2010), production of siderophore was induced in certain rhizospheric microbes in presence of heavy metal contamination. Siderophore is an iron chelating compound produced when there is shortage of the metal around the vicinity of rhizosphere. Iron (Fe) is an important element for plant growth, and its deficiency in plants grown in heavy metal-contaminated soil leads to symptoms like chlorosis and inhibits chloroplast development and chlorophyll biosynthesis (Imsande 1998). These microbes transform insoluble iron (Fe^{3+}) into its soluble form (Fe^{2+}). Siderophore produced by *Pseudomonas fluorescens* exhibited an enhanced uptake of Fe in mung bean and maize plant (Sharma and Johri 2003a, b). The sequestration by siderophore is not only limited to Fe, rather it is also reported to make complexes with other heavy metals such as Cd, Mn, Pb, Zn, and Al (Rajkumar et al. 2010). Siderophore produced by *Pseudomonas aeruginosa* was reported to significantly enhance the uptake of Pb and Cr in maize (Braud et al. 2009).

Exopolysaccharides (EPS) production by several rhizospheric bacteria also helps in sequestering of metal ions and forms sheath to protect them and plant roots from metal toxicity (Mishra et al. 2017). *Pseudomonas* sp., *Arthrobacter* sp., *Azotobacter* sp., and rhizobia are some common examples of bacteria producing EPS (Gupta and Diwan 2016). Joshi and Juwarkar (2009) reported Cd (15.2 mg gm^{-1}) and Cr (21.9 mg gm^{-1}) immobilization by inoculation of EPS producing *Azotobacter* sp. Some AMF have been reported to form complex with heavy metals (Pb, Cu, and Cd) by production of EPS and glomalin and could play an important role in rhizoremediation of metal-contaminated soil (Gonzales-Chavez et al. 2004). EPS produced by rhizobacteria binds and entraps precipitated metal sulfides and oxides by forming EPS-heavy metal complex resulting in heavy metal remediation (Xu et al. 2012; Kaushal and Wani 2016). Electrostatic interaction is another attribute which plays a very important role in detoxification of heavy metal stress by EPS (Costa et al. 2018). Rhizobacterial EPS have also been reported to increase root and shoot length of plants under stressed conditions (Hussain et al. 2014).

Production of biosurfactant, an amphiphillic surface active compound, by rhizospheric bacteria is another mechanism by which these microbes reduce the harmful effects of heavy metal pollution in soil (Sachdev and Cameotra 2013). Biosurfactants form complex with heavy metals and lower the solution activity of the metal, thereby increasing their desorption which makes them available to the plants (Gupta and Kumar 2017). Rhamnolipid, a biosurfactant produced by *P. aeruginosa*, is one of the examples which increases the solubility of heavy metals in soil (Maeir and Soberon-Chavez 2000; Rufino et al. 2014). Biosurfactant producing endophytic *Pseudomonas* sp. LK9 isolated from metal contaminated site was found to increase shoot dry mass of *Solanum nigrum* by 14% and Cd uptake by 46.6% (Chen et al. 2014). Rhmanolipids along with surfactins have also been reported to detoxify effects of heavy metals, such as calcium (Ca), Ba, Li, and Zn (Nielsen and Sørensen 2003; Mulligan and Wang 2004). Increased biomass production was achieved in maize, rape, sundargrass, and tomato plant after inoculation of biosurfactant producing rhizospheric *Bacillus* sp. J119 under metal-stressed soil (Sheng et al. 2008).

Heavy metal pollution also induces the production of an enzyme ACC deaminase to regulate the synthesis of ethylene, a stress hormone. During abiotic stresses (including heavy metal stress), plant releases ethylene reducing the growth and root elongation in plants (Khalid et al. 2006; Arshad et al. 2007). To converse the situation, PGPR produce ACC deaminase to regulate the biosynthesis of ACC, a precursor of ethylene which in turn balances the amount of ethylene helping plant to combat the stress caused during such conditions. Phytohormone indole acetic acid (IAA) is also released by microbes which helps plant to withstand the adverse effects of heavy metals by inducing root elongation in them. *P. aeruginosa* MKRh3 was reported to enhance growth of *Vigna mungo* under Cd contamination and also reduced metal accumulation by production of IAA and ACC deaminase (Ganesan 2008).

Some metal-binding peptides, i.e., metallothioneins (MTs) and phytochelatins (PCs) (synthesized from the tripeptide glutathione or by enzymatic reactions involving PCs synthase), are reported to eliminate free metal ions by transportation, sequestration, or compartmentalization (Cai and Ma 2002; Solanki and Dhankar 2011). The expression of a metal-binding peptide (EC20) in rhizobacteria *Pseudomonas putida* improved cell growth in Cd-contaminated soil, and binding of Cd ion was also enhanced in sunflower seedling (Wu et al. 2006). Some of them, e.g., *Azotobacter vinelandii*, are also reported to produce metallophores (protochelins and azotochelins), the organic ligands, which also increase the bioavalibility of some cations and oxoanions (Deicke et al. 2013).

Microbial methylation also helps in reducing the toxicity of heavy metals. Bacterial species of *Pseudomonas*, *Bacillus*, *Clostridium* are known to perform biomethylation of Hg into gaseous methyl mercury (Pongratz and Heumann 1999). There are also some PGP bacteria which can transform metals such as Pb, selenium (Se), tin (Sn), and tellurium (Te) into gaseous state through addition of methyl groups which diffuses methylated metals away from cell wall due to volatility. This creates a reduction in toxicity (caused by heavy metals) in their niche (Etesami 2018).

Rhizospheric microbes are also known to secrete organic acids (e.g., oxalic acid, gluconic acid, citric acid) which mobilize or solubilize the heavy metals present in the soil (Rajkumar et al. 2012; Ullah et al. 2015). *Gluconacetobacter diazotrophicus* produces 5-ketogluconic acid, a derivative of gluconic acid which was found to enhance the solubility of Zn compounds (Saravanan et al. 2007). In addition, microbes also possess mechanisms involving reduction of metal ions which not only impart resistance to microorganisms against metal toxicity but also soothe plants against the same. Cr (VI), a hexavalent metal ion, is a highly toxic element present in soil and is known to produce free radicals and reactive oxygen species (ROS) by their easy diffusion into cell membranes. In this regard, PGPR have been documented to reduce the toxicity caused by Cr(VI) compounds and also increase plant biomass. *Pseudomonas, Bacillus, Ochrobactrum, Delftia, Rhodococcus* are few of the genera which are known to reduce this metal ion into less harmful form (Ahemad 2014; Upadhyay et al. 2017).

2.2.2 Polyaromatic Hydrocarbon Contamination

Polyaromatic hydrocarbons (PAHs) are one of the most hazardous and most common classes of organic contaminants. These are distributed widely and are produced due to partial combustion of some organic products like oil, coal, or wood and certain waste materials (Finlayson-Pitts and Pitts 2000). They have lower solubility and hence are persistent in environment for a longer period of time (Shin et al. 2006; Balakrishna 2016). The concentration of PAHs in soil and sediments varies from 1 μg/kg to 300 g/kg (Kanaly and Harayama 2010). They are categorized in two forms based on molecular structure, i.e., low molecular weight (LMW) and high molecular weight (HMW). Those PAHs having two or three aromatic rings in their molecular structure are termed as LMWs and those with four or more aromatic rings are known as HMWs. Solubility of PAHs decreases with increase in molecular mass (Heitkamp and Cerniglia 1989; Wild and Jones 1993). PAHs can be carcinogenic, mutagenic, and teratogenic in nature and cause irreversible changes in living beings which could be fatal if consumed (Wilson and Jones 1993). According to US Environmental Protection Agency (USEPA), there are 16 PAH compounds that have been listed as priority pollutants, namely, naphthalene, acenaphthalene, acenaphthylene, phenanthrene, fluorene, anthracene, benz[α]anthracene, chrysene, pyrene, fluoranthene, *benzo*[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[α]pyrene, indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene, and dibenz[*a,h*]anthracene (Yan et al. 2004).

Release of PAH in environment takes place via natural and anthropogenic activities. Natural phenomena include forest fires and volcanic eruptions, while anthropogenic activities include emissions from vehicles, leakage from petroleum industries, combustion of organic compounds such as wood and other fossil fuels, manufacturing of coal tar, and so on (Ravindra et al. 2008). Due to these ill properties, PAHs have become a global concern and demand an efficient remediation technology (Isaac et al. 2013). Rhizoremediation has emerged as a potential tool for reclamation of such compounds from polluted soils (Cook and Hesterberg 2013). The synergism between plants and microbial community plays a vital role in degradation of these xenobiotic compounds present in soil (Barac et al. 2009; Hong et al. 2011). An increased rate of PAH degradation was observed in vegetative soil compared to nonvegetative soils specially near immediate vicinity of roots (Aprill and Sims 1990; Daane et al. 2001), e.g., a higher bacterial gradient was observed near the root of ryegrass for the degradation of phenanthrene (up to 86% in 0–3 mm layer from root) (Corgie et al. 2003).

The root-colonizing microbes carry out degradation by mineralization of PAH compounds into corresponding diols and organic acids which are brought about by cleavage of aromatic ring structure by dioxygenase enzyme (Brezna et al. 2003; Peng et al. 2008). The bacteria which help in assisting the process of rhizoremediation of PAHs belong to genera *Pseudomonas, Bacillus, Burkholderia, Alcaligenes, Sphingomonas, Rhodococcus*, and *Mycobacterium* (Moody et al. 2001; Bisht et al. 2015). Phenanthrene degradation was also observed by the mentioned bacterial species in ryegrass rhizosphere using it as carbon source, of which, *Pseudomonas* and *Arthrobacter* were the best degraders (Radwan et al. 1998; Seo et al. 2006; Cébron et al. 2011). It was observed that such bacteria contain *nid A, nid B, pdo A2*, and *pcaH* genes which help in the oxidation of the aromatic ring of PAH compounds (Kim et al. 2007; Pagnout et al. 2007). Genes *nidA* and *nidB* encode for dioxygenase enzyme responsible for initial degradation of aromatic ring, whereas *pcaH* was found significant in β-ketoadipate pathway which belongs to a major class of non-heme-containing dioxygenase (Brezna et al. 2003; Peng et al. 2012). On the other hand, *pdo* gene helps in detoxification of catechol of PAHs into water-soluble compounds (Pagnout et al. 2007; Kim et al. 2007). In a study conducted by Darmawan et al. (2015), *Burkholderia fungorum* and *Mycobacterium gilvum* were reported to possess similar mechanisms involving these genes in the degradation of pyrene and fluoranthene. Rhizobia have also been reported to show tolerance to PAH contamination and have been shown to aid in their degradation by stimulating other PAH degraders present in the rhizosphere of contaminated soils. This was supported by a study in which PAH degradation was observed in the ryegrass and white clover (*Trifolium repens*) after inoculation of *Rhizobium leguminosarum*. The experiment showed that degradation of PAH was enhanced in the soil that received rhizobia inoculation compared to those which were uninoculated (Johnsen et al. 2005). The efficiency of rhizoremediation was studied in another experiment conducted by Daane et al. (2001) in which a variety of PAH degraders (*P. putida* NCIB 9816-4, *Comamonas testosterone* GZ42, *Sphingomonas yanoikuyae* B1, *Myobacterium* sp. PY01, and some *Paenibacillus*) from rhizosphere of salt marsh grasses were isolated and showed an excellent result in degradation of these pollutants which could hold a promising way in restoration of contaminated coastal environments and sediments. Root exudates play a significant role in enhancing viability of rhizospheric ecosystem contaminated with such compounds. Exudates secreted by plant roots reduce the toxicity of pollutants by mineralizing them and hence improve the heterogeneity of the microenvironment. This helps microbes in carrying out the degradation of PAHs efficiently and hence increases the efficiency of rhizoremediation (Hamdi et al. 2007; Cook and Hesterberg 2013). Oxidoreductases (dehydrogenases, peroxidases, catalases, and polyphenoloxidases) are some important root exudates which when secreted in the rhizosphere stimulate the soil microbes to increase the rate of degradation of PAHs in soil (Liu et al. 2014, 2015). Fire phoenix (plant) and their associated microbial communities are the excellent examples which were reported to degrade approximately eight PAHs (fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene) by up to 99.40% in 150 days, and those consisting of four to five aromatic rings were decreased to as low as 0.47 mg kg^{-1}. This was due to an increased secretion of these oxidoreductases which in turn resulted in an upward shift in degradation of eight PAHs. *Gordonia* sp. were among the potential degraders based on the results of DGGE plastic cutting sequencing in this study (Liu et al. 2014). Many rhizospheric bacteria particularly *Pseudomonas* sp. and *Bacillus* sp. have been reported to produce certain secondary metabolites which help in solubilization of PAHs in rhizospheric soil. Rhamnolipid biosurfactants are one such example which have been reported to help in transfer of PAHs from solid to liquid phase and become available to root colonizing bacteria (Zhang et al. 1997; Cui et al. 2008). Some root-colonizing fungi, e.g., ectomycorrhiza, have also been reported as excellent drivers of PAH degradation (Levyal and Binet 1998). The hyphae of mycorrhiza are much more extensive than plant roots and are capable of penetrating even to smaller pores of soil. AMF which are comparatively less aggressive are also capable of degrading PAHs in an indirect way, i.e., by inducing bacterial community residing in the rhizospheric region (Joner et al. 2004; Joner and Levyal 2003). Similar mechanisms of degradation have been observed in white rot fungi which secrete lignin-degrading extracellular enzymes to perform degradation of PAH (Anderson and Coats 1995). Furthermore, the phenomenon of lateral gene transfer has also been observed by Wang et al. (2014) using *P. fluorescens* TP13, a strain which has C230 gene (catechol 2,3-dioxygenase) carrying TOL plasmid (involved in expression of degradation pathway for xylene and toluene). When this strain was inoculated in the rhizosphere of tomato farmland contaminated with hydroxybenzene (phenol), it increased the number of bacteria carrying the same gene after its colonization and reduced the toxicity of phenol. The study proved that transfer of phenol-degrading gene took place among bacteria residing in the rhizosphere of tomato and helped in lowering the phenol contamination (Wang et al. 2014). In this way, the above mentioned reports reveal that the use of rhizoremediation technology is a successful method of reclamation of PAH contamination, and further studies on plant-microbe interactions would give far better results for mitigating this kind of soil pollution.

2.2.3 Pesticide Contamination

Pesticides are categorized as substances or a group of substances used to control pests and include insecticides, herbicides, or fungicides (Hernandez et al. 2013). Agricultural practices and industries producing them are their major sources, and these cause pollution of soil, water, and air. According to the reports, less than 5% of these compounds are able to reach the targeted organisms and rest of them get accumulated in soil (Pimental and Leviton 1986). The Stockholm Convention on Persistent Organic Pollutants (2011) (POPs) stated that out of 12 organic chemicals which persist in environment, 9 are organic pesticides. Some of them are organochlorine, organophosphate, organometallic, pyrethroids, and carbamates (Gilden et al. 2010). As per the reports of FAO (2017), increase of about 11% per year in pesticide usage has been estimated escalating the amount from 0.2 million tons in the 1950s to 5 million tons in 2000. The pollution caused by such persistent compounds leads to dangerous outcomes to living beings including plants, microbial species and humans (Arora et al. 2010, 2018; Mishra et al. 2015). Pesticides get adsorbed in soil and form soil colloids through compartmentalization without changing its original structure. The deposited toxic chemicals ultimately enter the food chain through bioaccumulation in plants and cause serious health problems in humans which could also be carcinogenic in nature (Jayaraj et al. 2017). Therefore, release of such hazardous compounds is one of the major environmental issues which needs to be mitigated in earliest possible ways and hence has spurred the development of an environment-friendly reclamation approach, such as rhizoremediation (Fulekar 2014). Rhizospheric bacteria including species of *Pseudomonas*, *Bacillus*, *Burkholderia*, *Alcaligenes*, *Acinetobacter*, *Serratia*, *Streptococcus*, *Stenotrophomonas*, *Rhizobium*, and fungi such as white rot (basidiomycetes), mycorrhizae, *Phanerochaete chrysosporium,* and *Ganoderma australe* are some common examples of microbial species which take part in the pesticide degradation (Velázquez-Fernández et al. 2012). A successful degradation was achieved in case of 2,4-dichlorophenoxyacetic (2,4-D) using rhizoremediation technique in pea (*Pisum sativum*) and its endophytic bacteria that stimulated its degradation (Germaine et al. 2006). *Ganoderma australe,* a lignolytic fungus, isolated from stone pine (*Pinus pinea*) is another good example for the degradation of pesticide lindane (Rigas et al. 2007). In a study conducted by Yadav and Krishna (2015), a PGPR strain RB1 showed high tolerance to an organophosphate pesticide methyl parathion at 500 ppm and proved to utilize it as a nitrogen and carbon source and was reported to enhance the growth of mung bean (*Vigna radiata*) simultaneously. Degradation of pesticides like chlorpyrifos, cypermethrin, and fenvalerate was brought about at different concentrations using *Pennisetum pedicellatum* (desho grass) and its rhizospheric microbes in a study conducted by Fulekar (2014). The associated microorganisms which were the potential degraders were identified as *Stenotrophomonas maltophilia* MHF ENV 20, *S. maltophilia* MHFENV 22, and *Sphingobacterium thalpophilum* MHF ENV 23 for chlorpyrifos, cypermethrin, and fenvalerate, respectively. *S. maltophilia* MHF ENV 22 alone is capable of degrading cypermethrin up to 58% in 192 h at concentration of 100 mg kg^{-1} in the rhizosphere of *Pennisetum pedicellatum* (Dubey and Fulekar 2012). A study conducted by Pai et al. (2001) showed that degradation of Mefenoxam was achieved by *P. fluorescens* and *Chryseobacterium indologenes* in the rhizosphere of *Zinnia angustifolia* (Tropic snow), and a major part of it got converted into its primary degradation product, i.e., N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine, a free acid. Yu et al. (2003) demonstrated that degradation of butachlor was efficiently carried out in wheat rhizosphere by externally inoculating microbes capable of degrading target pesticide than in uninoculated rhizosphere. Fungi are reported to degrade these xenobiotic compounds to CO_2 and H_2O with the help of lignolytic and oxidative enzyme system (Fu and Viraraghavan 2001). *Coprinellus radians*, a fungus, was found to degrade dibenzofuran in a study by Aranda et al. (2010).

The degradation is brought about by biotransformation of such compounds in rhizospheric region in similar way as in the case of other xenobiotic compounds described above and results into complete transformation of toxic products into non-toxic inorganic forms (Eerd et al. 2003). The three major enzymes that are involved in biotransformation of pesticides are cytochrome P450, esterases, and glutathione S-transferases (Ortiz- Hernández et al. 2013). These enzymes are found in plants, bacteria as well as in fungi and help in synthesizing growth regulators and other secondary metabolites in them to reduce the stress caused by pollution (Eerd et al. 2003). Cyanobacteria such as *Synechococcus elongatus*, *Anacystis nidulans,* and *Microcystis aeruginosa* have also been reported in degradation of pesticides, e.g., organophosphates and organochlorine insecticides, and in turn provide vitamins, enzymes and hormones to the plant in order to enhance its growth (Meghraj et al. 1994; Rajkumar et al. 2012). Moreover, the lateral exchange of foreign genetic material in the rhizosphere could be another means for the successful accomplishment of rhizodegradation of pesticides (Miyazaki et al. 2006; Devers et al. 2007; Zhang et al. 2012), for example, transfer of *lin* genes (responsible for hydrocarbon degradation) from *Sphingomonas* isolated from a remote area capable of degrading hydrocarbon was identified (Böltner et al. 2007). Transfer of genes *tfdA* (responsible for degradation of 2,4-D) from respective bacteria to phenol degrading bacteria induced and enhanced the degradation of phenoxyacetic acid by the latter ones (Lipthay et al. 2001; Nielsen et al. 2017). Rhizobia are known to tolerate pesticide stress by synthesizing certain metabolites. *Mesorhizobium* sp. strain MRC4 was identified to progressively increase the amount of EPS production with increment in amount of pesticides (Ahemad and Khan 2012). The probable mechanism of EPS in pesticide contamination is to protect the bacteria from desiccation and shield them from the harmful effects of the stress caused (Tank and Saraf 2003). These findings depict that rhizoremediation could be envisaged as a gateway toward remediation of pesticides.

2.2.4 Other Pollutants

Other than the aforementioned compounds, there are various other pollutants responsible for soil contamination which pose adverse effects on the quality and productivity of soil. In this context, chlorophenols, dibenzofurans, dioxins, and polychlorinated biphenyls (PCBs) are among potent pollutants which are carcinogenic in nature with recalcitrant and bioaccumulating properties (Godheja et al. 2016). These pollutants also come in the category of persistent organic pollutants (POPs). They enter in the environment in the same manner like any other pollutants, have recalcitrant structure which permits them to reside in soil for a prolonged period of time and get bioaccumulated in the food chain causing deleterious changes in the environment (Vergani et al. 2017). The remediation of such POPs has also been reported to occur by the synergistic mechanism of plants and their associated rhizobacteria, and by ectomycorrhizal fungi (Meharg and Cairney 2000; Chaudhry et al. 2005; Afzal et al. 2014). Rhizospheric microbes carry out the degradation process by two major mechanisms (especially in case of PCBs and dioxins degradation): (i) anaerobic reductive dechlorination and (ii) aerobic oxidative degradation of biphenyl ring. Anaerobic reductive dechlorination takes places in anaerobic environment like rivers, sediments, and paddy fields. In this process, chlorine is replaced at *ortho* and *meta* positions by hydrogen atom by halorespiring bacteria (e.g., *Dehalococcoides*), and the product is converted to lesser toxic form which is further degraded by aerobic microbes. In second mechanism, i.e., aerobic oxidative degradation of biphenyl ring occurs by the expression of *bph* gene clusters in aerobic bacteria which induces catabolic enzymes for the degradation process. *Burkholderia xenovorans* LB400, a rhizospheric microbe, is among the most studied bacterial species under this mechanism. Similarly, elimination of dioxins was reported by *Comamonas* sp. strain KD7 associated with white clover rhizosphere (Wang and Oyaizu 2011).

Potential toxicity of azo dyes in soils is another environmental concern which requires degradationand reclamation as their persistence causes several mutagenic effects in living beings (Saratale et al. 2011; Khandare and Govindwar 2015). Mitigation and restoration of such contaminated soil have been reported in a study conducted by Sinha et al. (2019) in which a PGPR strain *Klebsiella* sp. VITAJ23 was observed to degrade a reactive green dye by up to 79% and enhance plant growth of *Alternanthera philoxeroides.* Similarly, in another study by Shafqat et al. (2017), out of seven isolates, three rhizospheric bacteria, namely, *Achromobacter xylosoxidans, Burkholderia ginsengisoli,* and *Pseudomonas alcaligenes*, showed significant removal of azo dyes and increased plant biomass as compared to control.

Introduction of explosives into the environment has also caused some serious issues like contamination of land and other ecosystems posing serious threat. One of the examples of such explosives is 2,4,6-trinitrotoluene (TNT) which has been causing severe soil pollution in various military areas around the world and needs appropriate reclamation strategies which are cost-effective and ecofriendly (Mulla et al. 2013). Thijs et al. (2018), in their study, developed a 12-member rhizobacterial consortium having PGP characters and TNT detoxifying ability which significantly enhanced growth of maple (*Acer pseudoplatanus*) in comparison with uninoculated plant. A methylotrophic endophyte isolated from a hybrid poplar tree showed efficient degradation of explosives, namely, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), by up to 60% in 2 months suggesting their potential to remediate these explosives from soil (Van Aken et al. 2004a, b).

Mineral oil contamination due to accidental oil spills is another environmental hazard and a common soil contaminant at global level (Atlas and Hazen 2011; Lacalle et al. 2018). Mineral oils come in contact with soil surface, form a thick layer over the top, and make the nutrients present in the soil less available to plants and microbes rendering the soil infertile (Meghraj et al. 2011; Arora 2018). Successful remediation of several oil spillage sites has been achieved in recent past using rhizoremdiation. Endophytic strains, *Pseudomonas* ITRI53 and *Rhodococcus* ITRH43, showed better colonization in *Lolium multiflorum* (Italian ryegrass) when percentage of diesel contamination was increased from 1% to 2%. Moreover, expression of gene alkane monoxygenase *alkB* (help in degradation of hydrocarbons) was also found to be higher in high diesel-contaminated soil depicting an increased level of degradation in such habitats (Andria et al. 2009). Similarly, Arslan et al. (2014) in their study were able to degrade 85% of total petroleum hydrocarbons in Italian ryegrass (*L. multiflorum*) rhizosphere, inoculated with endophytes and amended with nutrients. Likewise, a PGPR inoculum was reported to reduce aliphatic hydrocarbon contamination by 50% in rhizosphere of *Festuca arundinacea* (Hou et al. 2015).

Contamination of salts in soil due to intensive irrigation practices in agriculture has become another menace degrading the physical and chemical composition of soil and causing huge loss to agriculture and ecosystem services (Qadir et al. 2014). Soil liquid phase requires a specific concentration of salts exceeding which may be injurious to the well-being of soil microbiota and hence to the plants. Irrigation waters can contain high levels of Ca, Mg, and Na ions which get accumulated in soil leading to soil salinization (Hanin et al. 2016). Approximately 20% of the world's irrigated land is facing the problem of soil salinity which is nearly equal to the area of France, i.e., 62 million hectares (UNU-INWEH 2014). Microbial tolerance to high concentration of salts is a well-known phenomenon and could be exploited using rhizoremediation technique (Arora et al. 2012; Tewari and Arora 2016). In a study conducted by Chang et al. (2014), rhizospheric *Pseudomonas* strains UW3 and UW4 increased oat biomass and almost completely alleviated inhibition caused by salinity. Khare et al. (2011) demonstrated the PGP activity (IAA, siderophore, and pyocyanin production) of a fluorescent *Pseudomonas* strain EKi under saline conditions and observed that the strain was able to tolerate NaCl up to 1550 mM concentration and also showed biocontrol activity against *Macrophomina phaseolina*, a phytopathogen by up to 76% under 400 mM salt concentration, as well as showed significant growth promotion of chickpea. Such a PGPR strains could prove beneficial to associated plants in reducing the incidence of diseases caused by phytopathogens during salinity stress and will also help in enhancement of plant growth lowering the impact of salt stress simultaneously resulting in soil reclamation.

2.3 Rhizoremediation for Enhancing the Productivity of Soil

Impairment in the fertility of soil due to above mentioned contaminants caused by anthropogenic activities creates a huge pressure on the agricultural production due to continuous growing demand of the ever-increasing population. Rhizoremediation not only ensures environmental safety, causing no harmful effects, but also increases the nutrient supply in soil by making use of biogeochemical processes and more importantly adds to the biodiversity and organic matter (Bello-Akinosho et al. 2016). There are several studies conducted by various group of scientists which give evidences of beneficial outcome of rhizospheric microbes residing in contaminated soils and how they tend to increase the growth and yield of plants as well as improve health of soil and its associated microbiota (Ma et al. 2016) (Table 2.1). Nutrient supply especially for elements like N and P in a degraded soil area through an improved soil management technique is an important parameter. In this regard, mycorrhizal fungi play a crucial role for the nutrient-deficient soil by colonizing the root cortex and developing mycelia with extra matrices to reach out to better water supply and nutrition (Azcón-Aguilar and Barea 1997; Hrynkiewicz and Baum 2011; Deguchi et al. 2012). Some AMF are reported to form beneficial interactions with other soil microbes to improve soil quality and fertility by aggregation of soil and are also reported to maintain hydrophobicity in soil particles by producing glomalin, a glycoprotein (Barea et al. 2002). An endophytic fungus, *Lewia* sp. associated in the rhizosphere of *Festuca arundinacea* found in the stressed soil is an example in this context (Cruz-Hernández et al. 2013). Similarly, there are beneficial rhizobacteria which also help in improvement of soil quality and increase biomass production in stressed environment. These bacteria are known to increase availability of nutrients by mobilizing them from organic matter through enzymatic actions and

Table 2.1 Rhizoremediation of soil contaminants by PGPR and their effect on productivity of crops

S. no..	Plants	Rhizospheric microbes	Pollutants	Results	References
1.	*Populus deltoides*	Endophytic *Bacillus* sp. SBER3	Anthracene and naphthalene (PAHs)	Showed significant increase in root and shoot length including plant biomass	Bisht et al. (2014)
				Degradation of PAH by 45.6% was identified in 12 days	
2.	*Festuca arundinacea*	*Bacillus licheniformis, Bacillus mojavensis*	PAH	Higher percentage of seed germination. High root and shoot biomass	Eskandary et al. (2017)
				Concentration of (Naphthalene, Phenanthrene, Benzo[a] anthracene and Dibenzo[a,h] anthracene) showed significant reduction in the rhizosphere	
3.	*Vigna unguiculata* (L.) Walp	*Achromobacter xylosoxidans* (JCp4) and *Ochrobactrum* sp. (FCp1)	Pesticide – chlorpyrifos	Increase in height, weight and leaf length of plant was observed	Akbar et al. (2015)
				93–100% reduction in chlorpyrifos within 42 days	
4.	*Sorghum bicolor* cultivar: CSH – 16	PGPM consortia of (PGPM2 a diazotrophic bacterium, PGPM9 a fluorescent Pseudomonad) and one fungal species (T103 a biocontrol fungus)	Organophosphate pesticides malathion and methyl parathion (MP)	More than twofold increase in root length and growth parameters were reported on consortium inoculation	Mishra and Sundari (2015)
				28% increase was identified in case of inoculation of consortia in contaminated soil compared to uninoculated ones	

(continued)

Table 2.1 (continued)

S. no..	Plants	Rhizospheric microbes	Pollutants	Results	References
5.	*Brassica napus*	*P. fluorescence* G10, *Microbacterium* sp. G16 (EN)	Lead	Increment in root length, shoot length, and dry weight Increased uptake of Pb in shoots	Sheng et al. (2008)
6.	*Pisum sativum*	*Microbacterium*sp., *Rhizobium* sp.	Chromium (VI)	Enhancement in nitrogen concentration by 54% in plants Reduction in Cr toxicity Increased rate of phytoremediation	Soni et al. (2014)
7.	*Medicago sativa*	*P. aeruginosa*	Petroleum hydrocarbon and heavy metal contamination	56% root and 105% shoot growth was observed Decrease in metal concentration. 68% removal of PAH	Agnello et al. (2016)
8.	*T. aestivum* and *Arachis hypogaea*	*Alcaligenes faecalis* RZS2 and *P. aeruginosa* RZS3	Heavy metal ions ($MnCl_2.4H_2O$, $NiCl_2.6H_2O$, $CoCl_2$, $CuCl_2$, and $ZnCl_2$	Significant increment in number leaves, shoot length, and overall plant growth were observed in both wheat and peanut Effective heavy metal remediation were reported as compared to chemical ion chelators like EDTA and citric acid	Patel et al. (2016)
9.	*Robinia pseudoacacia*	AMF	Pb	Total increment of plant biomass Pb uptake in root and shoot confirming the translocation of metal from soil	Yang et al. (2016)

(continued)

Table 2.1 (continued)

S. no..	Plants	Rhizospheric microbes	Pollutants	Results	References
10.	*T. aestivum*	Mycorrhiza *Funneliformis geosporum*	Zn	Enhanced fresh and dry weight of *T. aestivum*, improved photosynthesis	Abu-Elsaoud et al. (2017)
11.	*Prosopis juliflora* L.	*Rhizobium* strain	Fly ash and heavy metals	Increased plant biomass, photosynthetic pigment, and nitrate reductase activity Translocation of metals in above grounds parts were reported	Rai et al. (2004)
12.	*Sedum alfredii*	*Pseudomonas sp.* DDT-1	DDT and its metabolite and Cd	Enhanced root biomass of the plant Reduction in levels of DDT and its metabolites by 53.6% and of Cd by 31.1% were noticed	Zhu et al. (2012)
13.	*Brassica napus*	*Pantoea* sp. FC 1	Phenol and Cr	Promoted plant growth Reduced Cr (IV) to Cr (III) Phenol was used as sole carbon source by the bacterium	Ontañon et al. (2014)
14.	*Lotus corniculatus L.* and *Oenothera biennis* L.	26 endophytes belonging to genera *Rhizobium*, *Pseudomonas*, *Stenotrophomonas*, and *Rhodococcus*	Hydrocarbon	Plant growth promotion Effective root colonization by bacteria Significant degradation activity of petroleum was observed by the bacterial isolates	Pawlik et al. (2017)
15.	*Trifolium repens* and *Lolium perenne*	AMF *Glomus mosseae*	PAH	AMF inoculation enhanced root, and shoot and dry weight Natural attenuation of organic pollutants was observed	Joner and Levyal (2001)

siderophore production, regulate and modify stomatal opening of plants, contribute to the necessary vitamins and help in adaptation of root morphology (Bauer et al. 2013; Vacheron et al. 2013). *Acinetobacter, Arthrobacter, Rhizobium, Bacillus*, and *Pseudomonas* are among the best rhizoremediation candidates which are reported to show PGP characters in contaminated soils in association with suitable plant species (Xun et al. 2015). *Rhizobium leguminosarum bv. trifolii* was able to fix nitrogen efficiently in metal-contaminated soil, thereby increasing plant biomass (Nonnoi et al. 2012). Such rhizobacteria are also known to produce extracellular degrading enzymes which help in increasing decomposition of organic matter and also in soil aggregation (Johansen and Binnerup 2002). Some phosphate solubilizing bacteria help in release of phosphate ions by production of organic acids in soils with low phosphate levels (Vessey 2003). Similarly, deficiencies of Ca and Mg are also overcome by colonization of mycorrhizal fungi. Some fungi and bacteria are also well known in balancing pH of disturbed soils by decreasing toxicities of metals like Al and Hg which are known to disturb pH of the soil. Some other rhizospheric bacteria are reported to produce 2,4-diacetylphloroglucinol (DAPG), an antimicrobial metabolite in order to maintain balance of the stressed soils (Mendes et al. 2013). DAPG-producing *P. fluorescens* strain associated to corn rhizosphere was reported to reduce leaf lesions in a low pH soil caused due to the abiotic stress. The study indicated that treatment of plant with DAPG in contaminated soil can minimize or remove the negative effects caused by low pH in soil (Raudales et al. 2009). *Cellulosimicrobium funkei* isolated from rhizosphere of *Phaseolus vulgaris* has been reported to produce IAA, EPS, siderophores, biosurfactants and showed phosphate solubilization under Cr stress and improved root elongation of the plant (Karthik and Arulselvi 2017). On the other hand, there are PGPR which have been reported to increase quality of saline soils by enhancing productivity of crops grown in such stressed environment. In a study conducted by Tewari and Arora (2014; 2018), a PGPR strain of *Pseudomonas* PF23 showed significant enhancement in the growth of sunflower plant grown in a semiarid region with high salinity and showed potent biocontrol activity against the endemic phytopathogen, *M. phaseolina.* The study showed how repeated use of this useful microbe on such barren lands could alter its negative impacts making soil healthy and productive. Efficient strains of microbial consortia are also being extensively used to increase efficacy, reliability, and consistency of rhizoremediation technique under various environmental stresses (Stockwell et al. 2011). Use of consortia of rhizobacteria with AMF is now being exploited for enhanced cleanup of heavy metals and to increase solubility of other contaminants in soils (Khan 2014). They also have an advantage of enhanced biocontrol activity by increasing competition among other microbes residing in contaminated soil (Isaac et al. 2015). Earlier studies have shown use of microbes such as *Rhizobium*, *Bacillus, Pseudomonas, Trichoderma, Glomus* as excellent candidates for development of consortia (Rajasekhar et al. 2016). Studies have shown that microbial consortia have significantly increased growth of crops in stressed soils thereby enhancing their productivity and maximum degradation of pesticides have been achieved when seeds were treated with consortia (Gangola 2014, 2015). The co-inoculation of a PGPR, *Paenibacillus mucilaginosus*, and a rhizobial isolate

Sinorhizobium meliloti was reported to alleviate metal toxicity and also increased NPK content in tissues of alfalfa. Moreover, the co-inoculation of the PGPR and rhizobia was found to show increment in the soil organic matter, plant biomass, and nutrient content in soil suggesting the potential of consortia in stressed environment (Ju et al. 2019).

These studies depict that these microbes along with their compatible hosts, i.e., plants, are the effective candidates which are well adapted to adverse soil conditions, thereby improving quality of soils and increasing productivity by enhancing plant growth.

2.4 Future Prospects

It is clear that rhizoremediation is one of the most promising methods for the eco-restoration of contaminated sites but further research and trials at bigger levels are also required. To avoid any inconsistent performance during field applications, it has become an indispensable need to tend toward future avenues, elucidate mechanisms, metabolites/pathways/genes using latest omic technologies. Recent advances in area of biotechnology have enhanced our knowledge in efficient remediation of such pollutants through various mechanisms involving plants and microbes (Malla et al. 2018). Metabolomics, transcriptomics, proteomics, and genomics can be used to understand the biochemical and molecular aspects of agroecosystems resulting in leads for a reliable technology. These techniques can help in determining novel metabolites and pathways involved in degradation of pollutants through plant-microbe interactions. These techniques will also give better insights about structural and functional aspects of the microbial population and can be used to predict their linkages to various ecological processes (Aguiar-Pulido et al. 2016). Next generation sequencing (NGS) is another method which could be used in the mitigation of contaminants by increasing efficiency of bioremediation processes by piecing out desired microbial profile and other required information produced from databases in form of short reads. In this high-throughput technique, editing of genes is carried out which is cost-effective and is precise (Lowder et al. 2015). CRISPR-CAS9 genome editing is one such example in this context (Schiml and Puchta 2016; Arora et al. 2018). Transposon-mediated ISMoB technique would also be helpful for remediation purpose in which microbes with resistant transposons are transferred in contaminated sites which further donates them to others through horizontal gene transfer (Garbisu et al. 2017). A new method of genetic engineering in which regulation of gene expression is achieved using siRNAs, ribozymes, and riboswitches is evolving in which cellular processes of microbes such as combating biotic and abiotic stresses could be controlled and degradation of contaminants can be accomplished by modifying the gene of interest in microbes hence making rhizoremediation method more efficient (Basu et al. 2018). Another breakthrough method is the study of hologenome of the microbiome of plant and its rhizospheric microbes which could be used in manipulation of niches of microbial community and hence tolerance against several environmental issues including soil contamination (Mueller and Sachs 2015). Tailoring these microbes in order to increase the downstream processing of metabolites could also enhance the process of bioremediation. A multidimensional and skilled approach is further needed to study the relationship between plant-microbe interactions to make better use of contaminated lands (Verma et al. 2019). Also preservation of degrading genes which help in rhizoremediation method should be done in order to make a reservoir of novel genes which are better adapted to the residential environmental conditions (Thijs et al. 2016). Another technique termed as nanoremediation has come up in which nanoparticles are integrated with microbial cells to carry out remediation process more efficiently at sites with high metal concentrations (Mukherjee et al. 2017). Moreover, well designed and large-scale field trials are required in order to evaluate the feasibility of latest techniques and methods (Etesami 2018). Last but not the least, participation of consumers, researchers, industrial sectors, and other responsible authorities through communication channels and debates should be opened to discuss the importance of these organic methods (Prasad et al. 2017). This is possible when there is spread of knowledge regarding our dependence on nature and how its conservation is the need of the hour (Gomeiro 2016). This would open new routes of economic development and human nutrition worldwide. In this way, these solutions should be exploited in the future which would in turn help us in paving way towards a better system which promises to maintain quality of our pristine environment.

2.5 Conclusion

In the above sections, we discussed about a green technology, i.e., rhizoremediation, and how, it has proved itself as a better method of soil management compared to conventional methods of remediation. Various contaminants and their rhizoremediation has now been done successfully in order to minimize or nullify these harmful pollutants, thereby enhancing the quality of soil and increase its productivity. However, nature harbors huge microbial diversity, and a lot has to be discovered and exploited about the plant-microbe interactions, which is largely unexplored. Unraveling these interactions and accordingly manipulating the efficacy of rhizoremediation method would further help this sustainable technology to achieve its due success and reliability.

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Chapter 3
Phycoremediation of Pollutants for Ecosystem Restitution

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Abstract Due to rapid industrialization and urbanization, huge amount of production as well as accumulation of toxic and organic contaminants can lead to serious environmental problems. Unwanted buildup of untreated effluents and their discharge into water bodies negatively affects the stability and wholesomeness of natural aquatic ecosystems and may also become a reason for causing adversarial health effects to humans and the environment. To overcome this problem, many conventional approaches are in use, such as physical, chemical, reverse osmosis, electrodialysis, ultrafiltration process, ion exchange, and precipitation using chemicals, but these methods have their own limitations. To overcome these limitations, researchers diverted their attention towards biological means, that is, application of bacterial, fungal genera, plants and algae to eradicate, biodegrade, or render unhazardous inorganic and organic pollutants in water bodies. The purpose of using algae in wastewater treatment is that algae uses the waste as carbon source and energy, generating oxygen, and some species also accumulate lipids in their cell wall. These lipid molecules are then removed from the algal cell and are used as biofuel. Nowadays, several algal genera play a significant function in biomonitoring and controlling the organic contaminants in aquatic environment. Since long, all over the globe, people have employed and studied in detail the role of bacteria alone or in association with plants in pollution regulation. On the other hand, the relevance and function of varied algal genera in controlling and restoration of organic and inorganic contaminated aquatic ecosystem are also in practice.

Keywords Algae · Bioremediation · Ecosystem · Pollutants

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3.1 Introduction

In bioremediation process, the biotechnological potential of algal research is crucial and is in routine owing to its advantages as compared to other microbes. Vital roles are played by the members of the algal group in biodegradation and bioremediation of complex substrates (municipal rejections, effluents, ashes, etc.) (Aarti et al. 2008) and chloride in wastewater (Ramíreza et al. 2018). Persistent organic pollutants (POPs) can be defined as organic compounds which are non-susceptible to environmental degradation using biological, chemical, and photolytic processes. These are one of the probable sources of non-susceptible (resistant) chemicals in aquatic systems which require instant rectification for a feasible degradation. Because of their tenacious nature, POPs occur in aquatic bodies with some silent as well as detrimental impacts on natural environment as also on the human and animal health (Chekroun et al. 2018). The gradual buildup of these inorganic and organic chemicals in the natural environment causes long-term risk to the health and safety of human beings as well as to that of aquatic creatures (Pavlostathis et al. 2001; Nanda et al. 2010; Abdel- Raouf et al. 2012).

The xenobiotic contaminants which mainly are generated from several industrial sources, flow continuously or are discharged in the aquatic systems without any pretreatment (Zhang et al. 2008). This chapter tries to put an attempt to explain the intended possibilities to bioremediate the pollutants by using algal species (Martínez- Jerónimo et al. 2008; Ahmad et al. 2013). The organic and inorganic chemical pollutants become a part of hydrogeochemical cycle and are absorbed or uptaken by the benthic organisms, via an interface of sediment and water (Akhtar et al. 2004; Perelo 2010).

Reportedly, the intoxicating hydrocarbons are quite hazardous and unsafe when present in active form especially when transferring from one trophic level to another. Moreover, several compounds are found to be carcinogenic in nature such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (IARC 1983). Predominantly, POPs have been produced intentionally by the industries on a large scale to fulfill the demands of several sectors, which rely on such products. These products include pesticides, PCBs, polychlorinated naphthalenes (PCNs), which are largely employed in agricultural sector (Priyadarshani et al. 2011).

On the other hand, there are some products which are produced un-intentionally or they are by-products, which are discharged at the end of a major organic product formation. Such by-products are polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and PAHs. The research community has been working to elucidate proper mechanisms for complete removal of the POPs from the environment. Three-tier assessment procedures have been laid by several research institutions which suggested the establishment of POP convention. According to the Stockholm Convention, database on POPs and major action was taken against a dozen of POPs, namely, Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Mirex, Toxaphene, PCBs, PCDDs, and PCDFs.

Table 3.1 Comparative lists of POPs selected for environmental and toxicological studies (Adapted from EUGRIS; http://www.eugris.info)

POPs selected at the Stockholm Convention (2001)	Organic pollutants (or proposed POPs) with an assigned TEF^{a} or REP^{b}	Emerging POPs
Aldrin	Polychlorinated biphenyls or (PCBs)	PBDEs
Chlordane	Polychlorinated dibenzodioxins PCDDs (or dioxins) / Polychlorinated dibenzofurans or (PCDFs)	PBDDs/ PBDFs
DDT (p,p-dichlorodiphenyltrichloroethane)	Polychlorinated naphthalenes or (PCNs)	PBBs
Dieldrin	Polybrominated diphenyl ethers or (PBDEs)	
Endrin	PBDDs/PBDFs	
Heptachlor	Polybrominated biphenyls or (PBBs)	
Hexachlorobenzene	Polyaromatic hydrocarbons or (PAHs)	
Mirex		
Toxaphene		
PCBs		
PCDDs/PCDFs		

^{ab}Toxic equivalent factor; Relative potency

There are several classes of organic pesticides such as organochlorine, organometallic, organophosphate, carbamates, and pyrethroids, which are potential violators of environmental issues (Gilden et al. 2010, Stockholm convention 2011). Table 3.1 shows the Stockholm Convention database on POPs; it was mentioned in published report that nine out of twenty one persistent organic chemical compounds are pesticides.

Nowadays, there has been an increasing keenness and interest in employing bioremediation strategies, which are known to be one of the most emerging and preferable techniques employed by plants (alive) as a model system. The commonly used term "phytoremediation" can be explained as the exploitation of green plants to eradicate, absorb, and contain toxic environmental pollutants from soil and water (Cunningham and Berti 1993). Several procedures for phytoremediation of polluted soils, lakes, and rivers have been undertaken and carried out successfully (Brooks 1998; Chaney et al. 1997; McIntyre and Lewis 1997; Flathman and Lanza 1998; Salt et al. 1998). With the development in this new period, various artificial (synthetic) products are being used in our day-to-day life that generate more confusing studies in name of xenobiotics (Kumar et al. 2015). Furthermore, the analysis on organic xenobiotics reveals that their worldwide application in agricultural regions has become one of the serious issues in aquatic ecosystems. To overcome this problem, bioremediation of these toxic pollutants using green algae can be of significant importance from environmental point of view (Jin et al. 2012). According to studies, algae proved to be beneficial and advantageous in hyperaccumulation of heavy metals as well as in degradation of xenobiotics (Azab 2002; Prna- Castro et al. 2004; Suresh and Ravishankar 2004).

Scientific development in recent tools and techniques, micro-algae have potentially become a “green- tool” in bioremediation of colored wastewater because of their pivotal role that is, fixation of carbon dioxide and oxygen generation. Moreover, the biomass of algae produced has huge capacity (as feedstock) for production of biofuel (Huang et al. 2010; Kshirsagar 2013, 2014; Tahir et al. 2018). Such bioremediation capabilities of micro-algae are helpful for sustainability of environment as well as for the sewage water treatment (Ellis et al. 2012, Lim et al. 2010; Sengar et al. 2011; Sharma and Khan 2013; Azarpira et al. 2014). Figure 3.1 shows the energy (biofuel) production by algal cells and Figs. 3.2, 3.3, 3.4 depict the structures of different persistent pollutants.

All over the globe, contaminated regions have been kept for “radio detection and ranging” (RADAR) to check its state in ecological pyramid in which trees, microorganisms, herbs, and grasses have been identified and accepted for native freshness. Therefore, in order to comprehend the complex mechanisms of remediation of wastewaters, a model system is needed using potential algal genera. Furthermore, an algal model system provides an opportunity in various compositions in which potential algal genera are being utilized commercially to treat wastewater and to fix atmospheric carbon dioxide (Watanabe 1997) and model system has swiftly provided a growing market potential (Flathman and Lanza 1998). On the other hand, the term “phyto-remediation” (treatment using plants) is still in fashion in the European Union and USA, where naturally growing plants are being used for treating the wastewater or sludge, and until 2007, the USA funded around $300 million in such projects (Campos et al. 2008). For the very first time, not very long ago, marine micro-algal species have been employed in the restitution of sediments which were highly contaminated with chemicals (Yamamoto et al. 2008). In essence,

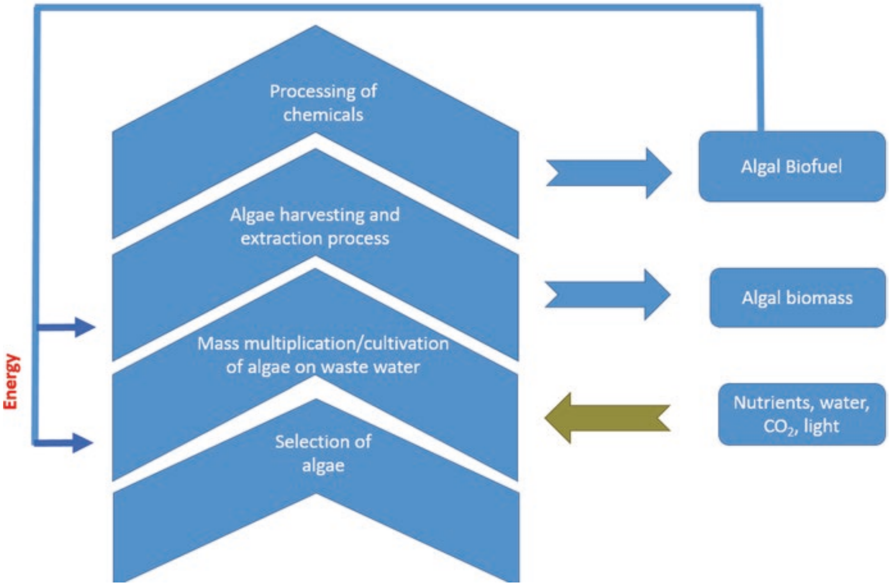


Fig. 3.1 Different stages of algal multiplication and biofuel (energy) production

Fig. 3.2 Polychlorinated biphenyls

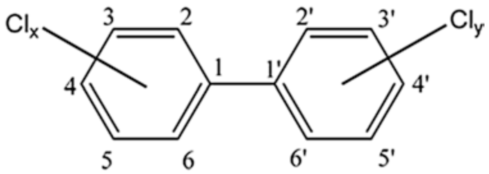


Fig. 3.3 Structure of organochlorine

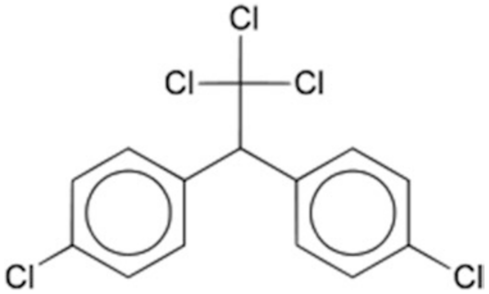
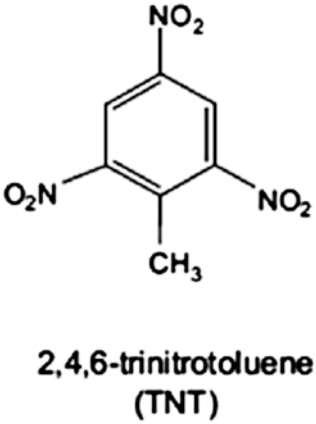


Fig. 3.4 Structure of trinitrotoluene (TNT)



this chapter focuses on understanding the role of some common algal genera for biological remediation of inorganic and organic pollutants.

3.2 Bioremediation of Heavy Metals

Many algal species have been reported to play a significant role in removal of heavy metals, such as arsenic, cadmium, nickel, molybdenum, copper, uranium, cobalt, lead, etc. from the terrestrial and aquatic ecosystems (Mane and Bhosle 2012; Zeraatkar et al. 2016). These algal species employ various means to uptake or remove these metals from the environment (Abbas et al. 2014). Table 3.2 shows the commonly used algal genera in removing the heavy metals from environment.

Table 3.2 Commonly employed algal spp. in metal removal

S. No	Algae	Function	Reference
1	*Chlorella* and *Scenedesmus* spp.	Metal removal	Brinza et al. (2007)
2	*Chlamydomonas reinhardtii, Chlorella salina, Chlorella sorokiniana*	As, Ni, Cd, Mo removal	Macfie and Welbourn (2000)
3	*Chlorella, Scenedesmus* and *P. tricornutum*	Cd, U and Cu removal	Perales-Velaetal et al. (2006)
4	*Porphyridium purpureum, Scenedesmus abundans*	Pb, Cd, Cr, Cu removal	Brinza et al. (2007)
5	*Tetraselmis suecica*	Cd removal	Pérez Rama et al. (2002)
6	*Chlorella vulgaris*	Cd(II) removal	Khan et al. (2008)
7	*Scenedesmus* spp., *Tetraselmis* spp.and *Chlorella* spp.	Cd removal	Sbihi et al. (2012)
8	*Spirogyra* spp., *Oscillatoria angustissima*	Co, Cd removal	Romera et al. (2007)
9	*Scenedesmus obliquus*	Zn removal	Monteiro et al. (2011)
10	*Spirulina* spp	Zn removal	Chojnacka et al. (2004)
	Spirulina spp	Cr, Cd and Cu removal	Chojnacka et al. (2005)
11	*Spirogyra hyaline*	Pb, Hg removal	Kumar and Oommen (2012)
12	*Oscillatoria laete-virens*	Pb removal	Miranda et al. (2012)
13	*Arthrospira platensis*	Pb removal	Ferreira et al. (2011)
14	*Spirogyra insignis*	Ni removal	Romera et al. (2007)
15	*Chlorella vulgaris*	Ni removal	Aksu and Dönmez (2006)
16	*Chlamydomonas reinhardtii*	Hg, Cr removal	Tüzün et al. (2005); Ibuot et al. 2017
17	*Asterionella Formosa*	Cu removal	Tien et al. (2005)
18	Spirogyra neglecta	Ni, Zn removal	Singh et al. (2007)
19	*Synechocystis* spp.	Pb removal	Tiantian et al. (2011)
20	*Closterium lunula*	Cu uptake	Yan and Pan (2002)
21	*Chlorella miniata, Clorella vulgaris*	Ni uptake	Wong et al. (2000)

3.3 Bioremediation of Petroleum Hydrocarbons

In emergent nations like India, exploration and production of "petroleum hydrocarbons" have been amplified by many folds, which eventually leads to the contamination at production sites, and some of the raw hydrocarbon materials do drift into aquatic streams; this situation occurs especially at offshore exploration sites. The inconsistent, unequal, and haphazard industrial dispersion leads to accumulation of such persistent hydrocarbons in the ecosystem that could trigger the instability of aquatic flora and fauna and further leads to substantial health impact on humans as well as on animals. Scientists are making consistent efforts to lay certain guidelines and checks so that such leakage or discharge of petroleum hydrocarbons may be reduced to stop entering the aquatic bodies. Still after taking all care and precautionary measurements, approximately 600,000 metric tons is the hydrocarbon flow per year with a startling amount of 200,000–2,000,000 metric tons leaked per year into the environment (Kvenvolden and Cooper 2003).

Bioremediation approach using live organisms offers a promising technology to aerobically treat shorelines that are contaminated by oils (Atlas 1991; Rosenberg et al. 1992; Venosa et al. 1992). To treat the hydrocarbon contaminated mangrove swamp areas with marine algae has been achieved successfully and this approach has been employed since long (Davies and Westlake 1979) and still is in use (Orji et al. 2012a). This bioremediation is attained by the aid of variety of microbiota (algae, fungi and bacteria) which produce efficient enzymes that have the capacity to degrade hazardous organic compounds into non-toxic ones. Owing to the production capability of efficient enzymes, which can biodegrade the hydrocarbons, they are being employed and considered as natural scavengers (Davies and Westlake 1979). It has been observed that in deficiency of vital elements like phosphate, nitrate; microbes are unable to utilize the efflux of such hazardous compounds present in large quantity and distributed over large area (Van Hamme et al. 2003).

According to another study, it has been found that protozoa and micro-algae are considered of great value in the community of microbes from ecosystem restitution point of view in aquatic as well as in terrestrial ecosystems. Although at present few reports are available with respect to protozoa and their participation in biodegradation of hydrocarbons (Jain and Bajpai 2012), on the other hand, use of bacteria having potential of biodegrading hydrocarbons in oil-contaminated regions does not promise the elimination of carcinogenic elements of crude oil. It means that it is still a great challenge to degrade some components namely alkanes of short and long chains: <C10 and C20- C40 and polycyclic aromatic hydrocarbons (PAHs) in crude petroleum hydrocarbons (Yuste et al. 2000).

Very few microbes can metabolize both i.e. chain- aliphatic and ring- aromatic hydrocarbons that are nonchlorinated. Though owing of their hydrophobic nature the hydrocarbons mixes slowly to the liquid phase where microbes are present to biodegrade the immisible hydrocarbons. Furthermore, Orji et al. (2012b) informed that at present the Remediation by Enhanced Natural Attenuation (RENA) is being employed as a cleaning technology in environments contaminated with petroleum hydrocarbons in the Niger Delta, which is comprised of mangrove ecosystems. RENA is a profound bioremediation technology in which contaminated soils, sediments, and sludges are intermittently tilled into soil to oxygenate the waste. This technology includes factors like evaporation, spreading, dispersion, emulsification, oxidation, and dissolution (Brusseau 1998).

3.4 Bioremediation of Polychlorinated Biphenyls (PCBs)

PCBs are organic chlorine compounds having the chemical formula: $C_{12}H_{10-x}Cl_x$. The chemical structure of PCB is shown in Fig. 3.2. They are considered among the worst pollutants since they have carcinogenic property, toxic in nature, vastly distributed, and slow biodegradation in the environment (Perelo 2010; Meagher 2000). A few PCBs that are monohydroxylated cause problems in endocrine system; on the other hand, it has also been observed that a few PCB metabolites that bear a hydroxyl (-OH) group at the meta- or para positions affect development and are neurotoxic (Maltseva et al. 1999).

Because of their thermal stable property, the PCBs have been utilized immensely in various products, by different industries (Dhankher et al. 2012). In addition, under different conditions PCBs can be degraded by a few microbes: aerobically or anaerobically (Borja et al. 2005; Pieper and Seeger 2008). Degradation of PCB is quite difficult as they exist in distinct and complex forms, which are quite stable. It has been observed that biodegradation of PCB is inhibited at a level of key enzyme named as dihydroxybiphenyl oxygenase. The activity of this enzyme is inhibited and inactivated by orthochlorinated form of PCBs (Dai et al. 2002). Degradation of lesser-chlorinated PCBs in presence of oxygen by using dioxygenases enzymes results in ring cleavage, finally resulting in complete mineralization of PCBs (Dhankher et al. 2012). Lynn et al. (2007) reported the vital role played by phytoplankton in regulating the hazardous consequences of persistent organic pollutants like PCBs in the water column that mimics marine as well as benthic aquatic systems.

For the very first time, benthic micro-algae have been employed for in situ remediation of the chemically contaminated sediments (Yamamoto et al. 2008). Earlier, Harding and Phillips (1978) recommended that aquatic organisms plus phytoplankton may uptake and assimilate various chlorinated hydrocarbons, thus reducing their concentration in the environment. Furthermore, another team of researchers observed that diatom *Stephanodiscus minutulus* absorbed one of the analogues of PCB (2,2′,6,6′-tetrachlorobiphenyl). The diatom was later fed to *Daphnia pulicaria* (zooplankton); it was observed that the uptake of nutrients by *S. minutulus* depends upon availability of nutrients, which ultimately influences zooplankton populations (Lynn et al. 2007). This study signifies that the PCBs trophic transfer may be modulated significantly by nutrients level of phytoplankton and the successive zooplankton rate of browsing and assimilation.

In two Wisconsin rivers, the association between live algal cells and PCBs was studied during four seasons at four locations. An encouraging relationship between PCBs associated with particle and algal carbon and chlorophyll-a concentrations showed that living cells of algae were a noteworthy organism for absorption of suspended PCBs (Fitzgerald and Steuer 2006).

In the past, Lara et al. (1989) described the potential of two brown algae namely, *Caepidium antarticum* and *Desmarestia* sp. and correlated the excretion of the algal exudates with pollutants like polychlorinated biphenyls (PCBs) in aquatic conditions. It has also been reported that exudates released by another brown algae—*Ascophyllum nodosum* and *Fucus* sp.–absorbed the organic pollutants in marine water samples. Interestingly, the fatty acids, amino acids, and sugars of brown algae also showed to bind PCBs. On the other hand, it has been observed that in marine algal organisms, PCBs are usually accumulated in the lipids reservoirs (Fitzgerald and Steuer 2006) and in case of phytoplankton of the 19 southern lakes of Sweden, content of lipid showed variation in concentration of PCBs (Berglund et al. 2001).

Recently, it has been reported that a most common drawback is that there is a lack of an effectual method of offering electron acceptors and donors to enhance PCBs biodegradation under in situ conditions. Moreover, the use of an electric potential to PCBs degrading and dechlorinating microbes, can be ecologically sustainable and cost-effective approach to bioremediate them under in situ conditions (Chun et al. 2013).

3.5 Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs)

In any aquatic ecosystem, polycyclic aromatic hydrocarbons (PAHs) are pollutants that are omnipresent. They naturally exist in fossil fuels like petroleum and coal; however, they are also produced at some stage of partial combustion of natural resources like wood, diesel, coal, and vegetation (Freeman and Cattell 1990; Lim et al. 2010). Basically, PAHs are a class of organic elements which comprise two or more number of amalgamated benzene rings organized in several structural forms (Bamforth and Singleton 2005). According to Cerniglia (1992), PAHs are extremely unmanageable molecules that could remain in the environment for long time owing to their hydrophobic property and less solubility. Further, Lima et al. (2005) added that PAHs are mainly formed during the combustion of fossil fuel plus they share numerous hazardous characteristics with PCBs, like toxicity, high boiling points, less solubility in water and high stability. The remediation of PAHs by green plants has minimal accomplishment as the pollutants of this particular class are highly toxic (Dhankher et al. 2012). With the utilization of two species of algae namely *Skeletonema costatum* and *Nitzschia* sp., the biodegradation and assimilation of two classic PAHs, specifically Phenanthrene (PHE) and fluoranthene (FLA), were evaluated. The researchers discovered that the assimilation and degradation potential of *Nitzschia* sp. were very much higher as compared to *S. costatum*. Moreover, it was found that the degradation of FLA was quite slow, which signifies that FLA was much obstinate compound of PAH. The species of micro-algae also demonstrated more or same efficiency in the biological elimination of PHE-FLA mixture; when grown on individually with PHE or FLA alone, this indicated that existence of one PAH triggered the degradation of second (Hong et al. 2008).

Muñoz et al. (2006) proposed that it is feasible to utilize micro-algae for the production of oxygen needed by bacteria to biologically degrade hazardous pollutants like PAHs, organic solvents, and Phenolics. Al- Turki (2009) and others stated that when the microbes uptake and biodegrade PAHs, they get triggered in the process of oxidative metabolism by incorporating two O_2 atoms by microorganisms (bacteria and green-algae) to form phenols or cis- dihydrodiols (Gattullo et al. 2012).

Another team of scientists described the naphthalene oxidation using microalgae and cyanobacteria. They stated that under photoautotrophic conditions, naphthalene could be oxidized by nine species of cyanobacteria, five species of green algae, one red alga, one brown alga, and two species of diatoms, revealing that the capability of oxidizing naphthalene is distributed globally among various algal species (Cerniglia et al. 1980).

The phenanthrene is extremely unsafe and toxic pollutant; it generally exists in aquatic environments, but its consequences on aquatic plant species have not been examined yet in detail (Burritt 2008). In addition, it was mentioned in literature that a partnership formed by algae and bacteria namely *Chlorella sorokiniana* and *Pseudomonas migulae* (phenanthrene degrading) has the ability to degrade 200–500 mg/L of PHE suspended in oil of silicone under some conditions of photosynthesis but lacking oxygen supply externally, thus proved that biosurfactants and oxygen released by micro-algae and may promote degradation of phenanthrene and bacterial growth (Muñoz et al. 2003).

There are numerous factors responsible that can affect the PAHs bioremediation. The common factors that are responsible for bioremediation are pH, oxygen, temperature, bioavailability, and toxicity of end-products. The temperature has a noticeable influence on the capability of microbes (in situ) to decay PAHs and, we understand that majority of the polluted areas during all months of the year would not be having most favorable temperature (Bamforth and Singleton 2005). The PAHs' solubility is directly proportional to the temperature (Margesin and Schinner 2001), which further promotes the bioavailability of molecules of PAH. The other factor that could affect biodegradation of PAH is pH of polluted regions, and the proliferation of microbes in such sites depends upon favorable pH. It was acknowledged that oxygen plays a crucial part in bioremediation of organic chemical pollutants like PAHs. This procedure of bioremediation can be carried out under both aerobic and anaerobic circumstances, provided other environmental conditions are favorable and the microbe is acclimatized to that particular environment (Bamforth and Singleton 2005).

3.6 Bioremediation of Pesticides

Contamination by pesticides (of aquatic ecosystems) is one of the most common and severe problem that ecosystem and any organisms may face and this needs utmost attention. The majority of highly cultivated rural and semi urban areas are the main regions of contamination of groundwater, since chemical pesticides and fertilizers slowly and continuously leach or percolate in the groundwater or nearby streams (Kloeppel et al. 1997; Olette et al. 2010). Moro et al. (2012) stated that the widespread employment of herbicides in agricultural area has augmented the risk of environmental contamination of marine ecosystems. In aquatic ecosystem, there is a probability that harmful chemicals might lead to harmful impacts on the health of aquatic micro- and macro-flora and fauna (Moore et al. 2007; Arora 2018).

Organochlorine- a group of pesticides are widespread as environmental contaminants and due to their persistence and lipophilicity nature, they are enriched in lipids and become a part of food chain (Loganathan and Kannan 1991; Voldner and Li 1995). In developing Asian countries, pesticides are considered the major reason of health hazards (approx. one million cases) and result in around 20,000 deaths per year (Duran- Nah and Colli- Quintal 2000). Researchers analyzed the buildup and toxic nature of cadmium (Cd) and lead (Pb) in the tissues of animals. Therefore, application of green algae such as *Cladophora fracta* played a significant role in accumulation of these toxic heavy metals. There are number of tactics and methods that involve management of heavy metals, such as disposal area (landfill) and incineration (burning) have come into action to eliminate such harmful pollutants, but these manual (physical) processes are neither efficient nor economical and sometimes leaching from landfills may result in contamination of ground and flowing water (Lamaia et al. 2005).

Hussain et al. (2009) mentioned that bioremediation by utilizing capable and prospective microorganisms have the potential of detoxifying the polluting pesticide plus they also offer a better way of remediating the pesticides by employing transgenic plants. In 2008, Olette (et al.) investigated that the following water plants namely *Lemna minor*, *Elodea canadensis,* and *Cabomba aquatica* have the potential to absorb and eradicate three major pesticides: (i) a fungicide and algicide- copper sulphate, (ii) a herbicide—flazasulfuron, and (iii) a fungicide—dimethomorph. The investigators noticed that the most effectual uptake potential had been shown by the plant *Lemna minor* followed by *Elodea canadensis* and after that *Cabomba aquatica*. Moreover, in 2010, Olette (et al.) stated that *Scenedesmus quadricauda* (a microalga) was much effective in the eradication of two general fungicides known as dimethomorph and pyrimethanil plus one herbicide called isoproturon from in and around their environment. Another researcher reported that *Gracilaria fisheri*—a marine alga—exhibited the biosorption of heavy metals (Cd and copper) (Chaisuksant 2003). Tahir et al. (2018) mentioned the common mechanism employed by algal cells in biosorption of metals from the environment.

On the other hand, it was demonstrated that *Chlamydomonas reinhardtii*; a green alga has enormous capacity to gather and breakdown prometryne (a commonly employed herbicide) into less toxic compounds. The uptake of prometryne and its metabolism by green alga resulted in quick elimination of the herbicide from media under laboratory conditions. This procedure of herbicide biodegradation could be inferred as algal internal system of tolerance, which means that the green algae are extremely effective and valuable for bioremediation of marine environment contaminated by prometryne (Jin et al. 2012).

In another interesting study conducted by Zhang et al. (2011), it was mentioned that similar species were spotted to uptake and absorb another herbicide fluroxypyr. The collected herbicide might get degraded rapidly in cells of algae, which proposes that the accumulation and breakdown of fluroxypyr takes place at the same time. Another promising species of algae known as *Monoraphidium braunii* has also been described for the bioremediation of water from Bisphenol A, though this organic synthetic compound is poorly soluble in water, though it has been reported in ground and surface waters (Gattullo et al. 2012). Nevertheless, it has also been reported that a micro-algae (freshwater) is able to convert Bisphenol A into its monoglucoside forms, which is known to be quite not dangerous (Nakajima et al. 2007).

Another study conducted by Moro et al. (2012) discussed the effect of chlortoluron and mesotrione, normally useful herbicides, on three different species of micro-algae, out of which two were from chlorophyceae (*Ankistrodesmus fusiformis*, *Pediastrum tetras*) and one was a member of diatom (*Amphora coffeaeformis*). It was found that the herbicide chlorotoluron demonstrated a notable inhibitory effect on the growth of *A. coffeaeformis*, though mesotrione enhanced the growth and density of cells in case of *A. fusiformis.*

3.7 Bioremediation of Heavy Metals

Biosorption, a novel technology is considered as another feasible and constructive strategy to eliminate heavy metal ions from wastewaters by employing mainly inactive biomass. The technique also uses living as well as non- living algal biomass (Colak and Kaya 1988; Dwivedi 2012). A few other researchers (Bitton 1990; El-Sheekh et al. 2005; Lamaia et al. 2005) reported that using living biomass of algae exhibited limited sorption potential as the ions of heavy metals poison the living cells frequently. Moreover, in particular live cells of algae may also be influenced by several environmental factors that coherently manipulate the potential of biosorption of metal ions. The mechanisms of absorption are quite complicated in biomass of live algal cells as compared to that of dead algal cells, as heavy metal absorption within live cells may depend upon external factors (Becker 1994). Furthermore, cells of non- living algae show simple biosorption by absorbing ions of metal on the surface of their cell membrane. This method is regarded as extracellular procedure (Godlewska-Zyłkiewicz 2001). The biomass of non- living cells of algae can be viewed as an assemblage of polymers like cellulose, carbohydrates, glycoproteins etc. that have the potential to bind to the cations of heavy metal like adsorbents, which results in economical treatment of wastewater (Volesky 2007; Arief et al. 2008; Choi and Lee 2012).

Kaplan (2013) reported that capacity of biosorption of heavy metal ions has been recognized as the phenomenon of different kinds of binding groups present on cell surfaces of algae like hydroxyl, sulfate, imidazole, carboxyl, sulphuryl, amines etc. FTIR (Fourier transform infrared spectroscopy) is a technique that can be employed to investigate the accessibility of functional sites for the uptake and binding of heavy metal ions in algal cells (Gupta and Rastogi 2008). The ability of cell surface of algae to attach to a particular ion depends on several factors like presence of number of functional groups in cells of algae, the affinity of binding groups towards metal ions, chemical structure of active sites etc. Usually, the availability of such binding groups' results in negative charge on surface of cell, which is linked to deprotonation of functional groups namely carboxyl and phosphate on surface of cell (Mehta and Gaur 2005).

For the biosorption of heavy metal ions, the algal cell wall is the foremost barrier. It has been reported that the cell walls of algae contain varied polysaccharides and proteins that carry many sites for metal binding (Schiewer and Volesky 2000). The potential for biosorption of metal ions by the algal mutant strains varied because of distinct distribution of proteins and polysaccharides and plenty of cell wall frameworks. Furthermore, brown alga has been considered as a better agent for biosorption of heavy metal ions as stated by Romera et al. (2006). On the basis of comparison of various strains of algae and affinity for biomass of metal ion, brown algae are considered as a great biosorbent candidates. Romera et al. (2007) also mentioned that brown algae bearing alginate in their cell wall composition have a high affinity for Pb ion biosorption. Polymers of alginate in brown algae make up the fundamental way for sorption of heavy metal ions plus their biosorption potential is directly proportional to the availability of binding sites on the polymer (Romera et al. 2006; Davis et al. 2003).

3.8 Bioremediation of Trinitrotoluene (TNT)

An explosive, Trinitrotoluene (TNT) has been frequently used for war fares and as an explosive in mines since decades. Owing to its use in mines and in military exercises, it has become a widespread contaminant in numerous sites of military throughout the world. It has been studied that for the treatment of TNT contaminated locations, in situ technologies such as bioremediation have been used. In aquatic environment, utilization of algae and plants are commonly used; it has been reported that numerous plants and algal species have the potential to breakdown TNT and some other nitro-aromatic compounds by excreting few enzymes like nitrate reductases (Subramanian and Shanks 2003; Wolfe and Hoehamer 2003).

Nitro-aromatics are synthetic organic compounds, which constitute a fundamental group of unmanageable or difficult to degrade xenobiotics. Though, these compounds are comparatively limitedly present in nature, but have been discharged in the environment predominantly through human activities (San and Parales 2010). Some nitro-aromatic compounds are generated by incomplete combustion of fossil fuels; other compounds are used as manmade intermediate products, chemical dyes, pesticides for agriculture, and commonly used explosives, employed by military and civil works (Spain 1995). It has been suggested that nitro-aromatic compounds may be biodegraded by the process of composting or microbial actions, microbes excrete certain enzymes, which break down the aromatic ring structure (Snellinx et al. 2002). The most toxic compounds found were 1,3,5-trinitrobenzene and 2,4,6-TNT followed by 3,5-dinitrophenol, 3,5-dinitroaniline and then 4-amino-2-nitrotoluene (Neuwoehner et al. 2007).

Ro et al. (1996) reported that the explosive TNT is moderately water soluble. Furthermore, Esteve- Núñez et al. (2001) added that TNT, in general, is obstinate to biological degradation as compared to the rest of xenobiotic compounds. As TNT is moderately soluble in water, therefore, it either gets absorbed in marine sediment or slightly dissolved in seawater that results in hazardous consequences for marine flora and fauna (Green et al. 1999; Nipper et al. 2001; Esteve- Nunez et al. 2001).

Earlier, Cruz- Uribe and Rorrer (2006) proposed that *Portieria hornemannii* (a marine red alga) has the potential to eliminate TNT from seawater. A reductase gene that is, Pentaerythritol tetranitrate (PETN) present in *Enterobacter cloacae* PB2 strain encodes for an enzyme that eliminates nitrate from TNT thus enabling the bacteria to utilize TNT as a source of nitrogen. A few species of plant were also capable of taking up moderately low levels of TNT, converting it to an organic compound called amino dinitrotoluene which further gets attached to glutathione or sugars and most likely gets deposited in the cell walls or vacuoles or secreted compounds (Dhankher et al. 2012). A novel genetic strategy was established by Altamirano et al. (2004) for the detection of TNT in media. In this approach, two distinct green microalga genotypes of *Dictyosphaerium chlorelloides*- a susceptible and a resistant strain were used as a biosensors of TNT. TNT inhibits the chlorophyll-a fluorescence of PSII and was considered as the biological signal. In TNT presence, resistant mutants consistently displayed high value of maximum fluorescence of light- acclimatized thylakoids (F'm) as compared to cells of wild- type that are susceptible to TNT presence, thus no emission of fluorescence. Biochip (microarray) and supplementary gene expression assays development has resulted in marking the presence of nitro-aromatics in the ecosystem (Dhankher et al. 2012; Gandia- Herrero et al. 2008).

3.9 Conclusions and Future Aspects

From the above discussion, it can be concluded that the relevance of micro-algae in biomonitoring and restitution of aquatic and soil systems further supports the phytoextraction and biological degradation of several organic pollutants, but there are a few organic compounds like POPs that are still quite challenging to degrade by micro-algae. To solve the above mentioned crisis, genetic engineering may provide a promising tool to bio remediate the numerous organic pollutants. Moreover, the genetically modified micro algae may possess the higher level of tolerance of recalcitrant pollutants (Arora et al. 2018). It is important additionally to realize and supervise various external parameters of aquatic ecosystems like pH, temperature, availability of nutrients, and other abiotic factors to enhance the uptake, transport in the algal cell, and biodegradation of various contaminants. Therefore, it becomes apparent for us to develop the algal organism or some better techniques to improve the process and duration of bioremediation in any soil and aquatic ecosystems. Biosorption processes have proved successful as they need economical biomaterials that show higher uptake of metal and selectivity on the basis of biochemical structure plus appropriate mechanical properties for practiced remediation methods. The main purpose of getting higher biomass of algae grown in wastewater has dual attractive applications such as cultivation of algae for wastewater treatment and oxygen as well as biofuel production.

Fact is that we also require much more knowledge and data on commonly employed algal-based procedures before being capable to predict reliably the short-, medium-, and long-term effect of contaminant stress on soil, sediment, and aquatic ecosystems. Further, it would be very fascinating to find out that some algal genera which are really capable to degrade herbicides, pesticides, and synthetic organic compounds in their natural ecosystems. This will help in protecting the natural flora and fauna from constant threat of hazardous pollutants, for example, protecting the phytoplanktons from organic pollutants in water bodies. Furthermore, the basic mechanisms lying behind the augmented pollutants degradation by algal and bacterial genera interactions also need to be revealed. Still there is uncertainity about the molecular and biochemical means and processes which these micro-algae perform to metabolize these toxic contaminants. Moreover, there is a need to precisely understand the final influence of these algal genera on the utilization of these toxic and unwanted compounds in the ecosystem. Therefore, further research work in this direction will certainly help us in developing efficient and competent micro-algal genera to remediate the toxicants and maintain the sustainability of ecosystems.

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Chapter 4
Phytoremediation of Heavy Metals and Pesticides Present in Water Using Aquatic Macrophytes

Sangeeta Anand, Sushil Kumar Bharti, Sanjeev Kumar, S. C. Barman, and Narendra Kumar

Abstract Heavy metals occurring naturally on the earth are used in various industrial activities, whereas pesticides are man-made products used for protecting the crop. Heavy metals are inorganic contaminants and aggravated due to their long-term persistence, whereas pesticides encompass a variety of different types of chemicals including herbicides, insecticides, fungicides, and rodenticides. Hence, remediation of water contaminated by heavy metals and pesticides seeks urgent attention. Phytoremediation is an efficient alternative and less expensive method to strip heavy metals and pesticides directly from the water. Some of the aquatic plants used for removal of heavy metals and pesticides from water are duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*), *Hydrilla* (*Hydrilla verticillata*), water spinach (*Ipomoea aquatica*), water ferns (*Azolla caroliniana*, *Azolla filiculoides*, and *Azolla pinnata*), water cabbage (*Pistia stratiotes*), etc. Molecular tools are used to understand the mechanisms of uptake, sequestration, translocation, and tolerance in plants. The purpose of this review is to assess the current state of phytoremediation as an innovative technology and potential of aquatic macrophytes in remediation of water contaminated by heavy metals and pesticides.

Keywords Phytoremediation · Heavy metals · Pesticides · Water · Macrophytes

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4.1 Introduction

Water is very valuable for agriculture and as a natural resource. Unfortunately, during the recent decades, overexploitation of natural resources by various human activities such as industrialization, urbanization, disposals of wastewater, and unplanned agricultural practices has resulted in enormous amount of contaminants in water. Heavy metals and pesticides released by anthropogenic activities beyond toxic limits are continuously threatening the life of human beings (Zhang et al. 2009; Ishaq and Khan 2013; Arora et al. 2018). The point source contaminants include metal smelting and mining effluent from industries, while nonpoint sources include fertilizers and pesticides from agricultural run-off (Kumar et al. 2018a). Each pollutant has its own deleterious effects on flora and fauna, but the addition of heavy metals and pesticides into the water is a growing concern. Heavy metals such as lead (Pb), mercury (Hg), arsenic (As), copper (Cu), zinc (Zn), and cadmium (Cd) and pesticides like endosulfan, dichlorodiphenyltrichloroethane (DDT), mevinphos, ethion, copper sulfate, are highly toxic when absorbed in plants and animals (Kumar et al. 2016, 2018b).

Elements having density between 5.306 and 22.00 g/cm^2 are termed as heavy metals and these originate both from natural and anthropogenic sources (Gall et al. 2015). These metals are leading contaminants for environment because of being non-biodegradable and can be transferred through trophic levels and accumulate in the biota insistently (Nancharaiah et al. 2016; Kumar et al. 2018c). Some metals such as manganese (Mn), Zn, cromium (Cr), molybdenum (Mo), iron (Fe), and nickel (Ni) are essential at low concentrations for healthy function of biota but toxic at higher concentration, and some are non-essential and extremely toxic even at very low concentration including Pb, Hg, and Cd (Nagajyoti et al. 2010; Prasad 2011; Chibuike and Obiora 2014; Rezania et al. 2016). Heavy metals such as Cd, Pb, Zn, Hg, Mn, Cu, Cr, Ni, and Fe released from various industries are toxic and hazardous. They enter into food chain and, if are beyond limits, then can accumulate in plants, animals, and humans causing serious health hazards (Babel and Kurniawan 2004; Barakat 2011; Sood et al. 2012). A summary of several anthropogenic sources of heavy metals, their effects on health, and the available control techniques are presented in Table 4.1.

Pesticides consist of a large group of chemicals that are used throughout the world as insecticides, herbicides, fungicides, molluscicides, rodenticides, nematicides, and plant growth regulators to control unwanted plants, pests, and diseases to improve the productivity of food (Agrawal et al. 2010). The major groups of chemical pesticides include organochlorines, organophosphates, carbamates, and pyrethroids. Pesticides target different types of pests and their constant exposure also impacts non-target species, and this can lead to induced toxicity once it crosses the threshold limit in the system and food chain resulting in depleted biodiversity and health of ecosystems including humans (Kumar et al. 2018b; Arora 2018a) (Table 4.2).

Table 4.1 Sources of heavy metals, their health effects, and control techniques available

Metals	Sources	Health effects	Available control techniques	References
As	Paints, dyes, drugs, soaps, fertilizer	Weakness, pigmentation, nausea, peripheral nervous system failure, cardiovascular failure, DNA breakdown	Reverse osmosis or nanofiltration	Duarte et al. (2009) and Vaclavikova et al. (2008)
Cd	Mining and smelting activities, pigments, paints, electroplating, batteries	Hypertension, weight loss, hypochromic anemia, pulmonary fibrosis	Anaerobic digestion activated sludge process	Jaishankar et al. (2014)
Pb	Pigment, paints, batteries, industrial smelting, ceramics, Ayurvedic herbs, Troy	Osteoporosis, inhibits formation of hemoglobin, loss of IQ, high blood pressure, anemia, gastrointestinal effects	Chemical precipitation	Singh et al. (2011a, b) and Naseem and Tahir (2001)
Hg	Pesticide, dental filling, switches, light bulbs, batteries	Damage brain, neurological and renal disturbances, tremor, memory problem, lung damage	Ion exchange process, carbon adsorption	Jaishankar et al. (2014)
Selenium (Se)	Plastics, paints, rubber, preparation in drugs, feed additive, anti-dandruff shampoo	Hair loss, stomach pain, difficulty in breathing	Biosorption	Jaishankar et al. (2014)
Cr	Rocks, electroplating magnetic tapes, paints, cement, rubber and paper, etc.	Nose ulcers, breathing problems, asthma, damage kidney and liver	Stabilization pond	Jaishankar et al. (2014)
Ni	Cigarettes, diesel exhaust, electroplating, pigment, arc welding, dental materials	Lung cancer, nose cancer, sickness and dizziness, respiration failure, asthma and chronic bronchitis, allergic reaction such as skin rashes	Activated sludge process	Cempel and Nikel (2006)

4.2 Conventional Methods Used for the Removal of Heavy Metals and Pesticides

In order to maintain water quality standards, it is essential to remove heavy metals from wastewaters. Various conventional processes are being used for removal of heavy metals from wastewater such as chemical precipitation, reverse osmosis, ion exchange, and electrochemical deposition. Toxic heavy metals required to be removed from wastewater include Zn, Cu, Ni, Cd, Pb, and Cr (Fenglian and Wang 2011). Conventional physical and chemical methods for removal of heavy metals

Table 4.2 Pesticides, their health effects, and available control techniques

Pesticides	Health effects	Available control techniques	References
Organochlorine (endosulfan, DDT, dieldrin, alachlor, atrazine, lindane, and methoxychlor)	Cancer, eye, liver, and kidney problem	Activated carbon adsorption	Mnif et al. (2011)
Organophosphate (malathion, ethion, and phorate)	Nervous system problem	Filtration and centrifugation	Sullivan and Blose (1992)
Carbamate (aldicarb, carbosulfan, carbofuran, and carbaryl)	Muscle weakness, dizziness, sweating	Activated carbon adsorption	Sullivan and Blose (1992)
Pyrethroid (permethrin, deltamethrin, bifenthrin, cyfluthrin, and barthrim)	Toxic to nervous system, nausea, vomiting, headache	Activated carbon adsorption	Mnif et al. (2011)

are costly and time consuming and result in formation of secondary pollutants apart from being non-sustainable as well (Namasivayam and Ranganathan 1995; Mishra et al. 2017). However, chemical precipitation is still the most widely used method for heavy metal removal from effluents. A summary of various conventional techniques used for these treatments of wastewater along with the associated limitations are presented in Tables 4.3 4.4, 4.5, 4.6, and 4.7.

Although these techniques are effective for remediation purposes, they have significant risks in the excavation, transportation, handling, and disposal of toxic by-products. Other drawbacks are the extremely higher operational cost and small-scale application; lack of knowledge, especially for incineration; and also increase in the exposure rate. Therefore, the restoration of contaminated aquatic ecosystems requires ecological and cost-effective remediation technologies. Phytoremediation is a technique in which plants are used for remediation of contaminated water, soil, and sediments (Kumar et al. 2013a; Bauddh and Singh 2015). This technology is used for the removal of heavy metals, radionuclides, nutrients (nitrate, phosphate, etc.), solvents, explosives, crude oil, and organic pollutants such as persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and pesticides from wastewater and soil by using plants (Kumar et al. 2013b; Arora 2018b). Phytoremediation is a novel, eco-friendly, cost-effective, solar-driven, and in situ applicable remediation strategy (Kalve et al. 2011; Singh and Prasad 2011; Sarma 2011; Vithanage et al. 2012).

In the last two decades, using plants for metal and pesticides removal has attracted more attention (Jha et al. 2010). According to sciencedirect.com, a total of 5647 articles are published containing the term “phytoremediation” since the last 16 years (Fig. 4.1). Phytoremediation is also a set benchmark to assess the patent and research article development compared with other alternative strategies. The average annual percentage of phytoremediation is higher in patents and research (12% and 24%) versus bioremediation (4% and 12%), remediation (6% and 12%), and constructed wetland (14% and 16%) from 1999 to 2011 (Keomel et al. 2015).

Table 4.3 Various applicable conventional methods of wastewater treatment and their associated disadvantages

Methods	Application	Disadvantage	References
Reverse osmosis	Separated by semipermeable membrane	Clogging of membrane and expensive	Singh et al. (2011a, b)
Chemical precipitation	Metals and pesticides are removed by addition of coagulants such as lime, alum due to its availability and low cost	Large amount of sludge containing toxic compound is produced	Aziz et al. (2008), Fenglian and Wang (2011) and Singh et al. (2011a, b)
Ion exchange	The electrostatic force was responsible for the ion exchange of the diluted metal solution	High cost, partial removal of certain ions	Singh et al. (2011a, b)
Activated carbon adsorption	Remove disagreeable taste and color, chlorine, pores trapped microscopic particles and large organic molecules	Can generate carbon fines which are corrosive and abrasive	Sivakumar et al. (2012)
Nanofiltration	Nano-sized reactive agent removes organic contaminants	Rapidly clump with soil and limit the dispersal to their target	Dialynas and Diamadopoulos (2009)
Coagulation and flocculation	Coagulation destabilized colloidal particles by adding chemicals and neutralized negative charges and flocculation destabilized in floc and settle by gravity.	Large-volume sludge production, high chemical consumption	Fenglian and Wang (2011)
Flotation	Solids are removed by attaching air bubble, decreasing its density and float.	Increasing of ion strength flotation efficiency decreasing, higher cost	Ahmad et al. (2016)
Membrane filtration	Process commonly based on molecular size, charge, chemical nature, affinity, etc.; transmembrane pressure acts as a driving force for contaminants to transfer across the membrane	Limited flow rates process, complex operating system, low selectivity, higher maintenance cost	Qin et al. (2007)

4.3 Uptake Mechanisms of Contaminants by Plants

Macrophytes have specific and effective mechanisms for the removal of contaminants which vary with the plant type and whether the pollutant is organic or inorganic. Inorganic uptake is driven via membrane transporters, while the organic contaminants by diffusion. These absorbed contaminants then get detoxified by biochemical reactions using enzymatic mechanisms in the plant. Uptake of inorganic compounds is facilitated by active or passive mechanisms, whereas organic compounds are generally governed by hydrophobicity and polarity.

Table 4.4 Phytoremediation and its techniques

Techniques	Mechanisms of action	Medium	References
Rhizofiltration	Metals are taken up in aquatic plants through the roots	Water	Rawat (2012) and Rezania et al. (2016)
Phytoextraction	Uptake and concentration of metals via direct uptake into plant tissue with subsequent removal of plants	Soil/ water	Ali et al. (2013) and Thakur et al. (2016)
Phytostabilization	Root exudates cause metals to precipitate and become less bioavailability	Soil/ water	Wuana and Okieimen (2011)
Phytovolatilization	Plants evaporate volatile metals (Se, Hg) from the surface of leaves	Soil/ water	Sharma et al. (2015) and Thakur et al. (2016)
Phytotransformation	Plants uptake and degradation of organics with the help of enzymes	Soil/ water	Cacadore and Durate, (2015)

4.4 Uptake Mechanism of Metals

Metal accumulations in aquatic macrophytes have been reported in literature (Zhang et al. 2009; Rahman and Hasegawa 2011; Revathi and Venugopal 2013). In aqueous ecosystems heavy metals are directly or indirectly present as free ions, insoluble and soluble forms such as oxides, hydroxides, carbonates, chlorides, and humic substances. The roots of plants accumulate these metals through the plasma membrane to the cells, where detoxification and sequestration of metals take place at the cellular level. The heavy metals uptake/translocation mechanisms are likely to be closely regulated. Metals bind with peptides and proteins in plants and this results in enhanced accumulation. These peptides or proteins are preferentially metal specific such that metals with toxic effects, i.e., Cd, Hg, and Pb are also sequestered. Detoxification or sequestration process occurs after translocation in which a huge amount of heavy metals concentrate in organs without suffering any toxic effects (Ryu et al. 2003). Malate and citrate are excretion of plants which acts as metal chelators. As the pH decreases, the plants simultaneously increase the bioavailability of the metals by strong chelating agents (Ross 1994). Many scientists explained that cytoplasmic Ni is sequestered by histidine while vacuolar Ni is detoxified by binding with citrate (Kramer et al. 1996; Dhir et al. 2009). Zn forms more stable complexes with citrate and oxalate, while malate returns to the cytoplasm. Oxidative stress by heavy metals occurs after the formation of reactive oxygen species, such as superoxide ions, hydroxyl ions, and hydrogen peroxide. These ions are deactivated by enzymes, i.e., superoxide dismutase, ascorbate peroxidase, catalase, guaiacol peroxidase, and glutathione reductase, and nonenzymes, i.e., glutathione, phenolic compounds, and ascorbic acid (Parvaiz et al. 2008; Azqueta et al. 2009). In detoxification process heavy metals form complex with chelators and remove meta-

Table 4.5 Historical advances of phytoremediation using macrophytes

Decades	Summary	Scope of works	Highlight	References
1970s	Due to massive capacity of nutrient uptake from wastewater, aquatic macrophytes used for remediation	Basically research on remediation potential of aquatic plants specially submerged plants	Potential of uptake	Boyd (1970) and Cowgill (1974)
	Submerged and floating quickly uptake pollutant from water			
	Levels of potentially toxic elements in the plants were at least an order of amount higher than in the supporting aqueous medium			
1980s	Floating and emergent plants can uptake contaminants through roots while submerged plants by root and leaves both	Study on emergent and floating plants capability	Species determination	Denny (1980, 1987)
1990s	Rates of toxic metal uptake and removal of plants are greater than 1000 μg g^{-1} called as hyperaccumulator	Research on metal accumulation through root and foliar parts of aquatic plants	Importance of various mechanisms	Outridge and Noller (1991) and Sharma and Gaur (1995)
00–10	Aquatic plants can efficiently remove heavy metals which is the largest category of pollutants	Monitoring the effective role of macrophytes and developing of hyperaccumulator plants	Effectiveness of species	Hu et al. (2003), Kamal et al. (2004) and Rai (2009)
	Aquatic macrophytes also used as nonliving, for removal and monitoring of heavy metals			
10–18	Uptake mechanisms of green plants which can accumulate pollutants with high ability	Focus on mechanism and improvement of techniques efficiency	Optimization toward implication	Sharma et al. (2012), Ali et al. (2013), Sasmaz et al. (2008) and Kumar et al. (2018a)
	Effectiveness of phytoremediation process (low-cost, low-energy consumption) in contrast with the conventional methods, and no special care is required			
	Chemical like chelating agents are used to enhance the remediation potential of hyperaccumulating plants			

Table 4.6 Macrophytes and their phytoremediation potential for various heavy metals

Plant species	Metals	Accumulation	References
Eichhornia crassipes	Cu	6000–7000 mg kg^{-1}	Molisani et al. (2006)
	Cr	4000–6000 mg kg^{-1}	Hu et al. (2007)
	Ni	1200 mg kg^{-1}	Low et al. (1994)
	Cd	2200 μg kg^{-1}	Zhu et al. (1999)
	Zn	1677 mg g^{-1}	Kamel (2013)
	As	909.58 mg kg^{-1}	Delgado et al. (1993)
	Hg	119 ng g^{-1}	Molisani et al. (2006)
	Mn	300 mg kg^{-1}	Dixit et al. (2011)
Azolla pinnata	Ni	16,252 1 μg g^{-1}	Arora et al. (2004)
	Cd	740 μg g^{-1}	Rai (2008)
	Cr	1095 μg g^{-1}	Rai (2010)
	Hg	940 μg g^{-1}	Rai and Tripathi (2009)
	Pb	1383 mg kg^{-1}	Thayaparan et al. (2013)
Azolla filiculoides	Cd	2608 μg g^{-1}	Arora et al. (2004)
	Cu	6013 μg g^{-1}	Zhang et al. (2008)
			Arora et al. (2006)
	As	>60 μg g^{-1}	Vesely et al. 2011
	Cr	12,383 μg g^{-1}	
	Pb	1607 mg kg^{-1}	
Azolla caroliniana	Cr	356 mg kg^{-1}	Bennicelli et al. (2004) and Zhang et al. (2008)
	Hg	578 mg kg^{-1}	
	As	284 mg kg^{-1}	
Hydrilla verticillata	As	231 mg kg^{-1}	Srivastava et al. (2010)
	Cu	770–30,830 mg kg^{-1}	Srivastava et al. (2011)
Typha latifolia	Ni	295.6 mg kg^{-1}	Afrous et al. (2011)
	Cu	1156.7 mg kg^{-1}	Nguyen et al. (2009)
Pistia stratiotes	Pb	519 mg kg^{-1}	Vesely et al. (2011)
Salvinia minima	Pb	5469 mg kg^{-1}	Vesely et al. (2011)
Salvinia natans	Cr	7.40 mg g^{-1}	Dhir (2009)
Lemna gibba	U	896.9 mg kg^{-1}	Mkandawire et al. (2004)
	As	1021.7 mg kg^{-1}	
Lemna minor	Pb	561 mg g^{-1}	Leblebici and Aksoy (2011) and Bokhari et al. (2016)
	Cu	34.6 μg g^{-1}	
Typha angustifolia	Mn	860 mg kg^{-1}	Sasmaz et al. (2008)
	Cu	50 mg kg^{-1}	
	Zn	56.47 μg g^{-1}	
Myriophyllum spicatum	Pb	8.94 mg g^{-1}	Kamel (2013)
	Zn	2.66 mg g^{-1}	
Ceratophyllum submersum	Pb	258.62 mg kg^{-1}	Kamel (2013) and Guo et al. (2014)
	Zn	1172.8 mg kg^{-1}	

(continued)

Table 4.6 (continued)

Plant species	Metals	Accumulation	References
Wolffia globosa	As	>1000 mg kg^{-1}	Zhang et al. (2009)
Phragmites communis	Fe	2813 μg g^{-1}	Chandra and Yadav (2011)
	Mn	814.40 μg g^{-1}	
	Zn	265.80 μg g^{-1}	
	Pb	92.80 μg g^{-1}	
Phragmites australis	Cu	16.55 μg g^{-1}	Salman et al. (2015)
	Pb	0.77 μg g^{-1}	
	Cd	33.115 μg g^{-1}	
Potamogeton pectinatus	Cd	964.75 μg g^{-1}	Salman et al. (2015)
	Cu	28.75 μg g^{-1}	

bolically active cytoplasm ions by moving them into vacuole and cell wall. In vacuoles hazardous metal ions are captured in limited sites. Therefore, other parts of the cell do not have access to these dangerous metal ions. Cd detoxification by inducing the synthesis of phytochelatins (PCs) forms a Cd-PC molecule, which is further transferred into the vacuoles by Cd/H antiport and ATP-dependent phytochelatin-transporter (Revathi and Venugopal 2013). MTP, a gene encoding a protein localized at tonoplast (separating vacuole from cell wall), is exceedingly expressed in plants of Zn/Ni hyperaccumulating plants (Dräger et al. 2004; Kim et al. 2004; Hammond et al. 2006; Gustin et al. 2009). It has been suggested that MTP play a major role in Zn tolerance and accumulation. Persant et al. (2001) explained that MTP also mediate the Ni vacuolar storage in *Thlaspi goesingense* shoots.

4.5 Uptake Mechanisms of Pesticides

Aquatic plants have capacity to uptake and accumulate organochlorine, organophosphorus, carbamate, and pyrethroid pesticides from water (Gobas et al. 1991; Rice et al. 1997; Macek et al. 2000). These pesticides pass through membrane between root symplast and xylem apoplast by diffusion and their entry depends on passive movement over membranes for their uptake into the aquatic plants (Nwoko 2010). No specific transporters are found in plants for these man-made compounds, so the speed of movement of pesticides in the plant depends to a large extent on their physicochemical properties. Three sequential phases are involved in metabolization of pesticides.

In the first phase, pesticides undergo hydrolysis, reduction, and oxidation (Eapen et al. 2007; Komives and Gullner 2005). Functional groups present in pesticides convert these into more polar, chemically active, and water-soluble compounds (Komives and Gullner 2005). In plants, oxidative metabolism is primarily mediated

Table 4.7 Macrophytes and their phytoremediation potential for various pesticides

Plant species	Contaminants	Accumulation	References
Hydrilla verticillata	Chlordane	1060.95 $\mu g\ L^{-1}$	Chaudhry et al. (2002)
Typha latifolia	Dieldrin	0.60 $ng\ g^{-1}$	Guo et al. (2014)
Pistia stratiotes	Chlorpyrifos	0.036 $mg\ g^{-1}$	Prasertsup and Ariyakanon (2011)
Lemna minor	Flazasulfuron,	27 $\mu g\ g^{-1}$	Olette et al. (2009)
	Dimethomorph	33 $\mu g\ g^{-1}$	
	Chlorpyrifos	0.23 g^{-1}	
Ceratophyllum submersum	Aldrin	0.38 $ng\ g^{-1}$	Guo et al. (2014)
	Endosulfan	0.73 $ng\ g^{-1}$	
Phragmites communis	α-HCH	0.89 $ng\ g^{-1}$	Guo et al. (2014)
	β-HCH	1.18 $ng\ g^{-1}$	
	γ-HCH	0.97 $ng\ g^{-1}$	
	DDTs	0.93 $ng\ g^{-1}$	
Schoenoplectus californicus	DDTs	30.2–45.7 $ng\ g^{-1}$	Miglioranza et al. (2004)
	HCHs	0.61 $ng\ g^{-1}$	
	Chlordane	4.04 $ng\ g^{-1}$	
Spirodela oligorrhiza	o,p′-DDT, p,p′-DDT	50–66%	Gao et al. (2000)
Plantago major	Cyanophos	76.91 $\mu g\ g^{-1}$	Romeh (2014)
Iris pseudacorus	Chlorpyrifos	1.88 $\mu g\ g^{-1}$	Wang et al. (2013)
	Triazophos	42.11 $\mu g\ g^{-1}$	Li et al. (2014)
Nymphaea amazonum	γ-Cyhalothrin	2.02 $\mu g\ g^{-1}$	Mahabali and Spanoghe (2014)
Eleocharis mutala	Imidacloprid	13.51 $\mu g\ g^{-1}$	Mahabali and Spanoghe (2014)
Cyperus rotundus	Triazophos	24.63 $\mu g\ g^{-1}$	Li et al. (2014)
Acorus calamus	Chlorpyrifos	15.3 $\mu g\ g^{-1}$	Wang et al. (2016)
Juncus effusus	Tebuconazole	720 $\mu g\ g^{-1}$	Lv et al. (2016)

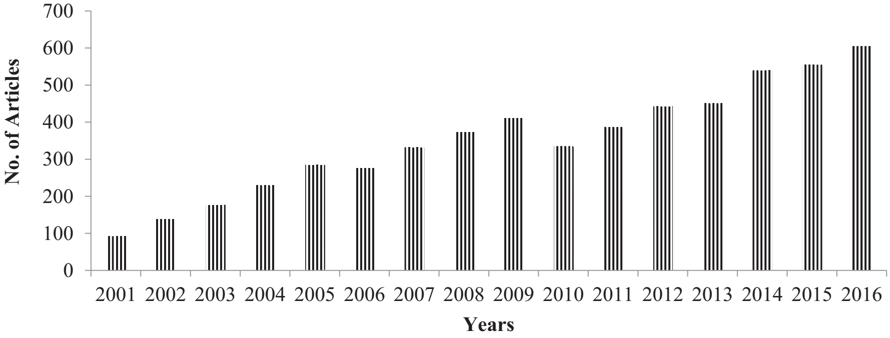

Fig. 4.1 Publications in the field of phytoremediation from the last 16 years (www.sciencedirect.com)

by cytochrome P_{450} monooxygenase (Sandermann 1994, Doty et al. 2007). These enzymes are very crucial during oxidative bioactivation process to emulsify the highly hydrophobic contaminants and convert them into chemically reactive electrophiles forming conjugates (Morant et al. 2003). In the second phase, conjugation takes place in the cytosol where pesticide gets conjugated with sugar, amino acids, and –SH group of glutathione and converts into hydrophilic forms. Conjugated compounds have a high molecular weight and are more polar and less toxic as compared to the parent compound. Transformation hydroxylation of organochlorine pesticides, i.e., 2,4-D, is followed by conjugation with glucose and malonyl and deposition in vacuoles. Every enzyme that participates in detoxification process has specific functions. Phosphatases that cleave phosphate groups from organophosphate are studied in *Spirodela polyrhiza*, and dehalogenases that cleave halogen group from organochlorine pesticides are noted in *Myriophyllum aquaticum* (Dhir 2009; Susarla et al. 2002). After this sequestration takes place. Capture of the pesticides such as organochlorine and organophoshate by plants includes physical (adsorption, absorption, partition) and chemical processes (complex formation), and reaction with cuticular membrane components helps in the sequestration of lipophilic organic compounds. Once man-made chemicals are taken up by plant, it can be transformed via metabolization, volatilization, lignification, and mineralization to carbon dioxide, water, and chlorides. Detoxification transforms the main chemical to non-phytotoxic metabolites, including lignin, that are stored in plant cells (Coleman et al. 1997; Dietz and Schnoor 2001). Then these metabolites are transported to the vacuoles by tonoplast membrane-bound transporters. Vacuolar compartmentalization is a major stage in detoxification of pesticides (Coleman et al. 2002).

4.6 Influencing Factors in Phytoremediation

There are several factors which can affect the uptake mechanisms of heavy metals and understanding about these factors can improve the metal removal capacity of plant. These factors are divided into two categories, biotic and abiotic, and are discussed below.

4.6.1 Biotic Factors

4.6.1.1 Plant Species

Phytoremediation techniques depend upon the suitable species that can accumulate heavy metals and produce higher biomass using established crop production and management practices (Rodriguez et al. 2005).

4.6.1.2 Plants Organs

Roots are important organs of the plants; they can absorb contaminants and bind to the cell wall or other macromolecules to prevent them from moving to other sensitive organs of the plant (Merkl et al. 2005). Zn and Cd get accumulated in the roots and the stem, while the accumulation of Cu was more in the leaves because the capacity of the roots gets exhausted due to the higher concentration of Cu in the wastewater (Rezania et al. 2015).

4.6.2 *Abiotic Factors*

4.6.2.1 pH

It is a very important abiotic factor controlling metal availability to the plant (Chen et al. 2015). Sanyahumbi et al. (1998) reported that Pb removal remained at approximately 90% between 10 °C and 50 °C and varied from 30% of the initial lead concentration at pH 1.5 to approximately 95% at pH values of 3.5 and 4.5. The impact of salinity on heavy metal uptake was investigated through *Potamogeton natans* and *Elodea canadensis*, and it was reported that metal removal efficiency increased with decreasing salinity and increasing temperature (Fritioff et al. 2005).

4.6.3 *Chelating Agents*

Chelating agents are commonly used to increase the bioavailability of heavy metals, accordingly enhancing their uptake by plants (Tangahu et al. 2011). Ethylenediaminetetraacetic acid (EDTA), a strong chelating agent and having strong complex formation capacity, has been widely used (Yen and Pan 2012). Phosphonates and phosphonic acids are also used as chelating agents in many applications, e.g., in paper, pulp, and textile industries and for heavy metals in chlorine-free bleaching solutions that could inactivate the peroxide (Gledhill and Feijtel 1992).

4.6.4 *Other Environmental Factors*

Climate is an important limiting factor for efficiency of phytoremediation at a particular site. Temperature is a key factor, affecting transpiration and growth metabolism, and ultimately leads to disruption of the plant's metal uptake capacity (Burken and Schnoor 1996; Bhargava et al. 2012). Removal efficiency of plants increases linearly with increasing temperature (Yu et al. 2011). The temperature affects the growth and consequently the length of the roots. The structure of the root under field conditions differs from that under greenhouse conditions (Merkl et al. 2005). Understanding mass balance analyses and the metabolic fate of contaminants in plants are the keys to maintain the applicability of phytoremediation (Mwegoha 2008). Metal uptake by plants depends on the bioavailability of the metal in the water, which in turn depends on the retention of the metal, as well as the interaction with other elements and substances in the water as well as on the prevailing climatic conditions (Tangahu et al. 2011).

4.7 Potential of Some Aquatic Macrophytes for Removal of Heavy Metals and Pesticides from Water

Macrophytes are a diverse group of photosynthetic organisms found in water bodies. They include bryophytes (mosses, liverwort, etc.), pteridophytes (ferns), and spermatophytes (flowering plants). Chamber et al. (2008) reported that macrophytes can be divided into seven different plant divisions: Spermatophyta, Pteridophyta, Bryophyta, Xanthophyta, Rhodophyta, Chlorophyta, and Cyanobacteria. Arber (1920) and Sculthorpe (1967) categorized macrophytes into four different categories depending on their growth forms.

1. Emergent macrophytes: Plants rooted in soil and also emerging to a significant height above water (e.g., *Typha latifolia*, *Phragmites australis*, *Sagittaria trifolia*, *Eleocharis*, *Cabomba aquatica*, *Polygonum hydropiper*, *Eleocharis plantagenera*, *Scirpus mucronatus*, *Alternanthera philoxeroides*).
2. Submerged macrophytes: Plants that grow below the surface of water and include a few ferns, numerous mosses, and some angiosperms (e.g., *Hydrilla verticillata*, *Ceratophyllum demersum*, *C. submersum Myriophyllum aquaticum*, *Elodea canadensis*, *Vallisneria americana*, *Utricularia vulgaris*, *Najas graminea*).
3. Free-floating macrophytes: Plants that are non-rooted to the substratum and float on the surface of the water (e.g., *Pistia stratiotes*, *Lemna gibba*, *Azolla pinnata*, *Salvinia molesta*, *Trapa natans*, *Eichhornia crassipes*, *Ipomoea aquatica*, etc.).
4. Floating-leaf macrophytes: Plants that are submerged or in sediment but with leaves that float with long flexible petiole on the surface (mainly include angiosperms, e.g., *Nymphea alba*, *Potamogeton crispus*, *P. natans*, *P. pectinatus*, *Nelumbo nucifera*, *Hydroryza aristata*). Boyd (1970), Stewart (1970), Wooten and Dodd (1976), and Conwell et al. (1977) were pioneers to demonstrate the pollutant removal potential of aquatic macrophytes. They considered these plants as important components of the aquatic systems in not only being food source, but because of the ability to act as effectual accumulators of heavy metals (Devlin 1967; Rai 2009; Deval et al. 2012; Sood et al. 2012).

Many scientists compared the efficiency of aquatic macrophytes for phytoremediation. Aquatic macrophytes absorb nutrients through their effective root systems. They are extensively used to remove nutrients, heavy metals, and pesticides from wastewater due to their relative fast growth rate and accumulation ability. Phytoremediation is an economic method with minimum maintenance and also helps in improving biodiversity. Several studies have revealed that aquatic plants are very effective in removing heavy metals and pesticides from polluted water (Khan et al. 2009; Yasar et al. 2013; Akter et al. 2014; Sasmaz et al. 2015). Discussed below are some important macrophytes which are potentially important for phytoremediation purposes.

4.7.1 Eichhornia crassipes

E. crassipes, commonly known as water hyacinth, is a rapidly growing aquatic macrophyte which can double its biomass in a few days and is one of the world's most troublesome weed. This quality has also made it an applicant for use in phytoremediation (Dhote and Dixit 2009). Many scientists proved that water hyacinth has high removal rates for various heavy metals like Fe, Zn, Cu, Cr, Mn, Hg, Cd, and As from aqueous solutions (Jadia and Fulekar 2009; Mohamad and Latif 2010; Priya and Selvan 2014; Rezania et al. 2015). The water hyacinths store metals in their bladders, followed by their translocation to stems, leaves, and roots (Rizwana et al. 2014). Mokhtar et al. (2011) used *E. crassipes* for the removal of Cd and Zn from water, as well measured the concentration of Cd and Zn absorbed in different parts of water hyacinth (leaves, roots, stem, and flowers). Ajayi and Ogunbayo (2012) studied the efficiency of *E. crassipes* in removing Cd, Cu, and Fe from water and found that transfer efficiency of Cd is more as compared to Cu and Fe. It was also investigated that this emergent plant is effective in removing mevinphos (insecticides) and ethion (phosphorus pesticides) from polluted water (Ramchandran et al. 1971; Wolverton 1975; Xia and Ma 2006).

4.7.2 Lemna

Lemna, commonly known as duckweed, is a free-floating macrophyte on the water surface. It is fast growing and adapts easily to various aquatic conditions and globally distributed in lakes, ponds, wetlands, and some effluent lagoons. It has been used to recover heavy metals since more than 30 years. Most of studies have been conducted with species *L. genus*, *L. minor*, and *L. gibba* (Guimaraes et al. 2012). The capacity of duckweed (*Lemna* sp.) to remove toxic heavy metals from water plays an important role in removal and accumulation of metals from contaminated water. *L. minor* can remove up to 90% of soluble Pb from water (Singh et al. 2011a, b). Sasmaz and Obek (2009) reported that the aquatic plant *L. gibba* was used for the accumulation of As, B, and U from secondary effluents as an alternative method for treatment. The results demonstrate that As was quickly absorbed by *L. gibba* in the first 3 days of the experimental study. Other studies on duckweed showed that an excess of Cu interferes in respiration, photosynthesis, pigment synthesis, and enzyme activity of the plants (Teisseire and Guy 2000; Prasad et al. 2001; Frankart et al. 2002; Babu et al. 2003). Olette et al. (2009) have found that *L. minor* can effectively accumulate pesticides, viz., copper sulfate (fungicide), flazasulfuron (herbicide), and dimethomorph (fungicide), from water bodies.

4.7.3 Typha

Typha is an ordinary wetland plant that belongs to family Typhaceae and grows widely in tropic and warm regions. Most of the studies have been done with the species *T. latifolia*, *T. angustifolia*, *T. domingensis*, and *T. angustata. T. latifolia* has a high capacity to transport heavy metals to its tissue. Therefore, it also tolerates higher levels of metals in its tissue without serious physiological damage. Dunbabin and Bowmer (2009) reported that metal concentrations increased in the order of roots > rhizomes > nongreen leaf > green leaf and found that the accumulation was highest in the roots and the green leaves had the lowest concentrations of Cu, Zn, Pb, and Cd. Chandra and Yadav (2010) also checked *T. angustifolia* for remediation potential of various heavy metals (Cu, Pb, Ni, Fe, Mn, and Zn) and resolved that it could be a possible phytoremediator for heavy metals from industrial wastewater under optimized conditions. Miglioranza et al. (2004) observed significant differences in the DDT level between root and shoot of *Typha* tissues, indicating the capability of the plant to uptake pesticide.

4.7.4 Azolla

Azolla is a small aquatic fern belonging to family Azollaceae with monotypic genus (Sood and Ahluwalia 2009). Azolla occurs in the symbiotic association with N_2 fixing blue, green alga *Anabaena azollae* (Mashkani and Ghazvini 2009; Sood et al. 2011). This fern can hyperaccumulate a variety of pollutants such as heavy metals and pesticides from aquatic ecosystems (Padmesh et al. 2006; Mashkani and Ghazvini 2009; Rai and Tripathi 2009; Sood et al. 2011). This fern has several features which prove it to be a better plant for phytoremediation, which include fast growth rate, nitrogen-fixing ability, and easy biomass disposal. Both living and dead biomass have been used for the removal of heavy metals (Rai 2008; Mashkani and Ghazvini 2009). Three species of water fern (*A. caroliniana*, *A. filiculoides* and *A. pinnata*) have been studied for heavy metal uptake from water. Rai (2008) reported that *A. pinnata* removed up to 70–94% of heavy metals (Hg and Cd) from chlor-alkali and ash slurry effluent in Singrauli region of UP (India). Deval et al.

(2012) concluded that *A. caroliniana* showed maximum efficiency toward the accumulation of Zn. Photosynthesis pigment of *Azolla* was also observed to increase under the influence of Zn and other contents of the effluents.

4.7.5 Hydrilla verticillata

H. verticillata is a submerged aquatic macrophyte that can grow on the surface and forms dense mats in water bodies. For removal of inorganic and organic contaminants, the whole plant plays an important role. Scientists explained that *H. verticillata* has strong appetite for As and Cd, but its appetite for Pb is not so strong (Ghosh 2010; Singh et al. 2011a, b, 2012, 2013). Dixit and Dhote (2010) studied Cr, Pb, and Zn uptake along with morphological changes in *H. verticillata* which indicate that uptake of metals is dose dependent.

4.7.6 Salvinia

Salvinia is a free-floating aquatic macrophyte of Salviniaceae family. It is widely distributed, having a fast growth rate and close relation with *Azolla* and *Lemna*. Genus *Salvinia* represents several species, i.e., *S. herzogii*, *S. minima*, *S. natans*, and *S. rotundifolia*, which show potential to remove various contaminants including metals from wastewaters (Nichols et al. 2000; Olguin et al. 2005; Sune et al. 2007; Sanchez-Galvan et al. 2008; Xu et al. 2009). *S. minima* is able to remove Ni, Cu, and As from water (Mukherjee and Kumar 2005; Rahman et al. 2009). Fuentes et al. (2014) indicated that *S. minima* are a hyperaccumulator of Ni, although higher concentrations may affect the physiological performance of the plant. Espinoza-Quiñones et al. (2008) demonstrated that *Salvinia auriculata* can be used as biosorbent for heavy metal removal from industrial effluents in wetlands.

4.7.7 Pistia

Pistia, commonly called as water lettuce, is a genus of aquatic macrophytes in the family Araceae. It floats on the surface of the water and roots are hanging beneath floating leaves. They are natural hyperaccumulators of many toxic heavy metals. Odjegba and Fasidi (2004) reported that *Pistia* is a potential candidate for the removal of Zn, Cr, Cu, Cd, Pb, and Hg. It accumulates Zn and Cd at high concentrations, whereas Hg is moderately accumulated and is poor in Ni accumulation (Guimaraes et al. 2012). Miretzky et al. (2004) mentioned that the percentage of removal by *P. stratiotes* was very high (>85% for Pb, Cr, Mn, and Zn). They also explained that it can almost completely eliminate the metals in the first 24 h of exposure. Prasertsup and Ariyakanon (2011) investigated potential of *P. stratiotes* for removal of chlorpyrifos (organophosphate pesticide) under greenhouse conditions and found it to remove the pesticide by 82% from water. Recently, Kumar et al. (2019) also reported the efficient removal of Cu^{2+}, Fe^{3+}, and Hg^{2+} from aqueous solutions by *P. stratiotes*.

4.7.8 Ipomoea aquatica

I. aquatica belongs to the family Convolvulaceae, originated in China, and is usually consumed as a green leafy vegetable. It is mostly found in southern Asia, India, and southern China. Chen et al. (2009) investigated that *I. aquatica* can remove Cr (III) from aqueous solution in the presence of chelating agent EDTA and chloride. Chloride can increase the solubility of Cr and enhance the bioaccumulation in shoots and roots of the plant. Gothberg et al. (2002) estimated the accumulation of Pb, Cd, Hg, and methyl mercury in *I. aquatica*. However, concentrations of Hg were higher in leaves than in stems. Chi et al. (2008) observed that accumulation of di-*n*-butyl phthalate (phthalic acid esters) in five different genotypes of *I. aquatica* with their potential of phytoremediation.

4.7.9 Myriophyllum

Myriophyllum is a submerged perennial macrophyte, found in stagnant and slow-moving waters in the southern hemisphere. Several studies on heavy metal biosorption ability of species *M. spicatum*, *M. triphyllum*, and *M. aquaticum* have been done. This is applied for biomonitoring and water purification by accumulating heavy metals in their tissues (Ngayila et al. 2007). Accumulating capacity of this plant is higher due to rhizomatous stem that are able to capture pollutants from water (Orchard 1981). Grudnik and Germ (2010) used it as indicator for pollution by metals in lake and reported the concentrations of metals in *M. aquaticum* were higher than other plants indicating the concentrations of the metal pollutants in the lake. Harguinteguy et al. (2016) showed positive correlation between Co, Cu, Mn, and Zn concentration in water and leaves of *M. aquaticum.*

4.7.10 Phragmites australis

P. australis is an emergent aquatic macrophyte commonly called as reed. They are grown under extreme environmental conditions in presence of nutrients and organic carbon (Quan et al. 2007; Bonanno and Giudice 2010). The root of this plant accumulates higher quantity of heavy metals in the cortex parenchyma cells with large intracellular air space (Sawidis et al. 1995). Bonanno and Guidice (2010) studied the heavy metal accumulation in *P. australis* organs and also evaluated its suitability for biomonitoring. Concentration of heavy metal in aboveground parts depends largely in growing season; particularly accumulation may increase simply at the end of the growing season (Brogato et al. 2009). Highest metal accumulation was recorded in roots and shoots in September and April, whereas leaves expressed higher value in February (Salman et al. 2015). Bananno and Guidice (2010) explained that the root of *P. australis* acts as a filter for Cu because it accumulates 70% (in roots). So, this filter effect is the most effective strategy for protection of shoots and roots from Cu-induced injuries. According to the recent studies, *P. australis* has many benefits, such as good growth, worldwide distribution, and high levels of heavy metal tolerance (Salman et al. 2015).

4.7.11 Ceratophyllum demersum

C. demersum is a submerged aquatic macrophyte which can grow in low light and muddy water, may be oligotrophic or eutrophic. Various studies of the phytoremediation have shown that *C. demersum* is effective for accumulation of heavy metals and pesticides (Krems et al. 2013; Guo et al. 2014; Chen et al. 2015). This plant has positive adaptive strategy in response to heavy metals and pesticides in in situ studies (Borisova et al. 2014). Rai et al. (1995) reported that *C. demersum* was able to remove >70% Pb from pond water in 15 days. Abdallah (2012) explained that chlorophyll is an important factor which is sensitive to heavy metal concentration. A decrease of chlorophyll proves the toxic nature of Cd, which interacts with –SH group of enzymes involved in chlorophyll synthesis. According to Saygidegs and Dogan (2004), *C. demersum* accumulated more Pb than *L. minor* and chelating agent EDTA has the ability to increase bioavailability of Pb to increase accumulation in plants. Guo et al. (2014) reported that organochlorine pesticides hexachlorocyclohexae (HCH), DDT, aldrin, dieldrin, endosulfan, etc. are accumulated in *C. demersum* tissues.

4.8 Management, Treatment, and Disposal of Phytoremediating Aquatic Macrophytes

It has been validated by various scientists that phytoremediation is a cost-effective and eco-friendly technology for rehabilitation of polluted environments as compared to conventional methods, but it has its own drawbacks (Rahman et al. 2009; Sood et al. 2012; Emmanuel et al. 2014; Sharma et al. 2015). For example, plant growth and biomass production are good, but seasonality and poor tolerance are constraints of the technology, and affective process should involve regular harvesting and disposal of macrophytes since they will decompose and release heavy metals back to the environment (Rai 2008). Only accumulation of metals in macrophytes is not enough implementation of this emerging technique. The proper disposal of these macrophytes after phytoremediation is very essential; otherwise, these macrophytes will act as another source of pollutants in the environment. There are several processes by which phytoremediating plants can be converted into economically beneficial material.

4.8.1 Biogas Production

Biogas is a clean and environmentally friendly fuel formed by the anaerobic digestion of organic wastes, i.e., animal dung, vegetable wastes, municipal solid wastes, and industrial wastes (Weiland 2010). Anaerobic digestion is a biological process in which organic matter is degraded in the absence of oxygen. The biogas generated can be used directly for various purposes, i.e., cooking, heating, or production of electricity. There is a comprehensive literature significantly describing the use of aquatic plants used as a potential store for biogas production due to high quantity, high carbon-nitrogen ratio, and good content of fermentable materials. *Eichhornia*, *Pistia*, *Typha*, and *Trapa* can be degraded easily and produce high biogas yields (Elhaak et al. 2015). Singhal and Rai (2003) showed the use of *E. crassipes* and *Panicum hemitomon*, for phytoremediation of industrial effluents and subsequent production of biogas.

4.8.2 Ethanol Production

Ethanol is a liquid fuel which can be produced from phytoremediating aquatic macrophytes through hydrolysis and fermentation which can make them a good substrate as well. Hydrolysis and fermentation require fermentable sugars, which may be available in very small amounts in aquatic plants, so pretreatment is necessary for making sugar more easily available for chemical hydrolysis (Gunnarsson and Petersen 2007). Scientists generally follow three steps for production of ethanol from aquatic plants. In the first step, the cellulase enzyme was produced by the isolation and qualitative screening of microorganisms in the excreta of cow, pork, goat and municipal waste. However, this enzyme has also been produced by the addition of dry aquatic plants and micro-organisms. In the last step, ethanol is produced through fermentation process by hydrolysis of cellulose present in aquatic plant by the fermenting organism (Randive et al. 2015; Patel and Patel 2015). Rezania et al. (2015) studied the use of barley and malt extract enhancer for ethanol production from *E. crassipes* and *P. stratiotes* and found that use of these substrates increases the production.

4.8.3 Incineration

Incineration is the combustion of waste material in the presence of oxygen. In this process the phytoremediating aquatic plants may be used for making charcoal and the by-products can be used as a fuel (Rahman and Hasegawa 2011). Sun drying and direct burning product of water hyacinth are used as fertilizer on a small scale in certain parts of the world (Gunnarsson and Petersen 2007).

4.8.4 Composting and Vermicomposting

Compost improves soil nutrient and structure; hence, it can be an option for management of harvested macrophytes in developing countries, where chemical fertilizers are not affordable. Macrophytes contain nutrients like nitrogen, phosphorus, and potassium, and converting them into compost takes less than 30 days (Newete and Byrne 2016). This makes it feasible for farmers for improving soil condition by swiftly utilizing the waste converted to compost. Hussain et al. (2016) formed vermicompost by using *Salvinia* and *Eisenia fetida* and concluded that it is an effective technique to convert *Salvinia* into value-added product.

4.8.5 Other Uses

Many macrophytes such as *Eichhornia*, *Typha*, and *Cyperus* have been directly collected from experimental sites and used for making mats, hats, bags, baskets, and spoon holders in weaving industries. Stem of *Scirpus grossus* is used in manufacturing of hard rope and fine mats. These plants also reduce wave action impacts and hold the bottom sediments more efficiently which helps to reduce turbidity and suspension of nutrients bound in the sediment.

4.9 Conclusion

Since contamination of water by toxic heavy metals and pesticides is a serious environmental problem, therefore, effective remediation methods are necessary. Conventional methods for clean-up and restoration of heavy metals and pesticides from contaminated water have limitations like high cost and creation of secondary pollutants. Phytoremediation is a promising technology that can become a reliable, efficient alternative for remediation of contaminated water. Plants can take up heavy metals and pesticides by their roots, stems, and leaves and accumulate them in organs. The knowledge of several factors which affect the uptake mechanisms of heavy metals, like plant species, addition of chelating agents, and physical and climatic conditions can help in improving the efficiency of the process. It is now proven that many aquatic plants such as *Eichhornia*, *Pistia*, *Lemna*, *Salvinia*, *Typha*, and *Hydrilla* are capable of accumulating heavy metals and pesticides. The roots of these plants naturally absorbed heavy metals from water. Accumulation and

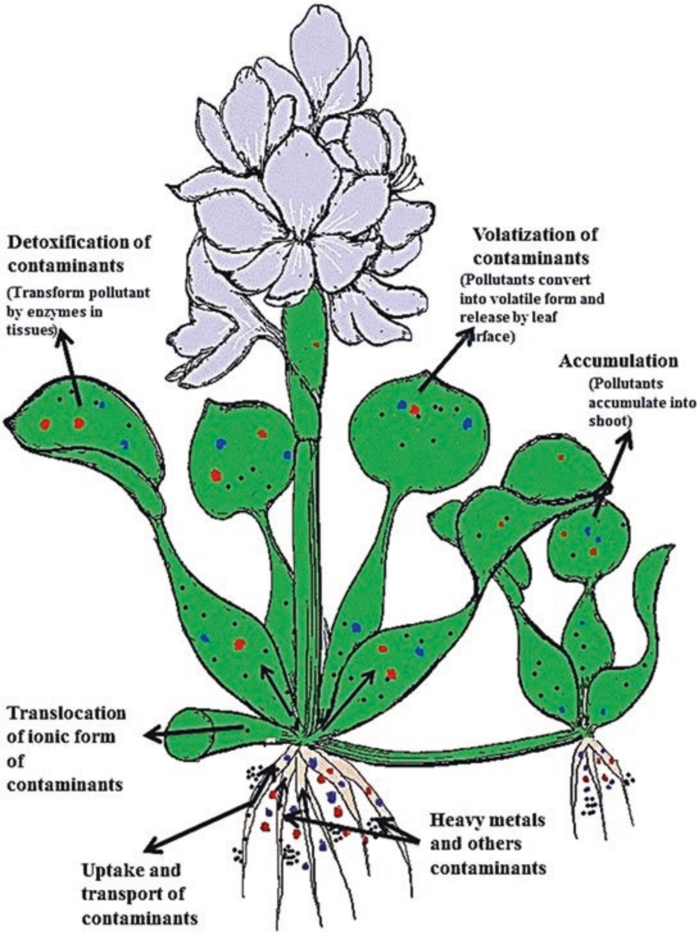

Fig. 4.2 Phytoremediation mechanism and its techniques in plant tissues

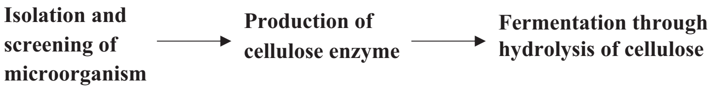

Fig. 4.3 Flow diagram for the three steps for production of ethanol from aquatic plants

remediation of heavy metals and pesticides are not enough for implantation of phytoremediation. Management, and treatment of the end product, i.e., the biomass is also a major concern. Some studies have now shown that there is possibility to use macrophytes' biomass for production of biogas, bioethanol, etc. This can pave the way for effective utilization of this technology for cleaning the contaminated sites by an eco-friendly and effective approach (Figs. 4.2 and 4.3).

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Chapter 5
Plant Growth-Promoting Rhizospheric Microbes for Remediation of Saline Soils

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Abstract Salinity is responsible for lowering the quality of soil and reducing the agricultural productivity, continuously increasing the area of marginal lands across the globe. Among the biological solutions, application of rhizobacteria has emerged as the effective strategy for promoting plant growth and enhancing the fertility of saline soil. Various direct and indirect plant growth-promoting attributes including phytohormones, exopolysaccharides, osmolytes, antioxidants, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase production help to combat the negative impact of salinity on plants. Indirect mechanisms such as synthesis of hydrogen cyanide (HCN), antibiotics and hydrolytic enzymes help in inhibiting the growth of phytopathogens in saline conditions. These beneficial microbes also improve the structure and quality of soil, rejuvenating their productivity index. Henceforth, the identification and selection of halotolerant microbes and studying the mechanisms involved in salt tolerance would provide a better future in designing amelioration strategies for saline soils.

Keywords Salinity · Rhizospheric microbes · PGPR · Rhizoremediation

5.1 Introduction

Soil salinity is a major issue across the globe increasing by the day in irrigated, semiarid, and arid regions. Salinity has degraded almost one billion hectares of land across the globe, siphoning the fertility and productivity of soil leading to substantial loss of crop productivity (FAO 2015). The scenario is worsening in arid and semiarid areas where each day 7.7 square miles of land is lost due to salinization (Technical Report on Soil Degradation 2000). The impact of global climate change,

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irrigation with low-quality waters, use of chemical pesticides/fertilizers, and traditional farming techniques with poor drainage has increased the exposure of land to salinity stress (Egamberdieva and Lugtenberg 2014). The salinity losses in irrigated areas of about \$ 11.4 billion are much higher than those in non-irrigated areas (\$ 1.2 billion), and the cause is inappropriate irrigational methods (Zhu et al. 2005). Peaking figures of salinization were observed since 1945 and the reason being injudicious use of chemical pesticides and fertilizers during green revolution; till date 17% of agricultural land has been lost to salinity (Mishra et al. 2018). Arora et al. (2018a) reported the positive correlation coefficient (r) of 0.928 for human population and salinity and of 0.9996 between CO_2 and salinity, clearly describing the influence of human-induced factors in increasing salinity stress across the globe. The stats show that if amelioration actions are not planned now, then about 50% of global area would be under the menace of salinity by 2050 (Bartels et al. 2005).

Saline soils have been classified on the basis of their electrical conductivity (ECe) and soils having ECe >4.0 dS m^{-1} are categorized as salt affected (Rengasamy 2006). Saline soils are characterized by low structural stability, low water holding capacity, poor infiltration rate, and loss of beneficial microbial population (Tanji 2002). The major accumulated salt constituents are Na^+, Ca^{+2}, Mg^{+2}, Cl^{-1}, SO_4^{-2}, and CO_3^{-2}, and their varying concentrations define the extent of salinization in soils (Volkmar et al. 1998). Among these mobile salts, Na^+ causes maximum toxicity in plants and impairs their health and productivity (Khorasgani et al. 2017). The negative impacts of salt on plants include osmotic imbalance, reduced photosynthesis, low nutrient uptake, impaired nitrogen fixation, poor root and shoot growth, inhibition of seed germination, low water retention, cell plasmolysis, cell membrane flaccidity, increased ethylene production, stomatal closure, and so on (Arora et al. 2016, 2018a). The detrimental responses on legume nodulation due to salinity reported by Arora et al. (2018b) showed that in saline regions of Kanpur, Uttar Pradesh, 80% of pigeon pea was either not nodulated or poorly nodulated. Thus, poor cultivation in such fields increases the area of marginal lands and the unbound salty soil are exposed to wind and water erosion, further affecting the adjoining areas.

Traditional physical and chemical methods of saline soil reclamation are unsustainable and cost-ineffective approaches. These techniques thus cannot be regarded as large-scale remediation strategy particularly by farmers of poor or developing countries. Physical methods including drainage of excess soils by construction of canals, subsoiling, plowing, etc. are far more expensive (Raychev et al. 2001). Similarly, application of chemicals causes ecological imbalance and loss of beneficial microbes which would otherwise support the growth of plants and fertility of soil. Contradicting to these unsubstantial strategies, use of rhizospheric microbes has been emerging as a cost-effective and sustainable solution in remediating saline soils and rejuvenating their fertility and productivity. The market of rhizoremediation is increasing at an exponential rate with estimated value of US\$ 32.2 billion in 2016 and expected to reach US\$ 65.7 billion by 2025 at a CAGR of 8.3% from 2017 to 2025 (Transparency Market Research 2018; Arora et al. 2018a). The beneficial microbes commonly referred to as plant growth-promoting microbes/bacteria (PGPM/PGPR) survive in the vicinity of plant rhizosphere and form profitable association with plants increasing their growth and also enriching the rhizoplane under hospitable and inhospitable conditions such as salinity (Rodriguez and Redman 2008). PGPM provide the plants and soil with nutrients, water, and other essential factors to combat the adversity of salt stress and improve the structure of soil and plant health. Rhizoremediation of saline soil involves the consecutive application of these beneficial microbes improving the soil quality through aggregation of soil particles trapping water and nutrients, protecting from erosion and top soil loss, and also promoting cultivation on these fields by stimulating plant growth. Various studies denote increase in plant growth and productivity upon inoculation with PGPM under salt stress conditions. However, large-scale application of these microbes is still in introductory stage. The potential of salt-tolerant PGPM for remediation and increasing the productivity of saline soils is discussed in this review.

5.2 Beneficial Rhizospheric Microbes and Their Role in Rhizoremediation of Saline Soil

Rhizoremediation is defined as the sustainable approach for ameliorating polluted or stressed soils using population of rhizospheric microbes along with their symbiotic partner which are either indigenously present in the soil or are applied externally to restore its quality (Anderson et al. 1993; Schwab and Banks1994). The rhizosphere of plants is enriched with a nexus of beneficial free-living or symbiotic microbes known as PGPM which thrive as integral part of plant root and exert useful activities regulating healthy plant-microbe interactions. The advancing research beginning with classical study of legume-rhizobia interactions has now denoted the action of various PGPM in combating the stress and establishing rhizoremediation. PGPM follow various direct and indirect mechanisms in supporting plant growth under stressed conditions to dilute the negative responses of salinity in plants (Fig. 5.1). The primary action of PGPM is investigated as their role in maintaining osmotic balance, ion homeostasis, and turgor pressure to combat the toxicity of salinity in plants (Ilangumaran and Smith 2017). The other mechanisms involve the initiation of complex cascade of events maintaining the plant-microbe interactions, stress alleviation, and plant growth promotion (Smith et al. 2017). Table 5.1 shows the plant growth-promoting traits of microbes involved in salt stress amelioration and their role in promoting the plant growth. The subsequent sections mention the role and action of PGP attributes in mitigating salinity stress and improving the quality of soil.

5.2.1 Phytohormone Production

Abnormal incitation of phytohormone production in plants under salt stress highly depresses their growth. Contrasting the scenario, the application of salt-tolerant PGPM stimulates the source-sink communication by evoking hormonal signaling of

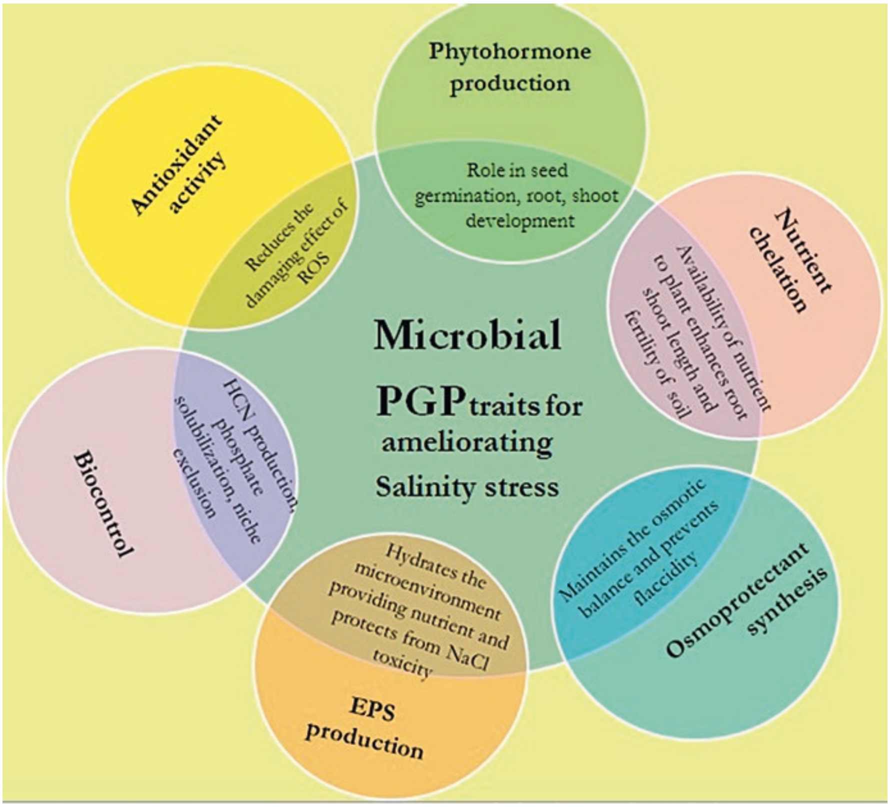

Fig. 5.1 Plant growth-promoting traits of microbes and their role in ameliorating salinity stress

various classes of plant hormones including indole acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and cytokinins, supporting the increased root and shoot length, root surface area, root hair tips, shoot length, shoot biomass, enhanced stomatal conductance, etc. (Dodd and Pérez-Alfocea 2012; Khare et al. 2018).

Production of auxin derivatives like IAA, is a very common attribute of PGPM which counteracts the salinity stress in plants by activating the radicular systems, elongating the apical meristem, increasing root and shoot length, thereby coordinating root and shoot responses to environmental stress stimuli (Sachs 2005). IAA also acts as negative signal suppressing cytokinin synthesis and transportation which increases root elongation (Nicolás et al. 2004). Correspondingly, Sadeghi et al. (2012) found that inoculation of wheat with *Streptomyces* promoted growth by enhanced synthesis of IAA in the presence of salts. Similarly, Orhan (2016) concluded that halotolerant and halophilic PGPR increased the growth parameters of wheat by IAA production under salt stress. Nadeem et al. (2016) denoted the mitigation of salinity stress in *Cucumis sativus* by *Pseudomonas fluorescens* through increased accumulation of IAA promoting root and shoot length and total plant

Table 5.1 Salt-tolerant microbes and their role in promoting growth of associated plants under salinity stress

S. no.	Microbes	Associated plants	Mechanism of action	Plant responses	Reference
1.	*Curtobacterium flaccumfaciens* E108	*Hordeum vulgare*	Phosphate solubilization, IAA production	Increased salt resistance, water accumulation in leaf and stem, germination rate, and other growth parameters	Cardinale et al. (2015)
2.	*Lactobacillus* sp., *Azotobacter chroococcum*, and *Pseudomonas putida*	*Raphanus sativus, Lactuca sativa*	IAA production	Higher plumule and radicle lengths, germination rate, and early germination	Hussein and Joo (2018)
3.	*Bacillus alcalophilus, Bacillus thuringiensis,* and *Gracilibacillus saliphilus*	*Arthrocnemum macrostachyum*	IAA and siderophore production, phosphate solubilization, nitrogen fixation, ACC deaminase activity	Improved phytoextraction capacity, increased germination rate, osmotic adjustment, stomatal regulation, and enhanced uptake of minerals	Navarro-Torre et al. (2017)
4.	*Bacillus endophyticus, Bacillus tequilensis, Planococcus rifietoensis, Variovorax paradoxus,* and *Arthrobacter agilis*	*Salicornia europaea*	IAA, ACC deaminase, production, phosphate solubilization	Increased germination percentage, root shoot length, and fresh weight	Zhao et al. (2016)
5.	*Virgibacillus marismortui, Virgibacillus pantothenticus, Bacillus clausii*	Wheat	Ammonia, IAA and ACC deaminase production, gibberellic acid producer, phosphate solubilizer, nitrogen fixation	Increased plant stress tolerance level, higher yield, better root growth, and development	Torbaghan et al. (2017)
6.	*Planococcus rifietoensis*	Wheat	IAA, ACC deaminase production, phosphate solubilization	Improved growth and stress tolerance level of wheat plant	Rajput et al. (2013)

(continued)

Table 5.1 (continued)

S. no.	Microbes	Associated plants	Mechanism of action	Plant responses	Reference
7.	*Cunninghamella elegans*	Cowpea	Chitosan (natural polymer) and phosphate production (chelating phosphorous), nitrogen fixation	Increased NPK content in soil and plants, stimulated plant growth and yield	Berger et al. (2013)
8.	*Glutamicibacter halophytocola* strain KLBMP 5180	*Limonium sinense*	Synthesis of proline, antioxidant enzymes, flavonoids, ACC deaminase, IAA gibberellin, abscisic acid, brassinosteroid, jasmonic acid, and salicylic acid production	Increased stress tolerance, chlorophyll content, K^+ concentration, and reduced Na^+ concentration	Qin et al. (2018)
9.	*Halomonas sp.*	*Salicornia strobilacea*	IAA, siderophore, EPS production, phosphate solubilization	Increased plant growth parameters	Marasco et al. (2016)
10.	*Sphingomonas* sp. LK1, *Sphingomonas* sp. LK11	*Solanum lycopersicum*	Production of gibberellins	Increased root shoot length and biomass	Halo et al. (2015)
11.	*Brevibacterium linens RS16*	*Oryza sativa*	Induction of various primary and secondary metabolic pathways combating salinity stress	Increased photosynthetic activity	Chatterjee et al. (2018)
12.	*Pseudomonas anguilliseptica SAW 24*	*Vicia faba L.*	Exopolysaccharide production and biofilm formation	Elevated plant height and fresh and dry weight	Mohammed (2018)
13.	*Enterobacter asburiae, Moraxella pluranimalium, Pseudomonas stutzeri*	*Triticum aestivum L*	Synthesis of auxin and ACC deaminase	Increased number of tillers, grain weight, and yield	Raheem and Ali (2015)
14.	*Bacillus spp.*	Wheat	IAA, lipase, protease, siderophore production, P solubilization	Increased germination rate, root length and weight, and panicle weight	Ravari and Heidarzadeh (2014)
15.	*Arthrobacter protophormiae (SA3) and Dietzianatrono limnaea (STR1)*	Wheat	Enhanced auxin level and decreased ACC and ABA levels	Increased growth parameters and stress tolerance of plant	Barnawal et al. (2017)

biomass. Recently, Mishra et al. (2017) depicted the concept of growth promotion under salt stress by PGPR *Pseudomonas sp*. through IAA production in *Zea mays*.

GA is another plant growth regulator inducing various physiological responses and promoting plant growth by increasing cell proliferation and elongation (Seckin et al. 2009). The improvement in plants against salt toxicity contributed by GA producing PGPM involves chlorophyll synthesis, antioxidant activity, preventing lipid peroxidation, enhanced photosynthetic rate, root and shoot length, and plant biomass (Maggio et al. 2010). Khan et al. (2011) reported that under 70 and 140 mM salt stress, soybean plant inoculated with GA-producing endophyte *Aspergillus fumigatus* sp. LH02 promoted shoot length, shoot fresh and dry biomass, chlorophyll content, photosynthetic rate, and leaf area, alleviating salinity stress in comparison with intolerant non-inoculated plants. *Penicillium janthinellum* LK5, an endophytic habitant of *Solanum lycopersicum* Mill (tomato plant), produced bioactive gibberellins GA3, GA4, and GA7 and improved growth of rice seedlings and tomato, reducing sodium toxicity (Khan et al. 2013). *Burkholderia cepacia* SE4, *Promicromonospora* sp. SE188, and *Acinetobacter calcoaceticus* SE370 mitigated the salinity stress through production of GA in *Cucumis sativus* (Kang et al. 2014).

ABA, like other phytohormones, is also responsible for stress adaptation and growth promotion. ABA plays a major role in inducing and mediating signals responding to expression of stress-associated genes (Sah et al. 2016). ABA also improves root growth, water content by stomatal closure, and protection against oxidative damage caused by salinity (Cutler et al. 2010). *Dietzia natronolimnaea* STR1, a halotolerant strain, showed elevated salinity tolerance level in wheat *via* initiation of ABA signaling cascades upregulating various stress-responsive genes (Bharti et al. 2016). ABA production by *Bacillus licheniformis* and *P. fluorescens* aided in growth promotion of grapevine under water and salt stress (Salomon et al. 2014). Similarly, Shahzad et al. (2017) concluded that production of ABA by *Bacillus amyloliquefaciens* RWL-1 under both stressed and non-stressed conditions increased root and shoot growth and SA concentration in rice.

Cytokinins are purine derivatives involved in differentiation of root callus and shoot formation, maintaining the totipotent stem cells in root and shoot meristem (Howell et al. 2003; Leibfried et al. 2005; Numan et al. 2018), enhanced root hair formation and embryo vasculature formation, and promoted seed germination, chlorophyll content, leaf expansion, nutrient signaling, etc. (Amara et al. 2015; Jha and Saraf 2015; Wong et al. 2015; Goswami et al. 2016). Cytokinin production by PGPM interlinks with plant growth promotion. López-Bucio et al. (2007) found that cytokinin receptors in *Bacillus megaterium* UMCV1 had role in improving biomass of bean and *Arabidopsis thaliana* under stressed conditions. Egamberdieva (2009) reported rice growth elevation by up to 52% in comparison with control by inoculation with cytokinin producing strains, viz., *Pseudomonas aurantiaca* TSAU22, *Pseudomonas extremorientalis* TSAU6, and *P. extremorientalis*TSAU20 under stress conditions.

Thus, the upregulation of phytohormones' levels by salt-tolerant PGPM induces the growth and enhances the level of tolerance in plants. The developed root and shoot systems increase the level of nutrients in plants and henceforth check the impact of salinity.

5.2.2 Nutrient Acquisition

The toxicity of higher level of salts, Na^+/Ca^{2+}, Na^+/K^+, Na^+/Mg^{2+}, Cl^-/NO_3^-, and $Cl^-/H_2PO_4^-$ causes nutritional imbalance, decreased infiltration, and thereby low uptake of nutrients by plants (Chaichi et al. 2016; Mishra et al. 2018). Biological nitrogen fixation is the most important biochemical process which regulates the availability of nitrogen to plants and defines the fertility of soil. The mechanism of symbiotic (by rhizobia) or free-living nitrogen fixation (by *Azotobacter, Acetobacter, Azospirillum, Burkholderia, Enterobacter, Pseudomonas,* and cyanobacteria) controls the nitrogen content, hydration capacity of soil, and plant growth and thereby can be a reclamation strategy for environmentally stressed soils (Verma et al. 2019). Although salinity negatively influences the population and distribution of rhizobia, yet there have been reports where several salt-tolerant rhizobia have been associated with various legumes including *Pisum sativum*, *Vicia faba* (Cordovilla et al. 1999), and *Phaseolus vulgaris* L. (Fukami et al. 2018) and non-legumes like *Lactuca sativa* L. (Han and Lee 2005). The inoculation of these salt-tolerant rhizobia increased nitrogen-fixing ability and nodulation and revived the lost nutrient status of arid soils confirming reclamation for future use (Zahran 1999). Mashhady et al. (1998) found that *Rhizobium meliloti* successfully formed nodules in *Medicago sativa* under saline conditions (100 mM NaCl). Similarly, Garg and Singla (2005) showed increase in number of nodules and biomass in chickpea upon inoculation with salt-tolerant *Mesorhizobium ciceri.* Kumar et al. (1998) reported salt-tolerant rhizobia nodulating *Acacia catechu* and found that there was no reduction in nitrogenase activity from 0 to 50 mM NaCl. Arora et al. (2000) mentioned the growth promotion and nodulation of *Mucuna pruriens* even at 850 mM NaCl, when inoculated with *R. meliloti.* Zahran et al. (1997) reported that *Azotobacter*, a free-living nitrogen fixer, can fix nitrogen even at 5–10% NaCl levels. Relatively, Silini et al. (2016) elucidated the positive impact of *A. chroococcum* AZ6 on growth parameters of durum wheat under saline conditions. Khalid et al. (2017) recently reported the mitigation of salinity stress in *Trifolium repens* by inoculation with *A. brasiliense* and increased root shoot height, dry and fresh biomass, leaf area, and chlorophyll content in comparison with control. The potentiality of *Azotobacter* and *Azospirillum* as salinity ameliorating candidates confirms them as biofertilizers for agriculture in saline soils (Akhter et al. 2012).

Phosphate is another limiting nutrient in saline soils and in stressed plants, causing abnormal growth and disturbed physiological and developmental processes. Microbes have been found to be efficient chelators of phosphate, availing them to plants for their growth and development and rejuvenating the fertility index of saline soils. Microbes converting the insoluble complex phosphates to soluble forms are known as phosphate solubilizing microorganisms (PSMs). Application of PSMs minimizes the use of chemical fertilizers in saline agricultural soils thereby offering a better sustainable solution. The mechanism behind phosphate chelation involves acidification, chelation, ion exchange reactions, production of organic acids (gluconic acids, citric acid, oxalic acid), and enzymes like acid phosphatases (Numan et al. 2018). There are reports signifying the isolation and application of salt-tolerant PSMs to enhance the quality of soil and growth of plants under stressed environment. Saghafi et al. (2018) showed the efficacy of *Rhizobium* strains in promoting growth of *Brassica napus* L. under salt stress by increasing the phosphorous content in shoot by about 24% as compared to control. Similarly, Hahm et al. (2017) found that phosphate solubilizing strains *Microbacterium oleivorans* KNUC7074 and *Rhizobium massiliae* KNUC7586 accounted for overcoming the salt stress responses in *Capsicum annum* L. and promoted their growth in comparison to un-inoculated plants.

Iron (Fe) is also a limiting factor in defining the growth and productivity of soil and plants under salt-stressed conditions (Turan et al. 2012). To increase the uptake by plant's root and shoot, microbial systems synthesize low molecular weight compounds known as siderophores which chelate the soluble form of Fe from insoluble complexes (Arora and Verma 2017). The growth promotion attribute of siderophore-producing strains was observed by Panwar et al. (2016), concluding that application of halotolerant, siderophore producing *Enterococcus* and *Pantoea* sp. strains on salt-stressed mungbean (salinity condition 5 and 10 dS/m) improved plant growth and decreased NaCl uptake. Similarly, Mahmood et al. (2016) showed the outstanding performance of strain *Bacillus drentensis* P16 over strain *Enterobacter cloacae* P6 under salt stress condition due to the property of siderophore production, thereby significantly improving the growth of stressed mungbean. Sadeghi et al. (2012) showed that siderophore producing strain of *Streptomyces* increased the growth of wheat under saline conditions. Chakraborty et al. (2011) reported siderophore production by highly salt-tolerant (up to 10% NaCl) strain *Bacillus cereus* S4 which increased the growth of *Cicer arietinum, Vigna radiata*, and *Oryza sativa* under salt stress.

Zinc (Zn) is an essential micronutrient found in the range of 10–300 ppm in the soil (Lindsay 1992). Zn is required by plants for various metabolic processes including photosynthesis, disease resistance, gene expression, enzyme activity, protein synthesis, etc. About 50% of soils are reported to be Zn-deficient (Review 2008). The nutrient-limited condition in saline soil further worsens the scenario and affects the growth of plants. The application of Zn solubilizing rhizospheric microbes promotes the plant growth by availing the deficient nutrient, thereby promoting their survivability under saline stress conditions. Sapre et al. (2018) reported plant growth promotion in oat seedlings upon inoculation with zinc solubilizing strain *Klebsiella sp*. IG 3 under salt stress. Mumtaz et al. (2017) summarized biofortification in maize using Zn solubilizing *Bacillus spp.* as potential candidates. Ramesh et al. (2014) found *Bacillus aryabhattai* strains MDSR7 and MDSR14 as efficient biofortifying agents supporting increased concentration of Zn in soybean and wheat. Hence it can be concluded that the application of these nutrient chelators can be a sustainable and cost-effective solution in reclamation of saline soils reviving their fertility.

5.2.3 Osmoprotectants/Osmoregulants/Osmolytes/Compatible Solutes

For defending the osmotic imbalance, diluting the toxicity of ions like Na^+ and Cl^-, and effluxing the essential ion K^+, PGPM are known to accumulate low molecular weight intra-cytoplasmic soluble sugars (sucrose, trehalose, gentiobiose, maltose, cellobiose), heterosides (glucosylglycerol and mannosucrose), amino acids (alanine, threonine, serine, aspartic acid), quaternary amines (lycine betaine, carnitine), polyols (glycerol, arabitol, and inositol), and tetraydropyrimidines (ectoines) (Qurashi and Sabri 2013; Nabti et al. 2015). The roles of these compatible solutes is to maintain the osmotic equilibrium across the membrane, stabilize the turgor pressure and cell osmotic potential, ensuring the correct protein folding, stabilize biomolecules (DNA, membranes, enzymes) and alleviating NaCl toxicity (Lacerda et al. 2005; Paul and Lade 2014). The transport of osmoprotectants involves specific uptake systems, stretched activated channels, aquaporins, and efflux systems. These accumulated osmoprotectants are liberated into the surrounding environment where they are taken up by plants through active transport in exchange of energy, mitigating the osmotic stress (Miller and Wood 1996). Thus, this transfer of osmolytes from producers (microbes) to consumers (plants) involves a series of cycles so that plants which are unable to synthesize them de novo can uptake and improve their growth under inhibitory conditions (Arora et al. 2013). According to Paul (2013), glycine betaine or trimethylglycine is the preferred osmolyte for most of the bacteria. *Halomonas elongate* upon high and low osmotic shocks caused the biosynthesis of osmoprotectants which were released into the environment from where they were taken up by crops, such as potato, wheat, barley, and tobacco, transferring the halotolerance (Ashraf 2009; Bougouffa et al. 2014). Chen et al. (2007) claimed the salt tolerance in transgenic *A. thaliana* impregnated with proBA genes derived from *Bacillus subtilis* initiating proline synthesis. Similarly, Nautiyal et al. (2013) confirmed the modulation of osmotic and stress-responsive genes in rice by inoculation with *B. amyloliquefaciens* SN13. Overexpression of trealose-6-phosphate in *Phaseolus vulgaris* was observed by co-inoculation with *Rhizobium tropici* and *Paenibacillus polymyxa* strains which thereby increased the nodulation, N content, and plant growth under salt stress. The microarray analysis of nodules suggested that upshift of stress-responsive genes was because of the uptake of extracellular trehalose accumulated by bacteria (Figueiredo et al. 2008). Jha et al. (2011) reported that inoculation of *Oryza sativa* with endophyte *Pseudomonas pseudoalcaligenes* and rhizospheric microbe *B. subtilis* showed better stress responses by increasing the intracytoplasmic accumulation of osmoprotectants. Lópéz-Leal et al. (2014) found that *Rhizobium etli* uses *tre*YZ pathway synthesizing sucrose and trehalose during salt stress conditions.

The adverse effects of salinity also increase the chances of destabilization of membranes, desiccation, defoliation, impacting nodulation, photosynthesis, and other physiological processes. The application of PGPM improves the growth of plants by accumulating compatible solutes which restricts the influx of Na^+ or Cl^-

and stabilizes the intracellular ion level. Correspondingly, Rojas-Tapias et al. (2012) declared that there was significant decrease and increase of Na^+ and K^+ ions, respectively, in *Zea mays* upon inoculation with *Achromobacter chroococcum* C5 under salt-stressed environment. Similarly, increased accumulation of free amino acids was found in groundnut when inoculated with *Pseudomonas aeruginosa* AMAAS57 (Ghorai et al. 2015). Arif and Ghoul (2018) also found the survival ability of fluorescent pseudomonads (isolated from wheat rhizosphere and as endophyte of *Atriplex halimus),* by producing osmoprotectants at various NaCl concentrations (100 mM, 300 mM, 500 mM, and 800 mM). Thus, osmolyte accumulation is an important property of PGPM by which they enhance the growth of plants and in return remediate the saline soil by increasing the vegetation cover.

5.2.4 Polysaccharide Production

Salinity stress exposes the plants and microbes to hypo-osmotic conditions which increase the risk of cell flaccidity, exosmosis, and denaturation of proteins or enzymes. The adaptive response of salt-tolerant microbes includes formation of organo-mineral sheath (biofilm) confining the physical and biochemical functionality of organisms against salinity stress (Awad et al. 2012). The components of this biofilm include the polysaccharides secreted by microbes. Bacterial polysaccharides include intracellular, structural, and extracellular or exopolysaccarides (EPS). Among these, EPS play the most important role in alleviating salinity stress and establishing beneficial plant-microbe and soil-microbe interactions stabilizing the growth of plants and fertility of soil (Koo et al. 2013). EPS production in saline soil by PGPM involves the formation of aggregates trapping water and nutrients, reducing evapotranspiration, forming micropores in silt soils, and enhancing vegetation index (Elsheikh and Wood 1990; Poli et al. 2010). Kohler et al. (2006) noticed stabilization of soil aggregates upon inoculation of EPS producing *Pseudomonas mendocina* along with an arbuscular mycorrhizal fungus (AMF), *Glomus intraradices* to *Lactuca sativa.* Similarly, Yang et al. (2016) confirmed improvement of plant-water relation when *Chenopodium quinoa* seeds were inoculated with *Enterobacter* sp. MN17 and *Bacillus* sp. MN54 under 400 mM NaCl concentration. EPS has also been useful in remediating polluted soils by trapping heavy metals and in a similar manner can be used to ameliorate saline soils by trapping NaCl.

Osmotic shock retrogresses the planktonic stage of microbes to sessile stage adhering to solid substrate, to overcome the competition of nutrients, water, and essential ions, and forming a micro-protected environment against the outside hyperosmostic conditions (Quarshi and Sabri 2012). The major role to form this watery-protected environment is played by EPS. This mantled layer leads to the immobilization of Na^+ ions by EPS thereby, reducing the availability of toxic ions to microbes and plants (Kumari and Khanna 2015; Ribeiro and Burkert 2016). EPS also have a significant role in protecting the bacteria against salt stress, helping in forming association between roots and bacteria known as rhizosheath. The rhizosheath is a protective zone and an active site of nutrient recycling, osmotic balance, nutrient chelation, cation uptake, and nodulation, aiding in the establishment of symbiotic relationship between plant and microbes (Ashraf et al. 2004; Bhargava et al. 2016). The EPS matrix is responsible for cohesion of microorganisms, adhesion of microbes to substrates, ensuring interaction among microbes and plant, finally forming a tridimensional gel like structure establishing stable consortia (Costa et al. 2018). Correlatively, Mahmood et al. (2016) reported altered rhizospheric structure when mung bean was inoculated with EPS-producing *E. cloacae* P6 and *Bacillus drentensis* P16 increasing nutrient and water availability to crops. Kumari and Khanna (2015) concluded that to resist the desiccation in plant and microbes, production of EPS increases in salt stress. Tewari and Arora (2014a, b) also showed plant growth promotion of sunflower under salinity stress by using talc-based EPS bioformulation extracted from *P. aeruginosa* PF07. Vivas et al. (2003) elucidated 5, 70, and 50% increment in N, P and K, respectively, in lettuce upon inoculation with EPS producing *Bacillus* sp. According to Hossain et al. (2016), the salt tolerance index of mung bean increased in relation to the increased EPS production by the applied bacteria. The inoculation expectedly altered the rhizospheric structure by forming biofilms on root and improved nutrient and water availability to plant. The reports of Na^+ trapping by PGPM postulate that increasing population of EPS-producing bacteria would highly reduce the Na^+ in soil to toxify plants. Tewari and Arora (2016) checked the efficacy of EPS bioformulation and concluded that it significantly increased the germination rate and growth parameters of sunflower under saline conditions.

The surface polysaccharides produced by rhizobia also play a major role in alleviating salinity stress and establishing a healthy association of rhizobia and legume. These rhizobial polysaccharides are succinoglycan, galactoglucan, neutral glucomannan, and gel-forming polysaccharides (Rüberg et al. 1999; Laus et al. 2006). EPS are important for invasion of bacteria in plants (infection), bacteroid and nodule formation, and resilience against salinity stress (Bogino et al. 2013). In *Sinorizobium meliloti,* two types of polysaccharides have been reported, i.e., succinoglycans and galactoglucans (Reuber and Walker 1993). Arora et al. (2018b) reported that pigeon pea was poorly or not nodulated due to salinity stress prevailing in the area in northern India. Same report suggested that upon inoculation of pigeon pea with salt-tolerant rhizobial strains, there was enhancement in nodulation and growth of the plant. Thus, EPS holds a very important role in ameliorating the saline soils and enhancing the sustainability and food security of these marginal lands.

5.2.5 Antioxidant Systems

The hyperionic and hyperosmotic conditions during salinity stress lead to oxidative stress in plants and microbes thereby generating reactive oxygen species (ROS) (Parvaiz et al. 2012; Arora et al. 2013). ROS are partially reduced oxygen such as superoxide radical (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2), mainly generated due to over-reduction of photosynthetic activity (Scandalios 2002; Misra et al. 2018). ROS are highly damaging to the membrane proteins, lipids, and nucleic acids, altering enzyme activities and cell homeostasis (Mhadhbi et al. 2011). ROS also have a negative impact on nodules repressing their tissue integrity and function, suppressing nitrogenase activity and protein and leghemoglobin content of nodules (Mhadhbi et al. 2008). Nitrogenase enzyme is oxygen sensitive (Puppo and Halliwell 1988), but due to salinity, the enzyme is exposed to ROS and the enzymatic activity declines. Impaired leghemoglobin activity also leads to poor bacteroid formation and cell respiration, as there is limited supply of oxygen to the nodules (oxygen carried by leghemoglobin) (Mhadhbi et al. 2015). These major depressing activities, due to salinity, affect the vegetation of saline soils and especially legumes which thereby reduces the nitrogen and other nutrient contents of the soil. Hence, ameliorating the salinity stress in plants can be a strategy to remediate these marginal lands. The detrimental responses of ROS have been reported to be combated by application of salt-tolerant PGPM which show antioxidant systems scavenging these notorious radicals. Developing the adaptive mechanisms in plants, PGPM increase the antioxidant enzymes as compared to untreated plants; these enzymes include catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), polyphenol oxidase, and guaiacol peroxidase, produced as secondary metabolites (Ghoulam et al. 2002). Correlatively, Wang et al. (2012) and Kumar et al. (2018) found that application of PGP strains of *Bacillus* and *Pseudomonas* under abiotic/salinity stress increased the activity of SOD, POX, CAT, and phytohormones resulting in enhancement of growth and yield of *Lycopersicon esculentum.* Similarly, Habib et al. (2016) found that *Abelmoschus esculentus* (okra) inoculated with PGPR *Enterobacter* sp. UPMR18 showed enhanced antioxidant activity when grown in 75 mM NaCl concentration. Subramanian et al. (2016) applied thuricin 17 purified from soybean endosymbiont *Bacillus thuriengenesis* NEB 17 to salt-stressed *A. thaliana* and found that activity of isocitrate lyase and glutathione-S-transferase antioxidant enzymes was increased. This adapting and inducing response of PGPM needs to be further studied to know the exact mechanism behind the change in pathways and up/down regulation of genes to advance the concepts and reasons of tolerance.

5.2.6 ACC Deaminase Production

Salinity stress upshifts the production of ethylene in plants leading to inhibition of root length, nodulation, premature senescence, and defoliation, thereby declining the growth and productivity of plants (Ma et al. 2002; Bari and Jones 2009). Classical triple response illustrated by biomarker or bioassay showed that under saline conditions, there was stunted seedling length, enhanced shoot diameter, and directional change of growth (Shaharoona et al. 2006; Ahmad et al. 2011). 1-amino cyclopropane-1-carboxylate (ACC) is the precursor of ethylene and increases with salinity stress. The alleviation strategy of PGPM includes the production of ACC deaminase which lowers the ethylene level by taking up ACC and converting it into ammonia and α-ketoglutaraldehyde and also providing energy (Selvakumar et al. 2012; Egamberdieva and Lugtenberg 2014). The model was first proposed by Glick et al. (1998) postulating the mechanism of ACC conversion and also showing that the mutant *Pseudomonas putida* GR12-2 strains devoid of ACC deaminase activity were unsuccessful in promoting the elongation of canola roots under stressed conditions. In contrast, the wild-type *P. putida* GR12-2 strains promoted the root growth of tomato, lettuce, wheat, and canola. ACC deaminase is synthesized by plants even under hospitable conditions, but under salinity stress (or other abiotic stresses) with the peaking concentration of ethylene (ACC), its production increases. The two responsive peaks in ACC deaminase production include first peak which acts as the first line of defense in which ACC deaminase is produced in low quantity and second peak producing the enzyme at higher concentration. When the level of ACC deaminase surpasses the upper limit, the second peak gets reduced by about 50–90% (Glick 2014). ACC deaminase confers growth and stress tolerance in plants by mitigating the ill effects of ethylene. The production of ACC deaminase by bacteria plays a very important role in promoting plant growth and feeding the increasing population of the world. The functioning of these ACC deaminase producing bacteria is interrelated to both plant and microbial auxin production. Details of the synergism are beyond the scope of this chapter and can be referred to publications by Glick (2005, 2014). Relatively, Yan et al. (2014) found that there was increased shoot growth after 6 weeks in *S. lycopersicum* when inoculated with ACC deaminase producing strain *P. putida* UW4 in saline conditions. *Pantoea dispersa* PSB3 increased plant biomass, pod number and weight, and seed number and weight of chickpea at 150 mM NaCl. Jaemsaeng et al. (2018) concluded that ACC deaminase-producing endophytic *Streptomyces* sp. GMKU 336 increased *Oryza sativa* growth, essential ionic contents (i.e., K^+, Ca^{+2}), water content, chlorophyll, and proline, with decreased ethylene level and ROS and balanced Na^+/K^+ ratio in comparison with plants inoculated with ACC deaminase-deficient mutant strains. Similarly, Saravanakumar and Samiyappan (2007) also showed that *Pseudomonas xuorescens* with ACC deaminase activity were better groundnut growth promoters as compared to mutant strains lacking the activity.

5.2.7 Biocontrol Activity

Saline condition drastically disrupts the physiology and metabolism of plants which makes them more susceptible to diseases and can be invaded by salt-tolerant fungi like *Verticillium, Macrophomina,* and *Fusarium* (Besri 1993; Coser et al. 2017). According to various studies, salinity increases the risk of plant infection to soilborne fungus including *Phytophthora* sp. (Sanogo 2004); *Pythium ultimum* (Rasmussen and Stanghellini 1988); and *Alternaria solani, Verticillum dahlia*, and *Verticillum* *alboatrum* (Nachmias et al. 1993; Howell et al. 1994). Daami-Remadi et al. (2009) reported increased sporulation of *Fusarium oxysporum f. sp. lycopersici* with high NaCl doses of 8 and 10 g/l. Goudarzi et al. (2011) also suggested that the saline conditions increased the infection of *Macrophomina phaseolina*, causal organism of charcoal rot in a number of plant species. To overcome the growth inhibition by pathogens, salt-tolerant PGPM are reported to exhibit biocontrol activity against the pathogenic fungi and bacteria at high salt concentrations (Arora et al. 2016).

Apart from directly influencing the growth of plants, PGPM also indirectly stimulate the plant productivity and resistance against pathogens under stressed conditions. The mechanisms behind biocontrol activity of PGPM involve the chelation of nutrients, restricting their availability to phytopathogen thus inhibiting their growth, competition for habitat, induced systemic resistance (ISR), antibiosis, etc. (Olanrewaju et al. 2017; Mishra et al. 2018). The metabolites involved in biocontrol include siderophores, organic acids, biosurfactants, EPS, volatile organic compounds (β-phenylethanol and dodecanal, HCN (hydrogen cyanide), acetoin and 2,3-butanediol) (Kai et al. 2007; Lugtenberg and Kamilova 2009; Banat et al. 2010; Mavrodi et al. 2012; Wang et al. 2015), δ-endotoxins or Cry protein (Loper and Gross 2007; López-Pazos et al. 2009), microbial structures including homoserine lactones, flagella, and lipopolysaccharides, and the enzymes involved include fungal cell wall-degrading such as lipase-degrading, β-1,3 glucanase-degrading, chitinase-degrading (Husson et al. 2017) and protease-degrading (Vaddepalli et al. 2017; Etesami and Beattie 2018). These enzymes act against fungal oomycetes, cell wall, their sporulation, and hyphal extensions (Saraf et al. 2014; Arora et al. 2020). The regulatory mechanism of antibiosis and lytic enzyme production involves GacA/GacS or GrrA/GrrS for quorum sensing (Haas and Keel 2003). Various types of antibiotics produced by microbes include tensin, tropolone, 2,4-diacetylphlorogluconol (DAPG), zwittermicin-A, kanosamine, cyclic lipopeptides, oomycin A, viscosinamide, butyrolactones, N-BBS, and pantocin A and B (Bhattacharyya and Jha 2012). δ-Endotoxins or Cry protein is toxic for various classes of nematodes/insects such as Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Isoptera, Orthoptera, Siphonoptera, and Thisanoptera (Schünemann et al. 2014) and has successfully dominated the market of microbial biocontrol agents. The biofilming property of EPS isolates the plants from the pathogens in a hydrated and nutrient-rich microenvironment (Mishra et al. 2018). Biosurfactants produced by PGPM are responsible for forming channels in the pathogens' cell wall and thereby disturbing their cell wall properties (Raaijmakers et al. 2006). ISR induced by microbes enhances the defensive mechanism of plants against the pathogens by increasing cell wall protection, altering various physiological and metabolic pathways, forming callose at the site of infection to restrict the entry of pathogens inside the plant and secreting various biocontrol-active compounds such as phenolics to provide defense against the pathogens even under stress conditions (Compant et al. 2005).

Sgroy et al. (2009) isolated the salt-tolerant strains, *Bacillus halotolerans* Ps9 and *Bacillus pumilus* Ps19 from halophyte *Prosopis strombulifera* which exhibited protease activity inhibiting growth of pathogenic fungi *Alternaria* sp. by more than 50%. Similarly, the halotolerant isolate *Pseudomonas* sp. from halophyte *Suaeda* *salsa* suppressed *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum f.* sp. *conglutinans* infecting the plants (Teng et al. 2010). Egamberdieva et al. (2017) found that *Pseudomonas chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 reduced the *Fusarium* infection by 23 and 14% under non-saline conditions and by 42 and 25% under saline conditions, respectively. Sharma et al. (2015) depicted the role of volatile secondary metabolite HCN, produced by *Bacillus* in biocontrol even at 4% NaCl conditions. ISR was reported in *Arabidopsis* under salt stress using *B. subtilis* GB03 (Zhang et al. 2008). EPS also plays an important role in biocontrol, explaining which Tewari and Arora (2018) summarized that EPS and salicylic acid producing *P. aeruginosa* PF23EPS$^+$ showed biocontrol against *M. phaseolina* upto 500 mM NaCl.

5.3 Future Prospects

The increasing concern of salinity is highly impacting the agriculture, biodiversity, and soil quality. The mitigation of this major issue, concerning the soils globally, requires a cost-effective and sustainable green solution. In this context, application of halotolerant PGPM is regarded good solution in ameliorating salinity stress in soil and plants. Prospering in the vicinity of plant roots, these microbes play a very important role in defining the quality and fertility of soils. One gram of soil holds a "biological universe," important for recycling the nutrients, upgrading the water holding capacity, aggregating the soil, and addressing the establishment of beneficial soil-microbe/plant-microbe interactions. Although the application of beneficial microbes is gaining popularity and there are a number of biofertilizers and biopesticides available in the market, yet there are no products for reclamation of saline soil. The problem associated with the large-scale application of beneficial microbes to remediate saline soils is the difference in results obtained at laboratory-scale and field conditions. This friction is due to the uncertainty in the biotic and abiotic conditions such as soil quality, irrigation method, native microflora, temperature, precipitation, etc. Therefore, the future of saline soil reclamation should include a prior optimization of microbes or the manipulation of applied microbes for specific soils with specific conditions (Bargaz et al. 2018). Another bottleneck of this microbial remediation strategy involves the lack of knowledge about various secondary metabolites involved in stress amelioration. Covington et al. (2017) suggested that there are various stress-activated gene clusters, whose products are not yet identified, and even some genes are not expressed at lab conditions. These adaptive and remediating properties of rhizospheric microbes have to be explored for a new and inter-disciplinary field of agricultural microbial biotechnology. Metaomic techniques can provide the future directions in decoding the expression of novel metabolites and also reporting the up or downregulation of various metabolic genes during stress conditions.

The words by Matt Kleinhenz "big potential in small packages" (Third world congress on the use of biostimulants in agriculture 2017, Miami) itself defines the potentiality of the microbial universe. Thus, besides unlocking the roles and mechanisms of rhizospheric microbes, there is also a need to advance the technical and ecological aspects. Application of proteomics, metagenomics, and transcriptomics can monopolize the research for getting more efficient microbes and the associated metabolites to remediate saline soil. The gradual advancement of microbiology introduced the use of culturomics to investigate the novel soil-microbes. These cultured or uncultured microbes are storehouse of various tolerance properties which can be applied to get more effective responses. The conventional approaches of developing tailor-designed media for culturing are tedious, and thus metagenomics can be applied to detect and purify novel microbes in the natural systems (Biswas et al. 2018). Metagenomics can describe the diversity of sample and also can provide the whole genomics of the isolates to detect the novel genes. Metatranscriptomics and metaproteomics can also elaborate the stress responses by assistance in understanding the expression of genes and corresponding proteins establishing a correlation between salinity stress and the associated responses in an easy way (Gutleben et al. 2018).

The amelioration of saline soils involves the use of bioformulations to achieve elevated growth of plants and fertility of soils. The traditional approach of using microbial cells in bioformulations can be avoided by replacing them with the essential metabolites involved in imparting stress tolerance. The advantage of using metabolites would result in reduced failure due to abiotic and biotic factors suppressing microbial growth, long shelf-life, cost effectiveness, easier application, and so on (Arora and Mishra 2016). Also there is a total lacking of bioformulation suitable for saline soils and hence, a whole field is unexplored and needs to be researched.

The future of this ameliorating strategy is incomplete without the adoption of collaborative mindset which can allow progression of this technique from researchers to farmers.

5.4 Conclusion

The problem of salinity has diminished the productivity of soils and challenged food security across the globe. The biological sustainable treatment involves the application of rhizospheric microbes which amend the structure of soil and reduce the toxicity of salts. The mechanism of amelioration by these microbes include the enhancement of plant growth which could thereby hold the soil as aggregates trapping the important nutrients and water molecules and also reducing the chances of soil erosion or spread of saline content to neighboring areas. The large-scale application of microbial systems to saline soil is still in its preliminary stage, and the amalgamation of omic-based techniques and the microbial systems can advance the approach and yield better results.

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Chapter 6
Plant-Microbe-Soil Interactions for Reclamation of Degraded Soils: Potential and Challenges

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Abstract Increasing rate of industrialization has been dramatically causing elevated release of chemicals such as heavy metals, pesticides, hydrocarbons and dyes into the environment leading to degradation of soil. However, soil microorganisms and plants have the potential to survive in polluted soils and aid in the process of soil reclamation. The responses of plant and soil microbial community are cooperative towards environmental stress and contamination. The rhizospheric microorganisms reside in rhizosphere of plants and compete with plants for their survival and in return, their unified interactions display a critical role in acclimatizing to the stressed environment, eventually explored to enhance phytoremediation processes. The plant-microbe-soil interactions are important for increasing soil fertility both in the healthy and degraded soil ecosystems. Nevertheless, the concept of plant-microbe-soil interactions in healthy soil system is required to be studied in degraded soils for their efficient reclamation. The current review aims to study the prospects of plant-microbe-soil interactions for reclamation of degraded soil ecosystems. In addition, the various causes behind soil degradation and different remediation techniques will be highlighted. The importance of plant-soil interactions for reclamation of degraded soils could be explored further for restoration of various contaminated environments.

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6.1 Introduction

Soil is one of the most important raw material used to fulfill the human and animal needs by producing various kinds of food, fiber, fuel and many different major items useful for the mankind. Approximately 90% percent of the total food production comes from soil ecosystems and the remaining 10% is produced from inland water and oceans (Philosophy and Kulkarni 2007). Soil degradation is the process of declination in the productivity of soil due to biotic and abiotic stress factors including variations in organic matter, decline in nutrient status, structural parameters alongside amounts of electrolytes and other toxic compounds (Kumar et al. 2018). It is defined as the rate of variation in soil properties due to human intervention processes resulting in deterioration in productive capacity of soil (Arora et al. 2018). Hence, it denotes decay in productivity of soil, decline in vegetation, degradation of soils, quantitative and qualitative decline of water resources, and air pollution. Soil degradation is an evolutionary point that leads to decline in resource generation. Approximately 7.40 mha land was found to be degraded annually due to either natural or human-made causes (Aulakh and Sidhu 2015). The phenomenon of soil degradation has been observed from the time of start of soil cultivation. Due to growing population of India, it is necessary to meet the food demand with the agricultural processes. According to United States Department of Agriculture, eco-reclamation process is defined as the restoration of a biological system to a nearby estimate of its natural condition (Covington et al. 1999). The process involves the remediation of various soil pollutants such as aromatic compounds, pesticides and heavy metals present in soil and enriching the nutritional content of degraded soil eventually reclaiming its characteristics. The remediation and reclamation of soil degraded by various industrial or natural activities pose numerous difficulties. Globally, numerous remediation techniques have been used for restoring and reclaiming properties of degraded soils, however, every technique has its own pros and cons associated with it (Achal et al. 2011). Recently, the modern bio-technological clean-up methods involving the application of plant-microbe interactions are found to be a successful hotspot for reclamation of degraded soil ecosystems (Gupta and Chugh 2016). With this background, the chapter highlights about different causes responsible for soil degradation and represent various reclamation techniques with main focus on reclamation with the application of plant and microbial communities present in soil.

6.1.1 Cause of Soil Degradation and Contamination

The main processes that result in soil degradation include those accountable for change of physico-chemical and biological characteristics of soil (intrinsic) and also accountable for deterrence of its usage owing to other reasons (extrinsic processes). The different classifications representing the cause of soil degradation are presented in Fig. 6.1. Soil degradation is contributed to both the anthropogenic as well as natural activities. Earthquakes, floods, tsunamis, droughts and volcanic eruptions are some natural phenomenon that causes soil degradation. Anthropogenic activities responsible for soil degradation include utilization of unsuitable agricultural practices, deforestation, mining activities and improper disposal of industrial waste (Upadhyay et al. 2016).

6.1.1.1 Chemical Soil Degradation

Among numerous types of soil degradation, the most studied and explored type is chemical incursion which causes degradation of soils due to toxic xenobiotics. As per observations of Logan (1990), chemical soil degradation has large importance. An evaluation reported by EC (2013) states that, nearly more than 200 years of

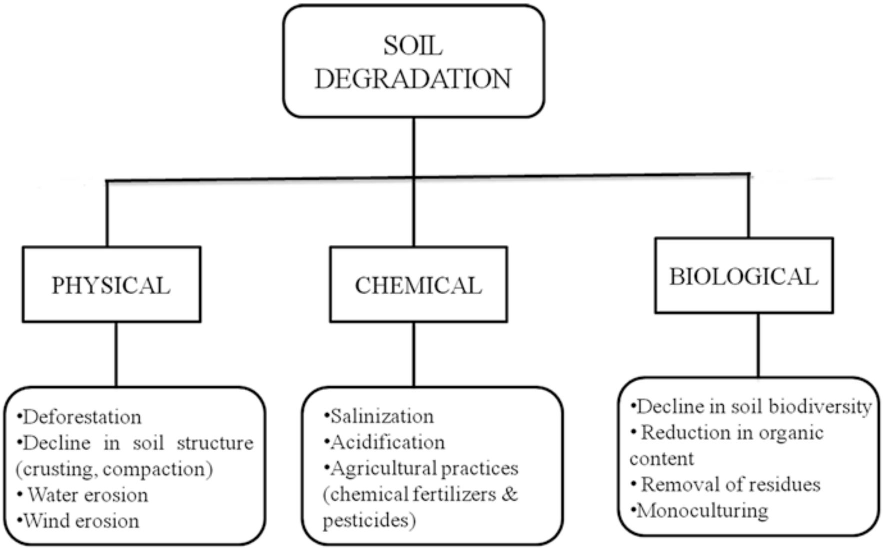

Fig. 6.1 Schematic representation of different processes responsible for soil degradation

industrialization has resulted in soil contamination, leading to soil degradation. Chemical degradation implies the amassing of harmful chemical compounds and impacting on the chemical properties which control the biological processes (Logan 1990). Suraj et al. (2001) specified that by altering these chemical properties either alone or in combination, leads to adverse impacts on the chemical soil fertility. Chemically degraded soils contain high concentrations of noxious chemicals intervening with soil ecosystem process and microbial activities. These contaminants also influence different biochemical processes in soil such as the nutrient uptake, availability and mobility. Different types of chemical soil degradation are explained in next sections.

6.1.1.2 Salinization

Salinization is phenomena of chemical soil degradation, defined as aggregation of water soluble salts on the soil surface or at the edge of the soil surface (Kavvadias 2014). It causes the accretion of various types of salts. However, the augmented content of transportable sodium (Na^+) ion in soil makes soil unfertile and the process is known as sodification (Kavvadias 2014). Sodification occurs through a number of processes which cause salt accumulation and amplification mainly due to anthropogenic actions. Hedge et al. (2011) examined that alongside irrigation water other sources of soluble salts in the soil include fertilizers, mineral weathering, atmospheric transfer of sea spray salts used on frozen roads, and movement of ground water from salt containing regions.

6.1.1.3 Acidification

Acidification causes alteration in the chemical nature of soil and results in the generation and distribution of heavy metals (Nagle 2006). The process has a negative impact on the soil ecosystem leading to plant damage and poor growth. The phenomenon cause changes in soil water potential and also results in decline in pH due to deposition of acids. Deposition of acids into soil results from the activities such as gas (SO_2) emission from motor vehicles, industrial activities such as mining and alteration in natural biogeochemical cycle due to rainfall (EC 2013). The process of acidification influences every type of soils but already acidic ones get even more affected. The extremely acidic soils can also be due to oxidation of pyrites (Dent 1986). Soils in waterlogged conditions are full of organic matter and therefore, accumulate pyrites and dissolved sulphates from salt water. Due to drainage, oxygen comes into the soil system and as a result of that pyrites gets oxidize into H_2SO_4 resulting into decline in pH to less than 4. Soil acidification hinder soil biochemical reactions and makes it deprived of some important minerals and nutrients by producing other forms which associate with soil particles and develop into complexes which are further leached down.

6.1.1.4 Chemical Fertilizers and Pesticides

Indiscriminate use of chemical-based pesticides causes environmental pollution by releasing toxic substances and heavy metals into soil and water ecosystems. As stated by Savei (2012), inorganic fertilizers majorly consist of nitrates and phosphates. The fertilizers also comprise of heavy metals such as mercury (Hg), hassium (Hs), cadmium (Cd), lead (Pb), nickel (Ni) and copper (Cu). Pesticides have immense contribution in the modern agricultural practices. The prospect of pesticides contaminating the soil and causing its degradation cannot be undervalued. These possibilities tend to increase upon application of pesticides at high proportions over the years (Hance et al. 2001) and result in increase in toxicity and loss of biodiversity and subsequently the organic matter. The concentrations of pollutants are higher in upper layers of soil, an area where most soil organisms live (Ulrich 1987).

Heavy metal contamination in soil occurs due to improper use of agricultural techniques and heavy exposure of agricultural pesticides. The commercially available fertilizers and pesticides contain a significant amount of heavy metals, which cause several environmental hazards and responsible for creating soil degradation. Research findings state that the influence of chemical fertilizers and pesticides on the soil is not immediately apparent due to strong buffering capacity of soils. Soil structure is one of the components which is degraded because of use of synthetic fertilizers and pesticides.

6.1.1.5 Physical Soil Degradation

The major cause of physical soil degradation includes unfitting utilization of land and poor soil management practices. All these practices finally result in soil degradation and reduction in productivity of soil. In developing countries, land unsuitable for cultivation, including dried lands, steep terrain, is progressively harvested. The practices of cultivation and husbandry linked to the land utilization are largely accountable for soil degradation. Physical degradation of soil results in reduced plant growth, soil productivity and crop yields (Hellin and Haigh 2002).

6.1.1.6 Compaction

Soil compaction disturbs the permeability, aeration, and water retention ability of soils, alongside root expansion and biological activity of soil, hence impacting the plant growth and agricultural yields (van den Akker et al. 2003). The factors affecting soil compaction include soil water content, mechanized farm operations, intensity of trafficking etc. (Hamza and Anderson 2005).

6.1.1.7 Soil Erosion

Soil erosion is a crucial environmental condition in all terrestrial ecosystems. Erosion imposes multiple and severe harms in various ecosystems such as pastures, crops, forests alongside natural ecosystems. Particularly, erosion results in reduction of water holding capability due to swift water overflow and reduction of organic content of soil. Consequently, nutritional compounds and other important biota are elated. Also all together, species diversity of microbes, animals and plants is considerably reduced (Zuazo and Pleguezuelo 2009).

6.1.1.8 Biological Soil Degradation

The main reason of biological soil depletion includes depletion of organic matter, reduced biodiversity, carbon cycling, and processes enriching soil with nutrients and water. Soil serves as habitat for a large number of flora and fauna which establish an important part of biodiversity. Soil organisms execute crucial biochemical reactions in maintaining the fertility of soils. The decrease in recycling of organics in soils is the main factor involved in reducing the structure and variety of soil microorganisms.

6.1.1.9 Others

The enhancement in the practices such as mining, overgrazing, urban growth and industrialization gradually acquire significant areas from grasslands, forests and agriculture and are responsible for causing soil degradation. According to the National Remote Sensing Agency (NRSA) more than 20% of wasteland is due to overgrazing and deforestation in different states of India. Mining activities are also identified to cause environmental hazards to natural ecosystems by affecting plant and microbial diversity (Gupta and Sinha 2006). Mining processes (most importantly open cast mining) cause deforestation and create an unwanted waste which consists of many heavy metals leading to heavy metal pollution both in the soil and water ecosystems. The opencast mining techniques also result in the creation of overburden (OB) dumps and removes the healthy soil and the aboveground vegetation and destroys the physico-chemical and biological characteristics of soil leading to its degradation (Ghosh 2002; Sahu and Dash 2011). In comparison with the underground mining, opencast mining produces approximately eight to ten times higher waste (Anon 2006). Approximately 90% of the mining waste is characterized as sulfides and high percentage of heavy metals predominantly chromium (Cr), Pb, Cu, zinc (Zn), Cd and arsenic (As) (Levy et al. 1997). Even though metals are also important for functioning of healthy ecosystem, their concentration above threshold level becomes detrimental to the system (Wang and Chen 2006).

6.1.2 *Current Scenario of Degraded Lands in India*

In India, the human population is increasing swiftly and according to the latest reports the population is approximately 1.2 billion and predicted to reach to 1.39 billion by 2025. To fulfill the requirement of the rapidly increasing population, approximately 320 million tones of food will be required by 2025. However, the growth in agriculture production is not increasing in accordance with the population growth. The globalization and increasing population are responsible for affecting both qualitative and quantitative proportions of land. India constitutes approximately 328.7 Mha of geographical area and out of which 304.9 Mha is reported to be utilized in agriculture, forestry and other practices (264.5 Mha) (Bhattacharyya et al. 2015). According to National Wasteland Development Board, 123 Mha of land are degraded which can very soon increase to 146.8 Mha as per the reports of National Bureau of Soil Survey and Land Use Planning (2005). The problem of soil degradation is not being effectively noticed, but is a crucial concern to raise awareness regarding soil degradation so that the upcoming land management practices may lead to a more sustainable and healthy agricultural systems.

6.2 Methods for Soil Reclamation

The process of soil reclamation refers to restore the degraded soils which were impacted either due to anthropogenic or natural causes so as to make the soil fertile and useful again. The application of land reclamation generally involves its utilization in agriculture, forestry, fishery, construction etc. (Shi et al. 2013). A comparison of various methods is depicted in Fig. 6.2.

6.2.1 *Physical and Chemical Methods*

The physical and chemical processes of soil reclamation comprise the most varied group of remediation processes. These do not involve the modification of the physicochemical characteristics of the soil contaminates. The techniques of physical soil reclamation include soil vapour extraction, storage of polluted soil, soil stabilization,

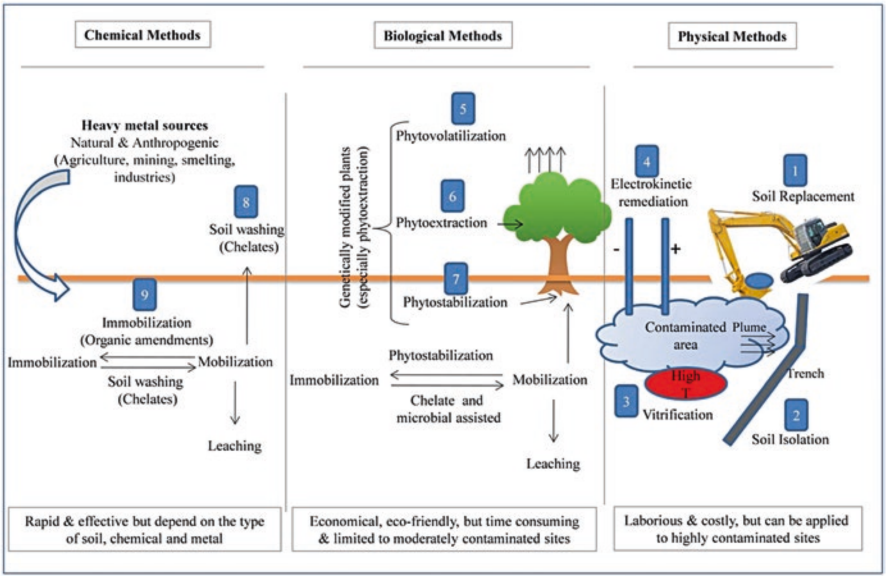

Fig. 6.2 Comparison of different soil clean-up methods. Soil remediation methods can be broadly divided into three categories: physical, chemical and biological. Physical remediation methods include (1) soil replacement, (2) soil isolation, (3) vitrification, and (4) electrokinetic; biological methods generally include (5) phytostabilization, (6) phytoevaporation and (7) phytoextraction, and chemical methods contain (8) immobilization and (9) soil washing. However, biological and chemical methods can be applied jointly depending on the type of metal, soil, plant and chemical reagent. Moreover, the effectiveness of different phytoremediation techniques can be enhanced by microbial-, chelate- and genetic-assisted remediation. (Adapted from Khalid et al. 2017)

soil flushing and electro-kinetic separation (Kueper 1997; Saichek and Reddy 2005). The advantages of physical methods include the opportunity to remove or dispose an extensive percentage of pollutants, however, the consumption and utilization of waste produced by these techniques is still an important issue to consider.

In comparison with the physical approach, chemical methods of soil remediation involve degradation of soil pollutants to a non-toxic or less toxic form to decrease the ecological hazards associated with the pollutants. The most important process of chemical remediation method is oxidation of the pollutants with the help of different chemical substances for example potassium permanganate (Hrapovic et al. 2005), and fenton’s reagent (Brown 2003). Other chemical processes such as reduction, extraction and precipitation are also utilized for the remediation of less soluble chemical compounds. Chemical remediation approaches are highly efficient and can be utilized for a wide range of pollutants but the application requires high cost and results in the production of secondary waste which creates additional hazard to the environment. According to Padmavathiamma and Li (2007), the utilization of these physical and chemical approaches for reclamation of soil requires 75–500 dollars for per ton of soil.

6.2.2 Biological Methods

The biological methods of soil reclamation have been recognized worldwide as an effective approach for reclamation of wastelands. The biological methods of reclaiming soil are based on the utilization of the microbial activity and biochemical activity of higher plants, having the potential to destroy the toxic contaminants of soil and their removal. The in situ bioremediation techniques comprise of monitored natural attenuation (MNA), bio-augmentation, biostimulation, phytoremediation and rhizoremediation (a process including microbial assisted remediation of pollutants in rhizospheric niche of plant) have been recognized as effective methods to rehabilitate contaminated sites (Fig. 6.2) (Ayoub et al. 2010; Truu et al. 2015; Prakash and Saxena 2017; Arora et al. 2018). The process of biostimulation and bio-augmentation involve the utilization of microorganisms to degrade and reduce toxic contaminants to non-toxic forms and recognized as an effective approach in recent years for remediating polluted ecosystems (Joutey et al. 2013). A successful example of microbial remediation includes the application of bacteria from the genus '*Acidithiobacillus*' responsible for the breakdown and conversion of heavy metals in acidic environment isolated from the mining environments (Malik 2004). Another example of microbial remediation is the reclamation of Cr contaminated coalmine soil by *Bacillus* spp. MNU16 (Upadhyay et al. 2017). The advanced bioremediation approach is phytoremediation, which is highly ecofriendly and inexpensive technique to remediate contaminated soils (Pollard et al. 2002) with the help of plants. In comparison with the physical and chemical techniques, biological methods are highly efficient and economic (5–40 dollars/ton) for reclaiming soil properties (Padmavathiamma and Li 2007).

6.2.3 Phytoremediation Method

The phytoremediation process is stated as the utilization of green plants to absorb, accumulate and detoxify the toxic compounds present in the contaminated soils (Fig. 6.2) (Ahemad 2015; Prakash and Saxena 2017). This technique is environment friendly, and increases the soil microbial population by releasing root exudates to maintain healthy ecosystems. The method utilizes the dominant plants or genetically modified plants which have the potential to directly take up the contaminants from the soil (Macek et al. 2000). A number of plants are recognized as appropriate for phytoremediation and these must have high biomass and enhanced metal tolerance and metal sorption activity (Kent and Triplett 2002). Phytoremediation has the applications for both the organic and inorganic contaminants found in liquid or solid conditions (Salt et al. 1998). The process of phytoremediation has the application of plants for removal of toxic contaminants and proceeds in several steps starting from uptake of contaminants, its translocation, transformation and transferring to different plant parts. However, in some cases it also results in mineralization (Schnoor et al. 1995).

6.2.4 Soil Reclamation by Trees and Grasses

Tree plantation is an essential step for protecting soil surface from erosion and also to allow accumulation of nutrients. Trees have tremendous capacity to stop or lessen degradation process through development of new and dense roots networks. These networks not only increase soil organic matter but also make soil pH adequate. Plants accumulate organic matter on the soil surface which become readily available for microbes for breakdown and make the nutrients easily available for the plant growth. There are some successful examples of phytoremediation reported by researchers (Table 6.1).

Selection of trees is very crucial for devastated lands. These plants must be able to survive and reproduce under severe natural conditions as well as stabilize soil organization. In general practice of plantation, plant varieties must be fast growing with having ability to grow on nutrient deficit soils and be drought tolerant. They should form dense top and roots. Plants improve soil quality through different procedures which include maintenance of organic content of soil, biological nitrogen assimilation, nutrients uptake, better water infiltration, progression in soil properties, and decrease in soil acidic content and advancement in biological activity of soil. Basically, selection of plant variety must be dependent on nature of degraded soil.

For an early vegetation purpose, we can consider grass as nurse crop. They are very effective for stabilization of soil. In fact, grasses may play a negative role as they compete with plants regeneration. But grasses which come under C4 category are better in terms of drought tolerance, require less nutrients and are able to withstand climatic stresses. This category of grasses have fibrous roots which help in formation of organic soil, conservation of moisture and also competition with weeds (Hao et al. 2004).

Some of the grass species for example *Setaria italica*, and *Lolium multiflorum* function in a way that protects the surface from water erosion by decreasing the consequences of falling rain drops lessening soil movement. The utilization of *S. italic* is chosen mainly in the spring or summer season whereas *L. multiflorum* are utilized in winter seasons. These grasses have the potential to prevent erosion, develop soil structure and their germination is fast and therefore, provides a healthy cover to the surface during the early years of vegetation. Therefore, it is of important concern to grow groundcover species not so tall or dense because it will hinder with the growing of trees by blocking the sunlight. Grasses like *Lolium perenne* and *Phleum pretense* have ability to grow on acidic and degraded soils. They are fast growing and established for several years and aid in growth and development of legumes or trees. Some of the grasses (e.g., *Dactylis glomerata*) have been utilized for providing advanced protection against erosion. *Eragrostis curvula* is an acid tolerant grass and able to germinate very quickly, therefore contributing to restrict early erosion (Burger and Zipper 2011).

Eroded soil lands nearby mines require specific addition of nutrients for re-vegetation. These soils require nitrogen as nutrient and healthy growth of plants are subjected to regular addition of this nutrient. If there is unavailability of nitrogen supplement or area is too large to give supplements then there is another way to

Table 6.1 Application of phytoremediation against soil contaminants. (Slightly modified from Masciandaro et al. 2013; Sabir et al. 2014; Chigbo and Nnadi 2014)

Plants	Pollutants	Results/mechanism	References
Indian mustard (*Brassica juncea*)	Cr	Significant increase in Cr accumulation in response to Cr stress	Diwan et al. (2010)
Tomato (*Lycopersiconesculentum*), maize (*Zea mays*), greengrocery (*Brassica chinensis*), cabbage (*Brassica oleracea*), Japan clover herb (*Kummerowiastriata*)	Cd, Pb, Cr, Cu, Fe	Greater amounts of heavy metals absorption in tomato plant species; the accumulation increased when tomato was intercropped with other plant species	An et al. (2011)
Willows (*Salix viminalis*)	TPH	Higher THP reduction (57% of original concentrations) in sediments planted with willows compared to unplanted sediments	Vervaeke et al. (2003)
Alfalfa (*Medicago sativa*)	PCB	Significant decrease in soil PCB concentration after one and 2 years of alfalfa planting (31.4 and 78.4%, respectively)	Tu et al. (2011)
Rye grass (*Loliumperenne*), white clover (*Trifoliumrepens*), celery (*Apiumgraveolens*)	PAH	Mixed culture of rye grass, white clover, and celery resulted more effective in removing PAH (52%) with respect to monocultures (45%) and to the control soil (30%)	Meng et al. (2010)
Silene vulgaris	Fe, Ni, cu, AI, Sn, Zn	Binding with a protein with oxalate	Bringezu et al. (1999)
		Oxidase activity in cell wall accumulation in cell wall as silicates	
Sedum alfredii H	Pb, Cd	Induction of glutathione biosynthesis that bind metals in roots	Anjum et al. (2012)
Athyrium wardii	Cd, Pb	Root retention of metals	Zhang et al. (2012)
Sorghum vulgare L.	Phenanthrene, Pyrene	Phenanthrene and pyrene dissipated	Xin et al. (2009)
Lolium arundinaceum, Lolium multiflorum, Cynodondactylon	Alkylated PAHs	Greater degradation for anthracenes and phenanthracenes	White et al. (2006)
Lolium perenne, *Trifolium repens* L	Chrysene	Degradation of chrysene	Johnson et al. (2004)
Vetiveria zizanioides	TNT	Removal of TNT helped by urea	Das et al. (2010)

introduce nitrogen in soil including plantation of legumes and incorporating species with the property of N-fixation. Nitrogen fixers maintain fertile status of soil and escape soil erosion by forming fine roots with nodules. These species also produce readily decomposable litter which is also rich in nutrients. Once this litter mineralizes it also helps in self-improvement of soil ecosystem. Leguminous crops showed better performance over non-leguminous crops for nitrogen fixation as well as soil reclamation (Singh et al. 2002).

Plantation has a significant part in shielding the aboveground surface of soil from erosion and permitting deposition of fine particles. They can reverse progression of pollution and soil degradation by steadying soils through enhancement of widespread root systems. As soon as the plants get established in the degraded soils, they aid in improving soil physicochemical properties in a way such as by enhancing soil organic matter, lessen bulk density, regulate pH of soil, and collect nutrients for the availability of plants. Their root systems help them to uptake of nutrients which are now available.

For reclamation, selection of trees must be future use oriented so that after some years the formed forest can be utilized in multiple ways. Two kinds of tree species are suggested; one are crop trees that are commercially valued and other are wildlife trees that are able to fix nitrogen and attract wild animals. Conditions and requirements for tree plantation are different from grasses and legumes because grasses have the toleration to more pH and salt concentration whereas trees can’t survive in these conditions. Moreover, survived trees will not grow well. Grasses can survive in thinner soils as they have short roots whereas trees require high depth of soil for survival. Yellow poplar (*Liriodendron tulipifera*), oaks (*Quercus* spp.), ash (*Fraxinus* spp.), maple (*Acer* spp.), and other tree species are generally implanted as crop trees on mine devastated lands. Before planting crop trees, at initial stage, soil needs to be enhanced for organic matter and nitrogen status. Nurse trees are perfect for playing this role. Also, they can be cut after few years when crop tree need more space. Some famous species are *Cornusflorida*, *Cerciscanadensis*, *Viburnum prunifolium*, *Rosacea* spp., *Caryaovata*, *Pinus strobus*, *Robinia psuedoacacia, Elaeagnus umbellata*, *Elaeagnus angustifolia* and *Lespedeza bicolor* (Miller et al. 2008).

6.3 Role of Plant-Microbe-Soil Interactions in Soil Reclamation

The process of rhizoremediation involves the utilization of complicated associations between soil present in the rhizosphere, roots, the compound secreted from root tips and microorganisms resulting in conversion of contaminants into compounds of lesser toxicity (Arora 2018). The rhizospheric microbial diversity is stimulated by the plant roots by carrying out aeration in soil and secreting exozymes along with the nutrients and minerals via root exudation as well as they provide surface and protection to the bacteria against dehydration and various stresses (Kuiper et al.

2004). These microbes bring out the plant growth promotion and development by number of phenomena viz. by fixing nitrogen, mobilizing and solubilizing phosphorous (nutrients), producing growth regulatory compounds, reducing levels of stress-producing hormones, protecting plants against pathogenic microbes and degrading contaminants in the vicinity of plants (Chaudhry et al. 2005; Segura et al. 2009; Upadhyay et al. 2017; Vishwakarma et al. 2018). Subsequently, these joint associations between the plant and microbe cause the elevation in diversity, number and metabolism of microorganisms in order to promote the degradation of pollutants and growth of plants in the neighborhood of roots in comparison to bulk area of soil (Ramos et al. 2000; Kent and Triplett 2002).

The creation of wealth of nutrients in the root neighborhood is specific to the classification of plant. The compounds present in root exudates comprise of sugars, organic acids, amino acids as well as some secondary metabolites (Jones 1998; Singer et al. 2003; Singh et al. 2004). The major parts of exuded organic acid are present in the form of dissociated anions in the rhizosphere (carboxylates) (Martin et al. 2014). It was perceived that around 10–45% of the carbon fixed by photosynthesis is emitted via rhizodeposition (Bais et al. 2006; Kumar et al. 2006; Singh et al. 2007; Kumar et al. 2009). Furthermore, microbes also degrade the contaminants through induction of expression of numerous catabolic genes particular for degradation (Singer et al. 2003). For instance, salicylic acid (a secondary metabolite) is thought to be involved in microbe-assisted degradation of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) such as chrysene, fluoranthene, naphthalene, and pyrene whereas terpenes aid in microbial degradation of phenol, toluene and tetrachloroethane (TCE) (Kim et al. 2002). The exuded components that are easy to degrade are also found to act as co-metabolites in process where the pollutants are not utilized as sole source of carbon; for instance, trichloroethylene is degraded aerobically (Hyman et al. 1995). It is considered to be an essential process where microbe can't depend upon gain in energy from the pollutant and co-metabolism is the only option left for degrading the contaminant. Rhizospheric bacterial population together with plant roots release biosurfactants and hence increase the bioavailability and take up of contaminants (Schwitzguébel et al. 2002). These functions are particularly seen in aged soils having less pollutant bioavailability and hence show much reduced response towards rhizo-mediated degradation than fresh soils (Dams et al. 2007; Gunderson et al. 2007).

In a recently developed strategy, endophytic bacteria have been incorporated to increase phytoremediation and to detoxify the pollutants (Khare et al. 2018). Such bacteria are termed as non-pathogenic and possess the pervasive existence in almost all species of higher plants. Generally, *Pseudomonas, Burkholderia, Bacillus* and *Azospirillum* genera belong to the endophytic bacteria residing in soil (Lodewyckx et al. 2002; Moore et al. 2006; Yrjälä et al. 2010). The utilization of endophytes in phytoremediation is preferred over rhizospheric; this is because the endophytic bacteria have tendency to inhabit the internal plant tissues naturally and hence cause reduction in competitive scenario between bacterial strains (Doty 2008; McGuinness and Dowling 2009). A number of studies have investigated that such bacteria might be utilized to balance the metabolic potential of the host plant via direct degradation

(Barac et al. 2004; Germaine et al. 2006: Phillips et al. 2008; Phillips et al. 2009) and may be involved in transferring degradative plasmids to another endophyte (Taghavi et al. 2005; Wang et al. 2007).

Due to the effect of environmental restrictions at field sites, the process of increased rhizoremediation slows down significantly as compared to the ex-situ treatments. Such restrictions may include challenges imposed by weeds which are much better adapted to site, restricted plant development in polluted soils, involvement of pathogenic species and action of abiotic and biotic stress factors (Gerhardt et al. 2009). Moreover, rhizoremediation can only be applied to area near roots and is not suitable for its utilization in deep sub-layers of soil. Although there are many limitations of rhizoremediation, this process has been emerging as one of the efficient methods to remediate the contaminated soils (Reichenauer and Germida 2008; Gerhardt et al. 2009, Martin et al. 2014; Azaizeh et al. 2011). In spite of many challenges in taking phytoremediation to field scale (from lab and greenhouse conditions), rhizoremediation has provided treatment to soils polluted with petroleum hydrocarbons, PAHs, trinitrotoluene (TNT), benzene toluene ethylbenzene xylenes (BTEX) and TCE (Truu et al. 2015). Till now, there are numerous studies focusing on phytoremediation of different pollutants by utilizing rhizospheric bacteria or endophytes.

6.4 Challenges

Microbe assisted remediation (rhizoremediation) of soil utilizes the biological complex connections of roots, exudates secreted in rhizospheric soil and microbes associated with it. These complex connections facilitate the conversion of pollutants into less hazardous compounds. The roots of plants promote the colonization of microorganisms by the means of providing useful nutrients for their growth and development and also provide biomes in order to protect microbes from different types of adverse conditions (Kuiper et al. 2004; Vishwakarma et al. 2016). Microbes present in close vicinity of rhizosphere in turn provide protection to plants by inhibiting the growth of pathogens and remediating the pollutants prior to their detrimental action on plant growth (Chaudhry et al. 2005; Segura et al. 2009). This type of mutualism in rhizosphere drives higher number of microbes, their variety and activities relevant to metabolism, which make them potent for degrading pollutants as well as promote their growth in rhizosphere compared to bulk soil (Vishwakarma et al. 2016; Ramos et al. 2000; Kent and Triplett 2000). The microbes present in the rhizospheric soil thus play crucial role in remediation of soil pollutants. However, due to resemblance of secondary metabolites with pollutants many genes get activated which are required for catabolism of pollutants (Singer et al. 2003). The compounds of root exudates which degrade easily can act as co-metabolites in systems where pollutants cannot be utilized as C source for energy (Reichenauer et al. 2008). In addition, roots of plants and microbes associated with it may release surfactants, facilitating the increased bioavailability and procurement of contaminants (Kuiper et al. 2004).

This soil-microbe assisted remediation process must deal with complex, heterogeneous, multiphasic atmosphere of soil frequently. Therefore, in order to make this process effective, interdisciplinary approach is important including ecological, microbial, chemical as well as geological studies. In addition, the net result of each remediation process rely on microbial cell concentration, diversity in microbial communities, enzymes secreted by them; physico-chemical feature, structure and amount of substrates; temperature, pH, moisture content, presence of oxidizing agents, carbon content of contaminants at remediation site. These factors determine the period of acclimatization of bacteria to the substrate.

Such parameters influence the acclimation period of the microorganisms towards substrate. Concentration and structure of pollutant have also been reported to affect the viability of soil reclamation process, the nature of transformation reactions and nature of compounds (as primary/secondary/co-metabolic substrate) (Boopathy 2002). The bioremediation process through rhizosphere means is one of the effective approaches for remediation of contaminants from soil. Table 6.2 describes the various bioremediation strategies along with benefits and limitations.

Table 6.2 Summary of bioremediation strategies

Technology	Examples	Benefits	Limitations	Factors to considers
In situ	In situ bioremediation	Most cost efficient	Environmental constraints	Biodegradative abilities of indigenous microorganisms
	Biosparging	Noninvasive	Extended treatment time	Presence of metals and other inorganics
	Bioventing	Relatively passive	Monitoring difficulties	Environmental parameters
	Bioaugmentation	Natural attenuation processes		Biodegradability of pollutants
		Treats soil and water		Chemical solubility
				Geological factors
				Distribution
Ex situ	Land farming	Cost efficient	Space requirements	As above
	Composting	Can be done on site	Extended treatment time	
	Biopiles		Need to control abiotic loss	
			Mass transfer problems	
Bioreactors	Slurry reactors	Rapid degradation kinetic	Soil requires excavation	Bioaugmentation
	Aqueous reactions	Optimized environmental parameters	Relatively high cost capital	Toxicity of amendments
		Enhances mass transfer	Relatively high operating cost	Toxic concentrations of contaminants
		Effective use of inoculants and surfactants		

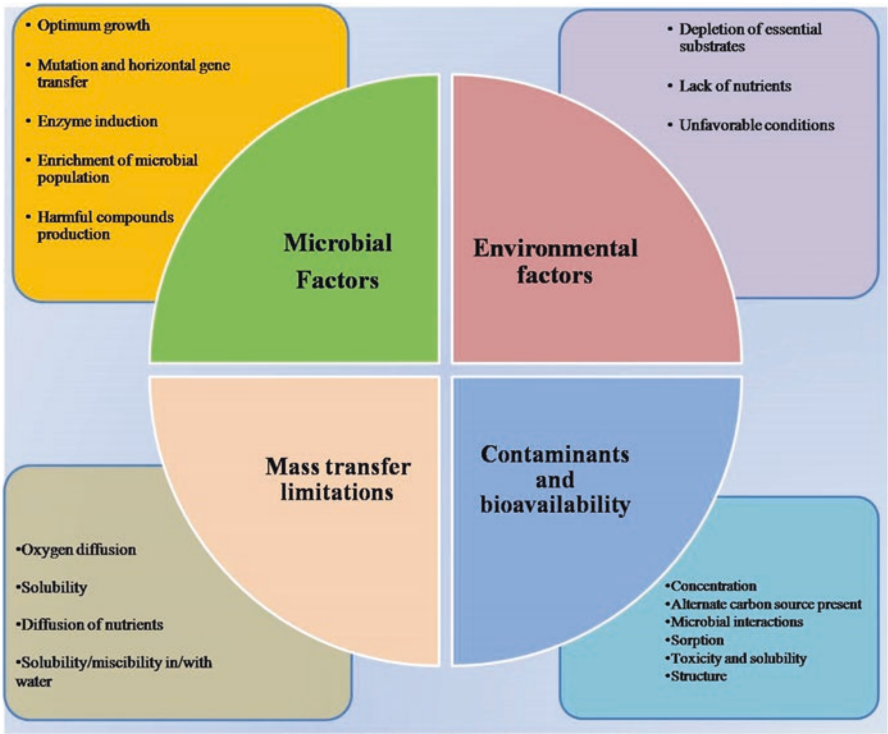

Fig. 6.3 Various factors affecting soil reclamation process through bioremediation (Modified from Boopathy 2000)

Still various factors restrict the applicability of plant-microbe-soil-interactions in reclamation of degraded soils (Fig. 6.3). These include:

6.4.1 Energy Source

The availability of carbon containing compounds and their capability of serving as source of energy determines the bioactivity of microbes present in rhizosphere. The oxidation state of carbon present in organic compounds determines whether contaminants can serve as effective energy source or not; higher the oxidation state, less the energy supplied for decomposition of pollutants by microbes.

6.4.2 Bioavailability

The rate of conversion of pollutants to less toxic compounds depends on procurement of pollutants by microbes, their metabolism and rate of movement to the microbial cells. The mass transfer rate acts as limiting factor for the site containing high pollutant accumulation (Boopathy and Manning 1999). Some of the pollutants present in soils do not get biodegraded even after many years. Methods involving thorough mixing and breaking of larger soil particles to smaller ones significantly promote the remediation process through biological means. The availability of pollutants to the microbes is regulated by processes like diffusion, dissolution, sorption, and desorption. Slow mass transfer decreases the rate of bioavailability (Luka et al. 2018). When rate of mass transfer becomes negligible then availability of pollutants becomes zero. The reduced rate of availability of contaminants during long time span is generally referred as weathering or aging. This may result due to oxidation reactions, low rate of diffusion, film formation which resists mass transfer. The problem of bioavailability can be sorted out by utilizing surfactants of biological origin which enhance the availability of pollutants for degradation mediated by microbes (Boopathy 2000; Boopathy and Manning 1999).

6.4.3 Biological Activity of Microbes

Biological activity of microorganisms involved in remediation process is considered the essential factor to estimate the efficiency of this process. Improvement of bioactivity means that conditions should be adjusted in such a way so that bioremediation process gets optimized (Blackburn and Hafker 1993). If certain rate of bioremediation process is required, then optimization of conditions for improving biological activity becomes an important aspect. Naturally, the ability of microbes to convert simpler as well as complex compounds varies. The evaluation of affectivity of conversions via microbial activity is done on the basis of removal of elements from parent molecules of contaminants, degree of production of toxic compounds and generation of metabolites in measurable amount through biochemical process. The biological activity of microbes during remediation process can be regulated under in situ system by optimizing the conditions to attain desired outcome. Moreover, plant growth promoting rhizobacteria (PGPR) can also be utilized for reclamation of soil and removal of plant-soil related diseases (Upadhyay et al. 2017; Verma et al. 2018).

6.4.4 Lack of Commercialization

The interaction between soil-microbe has been utilized frequently and efficiently for remediation purposes. However, this technology of soil-microbe interaction has not been utilized commercially. Presently, bioremediation approaches rely on application of genetics, physiology and biochemistry to increase the tolerance level of soil/ microorganisms against contaminants and possible degradation of organic pollutants (Dowling and Doty 2009; Wenzel 2009; Conesa et al. 2012). Due to low revenue generation via phytoremediation, problems related to commercialization still remains. Therefore, it is suggested to use this methodology as a means of gaining some profits. One of the primary constraints in commercial usage of remediation process is the lack of studies related to economical prospects. Recently various efforts have been made to evaluate economic earnings from bioenergy production at polluted sites. Such remediation processes are time consuming and impose extra cost, making the application of remediation technique unpredictable and toughen the evaluation.

6.5 Overcoming the Challenges

In order to mitigate constraints of reclamation process many technological advancements have been made to improve its cost, speed and efficiency. These advancements include application of novel ideas of recombinant technologies for reclamation purposes. The natural potential of organisms originated as a result of evolutionary processes to get adapted under harsh chemical conditions. This process is efficient but the adaptation takes long time. The proper understanding of the pathways that are involved in degradation of harmful chemicals is still lacking. Furthermore, the other pathways involve incomplete transformation releasing contaminants that accumulate and inhibit degradation pathways (Jones and de Voogt 1999). In addition, the degradation rate of contaminants by microbes naturally is slow due to harsh environmental conditions; therefore, the new and advanced techniques are gaining pace in research and presenting promising ways to improve overall performance of remediation process.

6.5.1 Metagenomic Approaches

Microbes present in environment are the main source of variety of genetic material having biotechnological implications including remediation processes. Majority of microorganisms (>99%) are uncultivable under normal laboratory conditions which restrict numerous industrial processes that include the utilization of such microbes. Metagenomics approaches lead to the identification of microbes in a given sample and with the help of this information; genes related to specific function of remediation can be genetically engineered in microbes. Recently many genes and enzymes of uncultivable microbes have been identified from metagenomic approaches. These genes or their products can be utilized for soil reclamation processes.

6.6 Metabolic Engineering

Understanding the metabolic pathways involved in degradation of hazardous materials is one of the important aspects in bioremediation process in order to ensure that metabolites generated after degradation are non-hazardous and least reactive and do not cause any threat to environment. Current practices under metabolic engineering focus on utilization of pre-existing metabolic system in relation to degradation of hazardous compounds. It also involves the designing of novel, effective and efficient pathways having superior degradation of hazardous contaminants (Erb et al. 1997). Metabolic engineering can also be employed for generation of engineered microbes which have improved enzymatic activity, capability to survive in harsh conditions (Marconi et al. 1997) and potential for producing surfactants under circumstances when there is limited bioavailability (Pei et al. 2010). Deletion strategies have also been employed for removing competitive chemical reactions (Kind et al. 2010).

6.6.1 Enzyme Engineering

The stability, specificity of substrates, kinetic parameters of proteins and enzymes can be improved by protein engineering methodologies by DNA shuffling (Kuchner and Arnold 1997), random priming (Shao et al. 1998) and staggered extension process (Zhao et al. 1998). Another approach includes the production of chimeric enzyme having superior qualities than original ones (Beil et al. 1998). In addition, catalytic activity of enzyme can be enhanced by incorporating multi-binding sites within one peptide facilitating the attachment of small molecules/co-factors. It was also established as a multipurpose strategy for elimination of metallic wastes (Pazirandeh et al. 1998). In addition, multidisciplinary approach including application of metagenomics, protein engineering, and nanotechnology could be utilized to mitigate main constraints of remediation and to enhance the efficiency of biocatalysts in remediating polluted areas (Gopinath and Kumar 2017). To achieve an efficient and successful bioremediation, it is necessary to explore the biological, chemical and physical indicators of the associated ecosystem (Das and Dash 2014).

Along with genetic/metabolic engineering, enzyme-engineering techniques have also found to be involved in boosting the shelf life, stability and performance of enzymes significantly (Sharma et al. 2018). Enzyme engineering is considered to involve the structural alterations in amino acids of enzyme for improving its activity, increasing tolerance to stress, temperature, salt etc. (Rayu et al. 2012). Enzyme engineering utilizes recombinant DNA technology for changing the amino acid sequences of an enzyme (Singh et al. 2013). Now-a-days, enzyme engineering is utilized in highly selective bioremediation of contaminants such as radionuclides and heavy metals (Dhanya 2014). For instance, catalytic activity of horse radish peroxidase has been increased significantly in a work by Huang et al. (2018). Moreover, nano-size based enzymes termed as nanozymes are now offering high potential of remediation for wide range of contaminants. Such nanozymes found their broad uses in the area of bioremediation. For example, they have been utilized in detecting and degrading contaminants viz. lignite wastes, dyes, organic materials etc. (Xu et al. 2012; Liang et al. 2017). Nanozyme-based technology has come to be inexpensive, simple and powerful method for degrading and mineralizing organic dyes released from industry based processes (Xu et al. 2017).

6.7 Future Prospects and Conclusion

Soil degradation results in loss of vegetation cover; affects plant growth and generate risk for terrestrial ecosystems. Soil degradation is an important issue of concern; therefore, sustainable and effectual remediation techniques are essential. Rhizoremediation is an ecofriendly approach involving the interaction of plants and associated microbial community in rhizosphere for remediating soil contaminants and enriching soil nutrients; which is widely accepted for majority of the restoration studies. The beneficial role of plants and rhizospheric microorganisms in assisting microbial degradation for the in situ remediation of contaminated soils is significant. To utilize native species for reclamation requires proper examination because some of the species might create threat in other conditions. The plant-microbe-soil interactions facilitate several important biochemical reactions in the rhizosphere; for example nutrient cycling, carbon sequestration and ecosystem functioning. Microorganisms reside in soil and enhance the potential of a plant to uptake essential nutrients and minerals. The fate of the various organic and inorganic soil contaminants should be studied properly during the process of rhizoremediation so that the undesired effects can be avoided during field experiments. Study of molecular interactions between plants and microorganisms and utilizing these interactions for enhancing bioremediation activities is an interesting area of research. The utilization of metagenomic techniques in biodegradation studies will provide a new way by introducing effective genes into different beneficial strains so as to improve remediation. Further understanding of the critical factors influencing the plant-microbe-toxicant interaction in soils will permit more rapid realization of this new approach of in situ bioremediation.

Soil remediation is a technology whose full potential has not yet been realized. As the limitations of soil cleanup technologies become more apparent, research into alternative technologies will intensify. In future, increase in research has to be implied in evaluating the prospects of bioremediation that will include evaluating protocols for treatment of contaminants such as petroleum hydrocarbons, chlorinated solvents, PCBs, and metals as well as the site that needs to be treated. With the coordination of government, academia and industry, such procedures have to be tested in fields followed by proper reviewing process. Furthermore, reliable, fast and inexpensive site characterization techniques should be adapted for easing bioremediation evaluation. Developments in mathematical models to link understanding of chemical, physical, and biological phenomenon associated with reclamation processes should also be targeted.

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Chapter 7
Lichens as Sinks of Airborne Organic Pollutants: A Case Study in the Natural Ecosystem of Himalayas

Vertika Shukla, Ankita Asati, Devendra K. Patel, Manoj Semwal, and Dalip K. Upreti

Abstract Persistent organic pollutants (POPs) are predominantly released into the atmosphere due to anthropogenic activities. POPs pose risk to the global environment as they are persistent having long-range transport potential. In the developing nation like India, anthropogenic activities (mainly due to diesel-driven vehicles and industrial activity) are the major contributing factor of POPs in the environment and its dispersal in the air. Due to long-range transport of pollutants, deposition of POPs affects high-altitude ecosystems especially the Himalayas, which needs to be investigated as Himalayas are source of major perennial rivers and have rich biodiversity. Lichens having peculiar characteristics serve as potential sinks/accumulators for range of pollutants and are well-recognised biomonitors of pollutants including POPs.

The case study discussed in the present chapter is pioneer regarding the levels of PCB congeners as well as N-PAHs levels in lichens from Indian Himalayas. The levels and distribution of POPs, viz., polychlorinated biphenyls (PCBs) congeners, polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs (N-PAHs), together with heavy metals, were analysed in lichen *Heterodermia diademata* samples from protected forest area of Lansdowne in Garhwal Himalaya, Uttarakhand, India. The result provided valuable information on the concentration of different POPs in lichen samples from Garhwal Himalayan region. Concentrations detected in lichens

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were further utilised for source apportionment, detecting the spatial behaviour of individual POPs. The study clearly indicated the influence of long-range transport of the PCB congeners, PAH and N-PAHs, as well as local practices of excessive usage of wood, coal and practice of forest fire as the major factors affecting the air quality of the Garhwal Himalayan forests.

Keywords PCB congeners · PAHs · N-PAHs · Metals · Topography · Meteorological condition

7.1 Introduction

Persistent organic pollutants (POPs) are semi-volatile organic compounds based on their physicochemical characteristics, which occur either in vapour phase or are adsorbed on atmospheric particles, facilitating long-range transport through the atmosphere (Augusto et al. 2016; Escartin and Porte 1999). Being environmentally stable and semi-volatile and having tendency to bioaccumulate, POP compounds are distributed throughout the world including remote regions of Arctic and Antarctic (Fernandez et al. 1999; Park et al. 2010; Lo Giudice et al. 2019). Because of mutagenic and carcinogenic properties, the airborne POPs, like polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and N-PAHs, are great cause of health concern (Augusto et al. 2013a)

PCBs (among POPs) are major contaminants, which find use in numerous industrial applications (in production of dielectric fluid for electrical transformers and as flame retardants). PCB congeners are persistent and highly hydrophobic, thereby resulting in ubiquitous distribution in aquatic as well as terrestrial environments (Augusto et al. 2013a; Klánová et al. 2007).

PAHs are the compounds which are formed during incomplete combustion from natural and anthropogenic sources. High molecular weight PAHs are mutagenic and carcinogenic. Physicochemical properties (vapour pressure and aqueous solubility), semi-volatile nature and hydrophobic character typically influence its environmental fate (Srogi 2007). Vehicle emissions, use of fuel for cooking, electricity generation and waste incineration dominate the PAH input from diverse sources. Burning of biomass and vehicular exhaust significantly contribute towards PAH input to the ecosystem both qualitatively and quantitatively (Korenaga et al. 2000; Kumar et al. 2017).

Particulate-associated PAHs when reacting with N_2O_5 or HNO_3 produce N-PAHs, which are classified as potential mutagenic and carcinogenic compounds, whereas direct emissions of N-PAH pollution result due to emissions from diesel engine, vehicular exhaust and residential heating (Nielsen et al. 1999). More N-PAH isomers are formed during gas phase reactions (radical-initiated reactions) as compared to those formed via particle phase reactions or due to release from primary sources (electrophilic nitration) (Atkinson and Arey 1994; Feilberg and Nielsen 2000).

The assessment of global exposure to organic pollutants is pertinent owing to their eco-toxicological potential because of long term exposure through biomagnification and mutagenic properties, which pose health risk (Talaska et al. 1996; Kumar et al. 2018). Accumulation of POPs in plants could provide direct evidence about their level in ambient air and therefore associated health risks may be assessed (Talaska et al. 1996).

7.2 Lichens as Indicators

Monitoring airborne metals, PAHs and other contaminants with living organisms (biomonitoring) is a technique which is in use since 1960s (Garćia et al. 2009). Bioindication and biomonitoring studies are an authentic and cost-effective alternative to assess level of contaminants in the air and its impact on the ecosystem (Markert et al. 2003). Assessment of air quality in multisource environment requires extensive sampling along with proper analytical methodology while biomonitors respond well with the slight changes in microclimatic conditions, both as indicators and accumulators of pollutants (Conti and Cecchetti 2002). Lichens are symbiotic organisms having cuticle less thallus surface having dense hyphal network which facilitates direct adsorption of pollutants from the atmosphere (Elix 2011). In recent years, POP biomonitoring studies using lichens and aquatic mosses have proved to be a reliable tool (Augusto et al. 2013a) as it provides details about deposition pattern and its effect on the ecosystem. Seasonal variation is known to affect concentrations of Σ16 EPA-PAHs in lichens, with highest values during winter and lowest values during summer, while meteorological variables – temperature, atmospheric pressure, relative humidity and wind speed – are also known to significantly influence POP concentrations in both lichens and air (Augusto et al. 2013b). An added advantage of employing biomonitors lies in the fact that it enables sampling in remote areas where huge air volume samplers and other equipment cannot be installed due to difficult terrain (Blasco et al. 2008). By comparing and correlating POP concentration in lichens to the atmospheric equivalents, biomonitoring may be effectively utilised for environmental management. In a study, significant positive correlations between high molecular weight PAHs, Σ16 EPA-PAHs and BaP equivalent concentrations in lichens and corresponding values in air were observed (Augusto et al. 2013b).

Although lichens are excellent biomonitors of both organic and inorganic metals together with metalloids and radionuclide (Shukla and Upreti 2008; Shukla et al. 2013a, b), however, no reports regarding accumulation of POPs in lichens are available in India especially for PCBs and N-PAHs.

In order to understand environmental factors affecting fate and transport of POPs, high altitudes provide vertical gradients on a small scale to observe the chemical behaviour of POPs globally (Negotia et al. 2003). The assessment of concentration changes along an altitudinal gradient is practically considerably simpler to undertake in comparison to global studies, which may assist in understanding the influence of various meteorological parameters on spatial behaviour and bioaccumulation factors (Daly and Wania 2005). POPs and other organic substances have been measured at high altitudes in Europe and western North America (McLachlan 1999; Sehili and Lammel 2007; Lammel et al. 2009); however, only few reports are available with reference to monitoring of POPs in the Indian Himalayan region. Hence the present study aims to quantify organic pollutants such as PCBs, PAHs and N-PAHs as well as inorganic metallic content, along an altitudinal gradient of 700–1850 m a.s.l. in Garhwal Himalaya of India, to decipher any correlation existing between organic pollutants and metallic content and to validate the theory of long-range transport of organic pollutants.

7.3 Sampling Procedure

7.3.1 Area Surveyed

Lansdowne, the study area, is located at 29°50′N 78°41′E 29.83°N 78.68°E with an average elevation of 1850 m and having dense forest canopy (Table 7.1; Fig. 7.1). Lansdowne is one of the undeterred hill (minimum human interference) stations of the Garhwal district in Uttarakhand state located within protected forest area of Garhwal. As of 2001 India census, Lansdowne had a population of 7902. Study was undertaken in the forest areas of Lansdowne as the area has thick canopy forest with minimum anthropogenic activity.

7.3.2 Sampling Technique

Lichen samples were collected during April 2015 from Lansdowne region of Garhwal Himalaya between altitudinal ranges of 700–1850 m. Among epiphytic lichens, *Heterodermia diademata* was selected for quantification of the metabolites,

Table 7.1 Localities surveyed for lichen collection with aspect and slope of the site

Site no.	Locality	Latitude (N)	Longitude (E)	Aspect	Altitude (m)	Humidity (%)	Temperature (°C)
1	Lansdowne	29°50′26.5″	078°40′ 48.7″	Southwest	1688	58	22.2
2	Tip in top	29°50′ 59.1″	078°40′44.9″	Northeast	1786	56	22.1
3	Tarkeshwar	29°50′ 20.0″	078°47′33.7″	North	1778	48	27.4
4	Lansdowne 15 km	29°49′02.7″	078°43′19.2″	South	1061	50	24.3
5	Jaiharikhal	29° 51′34.8″	078°40′46.7″	Southeast	1641	52	27.7
6	Jaiharikhal	29°51′21.8″	078°40′38.7″	Northeast	1711	44	28
7	Sunset point	29°50′58.2″	078°40′30.9″	South	1805	54	25.5
8	Fatehpur	29°48′55.4″	078°38′ 14.1″	South	915	57	26.9
9	Sendhikhal	29°47′29.7″	078°38′ 28.7″	North	959	46	29.5

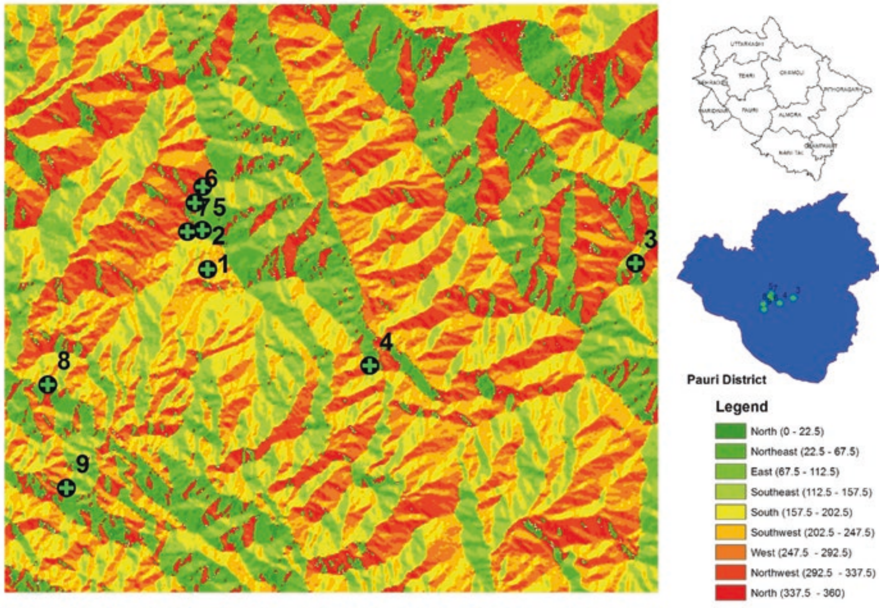

Fig. 7.1 Map of Lansdowne forest area in Garhwal Himalaya surveyed for collection of lichen samples

based on its wider distribution at all the locations and abundance. Its abundance and occurrence at various environmental niches indicate that the species may be used for providing ‘contaminant signatures’ at various altitudinal levels. The collected samples were wrapped in aluminium foil and kept in dark at −4 °C till the analysis.

7.3.3 Instrumentation Techniques Employed for Characterisation of Pollutants

7.3.3.1 Metal Analysis

The dried lichen and bark samples were ground to powder in manual mortar; ≈0.1 g each was dissolved in 5 mL mixture of concentrated HNO_3 and $HClO_4$ (v/v 5:1) (beakers covered with watch glasses) for 1 h and then heated until close to dryness. Residues were dissolved with 1% HNO_3 (2 mL) followed by filtration through Whatman Filter Paper No. 42 and diluted to 10 mL with 1% HNO_3. Analysis was done with flame atomic absorption spectrophotometer (Avanta-Σ, GBC, Australia). Stock standards were used from Merck India, traceable to NIST (National Institute of Standards Technology). Quality assurance (QA) and quality control (QC) along with linearity and limit of detection (LOD) of the method are as in Table 7.2.

Table 7.2 QA and QC parameters for PCBs, PAHs, N-PAHs and metals detected in lichen samples

					0.1 µg g^{-1} ($n = 3$)			0.05 µg g^{-1} ($n = 3$)		
		Linearity	LOD	R^2	Recovery (%)	Intraday	Interday	Recovery (%)	Intraday	Interday
PCBs										
2,2′,5,5′-Tetrachlorobiphenyl	PCB52	0.031–0.500	0.015	0.996	92	3.4	7.2	85	2.4	6.2
2,2′,4,5,5′-Pentachlorobiphenyl	PCB101	0.031–0.500	0.012	0.998	98	2.2	8.1	95	1.7	5.3
2,2′,3,4,4 5′-Hexachlorobiphenyl	PCB138	0.031–0.500	0.019	0.999	105	3	6.7	102	0.9	2.8
2,2′,4,4′,5,5′-Hexachlorobiphenyl	PCB153	0.031–0.500	0.017	0.997	96	2.5	6.3	89	1.5	4.1
2,2′,3,4,4′, 5,5′-Heptachlorobiphenyl	PCB180	0.031–0.500	0.014	0.998	95	1.4	5.1	92	3.2	7.6
Metals										
					1 ppm ($n = 3$)	RSD %		5 ppm ($n = 3$)	RSD %	
Cadmium	Cd	0.250–1.000	0.100	0.997	92	2.1	5.6	97	2.7	5.2
Nickel	Ni	1.000–3.000	0.100	0.999	96	2.5	4.7	99	3.5	5.5
Lead	Pb	1.000–3.000	0.100	0.998	95	3.0	4.4	100	3.9	6.0
Zinc	Zn	0.250–1.000	0.100	0.999	107	3.5	6.2	102	1.5	3.4
Copper	Cu	0.250–1.000	0.100	0.997	99	3.2	4.3	98	2.3	3.8
Chromium	Cr	0.250–1.000	0.100	0.998	97	2.9	4.1	95	3.3	5.0
Iron	Fe	0.250–1.000	0.100	0.999	100	3.1	2.7	102	2.0	5.3
PAHs										
					0.1 µg g^{-1} ($n = 3$)			0.05 µg g^{-1} ($n = 3$)		
Naphthalene	Nap	0.01–0.5	0.009	0.999	99	2.0	5.2	97	2.7	5.6
Acenaphthene	Ace	0.01–0.5	0.008	0.999	100	2.6	5.6	106	3.1	4.5
Fluorene	Flu	0.03–0.25	0.056	0.998	98	1.0	3.4	94	2.0	4.1
Phenanthrene	Phen	0.01–0.5	0.008	0.997	95	1.8	4.9	99	1.5	3.9
Fluoranthene	Fluo	0.002–0.5	0.004	0.999	105	2.3	4.6	108	3.0	4.7
Pyrene	Py	0.002–0.5	0.005	0.997	92	4.4	5.4	101	2.9	4.9
Benz(a)anthracene	BaA	0.002–0.5	0.006	0.998	96	3.4	5.8	89	2.5	3.4

Chrysene	Chy	0.01–0.5	0.009	0.999	102	4.1	6.2	105	3.2	5.2
Benz(k)fluoranthene	BkF	0.002–0.5	0.005	0.999	97	2.7	4.4	95	1.0	4.4
Benz(b)fluoranthene	BbF	0.002–0.5	0.006	0.998	98	3.0	4.8	101	2.1	4.0
Benz(a)pyrene	BaP	0.002–0.5	0.005	0.999	100	0.9	2.1	106	1.8	3.9
Dibenz(a,h) anthracene	DahA	0.01–0.5	0.008	0.998	97	1.0	4.7	99	2.2	4.1
Indeno(1,2,3-c,d)pyrene	IcdPy	0.01–0.5	0.014	0.998	94	2.9	3.5	95	2.2	4.6
Benzo(g,h,i) perylene	BghiPyl	0.01–0.5	0.017	0.997	96	3.5	5.9	100	1.5	3.5
N-PAHs										
					0.1 μg g^{-1} ($n = 3$)			0.05 μg g^{-1} ($n = 3$)		
5-Nitro-1,10 phenanthroline	5-N1,10Ph	0.016–0.50	0.001	0.997	101	1.8	4.5	98	2.7	6.2
3-Nitroaniline	3-NA	0.004–0.25	0.002	0.999	105	2.5	4.0	102	1.0	3.5
8-Nitroquinoline	8-NQ	0.016–0.50	0.001	0.998	99	1.4	3.5	104	3.1	4.4
2-Nitroaniline	2-NA	0.004–0.25	0.002	0.999	95	1.9	3.2	98	1.0	6.8
2-Nitrofluorene	2-NF	0.002–0.50	0.003	0.997	99	1.5	4.9	102	1.8	3.1
1-Nitropyrene	1-NPy	0.016–0.50	0.002	0.998	102	0.9	3.1	104	2.1	4.9

7.3.3.2 PAH and N-PAH Analysis

Lichen samples (0.1 g from each site) were weighed, and thimble (Whatman No.42) was prepared for extraction of PAHs in Soxhlet apparatus with 100 mL of dichloromethane (Merck, AR) for 16 h according to the procedure of Environmental Protection Agency, EPA 8310 (US EPA 1986). The extract was passed through anhydrous sodium sulphate (Qualigen, AR) to remove moisture and concentrated up to 2 mL under vacuum in Buchi rotary evaporator (Rotavapor®). The extract was cleaned by silica gel (100/200 mesh size, Qualigen) column according to the EPA method 3630 (US EPA, 1986). Final extract was solvent exchanged to acetonitrile (Merck, AR) and final volume was made to 2 mL in umber-coloured volumetric flask. Sample was stored in dark at 4 °C till the analysis was performed.

Ultrahigh performance liquid chromatography (UHPLC, Thermo Dionex) comprises of PDA detector and reverse-phase C-18 column (Thermo scientific Acclaim™ 120; size 250 X 4.6 mm i.d. and 5 μm particle size), equipped with Chromeleon software for processing the UHPLC data. The PAH chromatogram was observed at 254 nm and NPAH at 230 nm. A total of 16 PAHs were separated with acetonitrile and Mill-Q water in ratio of 85:15 as a solvent at flow rate of 2 mL min^{-1} in isocratic mode at 27 °C temperature. Out of the 16 PAHs, 2 peaks merged, while 14 PAHs were identified by their retention time and were quantified by their respective peak areas. Standards of PAHs were procured from Sulpelco, USA.

Gradient elution of solvent A 100% acetonitrile and solvent B 0.2% TFA in MilliQ water at constant flow rate of 1 mLmin^{-1} was used for characterisation of N-PAHs. The solvent B was kept at 40% till 7 min then decreased to 20% for next 0.5 min, thereafter decreasing with solvent B up to 0% till 13 min and then system was re-equilibrated with 40% solvent B until 18.0 min at 30 °C. NPAHs were identified by their retention time, and were quantified by their respective peak areas. Standards of seven NPAHs were procured from Sulpelco, USA. QA and QC along with linearity and LOD of the method for detection of PAHs and N-PAHs are as in Table 7.2.

7.3.3.3 PCB Analysis

The procedure for the extraction of PCBs from lichen samples was as follows: 0.1 g of samples were loaded into a 10 mL conical flask. Samples were extracted with 10 mL acetone by 10 min vortex then 10 min ultra-sonication. After sonication sample was centrifuged at 3000 rpm for 4 min. The upper solution was transferred into a 10 mL glass test tube. After evaporation of the sample extract solvent by a gentle nitrogen flow, the residue was dissolved in 100.0 μL *n*-hexane. Aliquots (1.0 μL) of the *n*-hexane phase were removed by a microsyringe and injected into gas chromatograph (GC) for PCB analysis.

GC-ECD (GC with Electron Capture Detector) analysis of PCBs was performed on Agilent 7890A series GC with micro-ECD (μECD) (Agilent Technologies, Palo Alto, CA, USA). Injected volume was 1 μL in splitless mode, injection port temperature was adjusted at 300 °C, while detector temperature was adjusted at 300 °C with nitrogen as mobile phase at the flow rate of 24 mL/min. Makeup gas, nitrogen, was released at flow rate of 1 mL min^{-1} as carrier gas. Chromatographic separation was conducted on MS-5, 50% phenyl, 50% methyl polysiloxane (30 m × 0.32 mm i.d. × 0.25 m capillary column J&W Scientific Inc. Flsom, CA, USA). Column temperature was gradiently optimised as 140 °C for 5 min, rose to 200 °C at 5 min, 240 °C at 2 min, 280 °C at 10 min, and maintained at 300 °C for 2 min. Sample was injected 1 μL into GC-μECD through (Hamilton Reno, NV, USA) syringe. QA and QC along with linearity and LOD of the method for PCB quantification are as in Table 7.2.

7.3.3.4 Statistical Analysis

Data was treated with Microsoft office and Statistica 12.7 (Statsoft Software, USA). Data were subjected to principal component analysis (PCA).

7.3.3.5 Shuttle Radar Topography Mission (SRTM) Digital Elevation Data Analysis

In order to study the topography of the area aspect map (Fig. 7.1; Table 7.1) was generated from the SRTM datasets downloaded and processed for the study area using the ARCGIS 10.0 software.

7.4 Variation and Levels of POPs and Metals in Lichen *Heterodermia diademata*

The POP concentration was higher as compared to the metal content in the lichen samples analysed (Tables 7.2, 7.3, 7.4,and 7.5). The higher POPs concentration correlates with the physicochemical characteristics (persistence and lipophilicity) of individual POPs. The low molecular weight POPs dominates within the total pollutant profile of the area.

A total of five PCB congeners analysed (Table 7.2) exhibit ranges from 0.93 to 4.96 μg g^{-1} of which PCB 101 has the maximum concentration of 0.887–2.707 μg g^{-1}. The PCB concentrations of individual location provide substantial evidence about dominance of long-range transport of PCBs in the area. The most dominant congeners are tetra-, penta- and hexa-PCBs. The trichlorinated congeners have not been detected from any of the locations. The probable reason for absence of the trichlorinated PCBs may be due to its biodegradable nature (Park et al. 2010).

It is interesting to note that hexacongeners were reported only from urban location, thereby affirming their local anthropogenic source, while other remote locations exhibit higher concentration (more than 85%) of tetra- and penta-PCBs of the

Table 7.3 GC-ECD quantification of polychlorinated biphenyl (PCB) congeners detected (in µg g^{-1}) in lichen *Heterodermia diademata* from Lansdowne area of Garhwal Himalaya

Code name of PCB	IUPAC name of PCB	1	2	3	4	5	6	7	8	9
PCB52	2,2′,5,5′ -Tetrachlorobiphenyl	BDL	BDL	1.35	1.21	1.47	1.37	2.00	1.53	2.17
PCB101	2,2′,4,5,5′ -Pentachlorobiphenyl	0.93	0.89	2.25	2.73	2.41	1.89	2.34	2.71	2.32
PCB138	2,2′ ,3,4,4 5′ -Hexachlorobiphenyl	BDL	0	BDL	BDL	BDL	BDL	BDL	0.42	0.41
PCB153	2,2′,4,4′,5,5′ -Hexachlorobiphenyl	0.59	0.05	BDL	BDL	BDL	0.06	BDL	BDL	BDL
PCB180	2,2′,3,4,4′ 5,5′ –Heptachlorobiphenyl	BDL	BDL	BDL	0.24	BDL	BDL	0.11	BDL	0.06
Σ PCBs		1.52	0.93	3.59	4.17	3.87	3.32	4.45	4.65	4.96

BDL below detection limit

total congener concentrations indicating contribution of long-range transport of PCBs (Park et al. 2010). Since the study area is situated in the foothills of Himalayas, in proximity to Northern plains of India having higher anthropogenic activity, therefore, it receives tremendous pollution load (Wang et al. 2007).

The detection of highly persistent (hexa and hepta) congeners, such as PCBs 118, 153 and 180 in the lichen samples may be due to local contamination of soil caused by wildlife, such as animal excrements and carcasses (Park et al. 2010). Since the study area is situated in wildlife sanctuary of Uttarakhand in the foothills of Himalaya, therefore, detection of PCB 153 and PCB 180 at dense canopy sites may be well justified. The results are in conformity with the study conducted in Antarctica by Park et al. (2010), which suggests long-range transport potential of PCB 101 into the remote location of Antarctica.

The total PAHs profile (Table 7.3) in the study arearranged from 132.66 –682.30 µg g^{-1} dominated by fluoranthene, which is also one of the most common PAHs encountered in Garhwal Himalayan lichens (Shukla and Upreti 2009; Shukla and Upreti 2012; Shukla et al. 2012; Shukla et al. 2013a, b). The exceedingly high concentration of fluoranthene in the present study may be attributed to long range transport of PAHs in addition to local sources. Reports on Mt. Qomolangma (Mt. Everest) (8844.3 m a.s.l) in the Himalaya also support the results of this study, where fluoranthene accounted for more than 50% of total PAHs, which not only shows its spatial movement but also hopping potential too (Wang et al. 2007). The probable reason for high concentration of fluoranthene together with naphthalene may be due to the fact that both are semi-volatile in nature having long range transport potential to get transported from source to remote locations (Shukla and Upreti 2009; Shukla and Upreti 2012; Shukla et al. 2012; Shukla et al. 2013a, b). Detection of high molecular weight PAHs, benzo [*a*]pyrene, B(a)P between the range of 0.13 – 3.41 µgg^{-1} indicates the potential health risk, as it has been listed as an endocrine disruptor in the environmental hormone category and has been categorised as potential carcinogen (Korenaga et al. 2000).

Table 7.4 HPLC quantification of PAHs and N-PAHs content in lichen, *Heterodermia diademata* sampled from different localities in and around Lansdowne, Garhwal Himalaya in μg g^{-1}

Contaminant	Localities								
	1	2	3	4	5	6	7	8	9
PAHs									
Naphthalene	1.63	0.93	549.53	0.95	1.35	1.12	1.43	0.58	0.70
Acenaphthene	1.12	2.06	1.47	2.37	3.05	3.56	2.56	3.21	3.54
Fluorene	BDL	0.02	BDL	0.08	0.01	0.02	0.02	0.07	0.09
Phenanthrene	BDL	BDL	BDL	BDL	BDL	BDL	0.01	BDL	BDL
Fluoranthene	128.08	142.30	128.94	146.51	157.81	165.67	161.56	163.92	149.97
Pyrene	1.68	0.07	BDL	BDL	0.26	BDL	BDL	BDL	0.06
Benz(a)anthracene	0.01	0.03	BDL	0.01	0.04	0.02	0.04	0.03	0.03
Chrysene	BDL	BDL	0.61	0.16	0.05	0.07	0.03	0.04	0.28
Benz(k) fluoranthene	BDL	0.49	BDL	BDL	BDL	0.24	1.32	BDL	BDL
Benz(b) fluoranthene	BDL	BDL	0.03	0.29	0.48	BDL	BDL	BDL	BDL
Benz(a)pyrene	0.14	0.42	1.38	0.14	0.48	3.42	0.54	0.41	0.80
Dibenz(a,h) anthracene	0.01	0.01	BDL	0.01	0.87	0.02	0.08	0.01	0.02
Indeno(1,2,3-c,d) pyrene	BDL	0.08	0.34	BDL	BDL	0.07	0.05	0.09	0.08
Benzo(g,h,i) perylene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
∑PAHs	132.67	146.41	682.30	150.53	164.41	174.19	167.64	168.36	155.56
N-PAHs									
5-Nitro-1,10 phenanthroline	10.14	1.03	2.62	1.14	0.65	2.04	1.33	4.96	3.76
3-Nitroaniline	120.17	216.57	193.13	148.30	127.60	227.20	144.05	169.85	247.46
8-Nitroquinoline	499.56	105.91	353.36	65.36	93.54	91.44	94.91	84.06	92.47
2-Nitroaniline	12.21	2.69	18.98	2.24	2.12	5.01	4.02	2.39	2.23
2-Nitrofluorene	4.90	10.60	15.52	10.35	14.22	17.35	7.95	14.00	14.24
1-Nitropyrene	72.42	78.17	79.41	72.33	78.55	71.14	74.60	74.69	72.01
∑N-PAHs	719.38	414.97	663.02	299.72	316.68	414.18	326.85	349.96	432.17

Diagnostic ratios are frequently used to predict the PAH sources. The Fluo/Fluo+Pyr ratio presumes that ratios in the range ≥0.4 and ≤0.5 indicate petroleum combustion, ratio <0.4 indicates petroleum sources, while ratio >0.5 indicates grass, wood and coal combustion. The value of BaA/BaA + Chry ratio <0.2 indicates petroleum origin, ratios in the range ≥0.2 and ≤0.35 as mixed sources (pyrogenic as well as petrogenic origin) and >0.35 as combustion sources (Yunker and Macdonald 1995; Inengite et al. 2012). The Ind/Ind + BghiP ratio presumes that PAH ratios <0.2 indicate petroleum sources, ratio in the range ≥0.2 and ≤0.5 as petroleum combustion sources and >0.5 as grass, wood and coal combustion sources (Inengiteet al. 2012). In the present study, PAH ratios of Fluo/Fluo+Pyr; BaA/BaA + Chry And Ind/Ind + BghiP ratio have been calculated (Table 7.6). The PAH ratio of Fluo/

Table 7.5 Variation in the metal profile (μg g^{-1}) of lichen, *Heterodermia diademata* sampled from different localities in and around Lansdowne, Garhwal Himalaya

	1	2	3	4	5	6	7	8	9
Cadmium (Cd)	0.45	0.18	0.47	0.67	0.59	ND	ND	ND	ND
Nickel (Ni)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lead (Pb)	6.20	ND	ND	0.78	ND	ND	ND	ND	ND
Zinc (Zn)	69.71	48.63	27.60	82.30	63.64	45.89	32.99	67.20	59.63
Copper (Cu)	1.86	1.30	1.41	3.05	1.61	1.71	1.21	1.74	2.76
Chromium (Cr)	0.77	0.20	ND	0.10	0.17	0.37	ND	ND	0.44
Iron (Fe)	136.59	113.08	ND	153.85	131.13	139.93	ND	ND	156.86
ΣMetal	215.57	163.40	29.48	240.76	197.13	187.90	34.20	68.93	219.69

ND: Not Detected

Table 7.6 Values of diagnostic ratios of PAHs indicating probable source of PAH emission in Lansdowne area

Localities	Flu/Fluo + Pyr	BaA/BaA + Chr	Ind/Ind + BghiP
1	0.99	**1.00**	0.00
2	1.00	**1.00**	1.00
3	1.00	0.00	1.00
4	1.00	0.06	0.00
5	1.00	**0.47**	0.00
6	1.00	0.25	1.00
7	1.00	**0.57**	1.00
8	1.00	**0.44**	1.00
9	1.00	**0.11**	1.00
Major source	Grass, wood, coal combustion	Bold: combustion; normal: petroleum	Grass, coal, wood combustion

Fluo+Pyr and Ind/Ind + BghiP clearly indicates dominance of PAHs released due to grass, coal and wood combustion. The BaA/BaA + Chry ratio further affirmed that apart from grass, wood and coal combustion, petroleum combustion also contributes towards release of PAHs in urban location. Dense forest is surrounded by small village where wood and coal are frequently used for cooking purpose; moreover excessive practice of burning of forest at the onset of monsoon season could be the probable cause for detection of high concentration of PAHs.

The N-PAHs originates from both primary and secondary sources, such as emissions in diesel vehicle exhaust and reactions of the parent PAHs in the atmosphere (Söderström et al. 2005; Bamford et al. 2003). The N-PAHs ranged from 299.72 to 719.38 μg g^{-1}in the Lansdowne area of which 8-nitroquinoline is the most common N-PAH (Table 7.4). Among 1-nitronaphthalene (1-NN) and 2-nitronaphthalene (2-NN), only 1-NN has been detected in diesel exhaust (Feilberg 2000), while gas-phase reactions between naphthalene initiated by OH radicals produce 1-NN and 2-NN in nearly equal abundance (Ou et al. 2010). The concentration ratios of N-PAH may be used to discriminate sources (direct emissions or gas phase reactions) responsible for production of atmospheric N-PAHs. The ratio of 2-nitrofluoranthene to 1-nitropyrene (2-NF/1-NP) is generally used for source apportionment (Fielberg and Nielson 2000; Marino et al. 2000). 2-NF is solely produced from gas phase reactions between fluoranthene and NO_2, initiated by OH during daytime and by NO_3 during night time. 1-NP has never been detected as a product of gas phase reaction and is therefore considered to be the product of direct emissions (Albinet et al. 2008). In the present study, 2NF/1NP ratio below 1 clearly showed that direct emissions from diesel exhaust are the major contributor in the area, due to excessive usage of diesel-driven vehicles in the area due to tourist activity.

The total metal (iron (Fe), chromium (Cr), cadmium (Cd), nickel (Ni), lead (Pb), copper (Cu), zinc (Zn)) ranged between 29.48 and 219.69 $\mu g\ g^{-1}$ (dominated by Fe) (Table 7.5), while Cr, Pb, Cd and Cu ranged between 1.21 and 9.27 $\mu g\ g^{-1}$. Metal profile negatively correlated with PCBs, PAHs and N-PAHs indicating diverse sources of metals and POPs detected. Since metals analysed (mainly Pb, Ni, Cu, Zn, Cr) indicate vehicular activity responsible for the release of these metals, therefore, significant negative correlation ($P < 0.05$) shows minimum local contribution of vehicular pollution on the high level of POPs detected. It is interesting to note that excessively high concentration of POPs is present in lichen samples as compared to metals, which also indicates that apart from local contribution, long-range transportation may also affect the POP profiles.

7.5 Source Apportionment of POPs

PCA analysis of overall pollutant profile including metals (Fig. 7.2) clearly segregated nine sites based on land use and anthropogenic pressure. Location 1 is in the Lansdowne city and represents the urban emission related to human activities that include combustion of fuel together with vehicular activity, while location 3 having dense *Cedrus deodara* tree forest is a famous religious centre that has heavy vehicular activity especially diesel-driven vehicles. The other seven locations situated in forested area have minimum anthropogenic pressure except forest fire. Locations 2, 4, 5, 6, 7, 8 and 9 are exposed uniformly to local, and long-range pollution resulted in formation of a closed cluster at the intersection of the PC1 and PC2 indicating mixed origin of pollutants (long-range and local sources). Since locations 1 and 3 are situated in urban area, they represent local sources of POP emissions. Moreover, drifting of location 2 (northeast) from 1 (very close site, but topographically different, southwest) also shows that spatial and topographical features influence bioaccumulation.

Valuable information regarding diverse sources of each class of POPs and metals could be retrieved from the PCA analysis of individual pollutants (PCB, PAH, N-PAH and metal) (Fig. 7.3; Table 7.7). PCA provided useful information on the distinct sources (1) petrol-driven vehicles vs diesel-driven vehicles and (2) local vs long-range pollution. PC2 segregated pollutants based on local and long range pollution contribution of pollutants which clearly segregates PAHs and PCBs having major long range source while N-PAH and metal originating from local sources. Though both N-PAHs and metals have local sources, their emission sources are different (N-PAHs from

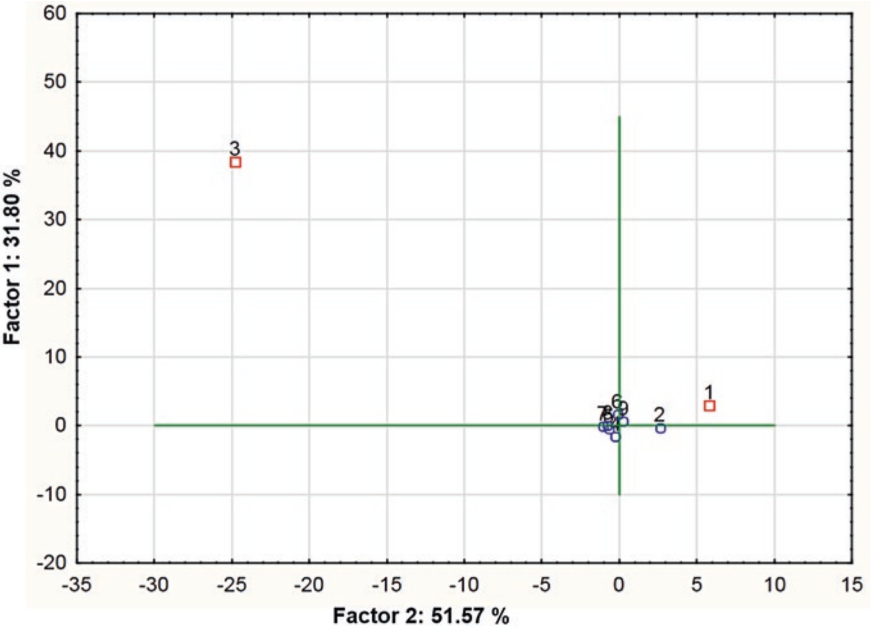

Fig. 7.2 Principal component analysis (PCA) of individual pollutants based on location shows spatial dispersal of pollutants causing formation of single cluster

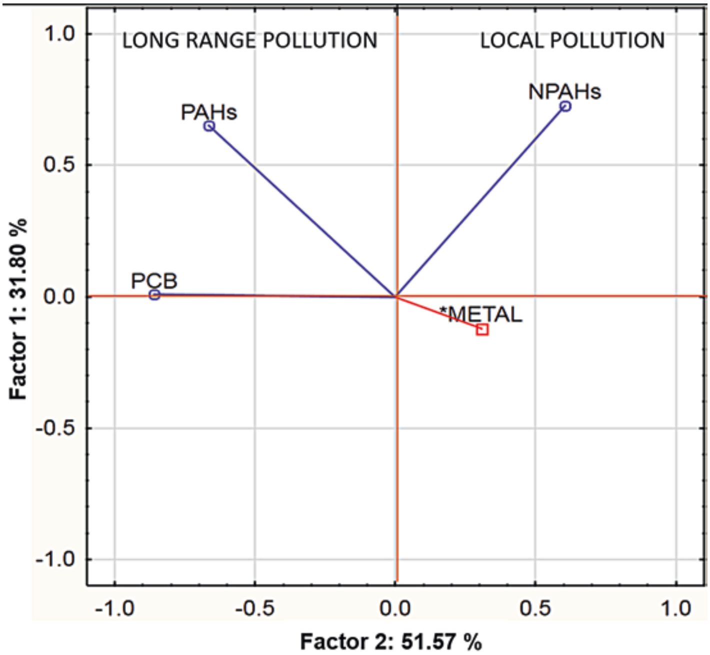

Fig. 7.3 Principal component analysis (PCA) of individual pollutants shows variation based on long range and local source of pollution

Table 7.7 The probable major local and long-range contribution of individual pollutant and its plausible sources based on the location in the foothills of the Himalaya

	Pollutants	Local sources	Long-range transport
1	PCBs	Wildlife	Industrial activity in the SEZ (special economic zones) regions of Uttarakhand located in foothills of Himalayas and air mass from Northern India
2	PAHs	Wood, coal and grass combustion owing to densely forested sites (frequent forest fire) and usage of wood and coal for cooking purpose	Low molecular weight PAHs (naphthalene and fluoranthene are major components of diesel exhaust)
3	N-PAHs	Diesel exhaust	Diesel exhaust (heavy tourist activity in and around the area
4	Heavy metals	Al and Fe are mainly geogenic in origin	No long-range metal contamination is detectable

diesel-driven vehicles and metals from petrol-driven vehicles or geogenic sources); therefore, it further segregated on PC1, where N-PAHs lie in the positive quadrate and metal in the negative quadrate of PC1. The PCA results thus provide statistical validation regarding possible sources of different class of pollutants.

7.6 Evidence of Influence of Long-Range Transport of POPs on Himalayan Ecosystem

The locations with higher concentration of POPs are situated in the lower altitudes in the valley area having dense tree canopy. Since the locations are situated in the Himalayan foothills, therefore, long-range transport from northern plains may also be the probable reason for the excessive bioaccumulation of POPs, as the westerly prevailing winds direct towards the high altitudinal ranges of the Himalayas (Wang et al. 2007). The dense forested vegetation with comparatively lower temperature inside the forest cover and humidity favours cold condensation, thus acting as a favourable sink for the POPs (Table 7.7). Another reason for the ongoing accumulation could be the climatic conditions (humidity ranging between 46% and 56%, temperature ranging between 22.1 and 29.5 °C) and topography which restricts further hoping of the POPs. The north-facing aspect of the hill from where most of the samples were collected has minimal diurnal temperature fluctuation. Since the adsorbed particulate bounded semi-volatile organic compounds (SOCs) remained for longer period of time in such habitats, therefore, it increases the chances of direct exposure or indirect exposure through biomagnification in the food chain. Detection of fluoranthene (PAH) and PCB 101 (PCB) indicates that compounds having semi-volatile characteristics tend to be carried to long distances of their source and accumulate when the favourable condition of temperature and other environmental factors is attained.

7.7 Future Prospects

Although in recent years understanding of POPs especially in mountain areas has significantly increased, still more research is required to be carried out in Indian Himalayas, as this region is exposed to organic contaminants due to major urban sources (air mass from urban cities mainly of India, Pakistan and China) and favourable meteorological conditions (monsoon system and westerly wind direction). It has been observed that the concentration of organic pollutants increases with increase in the altitude (indicating dependence on diurnal and seasonal temperature variation) (Daly and Wania 2005; Wang et al. 2007). The distribution of POPs in lichen samples correlates to a combination of factors including topography, source, aerosol dynamics, wind direction and atmospheric inputs (Bajpai et al. 2013). The POP concentration in lichen samples suggests influence of long-range transport of pollutants from source of origin. At smaller scale high-altitude systems provide clue regarding environmental behaviour of POPs depending upon meteorological conditions. These studies could be utilised to predict the global environmental behaviour of POPs on the base of latitudinal variation too. The estimation of pollutants such as POPs from extreme habitats such as high altitudes is very much neglected (Rajkumari et al. 2019). The present finding further emphasises the need to establish an integrated monitoring effort for persistent pollutants in the Indian Himalayan region. The detection of higher amount of POPs in samples collected from protected areas having complete absence of industrial or other anthropogenic activities clearly suggests long-range transportation of the detected POPs in the area. Study shows that extensive monitoring program should be executed and implemented for monitoring levels of POPs not only in and around the source but also in the remote areas and protected areas for any possible impact of long-range pollution on high-altitude ecosystem of the Himalayas.

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Chapter 8
Rhizoremediation of Polyaromatic Hydrocarbons (PAHs): A Task Force of Plants and Microbes

Ram Naraian, Roshan Lal Gautam, Siya Ram, and Manish Kumar Gupta

Abstract Polyaromatic hydrocarbons (PAHs) are a group of more than hundred highly toxic, recalcitrant, and carcinogenic organic compounds, generated after incomplete combustion of organics, and persist in the environment as very noxious pollutants. Generally, several anthropogenic activities often pollute the upper fertile and rhizosphere soil of earth that exerts multiple harmful influences on the ecosystems. The severe contaminations from PAHs cause ill health of soil by the damage of plant, animals, and microorganisms, which may result in imbalance in the ecosystem. Though various physical and chemical methods have been attempted, they were found inappropriate, non-economical, and non-ecofriendly. The rhizoremediation presents itself as a potential approach for the remediation of soil from the PAHs using plant and rhizosphere microbes. The synergistic interaction of plant and existing rhizosphere bacteria has a wonderful role in bioremediation of PAHs, which constitutes rhizoremediation as an excellent and very attractive green technology. Under the integrated rhizoremediation approach of PAHs, plant roots on one side secrete organic exudates, while on the other side, bacteria contribute various enzymes to degrade recalcitrant PAHs into non-toxic forms. Thus, this eco-friendly technology establishes an approach, which not only limits to PAHs but also has a broad spectrum of bioremediation for various other recalcitrant organic pollutants such as chlorinated phenyls, explosives, insecticides, fungicides, etc. In consequence, practical implementation of rhizoremediation at ground level for decontamination of highly polluted sites needs to be promoted. The present chapter emphasizes detailed account on rhizoremediation of PAHs using an integrated approach of plant and microorganisms.

Keywords Rhizosphere · Rhizoremediation · PAHs · Plant exudates · Soil microbes · Organic pollutants

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8.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are highly stable compounds with two or more fused benzene rings in linear, angular, or cluster formation (Muckian et al. 2007) and represent a diverse group of organic compounds. These compounds are ubiquitously distributed in nature. The PAHs are added in the environment by natural and various anthropogenic activities like forest fires, volcanic eruptions, vehicular emissions, residential wood burning, petroleum catalytic cracking, and industrial combustion of fossil fuels (Gan et al. 2009; Mumtaz and George 1995). The incomplete combustion and pyrolysis of organic matters is a well-known cause for PAH production. Contamination of soils with PAH compounds poses a significant risk to the soil ecosystems, because of their highly toxic, mutagenic, and carcinogenic nature (Cerniglia and Heitkamp 1989; Patnaik 1992). However, remediation of PAH-contaminated soil can be organized through the traditional mode such as simple incineration and via landfilling, but these processes exert additional side effects on the environment. The alternative biological technique with mutualistic associations between plants and microorganisms to decompose contaminants has become more applicable to clean up the environment (without any side effects) and is known as rhizoremediation (Nichols et al. 1997). The process of rhizoremediation has also established its effectiveness for a wide range of organic pollutants including insecticides (Kidd et al. 2008), explosives (van Dillewijn et al. 2007), polychlorinated biphenyls (Slater et al. 2011), and different petroleum hydrocarbons (Gaskin and Bentham 2010; Meng et al. 2011; Yateem et al. 2007). This most effective phytoremediation technology exploits plant roots and their associated microbial interactions in the rhizosphere for removal of organic contaminants (Ma et al. 2010).

Rhizoremediation is the consequence of an amazing relationship between plant and microbe interactions to degrade pollutants from contaminated sites to clean up the environment (Arora et al. 2018). The beneficial relationship between plant roots and soil microbes is recognized from the past century, and defined rhizosphere as the zone of soil where microbes are influenced by the root system (Martin et al. 2014). The plant rhizosphere is an ideal and dynamic micro-environment in which microorganisms interact very well and get benefited from root exudates (Clegg and Murray 2002). The phenomenon of rhizoremediation occurs naturally as the flavonoids and other compounds released by plant roots stimulate growth and activity of PAH-degrading bacteria (Thoma et al. 2003; Leigh et al. 2006; Jha et al. 2018). Furthermore, root growth and death of older roots promote soil aeration and result in facilitated oxidative degradation of PAHs (Leigh et al. 2002; Bisht et al. 2015).

The present chapter discusses the chemical nature of PAHs, their phytoremediation, biodegradation, mechanisms of rhizoremediation, and use of various microbes and plants working in rhizoremediation of carcinogenic PAH compounds.

8.2 Polyaromatic Hydrocarbons (PAHs)

PAHs are organic compounds consisting of more than one aromatic ring. These are generally produced through the incomplete combustion of organic substances, such as coal, oil, and gases. Such hydrocarbons directly pollute water, air, and soil, by means of emissions and agro-industrial production, as well as via irrigation of farmland using wastewater (Liu et al. 2014). Most often these are produced by several anthropogenic activities related to incomplete combustion of organic materials including wood and vegetation, coal, diesel, fat, and urban runoff (Bakeas et al. 2011; Singh and Tiwary 2017). In addition, various industrial activities such as coal liquefaction, coke production, petroleum refining, spillage of petroleum products, and waste incineration also facilitate considerable accumulation of PAHs in the environment, particularly at the sites closer to industries (Lim et al. 1999; Kanaly and Harayama 2000; Haritash and Kaushik 2009; Zhao et al. 2009; Bakeas et al. 2011; Singh and Tiwary 2017). PAHs exhibit very strong characteristics of carcinogenicity, mutagenicity, and teratogenicity (Menzie et al. 1992; Wilson and Jones 1993); therefore, the problem of PAHs has become a global environmental issue. These are extremely widespread and common industrial contaminants found in many scattered locations all around the world. In addition, hydrophobic nature of most PAHs facilitates them to bind with particulates of soil and consequently making their reduced biological uptake (Peng et al. 2008). Based on the survey of the literature, contaminations of PAHs are found to be very harmful to the health of biologicals including plants/animals and environment. Long time exposure of PAHs may cause birth defects, skin irritation, and disturbance of the immune system in animals including humans. Several studies performed on exposure of PAHs reported increased risks of cancer (Mastrangelo et al. 1996; Boffetta et al. 1997), enhanced risk of reproductive damage (Miller et al. 2004; Hsu et al. 2006), and suppression/damage of immune system (Sul et al. 2003).

8.3 Major PAH Compounds

Among a good number of recalcitrant petroleum compounds, PAHs comprise a wide range of hydrophobic members with two or more aromatic benzene rings (Chaudhry et al. 2005). In ecosystems, a broad range with more than 100 types of diverse PAH compounds are reported, and most of them persist to be solubilized and adsorbed on solid particles for several years (Volkering et al. 1993; Bosma et al. 1997). Among a wide range of PAH compounds, naphthalene and 2-bromonaphthalene with two benzene rings are the simplest compounds (Smith 1990). Based on the benzene rings (low-high) available and their nature of complexities, few important PAHs can be serially represented as anthracene, phenanthrene, tetracene, chrysene, pyrene, triphenylene, pentacene, benzo[a] pyrene, corannulene, coronene, ovalene, and benzo[c]fluorine.

8.4 Bioremediation and Biodegradation of PAHs

Microbial decontamination of soils polluted with the carcinogenic PAHs is an essential and relevant task for remediation of polluted soils (Peng et al. 2008). There are several important and potential methods for the remediation and control of soils contaminated with PAHs, including physical remediation, chemical remediation, bioremediation, and phytoremediation (Zhou and Sun 2004). In recent years, more attention has been paid on bioremediation technology due to its exceptional advantages with low cost (Liu et al. 2014; Arora 2018). It is established that greater the number of benzene rings in the structure of PAH, consequently the greater the hydrophobicity, toxicity, and environmental persistence, therefore, lesser bioavailability and biodegradability (Cerniglia 1992; Prak and Pritchard 2002; Seo et al. 2009; Singh and Tiwary 2017).

In general, PAHs are highly resistant to microbial attacks due to their low water solubility and complex structure with two or more fused benzene rings (Antizar-Ladislao et al. 2004). Many bacterial species degrade PAH compounds typically through an assimilative process to achieve carbon and energy for growth, as a result of mineralization of the complex PAHs (Kastner et al. 1994; Haderlein et al. 2006). In several studies, a group of microorganisms including bacteria, fungi, and algae have been used for the microbial degradation of PAHs. In this regard, several bacteria that generally employ intracellular dioxygenase enzymes were attempted for the successful degradation of PAHs (Johnsen et al. 2005). In addition, various ligninolytic fungi are also able to degrade and mineralize PAHs, and the mechanism is considered similar to that of lignin degradation (Sanglard et al. 1986). The oxidation of most of the PAHs is catalyzed by the laccases and peroxidases that results in the production of PAH-quinones and further oxidized into phenols using non-ligninolytic fungus *Cunninghamella elegans* (Cerniglia 1992).

8.5 Rhizoremediation of PAHs

Rhizoremediation is a biological technique, which includes the mutualistic relationship between plants and microorganisms to decompose complex pollutants for cleaning the environment (Mishra et al. 2017). In other words, rhizoremediation can be defined as the exploitation of beneficial plant-microbe interactions for the purpose of environmental cleanup. The interactions between plant root and microbial cells in rhizosphere offer a very useful way for remediation of environments polluted with highly recalcitrant organic compounds (Chaudhry et al. 2005). Therefore, rhizosphere regions are the ideal sites for experimental studies of competitive interactions of microorganisms. As it is obvious that without the microbial contribution, phytoremediation alone cannot be a viable technology for remediation of many hydrophobic organic pollutants (Chaudhry et al. 2005). Therefore, use of microbial community residing in the rhizosphere region of plants for the aim of bioremediation is referred as rhizoremediation (Anderson et al. 1993; Kuiper et al. 2004). The technique of rhizoremediation includes both phytostimulation and rhizodegradation activities describing the importance of both plant and microorganisms with beneficial interaction.

The technology of rhizoremediation is a very effective, non-destructive, and inexpensive process of bioremediation in a contaminated environment (Chaney et al. 1997). In comparison to few other methods of remediation such as mechanical and chemical methods, rhizoremediation is more cost-effective in terms of remediating many hazardous compounds from the environment (Pradhan et al. 1998). Besides, this wonderful technology has satisfactory social acceptance along with the potential support of regulatory agencies (Alkorta and Garbisu 2001). The most important aspect of rhizoremediation is characterized by the occurrence of plant growth-promoting bacteria growing in polluted soils. These bacteria promote the growth and provide various benefits to plants directly and indirectly. The important benefits to plants include providing compounds to protect plants from stress, chelators for delivering key plant nutrients, protection from plant pathogens, and degradation of pollutants before their negative impact on the plants (Balloiet al. 2010; Ma et al. 2011).

8.6 Task Force of Plant-Microbe Interaction in Rhizoremediation

Plant-microbe interaction in the rhizosphere region is a well-established association, which can efficiently execute the remediation of complex pollutants from contaminated sites. Pollutant-degrading bacteria are found associated with the roots in the rhizosphere of native plants. Plant roots provide a rich platform for bacteria to grow in the presence of root exudates; in consequence, bacteria working as biocatalysts remove pollutants (Segura and Ramos 2013) establishing a potential approach of rhizoremediation. Therefore, with rhizodeposition, plants bring a biostimulation activity to rhizosphere microbial population, in response to executing degradation of contaminants (Singer et al. 2003; Musilova et al. 2016). Hence, the intricacy of the advantageous relationships among plants and bacteria is very exciting (Leigh et al. 2006).

Moreover, the association of plant and rhizobacteria in promoting plant growth also assists the rhizoremediation of PAHs and many other contaminants. Subsequently, root-associated bacteria also provide various other benefits to plants through direct and indirect activities such as (a) synthesis of stress-reducing compounds to protect the plants, (b) production of chelators to deliver key plant nutrients, (c) protection from plant pathogens, and (d) degradation of pollutants before they impact the plants negatively (Balloi et al. 2010; Ma et al. 2011). In particular, plants growing in highly contaminated soils often have to face collective stresses of nutrient deficiency and chemical phytotoxicity (Mapelli et al. 2012). To overcome

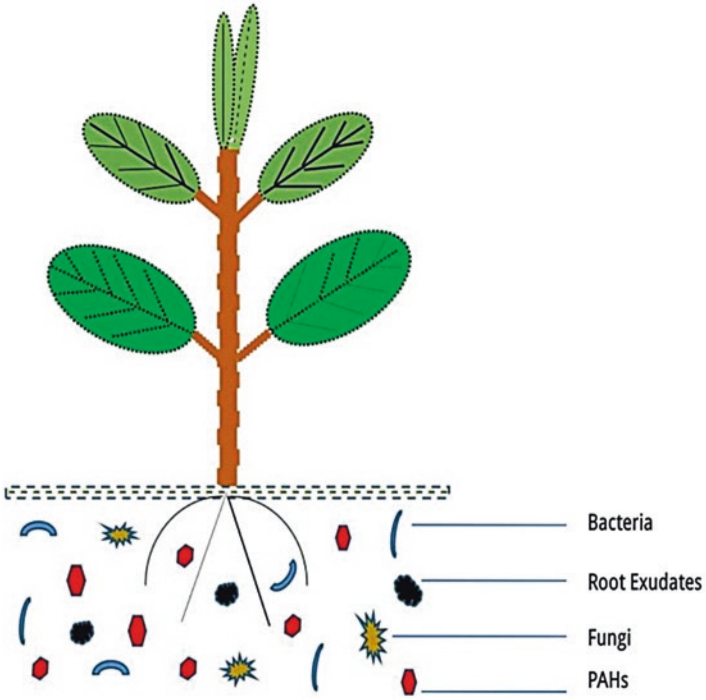

Fig. 8.1 Demonstrating rhizosphere region of a plant growing in the site contaminated with PAHs, where it contains bacteria, fungi, and secreted plant root exudates managing undertake for rhizoremediation

such challenges of plant stress during phytoremediation, rhizobacteria express 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Glick 2010). Generally, bacterial ACC deaminase metabolizes ACC and favors plant growth with the consequences of reduced stress susceptibility (Van de Poel and Van Der Straeten 2014).

Besides the growth of a plant, root promotes soil aeration and porosity, which results in the enhanced degradation of recalcitrant organic pollutants (Van Aken et al. 2010). The aromatic compounds released in root exudates often structurally resemble with many organic contaminants, and they can, therefore, stimulate the proliferation and activity of bacteria, acting as cometabolites by activating gene expression (Federici et al. 2012; Sylvestre 2013; Jha et al. 2015). Therefore, based on the advocacy of several studies, positive natural interactions between plants and bacteria can achieve the climax level of rhizoremediation to decontaminate from organic pollutants (Fig. 8.1).

The soil is known to be a very complex ecosystem with huge biodiversity of diverse microorganisms. The microorganisms including bacteria, archaea, and fungi conduct the maximum chemical and biochemical reactions (Nannipieri et al. 2003). Moreover, soil generally known to be poor in necessary nutrients and energy sources, besides contains hot spots, the zones of highest biological activities including plant and animals, animal manure, and rhizosphere (Nannipieri et al. 2003). The availability of basic nutrients is a critical factor for wood-degrading fungi to make them able to survive in soil (Baldrian 2008), including other microbes. Therefore, due to the limitation of nutrients, soil becomes the site of competition for nutrients and living space between rhizospheric microorganisms.

8.7 Phytoremediation of PAHs

Traditional physicochemical bioremediation of contaminated soils has encouraged the development of new remediation technologies (Huang et al. 2004). Therefore, phytoremediation emerged as an alternative technique with low cost and eco-friendly technology for remediation of soil and water pollutants. Phytoremediation has been now used as a technology for the control of environmental pollutants and their bioremediation, which is well established (Liu et al. 2014). Phytoremediation refers to transferring, accommodating, or transforming toxic and hazardous pollutants by the use of plants and thereby consequently making the pollutants harmless (Sun et al. 2008; Zhou and Sun 2004). This very unique and plant-based technology is a very promising strategy, which has been broadly used for its in situ removal of many organic contaminants through processes like phytovolatilization, phytodegradation, and phytoextraction by different plant species (Sandermann 1994; Schnoor et al. 1995; Cunningham et al. 1995). The plant species employed in the present green technology are known as hyperaccumulators, which often grow very well in contaminated areas with elevated concentrations of pollutants both in soil and in water. Therefore, this unique technology is applicable for the treatment of a wide variety of contaminants and is well suited for the decontamination of sites with contaminated soil, streams, and groundwater (Singh et al. 2003).

Phytoremediation of PAH-contaminated soils primarily includes few distinct mechanisms including (a) direct absorption of PAHs in plants, (b) plant volatility and adsorption, (c) degradation of PAHs by rhizosphere microbial communities, (d) and secretion of plant exudates plus enzymatic hydrolysis of PAHs (Liu et al. 2014). In literature several studies have reported different species of grass such as *Agropyron smithii*, *Bouteloua gracilis*, *Cynodon dactylon*, *Elymus canadensis*, *Festuca arundinacea*, *Festuca rubra*, and *Melilotus officinalis,* which are also known to degrade PAHs (Haritash and Kaushik 2009; McCutcheon and Schnoor 2003; Christensen-Kirsh 1996) from contaminated soil and water resources.

8.8 Microbial Bioremediation of PAHs

The understanding of the mechanisms of PAH catalysis through microorganism-mediated actions facilitated the development of microbial bioremediation of PAHs from the contaminated sites (Penget al. 2008). The PAHs characteristically are recalcitrant in nature, and only selected microorganisms have decent ability to decompose and utilize them as the sole source of carbon and energy under optimal conditions (Tam et al. 2003; Singh and Tiwary 2017). According to the reports of various researchers, it has been confirmed that a big number of aerobic microorganisms can very promptly degrade various PAHs. Based on recent studies, important bacterial members include *Pseudomonas* (Ma et al. 2013; Pawar et al. 2013), *Bacillus* (Bisht et al. 2015), *Mycobacterium* (Kim et al. 2007), *Stenotrophomonas* (Gao et al. 2013), *Sphingomonas* (Festa et al. 2013), *Streptomyces* (Ferradji et al. 2014), and *Martelella* (Cui et al. 2014). These bacteria of diverse genera contribute to the breakdown of aromatic PAH compounds and ultimately to the universal and ecological carbon cycle. The closed aromatic ring containing the structure of PAHs makes their degradation pathways much diverse and complex.

8.9 Basic Mechanisms of Rhizoremediation

The process of rhizoremediation is with the use of unique machinery of plant-microbe interaction in rhizosphere environment. Rhizoremediation of PAHs in soil is described as an effective remediation technology (Pant et al. 2016). In rhizoremediation, PAH-degrading rhizobacteria are stimulated by the root systems of host plant, and joint action of both consequently decontaminates the pollutants. Therefore, rhizoremediation strategy requires a balance between the knowledge of physiology of symbiosis, mechanism of bioremediation, and accurate bioformulation to apply in the contaminated sites (Singha and Pandey 2017). Bacterial succession, present in rhizosphere, is enormously stimulated by the presence of the plants. This stimulation is caused by the availability of complex carbohydrates in rhizosphere. The strategy of rhizoremediation is enhanced by utilizing rhizobacteria with the plant growth-promoting attributes, resulting in enhanced growth of host plant even in the presence of pollutant and creating a healthy root system for effective remediation (Bisht et al. 2015). Under this healthy rhizosphere environment, rhizobacteria, therefore, provide plant growth-promoting functions such as nitrogen fixation, acquisition of phosphorus, siderophore synthesis, IAA, HCN production, and enzyme ACC deaminase (Singha and Pandey 2017). In consequence, plants through its rhizospheric system remediate organic pollutants by the strategies primarily including direct uptake of contaminants, conversion into non-toxic forms, and accumulation in cells; secondarily the release of exudates to stimulate microbial enzyme activity and bio-transformation; and lastly enhanced mineralization in the contaminated rhizosphere (Mackova et al. 1997). Therefore, the whole phenomenon of rhizoremediation can be attributed to two major incidences including phytostimulation and rhizodegradation. In the process of phytostimulation, stimulation of microbial degradation by plant enzymes, secondary plant compounds (e.g., terpenes, saponins), and root exudates takes place (Fig. 8.2). However, under the subsequent process of rhizodegradation, the decomposition of various pollutants in the rhizosphere takes place due to microbial activities.

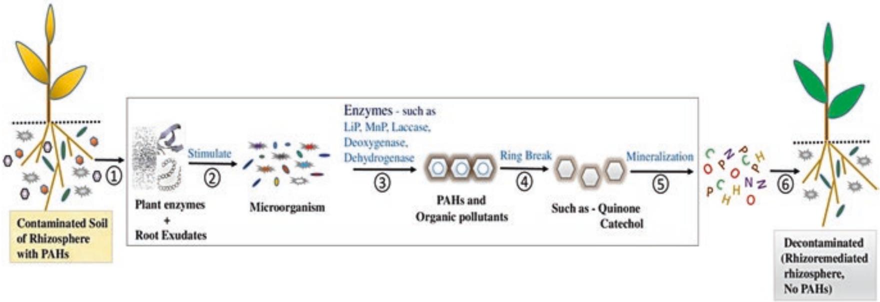

Fig. 8.2 Demonstrating multistep process of rhizoremediation occurring in the rhizosphere contaminated with PAHs. (*1*) Secretion of plant enzymes and root exudates, (*2*) stimulation of microorganisms by plant enzymes and exudates, (*3*) secretion of enzymes and attack over PAHs, (*4*) breakdown of rings of PAHs, (*5*) mineralization, and (*6*) rhizoremediated rhizosphere soil (free from PAHs)

8.10 Microbial Degradation of PAHs

Rhizoremediation of PAHs is a process requiring potent microorganisms and most often combined action of microbial consortia to decontaminate soils and waters. A variety of PAH-degrading microorganisms present in nature are actively involved in different environmental conditions playing an important role in decontamination of these pollutants. The unique catabolic characteristics of microorganisms establish the potential act of bioremediation. Different classes of PAH-degrading microorganisms including algae, fungi, and bacteria conduct the decomposition of PAHs. Of these, bacteria and fungi are extensively studied for their specific ability to degrade xenobitic compounds including PAHs (Haritash and Kaushik 2009). However, different microbial communities in the rhizosphere still are known to be unpredictable and, therefore, termed as black box (Dua et al. 2002).

8.11 Bacteria Involved in Rhizoremediation of PAHs

During the last few decades, many bacteria have been identified which are capable of degrading PAHs either through metabolism or co-metabolism. Bacteria in natural and laboratory conditions readily degrade the PAHs of low molecular weight such as naphthalene, phenanthrene, and anthracene (Cerniglia 1992; Sutherland et al. 1995). The viability of rhizosphere bacteria relies on plant root exudates that promote plant growth by increasing nutrient availability and assimilation as well as by reducing negative influences of pollutants and other environmental stresses on plants (Gerhardt 2009; Silambarasana and Vangnai 2017). There are various commonly studied PAH-degrading bacteria, which include *Pseudomonas* sp. *Mycobacterium* sp., *Haemophilus* sp., *Rhodococcus* sp., and *Paenibacillus* sp.

(Bisht et al. 2015). There have been multiple reports describing the microbial oxidation of four-ring PAHs. In a broad study, Aitken et al. (1998) isolated 11 strains from different contaminated sites with oil, motor oil, wood treatments, and refinery areas, which had the ability of degradation. Out of the 11 strains, 3 distinct species of *Pseudomonas*, as well as *Agrobacterium*, *Bacillus*, *Burkholderia,* and *Sphingomonas*, were identified (Aitken et al. 1998). In few other studies, benzo(a)pyrene has been reported to be degraded by the distinct bacterial isolates such as *Rhodococcus* sp., *Mycobacterium*, and co-culture of *Pseudomonas* and *Flavobacterium* sp. (Walter et al. 1991; Trzesicka-Mlynarz et al. 1995; Schneider et al. 1996). Several other bacterial members involved in the rhizoremediation and biodegradation of PAHs are compiled in Table 8.1.

8.12 Fungi Involved in Rhizoremediation of PAHs

Several fungi have been investigated to be capable of utilizing PAHs in contaminated sites. Fungal groups including filamentous fungi, basidiomycetes, white-rot fungi, and deuteromycetes have strong ability to remove PAHs competently in comparison to bacterial isolates (Cerniglia 1997; Zheng and Obbard 2003). The fungi have very strong machinery of lignocellulolytic extracellular enzyme production, which also helps in the degradation of PAHs. Few recent studies related to the rhizoremediation explaining the role of fungal members in rhizosphere are compiled in Table 8.2.

8.13 Factors Affecting Rhizoremediation of PAHs

Since the phenomenon of rhizoremediation is an act of plant-microbe interaction, therefore the level of association of both in the rhizosphere is considered to play a very critical role. The process of rhizoremediation is remarkably influenced by several physical, chemical, and biological properties of the rhizosphere (Al-Ameeri and Al Sarawi 2017), which include soil composition, temperature, pH, organic matter, and existing climate. Rhizoremediation establishes a reciprocal relationship between plant and their associated rhizosphere microflora, which determines the intensity of rhizoremediation.

The phenomenon of PAH biodegradation is also greatly influenced by many physicochemical factors such as nutrients, oxygen, pH of soil, composition, and concentration (Margesin and Schinner 2001). They suggested that the bioavailability of the pollutants, chemical and physical properties, and the pollution history of the contaminated sites also influence the process of pollutant degradation (Margesin and Schinner 2001). Various experts mentioned variability in microbial diversity and metabolic activities of microorganisms in the rhizosphere micro-environment as the rhizosphere effect (Salt et al. 1998; Kuiper et al. 2004; Pilon-Smits 2005;

Table 8.1 Summary of various important studies related to the process of PAH rhizoremediation using specific association of different genera of plant and bacteria

Plants	Bacteria	Different PAHs	References
Sugar beet	*Pseudomonas flourescens*	PCBs (polychlorinated biphenyls)	Brazil et al. (1995) and Bisht et al. (2015)
Calotropis	*Pseudomonas* sp.	Naphthalene and anthracene	Shukla et al. (2012)
Barley	*Burkholderia cepacia*	2,4-D	Jacobsen (1997)
Common reed	*Sinorhizobium meliloti* P221	Phenanthrene	Golubev et al. (2009)
Grasses	*Pseudomonas putida*	Napthalene	Kuiper et al. (2001)
Populus deltoides	*Actinomycetes*	1,4-Dioxane	Schnoor et al. (1998)
Festuca arundinacea	*Bacillus licheniformis, Bacillus mojavensis*	Naphthalene, phenanthrene, benzo[a]anthracene	Eskandary et al. (2017)
Ryegrass, red fescue, chickpea, red lentil	*Verrucomicrobia Actinobacteria*	Pyrene or phenanthrene	Kawasaki et al. (2012)
Clover root	*Novosphingobium* sp. (*HS2a*)	Phenanthrene	Conde et al. (2016)
Senecio glaucus	*Arthrobacter*	Oil	Radvan et al. (1995)
Populus sp.	*Pseudomonas alcaligenes, Pseudomonas stutzeri, Pseudomonas putida*	Naphthalene, phenanthrene, anthracene, and pyrene	Frerot et al. (2006), Shukla et al. (2011) and Shukla et al. (2013)
Populus deltoides	*Pseudomonas putida F1*	Anthracene and naphthalene	Paul et al. (2005) and Parales et al. (2000)
Coat poplar tree roots	*Burkholderia cepacia G4*	Trichloroethylene (TCE)	Plociniczak et al. (2011)
Meadow fescue	*Azospirillum* sp. (*Pseudomonas stutzeri)*	Anthracene, phenanthrene, and pyrene	Gałązka et al. (2012)
Brassica alba	*Rhodococcus* sp. (*IC10*	Phenanthrene	Kim et al. (2007)
Wheat	*Pseudomonas putida*	2,4-D	Kingsley et al. (1994)
Hordeum sativum, Brassica napus	*Pseudomonas fluorescens, Pseudomonas aureofaciens*	Phenanthrene	Anokhina et al. (2004)
Poplar	*Pseudomonas* sp.	(MTBE) methyl tert-butyl ether, TCE (trichloroethylene), BTEX (benzene, toluene, ethylbenzene, and xylene)	Germaine et al. (2004) and Moore et al. (2006)

(continued)

Table 8.1 (continued)

Plants	Bacteria	Different PAHs	References
Festuca arundinacea and *Festuca gigantea*	*Gordonia* sp.	Benzo(k)fluoranthene	Liu et al. (2014)
Yellow lupine	*Burkholderia cepacia*	Toluene	Barac et al. (2009)
Senecio glaucus	*Arthrobacter*	Oil	Al-Ameeri and Al-Sarawi (2017)
Corn	*Gordonia* sp. (S2Rp-17)	Diesel	Hong et al. (2011)
Pea	*Pseudomonas* sp.	Naphthalene	Germaine et al. (2009)

Table 8.2 Summary of various recent studies related to the process of PAH rhizoremediation using specific association of different plant and fungal strains

Plants	Fungi	Different PAHs	References
Oryza sativa	*Phomopsis* sp.	Phenanthrene	Tian et al. (2007)
Vetiveria zizanioides, Chloris barbata, Eleusine indica, Imperata cylindrica	*Glomus mosseae*	Crude oil	Kuo et al. (2014)
Bischofia polycarpa	*Ceratobasidum stevensii, Phomopsis* sp. ((B3)	Phenanthrene	Dai et al. (2010)
Phyllosphere	*Penicillium oxalicum*	Phenanthrene, naphthalene, toluene, xylene	Kannangara et al. (2016)
Brassica alba	*Aspergillus terreus, Penicillium* sp.	Phenanthrene	Kim et al. (2007)
Zea mays	*Glomus vesicular*	Atrazine	Nelson and Khan 1992)
Pinus pinea	*Ganoderma australe*	Lindane	Rigas et al. (2007)
Senecio glaucus	*Penicillium* sp.	Oil	Al-Ameeri and Al-Sarawi 2017)

Barea et al. 2005; Al-Ameeri and Al Sarawi 2017). In addition, it is also considered that the age of the plant, the availability of mineral nutrients, and the pollutants are important factors affecting the rate of root exudation (Chaudhry et al. 2005).

Moreover, the organic compounds in root exudates additionally provide carbon and nitrogen sources for the growth and survival of microorganisms capable of rhizoremediation of organic pollutants (Anderson et al. 1993; Kuiper et al. 2004). The exudation profile, therefore, extremely influences the microorganisms in the rhizosphere and eventually has a very considerable impact on the process of rhizoremediation of PAHs and other pollutants in nature.

8.14 Conclusion and Future Perspectives

Rhizoremediation is a green technology that synergistically employs plants and rhizospheric microbes to degrade complex and recalcitrant PAH compounds into nonhazardous simpler compounds. The polluted sites of PAHs can be cleaned using the rhizoremediation technique with the mutualistic approach of plant roots and associated rhizosphere microbes such as algae, fungi, and bacteria. Though the process of rhizoremediation and its machinery including plant and microbial community are greatly influenced by several factors, so with diversified conditions, selectively resistant and novel plant and microbes must be attempted for the success of rhizoremediation. Therefore, we suggest the large-scale use of rhizoremediation technique for the decontamination of polluted sites from harmful PAHs.

Nevertheless, for better achievements, further advancements including introduction of new and resistant plants with novel microorganisms for diverse environmental conditions are required. Therefore, further researches for the search and selection of suitable rhizosphere bacterial strains able to properly work with the root system for efficient rhizoremediation are urgently required. In addition, the development of genetically engineered and potent microorganisms and versatile plant varieties should be incorporated in the system of rhizoremediation.

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Chapter 9
Cadmium Stress Tolerance in Plants and Role of Beneficial Soil Microorganisms

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Abstract Heavy metal stress triggers anomalies in the plant metabolic activity thereby reducing the yield potential of the crop plants. Cadmium (Cd) is one of the toxic heavy metals that is continuously added to the soil through natural as well as anthropogenic means and once taken up by plants can inhibit plant growth and development. Cd toxicity causes problem in uptake and metabolism of essential mineral elements as a result of reduced enzyme activity and protein synthesis. It has been observed that essential mineral nutrients and the available soil Cd show direct competition for the transport proteins. In addition, Cd alters with the sulfhydryl group of proteins resulting in reduced enzyme activity. Soilborne microorganisms include all actively metabolizing organisms directly or indirectly associated with the improvement of soil health and the existing flora. Among the soilborne beneficial microorganisms, arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) have been widely accepted for their growth-promoting role. Optimization of important physiological and biochemical processes in plants can be achieved by the soilborne microorganisms. Hence exploiting their unique properties including stress tolerance via synthesis of compatible

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solutes and phytohormones, biocontrol agents, etc. should be exploited. The present review discusses the role of beneficial soil microorganisms in alleviating the effects of Cd stress in crop plants.

Keywords Stress tolerance · Plant microbiome · Arbuscular mycorrhizal fungi · PGPR · Cadmium

9.1 Introduction

Soil solution forms the key component for healthy plant growth and inhibiting microbes, and any alteration in its physical, chemical, and biological characteristics will have direct influence on the existing flora. Likewise, interactions triggered due to metal pollution affect the bioavailability of essential nutrients (Nazar et al. 2012; Ahmad et al. 2015; Kamran et al. 2015). Some plants modulate metal accumulation using various transporters either via active or passive mechanisms resulting in net change in the soil pH around root, i.e., rhizosphere. Alterations in the rhizosphere soil pH majorly affect the bioavailability of the pH-dependent nutrients in addition to the toxic metal ions (Hinsinger et al. 2006). As a common view, soil contains nutrients as well as toxic metals which often display interactions at precipitation, absorption, and the complex formations with the important organic compounds (Pantazis et al. 2007; Chibuike and Obiora 2014). Root secretions or exudates considerably influence Cadmium (Cd) bioavailability and its toxicity due to alterations in the pH, chelation, redox state, intensity, and the activity of soil microbes. It has been observed that the optimal availability of root-secreted organic substances like oxalic acid, citric acid, etc. in the rhizosphere has a key role in reducing the absorption of toxic metals like Cd by formation of metal-chelate complex.

Growth of plants is determined by several factors, and excess presence of a particular factor can create alterations in normal developmental and metabolic processes. Stresses, including biotic and abiotic, impart deleterious effects on the crop growth and hence result in significant yield losses. Between the abiotic stress factors, the presence of heavy metals in agricultural soils has been observed to restrict growth of major food crops. Cd is one of the toxic heavy metals easily taken up by plants and obstructs the metabolic processes. The most accepted reasons for the increased concentration of Cd in soils have been the greater usage of chemical fertilizers, sewage wastewater for irrigation, and rapid industrialization. All these sources result in triggering harmful effects on soil-plant environment system. Cd has been ranked among seven most toxic substances and the key reason for the alarming toxicity of being its prolonged lifetime in soil. It has been observed that the concentration of Cd in soils considered as suitable for normal growth is less than 0.5 mg kg^{-1} of soil (Vahter et al. 1991). Cd is considered as a non-essential element for the crop plants; however, it has high mobility between soil and plant system, thereby entering the food chain resulting in significant damage to plants as well as animals including humans. Different threshold levels for Cd accumulation in plants have been determined in crops like cereals (0.013–0.22 mg kg^{-1}) and grasses (0.07–0.27 mg kg^{-1}) (Kabata-Pendias and Pendias 2001). There are considerable evidences that excessive use of phosphate fertilizers or phosphorites also results in heavy accumulation of Cd in soils resulting in alteration of growth (Abd_Allah et al. 2015; Ahmad et al. 2015). It has been observed that accumulation of Cd causes several physiological, biochemical, and genetic alterations which are more often reflected on the structural makeup of the plant (Feng et al. 2010). Plants grown on Cd-rich soils display altered nutrient uptake, photosynthetic restrictions, hampered enzyme activity, altered stomatal functioning, and declined tissue water content (Abd_Allah et al. 2015; Hashem et al. 2016a, b). Moreover, excess Cd translocation into plants has been observed to reduce the activity of enzymes of carbon metabolism hence the productivity and biomass accumulation (Mobin and Khan 2007; Hashem et al. 2016a, b, c). Plants exhibiting higher intake of Cd and other heavy metals show greater generation of free radicals including superoxide, hydrogen peroxide, and hydroxyl due to the direct effect on the activity of the radical generating pathways mediated by enzymes like NADPH oxidase (Sirhindi et al. 2016). Cd hampers the cellular redox balance, thereby leading to obstructions in the normal functional patterns of major metabolic pathways (Ahmad et al. 2018; Alyemeni et al. 2017). In addition, the accumulated free radicals in Cd stress lead to enhancement in the rate of lipid peroxidation, and hence oxidative damage to plant tissue is initiated (Abd_Allah et al. 2015; Per et al. 2016).

For counteracting the negative effects triggered by Cd accumulation, numerous mechanisms are initiated, and these include greater synthesis and accumulation of compatible solutes, phytochelatins, upregulation of the antioxidant system, and effective partitioning of the toxic ions (Hashem et al. 2016a, b, c; Alyemeni et al. 2017). Antioxidant system neutralizes the toxic radicals to prevent oxidative damage to key molecules, and on the contrary, phytochelatins bring chelation of toxic metal ions thereby proving much effective by preventing the initiation of toxic effects of metal ions (Ahmad et al. 2018). Osmolytes, antioxidants, and phytochelatins in combination bring modulation of metabolic pathways by maintaining the tissue water content and structural integrity and preventing radical generation, respectively (Nazar et al. 2012). So, the net effect of Cd in soil-plant relationship is its influence on the physiological and biochemical processes primarily by affecting the enzyme activity, mineral nutrition and assimilation, photosynthesis, antioxidant metabolism, and redox homeostasis (Ahmad et al. 2018; Chiboub et al. 2018). In the first part of the present review, the growth and metabolic alterations triggered by Cd are addressed followed by the role of existing soil microorganisms in the amelioration of metal-induced effects focusing primarily on the importance of beneficial microorganisms in the alleviation of Cd toxicity and its ill effects.

9.2 Mechanisms of Uptake, Transport, and Accumulation of Cd

Uptake, transport, and accumulation or partitioning of Cd in plants have been studied in somewhat up to comparable levels. With the induction of Cd stress, plants have been observed to experience restriction on the uptake of other mineral elements by competing for the transport protein at the membrane levels (Nazar et al. 2012; Ahmad et al. 2018; Abd_Allah et al. 2017). Transport proteins including ABC and natural resistance-associated macrophage protein have been observed to carry out the transport of Cd ions in plants and their expression get upregulated in plant species exposed to Cd stress (Sarwar et al. 2010). Besides the activation of Cd transport proteins, the partitioning of accumulated Cd into various organs plays an important role in determining the intensity of Cd toxicity in plants under question. Several factors controlling the Cd uptake, accumulation, and toxicity include the concentration and availability of Cd in rhizosphere, symplastic or apoplastic space of Cd transport within the plant tissues, loading and translocation via xylem, and the accumulated Cd within vacuoles as cadmium-phytochelatin complex. In addition to the transport proteins and other mechanisms depicted above, the cation-exchange capacity of the cell walls of root tissues is also a key determining factor in the net uptake of heavy metal(loid)s. In the simplest way, greater action-exchange capacity reflects greater metal adsorption in the cellular walls, thereby making them easily available for the membrane transport with a significant reduction in efflux of metal ions. Such high ion-exchange capacity trigger metal accumulation in cellular components, including the cytoplasm, and severely affects metal tolerance potential of plants (Singh et al. 2015a).

In general, it can be quoted that transport proteins carrying out metal transport may be either general or specific. For example, among the key general metal ion transporters, NRAMP represents an important group of transporters which are transmembrane proteins implemented in transport and homeostasis of metal ions and are considered as responsible for the transport of Fe^{2+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} ions (Nevo and Nelson (2006). More precisely, the transporters that mediate transport of one ion may also carry the other ions as has been observed for uptake and transport of zinc (Zn) and Cd (Wong and Cobbett 2009). P1B-ATPase (HMA) – a specific metal ion transporter – is involved in the translocation of ions specifically Cd and Zn out of the cytoplasm into the vacuole, thereby reflecting to their efflux mechanism. It has been suggested that the efflux or export metal ion transporters are much more selective as compared to the ones involved in the import function (Kramer et al. 2007; Singh et al. 2015a). Overexpression of the exporter proteins derived from metal hyper-accumulators has been observed to improve tolerance of yeast to Cd and Zn (Papoyan and Kochian 2004). Such transporters are considered as potential candidates for exploitation as agents of improving Cd stress tolerance in plants. Expression of an iron transporter gene (IRT1), zinc-regulated transporter/IRT-related proteins (ZIP), can help in the removal of Cd from polluted soils. However, in *Arabidopsis* IRT1 transporters have affinity for a range of ions including Cd, manganese (Mn), Fe, and Zn (Korshunova et al. 1999). In addition to this action, the well-characterized proteins known as diffusion facilitator transporters are implicated in the vacuolar sequestration and transport of toxic metal ions (such as Zn, Cd, cobalt (Co), nickel (Ni), etc.) from the cytoplasm into the outer cellular compartments (Montanini et al. 2007). The isotopes of natural resistance-associated macrophage protein, i.e., NRAMP3 and NRAMP4, have been studied to mediate the efflux of cadmium from the vacuole (Thomine et al. 2003), and the overexpression of *NRAMP3* and *NRAMP4* in *Arabidopsis* resulted in greater Cd sensitivity due to disturbance caused in the Fe homeostasis after its release from the vacuole in addition to Cd. Beneficial soil microbes have the potential to restrict the uptake of Cd to upper plant parts (Abd_Allah et al. 2015; Hashem et al. 2016a).

9.3 Cd Toxicity in Plants

The effect of heavy metals in altering the soil characteristics is now well established; the influence of these metals on the essential mineral nutrition has been observed leading to impeded plant growth and development. The effects may vary with the concentration of metal and the type of existing plant species encountered. Recent studies of Abd_Allah et al. (2015) have demonstrated restricted growth in Cd-stressed *Helianthus annuus* and *Sesamum indium* L. due to impaired lipid metabolism and uptake of nutrients, respectively. While comparing the Cd tolerance in rice accessions, Ueno et al. (2009) noticed considerable variations in the concentration of Cd in shoot ultimately displaying completely vibrant tolerance potential (Fig. 9.1). Genotypes of the same species display considerable differences in the Cd metal tolerance (Khan and Khan 2014; Asgher et al. 2014; Alyemeni et al. 2017; Ahmad et al. 2018). These differences may be attributed to the inherent differential capacity of different species and varieties for Cd accumulation and partitioning in root and shoot and also on the ability to restrict Cd in roots. In *Glycine max*, De Sousa and Sodek (2003) observed the induction of the alanine aminotransferase and hexokinase activity after the plants were exposed to Cd (10 μM) resulting in improved tolerance due to greater production of reduced glutathione (GSH). Restricted functioning of the transport proteins in Cd-treated plants results in increased affinity for the sulfhydryl groups of enzymes, for example, the activity of H^+ - ATPase decreased significantly in the roots of papilionaceous plants due to Cd contrary to cucurbitaceous plants reflecting higher metal tolerance (Chetty et al. 1980; Janicka-Russak et al. 2012).

In addition, accumulation of Cd has been observed to impair the structural stability of DNA by inducing breaks in strands, causing oxidative damage, and also leads to chromosomal aberrations with increased programmed cell death (Tuteja et al. 2009). Cd has been reported to trigger cytotoxic as well as genotoxic effects in *Vicia faba* resulting in exchange of sister chromatids, and hence persistent DNA damage can occur (Unyayar et al. 2010). In connection to this, the protective mechanisms like osmolytes, phytochelatins, and antioxidant system have been essential in

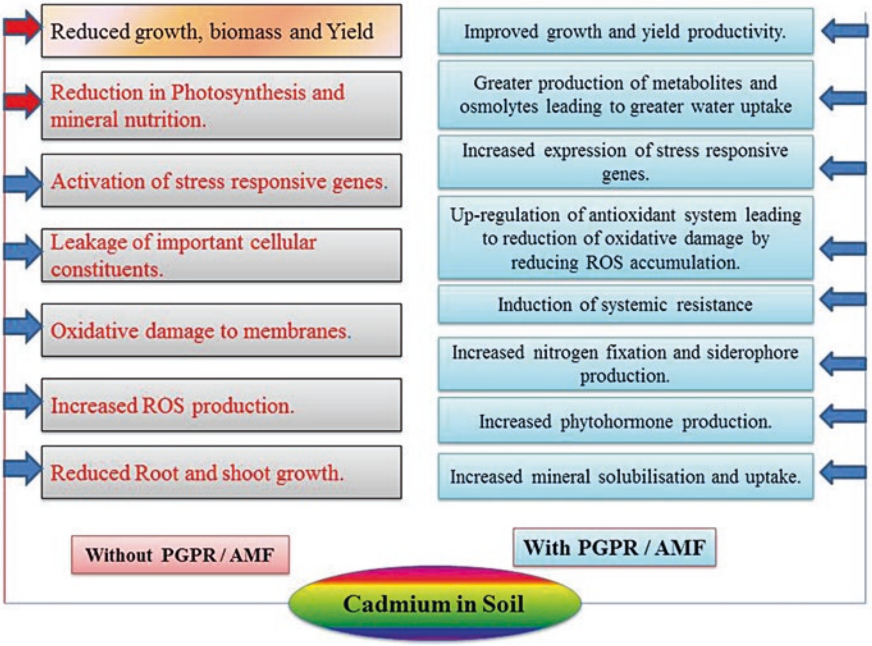

Fig. 9.1 Skeleton diagram describing the mechanisms involved in remediation and alleviation of adverse impact of cadmium stress by rhizosphere microorganisms

averting the genotoxic stress as observed in several crops subjected to Cd stress (Emamverdian et al. 2015). Future studies are required to unravel the exact mechanisms of protection from genotoxic effects of Cd and other metals. Thorough understanding about the cross talk between different protective molecules can be handy in strengthening our knowledge about signaling cascades and the apoptotic pathways involved in such conditions. While screening *Brassica juncea*, Blanvillain et al. (2009) identified a chromatin remodeling factor, i.e., OXS3, as key factor for improved Cd stress tolerance. Contrarily, OXS3 mutants exhibit extra sensitivity to Cd treatment which is usually overcome by its subsequent overexpression thereby leading to the protection of DNA (Verbruggen et al. 2009). No doubt plants tend to chelate most of Cd taken up; however, accumulated Cd is sequestered into the vacuole involving the active participation of transporters, e.g., CAX2 or CAX4 acting as Cd/H antiporters. Besides it has been confirmed that *Nicotiana tabacum* overexpressing the *Arabidopsis* tonoplast Cd/H antiporter exhibit greater tolerance to cadmium stress (Korenkov et al. 2007).

Most of the nutrients including nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), etc. are mobile within the plant system, i.e., in xylem and phloem tissues, compared to calcium (Ca), boron (B), sugars, etc. Heavy metals like Cd may compete with ions like Ca and Mg at the membrane level (Llamas et al. 2000) resulting in its uptake by the cation transport system (employed in the uptake of essential elements, e.g., transporters belonging to ZIP and NRAMP families and Ca channels (Perfus-Barbeoch et al. 2002)). Competition occurring between nutrients and the existing toxic heavy metals for the binding sites in different cellular compartments can affect the distribution of toxic metals, for example, absorption of Cd through Ca ion channel hampers the plant-water relationship (Perfus-Barbeoch et al. 2002) imparting hindrances in metabolism by inducing stomatal closure, lowered transpiration, and chlorophyll metabolism, hence restricting photosynthesis. Reduced uptake of sulfur (S) in *B. juncea* cultivars under Cd treatment restricts photosynthesis and growth (Asgher et al. 2014; Ahmad et al. 2015). Similarly, in chickpea, Cd stress reduces the uptake of key mineral nutrients leading to increased oxidative damage and growth restrictions. Reduced photosynthesis due to Cd stress has been attributed to the reduction in the activity of enzymes of the Calvin cycle, photosynthetic electron transport chain, PSII activity, CO_2 concentration, expression of Rubisco, and uptake and translocation of key mineral nutrients (Asgher et al. 2014; Per et al. 2016). Cd stress increased DNA polymorphism in rice and possibility of occurrence of breaks in the DNA (Aina et al. 2007). Cd affects the antioxidant system of plants and induces oxidative damage to key metabolic pathways (Hashem et al. 2015; Abd_Allah et al. 2015, 2017). Partly such negative effects of Cd can be ascribed to low mineral availability in the contaminated soils. Cd brings alteration in the protein conformation after binding with the sulfhydryl and carboxylic groups of proteins. According to the study of Yoshihara et al. (2006), exposure of tobacco to Cd stress induces iron deficiency by significantly increasing the expression of *NtFRO*1 and *NtIRT*1 in the roots. Transcription factors including *FIT*, *AtbHLH38,* and *AtbHLH39*, which belong to helix-loop-helix group, get upregulated in plants exposed to Cd stress for protecting the iron homeostasis. In *Arabidopsis*, overexpression of *FIT* either with *AtbHLH38* or *AtbHLH39* activates the expression of *MTP3*, *HMA3*, *IRT2,* and *IREG2* involved in the detoxification of heavy metals and further enhanced the expression of nicotianamine coding NAS1 and NAS2 genes thereby contributing to iron homeostasis.

9.4 Soil Microorganisms: PGPR and AMF

Already as mentioned above, plant development primarily depends on the characteristics of the rhizosphere soil, the existing environment, and lesser on the plant species. A galaxy of microflora interacting with their immediate neighbors for one or the other beneficial or neutral associations inhabit Earth. Species belonging to different bacterial and fungal genera form the vital components of soil. Among the key biotic activities is the nutrient turnover so as to make it sustainable for increased crop production (Hameed et al. 2014). Soil microorganisms bring positive changes in growth by mediating the mobilization of key nutrients and inducing the synthesis of endogenous plant growth regulators (Hashem et al. 2014; Abd_Allah et al. 2015), modulating the concentration of polyamines (Hashem et al. 2014; Upadhyay et al. 2012), inhibiting the growth of phytopathogens, improving soil structure, and removing the toxic heavy metals and xenobiotic compounds including pesticides (through sequestration) (Hayat et al. 2010; Singh et al. 2015a, b). It has been accepted that bacteria are inhabiting in the rhizosphere, i.e., rhizobacteria are the most versatile agents in transforming, mobilizing, and solubilizing the key mineral nutrients (Oteino et al. 2015). In connection to this, the rhizobacteria and arbuscular mycorrhizal fungi (AMF) are considered as the dominant driving forces for nutrient recycling and hence soil fertility (Li et al. 2017). Exploitation of soil microorganisms for remediation of toxic metals has been under intense research (Ma et al. 2016). The main aim of all the integrative biotechnological or agricultural approaches employed for improving crop production via biological means is gaining ground in the current era. In connection to this, rigorous research practices have been initiated worldwide with greater emphasis on exploring a range of rhizobacteria possessing novel traits like heavy metal elimination (Ma et al. 2011; Wood et al. 2016), pesticide degradation (Ahemad and Khan 2012a), salt tolerance (Upadhyay et al. 2012), and control of phytopathogens and insects along with the key plant growth controlling properties like phytohormone (Tank and Saraf 2010; Upadhyay et al. 2012), siderophore (Nadeem et al. 2014), 1-aminocyclopropane-1-carboxylate, hydrogen cyanate, and ammonia production in addition to nitrogenase activity, antioxidant enzymes (Stefan et al. 2013), and phosphate solubilization (Hashem et al. 2014) etc.

Plant growth-promoting rhizobacteria (PGPR) include both symbiotic and non-symbiotic bacterial strains, and key examples for symbiotic ones include rhizobia, while non-symbiotic ones are *Bacillus*, *Klebsiella*, *Pseudomonas*, *Azospirillum*, and *Azotobacter,* and AMF are being exploited worldwide as the bio-inoculants for promoting the growth and development of plants under a wide range of environmental stresses including heavy metals (Wani and Khan 2010; Abd_Allah et al. 2015; Hashem et al. 2016a, b, c), herbicides (Ahemad and Khan 2011a), salinity (Mayak et al. 2004; Alqarawi et al. 2014; Bharti et al. 2016), water stress (Alwhibi et al. 2017), insecticides (Ahemad and Khan 2011b), fungicides (Ahemad and Khan 2012b), etc. In mycorrhiza "myco" means "fungus" and "rhiza" means "root"; therefore it means fungi infecting roots (Hameed et al. 2014). AMF improve nutrient absorption, water holding capacity, and stress tolerance (Alqarawi et al. 2014; Candido et al. 2015). Nearly 250 species of AMF have been identified (Kruger et al. 2012). AMF isolates have been classified within six major genera under order *Glomales* also referred as *Zygomycota* (Ahanger et al. 2014). Advancement in the molecular techniques has enabled the direct identification of the AMF isolates infecting roots or rhizosphere, and the confirmation of the genetic diversity occurring within the different species has also been confirmed (Kruger et al. 2012). Therefore, both PGPR and AMF must have (1) efficiency to colonize roots and (2) potential to mediate growth promotion by modifying the metabolism (3) and must multiply and compete efficiently with the existing microbiota.

Despite having enormously available research findings on PGPR-AMF-induced stress mitigation, the exact mechanisms underlying plant growth promotion are not fully known. It has been observed that both PGPR and AMF exhibit beneficial properties for regulating the growth and development of plants positively (Khan et al. 2009; Hameed et al. 2014; Santoyo et al. 2016; Meena et al. 2017).

To be accurate rhizosphere can be the zone of soil, which is surrounding the plant root system, and rhizobacteria and AMF refer to cluster of microorganisms competently colonizing the roots (Kloepper et al. 1991). It has been reported quite often that plant roots synthesize and release an array of chemical compounds into the rhizospheric soils, which act as attractants for a wide variety of soil microorganisms (Walker et al. 2003). These exudates bring modification in the physical as well as chemical properties of the soil, therefore, determining the structure of existing soil microbial populace residing in immediate vicinity of the root surface (Baetz and Martinoia 2014). It must be pointed out here that some exudates repel microorganisms, while some attract which may vary with the composition of exudates, often dependent on the physiological status in addition to the type of plant and microbe species involved (Kang et al. 2010). Nardi et al. (2000) have suggested that exudates inhibit the growth of competing plant species as well and favor the symbiotic associations. However, the inhabiting beneficial microorganisms modify the root developmental patterns and mediate exchange of nutrients by making significant increase in the production of exudates (Neumann et al. 2014).

9.5 Plant Growth Promotion by PGPR and AMF Under Cd Stress

As already discussed PGPR and AMF trigger plant growth promotion by the alteration of the whole microbial community existing in the rhizosphere niche. Generally, the rhizospheric microorganisms (PGPR and AMF) improve plant growth by direct or indirect mechanisms. Enhancing the nutrient acquisition including N and P, modulating the endogenous hormone levels, and improving the enzyme activity are included in direct growth-promoting mechanisms while acting as biocontrol agents resulting in the reduction of the inhibitory effects the pathogens are involved in indirect mechanisms (Ahanger et al. 2014). However, both direct and indirect beneficial mechanisms are triggered in plants exposed to different stresses with specificity of response exhibiting certain degree of variation with type of stress imposed. Reports available depicting the beneficial role of soil bacteria and fungi in amelioration of different stresses have been described by various researchers all over the world (Al-karaki et al. 2004; Yedidia et al. 2001; Gamalero et al. 2009; Garg and Singla 2012; Gholamhoseini et al. 2013; Hameed et al. 2014; Hashem et al. 2014, 2015, 2016a, b, c; Yang et al. 2015; Abd_Allah et al. 2015, 2017; Wu and Zou 2017; Scagel and Bryla 2017; Mitra et al. 2018a, b) (Table 9.1). In the following sections, beneficial role of soil PGPR and AMF in growth regulation under metal stress will be discussed with special attention on Cd stress (Fig. 9.1).

Table 9.1 The effect of microbes on Cd stress of plants

Microorganisms	Plants	References
Bacillus amyloliquefaciens SAY09	*Arabidopsis*	Zhou et al. (2017)
Pseudomonas aeruginosa Burkholderia gladioli	Tomato	Kanika et al.
Agrobacterium fabrum, Stenotrophomonas maltophilia	Wheat	Zafar-Ul-Hye et al. (2018)
Enterobacter sp.	Rice	Mitra et al. (2018a, b)
Bacillus, Klebsiella, Leifsonia, Enterobacter	Maize	Ahmad et al.
Enterobacter aerogenes	Rice	Pramanik et al. (2018)
Burkholderia sp. D54	Tomato	Wei et al. (2018)
Burkholderia cepacia CS8	*Catharanthus roseus*	Khan et al. (2018)
Klebsiella michiganensis MCC3089	Rice	Mitra et al. (2018a, b)

9.6 Soil Microbes and Mineral Nutrition

Soil microbes including PGPR and AMF regulate the uptake of mineral ions in the host plants under normal and environmental extremes. Among the key mineral elements, the uptake of which is mediated by associated microbes, are N, P, Mg, S, etc. Nitrogen-fixing bacteria make the nitrogen available to plants for absorption and subsequent assimilation, while phosphate-solubilizing microbes preferentially assist in P uptake. Biologically fixed N accounts for at least two-thirds of the total fixed N even under extreme conditions like high temperature, salinity, metal stress, etc. (Raymond et al. 2004). Microorganisms including rhizobia, cyanobacteria, *Gluconacetobacter diazotrophicus, Azotobacter*, and *Azospirillum* may enter either in symbiotic or non-symbiotic relation with the host plant. Chen et al. (2003) have shown that soybean treated with Cd (10 and 20 mg kg^{-1} soil) exhibited a significant reduction in the nitrogen fixation by reducing the number of nodules resulting in considerable decline in the nitrogen assimilation. The specific type of nitrogen fixation genes (*nif* and fix genes) controls nitrogen fixation (Kim and Rees 1994). *Leucaena leucocephala* grown on metal-contaminated soils showed significant recovery in the decline of N assimilation by the presence of native rhizobia (Rangel et al. 2017). On the other hand, inorganic phosphate solubilization mediated by the associated bacteria involves the release of certain organic acids by the bacteria into the soil leading to solubilization of the complex phosphate compounds into orthophosphate, thereby making it easily available to plants for absorption and assimilation. Oteino et al. (2015) have demonstrated that endophytic bacteria have the potential to produce gluconic acid which initiates the solubilization of insoluble phosphate, thereby leading to significant improvement in growth of *Pisum sativum* L. Recently, Korir et al. (2017) reported that co-inoculation of rhizobia and other PGPR improved growth of common bean by increasing the uptake and assimilation of nitrogen and phosphorus. Greater uptake of minerals was found to be regulated by the impact of inoculants on the gene expression. In addition to N and phosphate assimilation, the homeostasis of other elements, including Zn, Ca, Mn, and Fe, has been observed to show close linkage with the Cd toxicity and tolerance (Wu et al. 2012; Zhai et al. 2016). Cd stress affects the transport proteins and PGPR, and associated beneficial microorganisms may have a key role in the modulation of their activity and expression; however, experimental studies confirming this are not available. It should be noted here that excess phosphate supplementation leads to disastrous effects on the soil fertility and plant growth due to considerably excessive accumulation of Cd. However, improving phosphate solubilization using PGPR is safe for the environment and plants. In this context phosphate-solubilizing microbes make the availability of P to the plants (Khan et al. 2006). Among the key phosphate-solubilizing microorganisms are *Bacillus, Beijerinckia, Rhizobium, Azotobacter, Enterobacter, Microbacterium, Flavobacterium, Pseudomonas, Serratia,* etc. making P availability easy for plants via the involvement of various mechanisms (Zaidi et al. 2009). It has been observed that bacterial strains show both phosphate solubilization and mineralization functions (Tao et al. 2008). Phosphate-solubilizing bacteria (PSB) are exclusively found in various soil types, and the performance of particular strain depends on the environmental factors (Ahemad and Khan 2012a, b). It should be noted here that the positive effects of the PSB either alone or with rhizobia under stress conditions have been reported by many workers (Zaidi and Khan 2005; Ahemad and Khan 2011a, b, 2012a, b; Poonguzhali et al. 2008; Chen et al. 2008; Zaidi et al. 2009).

9.7 Soil Microbes and Antioxidant System

It is well established that crop plants growing under stressed environmental conditions show upregulated antioxidant defense system for the protection of major cellular pathways from the free radical-triggered oxidative damage (Mittler 2002; Hashem et al. 2015). Antioxidant system includes enzymatic and non-enzymatic components working coordinately for bringing the stability to metabolism. Strengthening the antioxidant system with the help of soil beneficial microorganisms helps plants to neutralize the toxic ROS quickly and keep metabolism at optimal level (Abd_Allah et al. 2015; Alwhibi et al. 2017). Inoculation of Cd-resistant PGPR to stressed (50 μM $CdCl_2$) *Sulla coronaria* reduced the oxidative damage by upregulating the activity of antioxidant enzymes, SOD, APX, SOD, and GPOX (Chiboub et al. 2018) proving the beneficial impact of PGPR in protection of *S. coronaria* from heavy metals. Increased activity of antioxidant enzymes due to rhizobia and PGPR strains in metal-stressed *Vicia faba* reduces the oxidative damage by decreasing the accumulation of free radicals (Fatnassi et al. 2015).

Plants grown in Cd-contaminated soils show considerable increase in the generation and accumulation of free radicals such as superoxide, H_2O_2, and hydroxyl, leading to increased lipid peroxidation (Ouariti et al. 1997; Hossain et al. 2006; Ahmad et al. 2011, 2015; Abd_Allah et al. 2015, 2017). Metal stress-induced peroxidation of lipids is widely accepted as one of the important damaging aspects and a determining factor of the magnitude of environmental stress. The inoculation of AMF has been observed to improve the structural integrity of membranes by improving the polyunsaturated lipid components of the membranes (Alqarawi et al. 2014; Abd_Allah et al. 2015). It is established that the membrane lipids are very much sensitive to ROS and initiate their oxidation causing the accumulation of peroxide radicals. Cd stress-induced peroxidation of membrane lipids causes membranes to loose their functional integrity resulting in leakage of essential elements (Djebali et al. 2005). In addition to this, Cd and other stress factors triggered ROS accumulation and the lipoxygenase activity, deteriorating the membranes and other cellular components (Djebali et al. 2005). In rice, Kuo and Kao (2004) also demonstrated an increase in the production of H_2O_2 resulting in oxidative stress and reduced growth.

Barley root tips displayed a considerable increase in the expression of lipoxygenase activity after exposure to Cd resulting in membrane damage (Tamas et al. 2009). Zhang et al. (2014) have demonstrated a significant increase in the expression of antioxidant coding genes in *Populus* under Cd stress. In another study with *Chlorella vulgaris*, Cheng et al. (2016) have demonstrated that activity of SOD, CAT, POD, and GR increased with treatment of Cd resulting in the prevention of oxidative damage to some extent. Not only in plants Cd uptake by animals, including fishes like *Takifugu obscurus,* has been reported to improve the gene expression of important antioxidant genes and their isozymes including *CAT1*, *Cu/Zn-SOD*, *Mn-SOD,* and GR (Kim et al. 2010). The improving antioxidant system can prove exceptionally beneficial in promoting growth via protection of major metabolic processes. In context to this, increasing the indigenous antioxidant defense system has been the key target of present research for protecting the maximum crops from metal damage, and exploiting the potential of soil microbes is one such strategy (Fig. 9.1).

9.8 Soil Microbes and Osmolytes

The accumulation of osmotic solutes under different stresses including metal stress has been reported (Ahmad et al. 2015). The accumulation of osmolytes leads to osmoregulation and protection of major plant assimilatory pathways. Among the most important solutes are proline, glycine betaine, sugars, etc. The accumulation of solutes tends to bring the turgor maintenance via increased water uptake under stress by maintaining the water potential of tissue well below that of soil solution (Tester and Davenport 2003). Osmolytes maintain the ionic balance between vacuoles and cytoplasm. More importantly they do not hinder the normal metabolic reactions if accumulated in large concentrations (Zhifang and Loescher 2003). It has been reported that accumulation of osmolytes is proportional to change in the external osmolality, which varies with species, thereby leading to protection of key cellular structures and the maintenance of the osmotic balance for continuous water influx (Hasegawa et al. 2000). Though the majority of the compatible solutes implicated in osmoregulation are organic in nature, essential inorganic ion such as K^+

also serves as a major osmolyte (Yokoi et al. 2002). The type and concentration of solute accumulated vary with the type of stress and the species. Accumulated osmolytes may be simple sugars like fructose and glucose, sugar alcohols or complex sugars like trehalose and raffinose, quaternary amino acid derivatives like proline and glycine betaine, tertiary amines, and sulfonium compounds like choline (Yokoi et al. 2002). Among the main functions of osmolytes are the maintenance of cellular water status and the protection of subcellular structures, membranes, and proteins (Ashraf and Foolad 2007). It is now widely accepted that osmolytes including proline, glycine betaine, and sugars mediate ROS scavenging and signaling under stressful conditions (Kathuria et al. 2009; Szabados and Savoure 2010). Plants showing greater accumulation of glycine betaine have been reported to exhibit maintained activity of PSII complex and carboxylase activity of RUBISCO, thereby leading to improved photosynthetic efficiency (Papageorgiou and Murata 1995; Tian et al. 2017).

There are increasing research reports that crop cultivars exhibiting greater synthesis and accumulation of osmolytes are more efficient to combat stress as compared to the low accumulating mutants. Plants having increased expression of proline and glycine betaine coding genes accumulate significant concentrations of proline and glycine betaine (Verdoy et al. 2006; Giri 2011). Transgenic *Medicago truncatula* overexpressing delta(1)-pyrroline-5-carboxylate synthetase shows greater osmotic stress tolerance (Verdoy et al. 2006). Khan et al. (2015) reported that greater proline accumulation imparts Cd stress tolerance to *B. juncea*. Therefore, from the above brief discussion, it can be concluded that greater accumulation of compatible solutes is one of the key mechanisms or adaptations to combat stresses like Cd toxicity. PGPR and AMF inoculation increases the accumulation of proline, glycine betaine, and sugars in different crop plants resulting in growth regulation (Dimkpa et al. 2009; Abd_Allah et al. 2015; Vurukonda et al. 2016).

Increased accumulation of proline and other metabolites was observed in *Bacopa monnieri* after inoculation of PGPR (Bharti et al. 2013); similarly *Pseudomonas pseudoalcaligenes* colonizing rice seedlings resulted in enhanced synthesis of glycine betaine and glycine betaine-like quaternary compounds resulting in improved stress tolerance (Jha et al. 2011). It has been reported that inoculation of VOC-emitting *Bacillus subtilis* GB03 to *Arabidopsis* promoted the synthesis and accumulation of glycine betaine and its immediate precursor choline imparting greater stress tolerance (Zhang et al. 2010). However, it shall be noted that very scanty reports are available regarding the impact of soil microbes on the growth and tolerance of plants under Cd stress. Inoculation of *Pseudomonas putida* (ATCC 39213) to *Eruca sativa* declined the uptake of Cd (Kamran et al. 2015) and Ni (Kamran et al. 2016) by improving the accumulation of osmolytes. Haneef et al. (2014) have demonstrated reduced oxidative damage to *Plantago ovata* by the inoculation of AMF and *Azotobacter* via increased accumulation of osmolytes including proline. In *Cajanus cajan* inoculation of AMF improves growth and N metabolism by enhancing the accumulation of trehalose in the presence of Cd (Garg and Chandel 2011).

9.9 Soil Microbes and Phytohormone Production Under Cd Stress

Generally, it may be said that the phytohormones like indole acetic acid (IAA) synthesized by the rhizobacteria may interfere with the developmental processes of host plant altering the endogenous pool (Glick 2014). No doubt increase in endogenous concentration of hormones may initiate reciprocal signaling resulting in impeded gene expression; however, their role in initiating and regulating the rhizobacteria-AMF-plant interactions cannot be neglected (Spaepen and Vanderleyden 2011). Generally, plant hormones regulate so many processes including cell division and differentiation, germination, ripening, signaling, vegetative growth, root formation and development, and response to numerous biotic and abiotic stresses (Ahmad et al. 2011). It has been observed that the bacteria-derived phytohormones, e.g., IAA, result in increased root growth by enhancing the surface area and length of roots, thereby mediating improved growth of plants by improving the access to mineral nutrients. In addition to this, the root cell walls get loosened due to IAA facilitating increase in exudation, thereby providing nutrients for supporting microbes in rhizosphere (Glick 2014; Spaepen and Vanderleyden 2011). In *Leucaena leucocephala*, PGPR inoculation improved production of organic acids, IAA, siderophore production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and $Ca_3(PO_4)_2$ solubilization resulting in improved Zn and Cd tolerance (Rangel et al. 2017). Kotoky et al. (2019) reported enhanced growth of rice by IAA producing PGPR strain of *Serratia marcescens* even in the presence of high Cd concentrations. The synthesis of phytohormones by the microbes inhabiting the plants has been observed from times.

Another important phytohormone is ethylene which has been reported to get accumulated under stresses like waterlogging, salinity, heavy metal, drought, etc. resulting in significant decline in growth (Asgher et al. 2014; Khan et al. 2015). High concentration of ethylene induces defoliation, thereby reducing the crop performance (Bhattacharyya and Jha 2012). PGPR possessing ACC deaminase enzymes promote growth and development of plants by reducing the endogenous levels of ethylene to normal level (Zahir et al. 2008). *Achromobacter, Bacillus, Pseudomonas, Agrobacterium, Azospirillum, Burkholderia, Serratia,* and *Rhizobium* strains have been reported to show ACC deaminase activity (Nadeem et al. 2007; Kang et al. 2010; Glick 2014; Singh et al. 2015c). In general, phytohormones have been observed to mediate the signaling under metal stresses (Bak et al. 2014). The PGPR strains displaying ACC deaminase activity help host plants to prevent damage of several stresses including phytopathogenesis, heavy metals, radiation, salinity, temperature, and flooding (Glick 2014). Enhancement in the rhizobial nodulation and uptake of essential nutrients like N, P, and K in addition to healthy AMF colonization have been observed in crops infected by the ACC deaminase producing PGPR strains (Shaharoona et al. 2008; Glick 2014). In *Arabidopsis*, Zhou et al. (2017) demonstrated that inoculation with *Bacillus amyloliquefaciens* SAY09 improves Cd stress tolerance by regulating the synthesis of abscisic acid, IAA, and gibberellic acid resulting in optimizing signaling events.

9.10 Conclusion

The soil rhizospheric microflora including PGPR and AMF are much beneficial for the healthy maintenance of growth of plants, and most of them have been recognized for their beneficial role in regulating the growth and development of plants. PGPR and AMF benefit plants by enhancing the mobility of key nutrients into the plants concomitant with the reduction or restriction of toxic ions including metals. The beneficial role of soil microorganisms in ameliorating the ill effects of stresses via the modulations in the key defense mechanisms is now an obvious established fact. Future research shall remain focused to unravel the exact mechanism of soil microorganisms bringing amelioration of the metal stress. Making integration between the physiological, biochemical, molecular, and genetic approaches will be handy in identifying the key regulatory mechanisms induced by rhizospheric microorganisms in driving various known structural and functional integrities within the host plants for improved metal stress tolerance.

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Chapter 10
Acid Tolerant Microbial Inoculants: A Requisite for Successful Crop Production in Acidic Soils

Chandandeep Kaur, G. Selvakumar, and A. N. Ganeshamurthy

Abstract Acidic soils comprise more than 30% of the global land area and are characterized by a pH value lesser than 5.5 throughout the year. Acidic soils are characterized by the deficiency of beneficial elements such as molybdenum (Mo), phosphorus (P), magnesium (Mg) and calcium (Ca) and the abundance of aluminium (Al), iron (Fe) and manganese (Mn). Such soils are also home to unique species of microorganisms that have evolved several tolerance mechanisms in order to overcome the acidic environments. Such mechanisms range from maintaining the intracellular levels at near neutral levels to dispensing with peripheral cellular structures that are in direct contact with the soil acidity. But despite the global distribution and importance of these soils, microbial inoculants that are suited to acidic soil conditions have not received the attention of microbiologists and agronomists, thereby depriving these soils of the benefits of beneficial microbial inoculation. This chapter attempts to highlight the issues related to soil acidity with respect to their nature, nutrient availability, microbial diversity and the need for acid-tolerant microbial inoculants for use in acid soils.

Keywords Acidic soils · Acidity tolerance · Microbial inoculants

10.1 Introduction

Crop production and the subsequent food and nutritional security challenges are highly dependent on the nature and properties of the soil. But soil being a complex and heterogeneous ecosystem, its characterization still remains a challenge. Soil health is basically determined by its biological, chemical and physical attributes. Among the different parameters, the most important ones include soil bulk density (a measure of soil structure), soil reaction (indicates the soil pH and availability of various nutrients) and soil organic matter status (as a biological activity indicator). Apart from these, there are several inherent and dynamic parameters that influence

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soil quality. The inherent soil quality refers to the soil's innate capacity to function, whereas the dynamic soil quality varies depending on how a soil is managed (Ball and Rosa 2006).

The soil reaction is the association between the strength of H^+ and OH^- ions in the soil solution which determines whether the soil reaction is acidic or alkaline or neutral. The soil reaction can be divided into three classes, viz. acidic, alkaline and neutral, and therefore influences the biological composition and functional capabilities of the soil (Biswas and Mukherjee 1994). An acidic soil is one that has a pH value of less than 5.5 for most of the year. Acid soils occupy about 30% of the global land surfaces and are found predominantly in the humid temperate forests and humid tropics and subtropics. The tropical acid soils are spread over approximately two billion hectares, or 14%, of the total ice-free area of the world (Von Uexküll and Mutert 1995). In acidic soils, beneficial elements such as molybdenum (Mo), phosphorus (P), magnesium (Mg) and calcium (Ca) become less available to plants, while elements such as aluminium (Al), iron (Fe) and manganese (Mn) become more available and very often reach levels that are toxic to plants. Similarly, at acidic pH levels the distribution of microflora may be more skewed towards fungi which prefer an acidic pH. But despite the understanding of soil acidity and vast distribution, acid-tolerant microbial inoculants have received little or no attention. The microbial inoculants developed for normal soils may fail to establish or perform under the acid soil conditions that are unique, and therefore, it is necessary to develop acid-tolerant microbial inoculants for successful crop production in acid soils. This chapter attempts to explore the concepts of soil acidity, its effect on bacterial cell physiology, microbial diversity in acid soils and the need for the development of microbial inoculants for crop production in such soils.

10.2 Soil Acidity

Soil pH is a measure of the soil reaction and is universally defined as the logarithm of the reciprocal of the H^+ ion activity. It is expressed as moles per litre pH = log 1/ A_H+ or –log A_H+ where A_H+ is the hydrogen ion activity in moles per litre (Sorensen 1908). The pH of soil depends on the nature of the soils, e.g. the pH of mineral soils varies from 3.5 to 10.5, while the pH of peat soils may be 3.0 or lower, while alkali soils can have pH as high as 11.0. The main causes of soil's acidity can be leaching of basic minerals due to excessive rainfall, acidic parent rock material, continuous use of fertilizers conferring acidity, presence of aluminosilicate minerals, high concentration of carbon dioxide in soil and the presence of hydrous oxides like Fe and Al (Moore et al. 1998; Arora et al. 2018). These factors contribute to soil acidity either individually or in combination. Regions with high rainfall have an acidic soil reaction since basic minerals like Ca and Mg are leached away by rainfall, whereas the drier regions are more likely to have an alkaline soil reaction. Soils which develop from parental rocks like limestone and basalt have an alkaline reaction, while granite and sandstone impart an acidic reaction. Farming practices like liming reduce the acidic character of soil, while the use of manure, composts and nitrogen-containing chemical fertilizers progressively increase the acidity of soils over the years. There are two facets of soil acidity, viz. intensity and quantity. While the intensity refers to the ability to give up protons (hydrogen ions) which is identified by the measurement of H^+ ion activity and expressed as pH, the quantity is referred to as the amount of alkali required to neutralize the soil. The total acidity of soil is the sum of active acidity, exchange acidity and residual acidity (Amel'yanchik and Vorob'eva 1991).

The problems associated with acidic soil prevail worldwide. It is based in mainly two belts, viz. the northern belt in the cold humid temperate zone covering North America, South Asia and Russia and the southern belt in humid high rainfall tropical areas including South Africa, South America, Australia and parts of New Zealand (Von Uexküll and Mutert 1995). Around 3950 million hectares of land are afflicted by soil acidity. Data shows about 38% of farmland in Southeast Asia, 31% in Latin America, 20% in East Asia, 56% in sub-Saharan Africa and parts of North America are influenced by soil acidity (Wood et al. 2000; Hoekenga et al. 2006). Around 1616 million hectares of land in America is affected, whereas 239 million hectares of agricultural land in Australia and New Zealand is acidic. In China and India, 212 million ha or about 12% of agricultural land is classified as acidic (Von Uexküll and Mutert 1995) (Fig. 10.1).

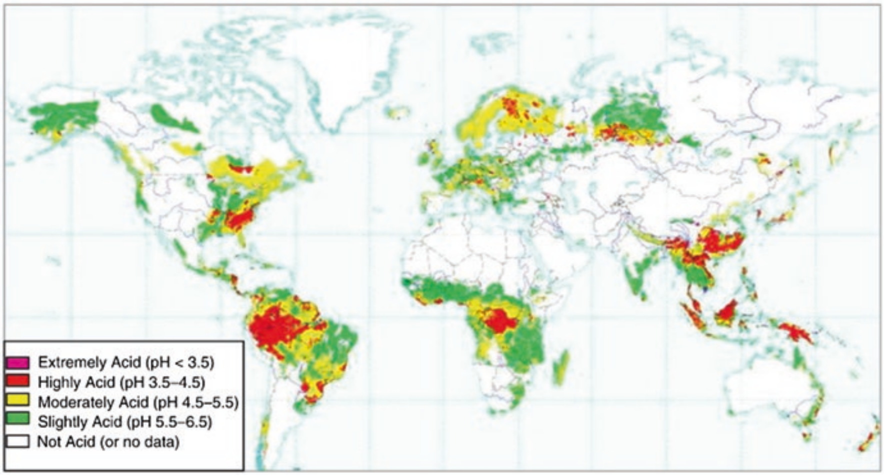

Fig. 10.1 World distribution of acid soils (Source: USDA, NRCS, world soil resources, Washington, DC) http://websoilsurvey.nrcs.usda.gov/. (Eswaran et al. 1997)

10.2.1 Diversity of Microflora in Acid Soils

The habitat available to a soil organism decides its structure and size, its interaction with other organisms and its overall impact on the soil health. Soil habitats differ greatly depending on the land use. For a similar soil type within the same climate zone, a forest soil will generally have a greater diversity of soil organisms than a cultivated agricultural soil. Factors like moisture, vegetation and pH and treatments like liming and organic manures show a considerable influence on the distribution of different microorganisms in the soil (Rangaswami and Venkatesan 1966; Rangaswami et al. 1967; Jha 1976). These differences are primarily associated with a greater diversity of plant species and heterogeneity of the soil and the characteristics of organic matter produced in natural and agricultural ecosystems. The abundance of bacteria is less in acidic soils than in soils of higher pH. Generally, there is not such a strong relationship between soil pH and fungi as there is between soil pH and bacteria, although individual fungi can have a marked pH preference. For a bacterium, important aspects of the soil environment include its microhabitat (soil structure), surfaces of soil particles, pore spaces, roots, dead organic matter (plant, animal or microbial), water films, etc. (Dinesh et al. 2003; Brussaard 2012).

Different species of soil biota have their own pH preferences. However, once soil pH changes from their preferred conditions, the species composition of the soil gets altered (Frey et al. 2004; Nilsson et al. 2007; Jenkins et al. 2009). Low pH results in a change in the microbial biodiversity, but potentially may impact decomposition processes essential for the release of nutrients from organic matter and in symbiotic relationships between native vegetation and soil organisms decreasing the survival of native vegetation (Fierer and Jackson 2006; Hartman et al. 2008; Jenkins et al. 2009; Lauber et al. 2009). Since soil pH dictates the biological diversity of soil, any management manipulation of soil reaction will also alter the population dynamics of the microbes in the soil (Jha 1976). Hence, liming soil to increase pH increases bacterial abundance relative to fungi (Rousk et al. 2010). Soil pH also affects the availability of nutrients and how the nutrients react with each other.

10.3 Influence of Soil Acidity on Plant Nutrition

Soil acidity has deep influence on the physical and chemical characteristics, charge on the surface of soil particles (positive or negative), degree of aeration, temperature and nutrients (Rengel 2011). The majority of effects of soil acidity on plant growth can be attributed to the changes in the levels of different nutrients in acid soils. At a low soil pH, beneficial elements such as Mo, P, Mg and Ca become less available to plants, while Al, Fe and Mn become more available, and very often Al and Mn may reach levels that are toxic to plants (Keyser and Munns 1979a, b). Soils with pH levels lesser than 5 result in chemical imbalances such as Al toxicity and deficiencies of P and trace elements such as Ca and Mo. Very low pH levels (usually lesser than pH 4) lead to the physical breakdown of soils, since the clay structure of the soil is broken down. Any deviation from the preferred pH range brings about a shift in the type of microbes that flourish in the soil and changes the rate of decomposition which leads to immobilization of important nutrients and affects the nutrient uptake in plants (Gyaneshwar et al. 2002; Hao et al. 2002). Soil pH affects the absorption efficiencies of major nutrients by plants. There are two forms of N which are taken up by plants, viz. ammonium (NH^{4+}) and nitrate ($N0^{3-}$). Most of the annual crops take up N as nitrate ($N0^{3-}$) form only. The process of conversion of NH^{4+} to $N0^{3-}$ is largely an autotrophic microbial process known as nitrification. Nitrification is favoured of at a soil pH range of 6.0–8.0, whereas in acidic soils, bacterial activity is reduced (Morrill and Dawson 1962, 1967). Soil pH is also known to influence N volatilization losses, since NH^{4+} and ammonia gas remain in equilibrium, and this equilibrium is dependent on the soil pH (Jayaweera and Mikkelsen 1990). In acid soils, the dissolved Al can be toxic to rhizobia and plant roots, thereby restricting the biologically fixed N (Bell et al. 1989; Bordeleau and Prevost 1994). Plant available P is usually found in the primary or secondary orthophosphate forms which are highly pH dependent. Plants acquire these forms of P from the soil solution, where these forms are present only in micromolar quantities (Zhou et al. 1992). The reason behind the low P availability is the fixation and formation of less soluble compounds. Under alkaline conditions, soil P forms more stable (less soluble) minerals through reactions with Ca and Mg, whereas under acidic soil conditions, soil P reacts with Al and Fe and forms more soluble Al-P and Fe-P compounds (Havlin et al. 1999; Gyaneshwar et al. 2002; Hao et al. 2002). In case of the third major plant nutrient, viz. potassium (K), pH is not a much concern, but several agronomists believe that liming affects the availability of soil K by increasing the pH (McKenzie 2003). Leaching is also known to reduce the availability of K in acid soils. Amongst the secondary nutrients, sulphur (S) exists in two forms, viz. elemental and the negatively charged SO_4^- ion. When the elemental S application to soil can result in the formation of sulphuric acid and subsequently reduce the soil pH, the application of SO_4^- containing compounds such as gypsum does not have the capacity to reduce pH of soils (Chien et al. 2011; Cui et al. 2004; Karimizarchi et al. 2014; Xiao et al. 2015) (https://www.canr.msu.edu/uploads/files/Lowering_Soil_pH_with_Sulfur.pdf).

10.4 Acidity and Soil Microbial Populations

The habitat available to a soil organism decides its structure, size, interaction with other organisms and its impact on the soil health. Amongst the soil dwellers, microbes are highly sensitive to pH levels and very often thrive within cardinal pH limits. Acidification of the soil changes the delicate balance between groups of living organisms in the soil. In acid soils, especially the presence of increased amount of Fe and Al causes toxicity to many microorganisms (Das 1994). Different species of soil biota have their own preferences for pH. The abundance of bacteria is less in acidic soils than in soils of higher pH since bacteria prefer neutral to alkaline soils, while fungi generally exhibit wider pH ranges for optimal growth (Rousk et al. 2010). There are some indications that the spatial patterns exhibited by soil fungal communities are not correlated, or less strongly correlated, with soil pH as observed with bacterial communities (Lauber et al. 2008).

The soil bacterial population is influenced by the microhabitat (soil structure), surfaces of soil particles, pore spaces, roots, dead organic matter (plant, animal or microbial), water films, etc. (Ladd et al. 1996; Oades 1984; Tisdall and Oades 1982). Soil acidity has deep influence on all these soil parameters besides influencing the charge (positive or negative) on the surface of soil particles, degree of aeration, temperature and nutrient availability.

Studies have demonstrated that changes in soil microbial communities across space are often strongly correlated with differences in soil chemistry (Frey et al. 2004; Nilsson et al. 2007; Lauber et al. 2008; Jenkins et al. 2009). It has been shown that the composition, and in few cases the diversity, of soil bacterial communities is often strongly correlated with soil pH (Fierer and Jackson 2006; Hartman et al. 2008; Jenkins et al. 2009; Lauber et al. 2009). However, it is not clear whether the microbial communities are directly or indirectly affected by pH, since indirect effects like modification of environmental factors like nutrient availability, organic C characteristics, moisture regime and the vegetation type also have a bearing on the microbial community structure. In highly weathered acidic soils, bacterial growth is inhibited by nutrient toxicities and low nutrient availability. Acidity is also known to alter the soil microbial community and decrease the root and rhizosphere effects. It decreases organic matter decomposition and nutrient transformation both of which are essential for nutrient uptake under strongly acidic nutrient poor conditions. Soil acidity decreases the activity of nitrifying bacteria, thereby reducing the levels of ammonium and nitrate for subsequent plant uptake. The metabolic activity and N fixation of cyanobacteria, which are an important group of N fixers, is known to be affected severely by the pH of the growth medium (Stewart 1969; Reddy et al. 1980).

Since soil pH dictates the biological diversity of soil, any management or manipulation of the soil reaction will also alter the population dynamics of the microbes in the soil. The low soil pH may impact organic matter decomposition and the symbiotic relationships between native vegetation and soil organisms, thereby decreasing the survival of native vegetation. The liming of soils to increase pH enhances the bacterial abundance relative to fungi. Low soil pH leads to the reduced growth of beneficial organisms such as rhizobia. If the pH of soil drops below 5, the number of nodules per soybean plant can drop by as much as 40–60%, compared to a soil having a pH above 6 (Lin et al. 2012). The main reason behind the inhibition of nodulation is due to the narrowing of the legume's capacity to release signal molecules that attract compatible rhizobia from the rhizosphere (Hungria and Stacey 1997). Apart from this minerals like calcium (Ca^{2+}) and Mo, which are indispensable for N fixation, become inaccessible at pH levels below 5, while the availability of Al and Mn which are toxic to legume–rhizobia symbiosis gets enhanced (Bordeleau and Prevost 1994). However, if the soil pH is strongly acidic, biological processes such as organic matter breakdown are slowed due to low fungal activity. In addition, the decomposition of plant residues by fungi under waterlogged conditions will lead to a further decrease in soil pH as toxic acetic acid is produced (Lynch 1995). The root mycorrhizal association flourishes in the slightly acidic pH range (Sylvia et al. 2005), and one of the major macronutrients, viz. P, which is usually fixed under acidic conditions, is mobilized for plant uptake by many mycorrhizal species (Seguel et al. 2013).

10.5 Effects of Acidity on Microbial Cellular Metabolism and Acid-Tolerant Mechanisms in Soil Bacteria

Bacteria growing in acidic environment need to maintain their intracellular pH (pH_i) in order to protect their internal cell components. Most bacteria that have been studied (Caldwell 1956; Waddel and Bates 1969; Booth 1985; Padan and Schuldiner 1986) are known to maintain a relatively constant internal pH over a wide range of external pH. This pH control involves one or more mechanisms for regulating the influx and efflux of protons across the cytoplasmic membrane (Glenn and Dilworth 1994). Cells of *Rhizobium meliloti* are known to maintain a slightly alkaline pH, even when the outside pH is acidic (O'Hara et al. 1989). It is not clearly established in root nodulating bacteria as to whether cells can function with an acid inhibited activity of their transporters or whether they have some compensatory mechanism of up-regulating their impaired transport systems or they synthesize acid adapted regulatory systems (Dilworth et al. 2001). Apart from this bacteria have to modify or abandon those external structures like chemoreceptors, periplasmic proteins, flagella, pili, exopolysaccharides, cell wall structures, etc. that are inevitably exposed to acidity and whose interaction with pH may be the actual determinant of survival or growth rather than H^+ toxicity per se (Dilworth et al. 2001). Motility in *Rhizobium leguminosarum* bv. *viciae* is dependent on pH, and it is observed that flagellar motility and chemotactic responses are lost by the organism at lower pH values (Bowra and Dilworth 1981). This places the cell at a disadvantaged position as it is no longer able to relocate itself in response to substrate availability and a suitable chemical environment.

Rhizobial cells grown in moderately acid conditions have been shown to exhibit greater resistance to severely acid conditions compared to cells grown at neutral pH (O'Hara and Glenn 1994). This phenomenon is called 'acid habituation' (Goodson and Rowbury 1989) or the 'adaptive acid tolerance response' (ATR) (Foster and Hall 1990; Foster 1991) and requires the synthesis of novel proteins that may be between bacterial species. Stationary phase cells of root nodule bacteria show decreased acid sensitivity compared to the log phase cells (Dilworth et al. 2001). An interesting observation that *Escherichia coli* cells develop an ATR that can immediately confer the resistance to cells grown at neutral pH via extracellular protein secretion holds importance in the development of acid-tolerant bacterial inoculant strains. Goodson and Rowbury (1989) confirmed this by proving that during growth of *E. coli* at pH 5.0, a component known as extracellular induction component (EIC) shows up in the media and is known for conferring acid tolerance induction.

10.6 The Need for Acid-Tolerant Microbial Inoculants

Approximately a quarter of the world's agricultural soils are acidic, and there is increasing concern about soil acidification (Munns 1986). Though acidic soils are widespread across the world, the quantum of knowledge on the microbial species that inhabit these soils and their utility is quite scarce. An important aspect of acid resistance in Gram-negative bacteria (including the root nodule bacteria) is the ATR, whereby cells grown at moderately acid pH are much more resistant to strongly acidic conditions than cells grown at neutral pH. The survival during pH shock is also markedly affected by the Ca concentration in the medium. It is therefore to be explored as to whether it is possible to improve the correlation between growth on laboratory media and performance in acid soils by determining the ATR of individual strains and screening on media with defined concentration ranges of critical metal ions (Dilworth et al. 2001). Most studies on acid-tolerant microbial inoculants seem to be carried out on *Rhizobium*. But it has been common experience that strains of root nodule bacteria selected for acid tolerance in the laboratory are not necessarily successful as inoculants in acid soils and the widely used technique of seed pelleting with lime to improve legume nodulation may not have a long-term benefit on highly acidic soils. In one of the successful instances, Howieson and Ewing (1986) used acid-tolerant strains of *Sinorhizobium meliloti* to develop medic pastures in the acid soils of Western Australia. It was also observed that strains of *R. meliloti* isolated on low pH media were, in general, more acid-tolerant than isolates from high pH media, when tested in both the laboratory and field. But there was a poor correlation between the growth ratings derived from the laboratory screening and acid tolerance as expressed in the field, and the link between polysaccharide production and acid tolerance was not strong (Howieson et al. 1988). The existence of acid-tolerant species of *Bradyrhizobium canariense* has been reported from the Canary Islands (Vinuesa et al. 2005). Appunu and Dhar (2006) isolated eight acid-tolerant strains of *Bradyrhizobium* from soybean plants grown on acid soils in Madhya Pradesh, India. They observed that all the tested isolates survived in acidic (pH 4–6) conditions, and their survival capacities were higher in soil than in nutrient medium at same levels of low pH. The strains tolerant to acidic stress were more effective nitrogen fixers in symbiosis with soybean in acid soil conditions. In a similar study, Appunu et al. (2009) isolated eight acid-tolerant strains of *Bradyrhizobium* from indigenous cowpea plants grown in acid soils of Varanasi, India. All these isolates survived under acidic (pH 3.5–6.5) conditions and showed variability in their symbiotic performances. The strains tolerant to acidic stress were more effective nitrogen fixers in symbiosis with cowpea under acid soil conditions.

The acid-tolerant rhizobial strain VRM3 obtained from the host grown in acidic soils demonstrated a comparative ability to nodulate and promote the growth and yield of green gram. The combined inoculation of this strain with other plant growth-promoting rhizobacteria (PGPR) resulted in improved crop yields under acid soil conditions (Vijila and Jebaraj 2008). But it is not clear as to what levels such acid-tolerant isolates have been put to commercial production. Pádua Oliveira et al. (2017) reported the symbiotic association between bean and an acid-tolerant strain of *Rhizobium* isolated from the Amazon. In all the cases the symbiosis between common bean and the acid-tolerant rhizobial strains resulted in satisfactory yields, with reduced levels of N application, thereby leading to better economics and environmental sustainability.

Few instances of isolation of beneficial microbes apart from rhizobia are documented. A super strain of *Bacillus firmus* (NCIM-2636) producing the phytohormone, indole-3-acetic acid (IAA), in addition to its high ability to solubilize insoluble inorganic phosphates when applied to the acid soils of Nagaland, India, in combination with rock phosphate produced more prominent effects in rice compared to the application of the same bacterium with Single Super Phosphate (Datta et al. 1982). Pal (1998) isolated a *Bacillus* strain (PAS-2) from a pasture and wastelands having a pH of 4.8. This strain had an acid tolerance rating of 42. The seed inoculation of this bacterial strain resulted in significant increases in grain and vegetative yield of finger millet, maize, amaranth, buckwheat and French bean with or without added P fertilizer sources. The *Kypad* soils of Kerala, India, which are saline-acidic in nature harbour sizeable populations of the N-fixing bacterium *Azospirillum*. Soil populations of *Azospirillum* were found to be more in the month of September, and their numbers declined drastically during the dry periods. The effect of *Azospirillum* inoculation on growth and yield of rice was studied over three seasons, and the results established that inoculation enhanced crop yield significantly over un-inoculated controls in the saline-acidic soils (Govindan and Varma 2010). A heterotrophic nitrifying bacterium *Arthrobacter* sp. capable of growth and nitrification at pH levels as low as 3.0 has been isolated from the forest soils (Brierley and Wood 2001). Vyas et al. (2007) isolated the fungus *Eupenicillium parvum* for the first time from the tea rhizosphere. The fungus solubilized North Carolina rock phosphate and Mussoorie rock phosphate and exhibited high levels of tolerance against desiccation, acidity, salinity, Al and Fe concentrations. This is one of the few instances of the isolation of a fungus with specific tolerance of acidity and metallic ions.

10.7 Future Prospects and Conclusion

Considering the extent of distribution of acid soils across the globe, it is not far-fetched to say that the microbiology of these soils has not received the attention they deserve. The microbes adapted to acidic soil situations are unique and have novel adaptation traits, which have not been elucidated in detail. The development of bioinoculants also seems to have been overlooked for these soils since most of the them are tailored to meet the needs of normal growing conditions, while acid soils require specific microbial inoculants. These have not been developed and utilized on a large scale with the exception of a few acid-tolerant root nodulating bacterial strains in Australia. Recently Egamberdieva et al. (2018) suggested the use of biochar-based inoculants for enhancing the productivity of stressed soils. Biochar can protect the inoculated microbe from extreme conditions and also acts as a soil conditioner. However, considering the extent and prevalence of acid soils across the globe, it can be rightly said that the efforts are inadequate. It is, therefore, imperative to develop microbial inoculants specific to different soil acidities and understand the underlying mechanisms that make them acid-tolerant, in order to achieve the overall goal of promoting microbial inoculants in mankind's quest for reduced chemical usage in agriculture. This can be achieved by exploring the amazing diversity of acid-tolerant microbes present in native acid soils for their plant growth promotion activities and utilizing the promising ones for bioinoculant production.

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Chapter 11

Plant Growth-Promoting Microbes as Front-Runners for On-site Remediation of Organophosphate Pesticide Residues in Agriculture Soils

S. Krishna Sundari, Anil Prakash, Pratibha Yadav, and Archana Kumari

Abstract Increasing population has led to increased demand of food supply which marks greater importance to the agriculture sector worldwide. Chemical pesticides applied with an intention to maximize agriculture productivity and crop yield are leaving high residues in the agricultural soils, influencing the soil health adversely. Plant growth-promoting microbes (PGPMs) are largely known to be applied for their role in enhancing plant growth, thereby becoming a favourable choice for developing the new age bioinoculants. Presently, many researchers are concentrating their efforts to explore the potential of PGPMs to fight this problem of chemical residue accumulation in soils. Employing PGPM for bioremediation process would provide dual benefits to agricultural fields.

In this chapter the focus would be on exploring the role of PGPMs to remediate widely applied organophosphate pesticides (OPPs) that belong to the group of second-generation pesticides. The chapter begins with details of OPP application worldwide and the toxicity issues raised by their usage. A consolidation of reports on pesticide-associated toxicity in humans/higher mammals and the possible route of accidental ingestion through consumption of pesticide-laden agriculture products is presented. The chapter also elaborates on the role of PGPMs in OPP bioremediation by means of employing different biomolecules and enzymes produced by them in response to pesticide stress. Further, the manuscript presents research reports on PGPM application for OPP remediation along with future scope of research in this field. The chapter presents an overall critical analysis on why PGPM-based residual pesticide remediation is the safest solution by far to minimize migration of toxic pesticide residues to the food chain and plug in the accidental ingestion risk of residual pesticides.

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11.1 Introduction

Increasing global food demand has led to an increase in global market for organophosphate pesticide (OPP) consumption in various sectors. The global market scenario as per several business reports stated consumption of organophosphate pesticides was hypothecated to be around 4.30 billion USD in 2015 with an estimated increase to 7.06 billion USD in 2017. Compound annual growth rate (CAGR) for the period 2018–2022 is predicted to be 5.5%. Asia-Pacific was found to hold maximum market share (40%) in OPP consumption rate, whereas North America and Europe were amongst the least consumers. The global annual OPP consumption for agricultural purposes during the period 2010–2015 has been compiled in Table 11.1. However this increasing global demand is getting affected due to changing government policies driven by awareness towards pollution in the environment and its control. To some extent these phenomena are bound to affect the consumption rate of OPP amongst different countries.

A large part of Indian economy is known to be dependent on agriculture. The agriculture sector contributes 27–30% of gross national productivity (GNP) to Indian economy. This fact is supported by several stats reported in the past stating dependency of almost 58% of the rural households on agriculture, as their principal means of livelihood. A report by the Government of India, Department of Agriculture, Cooperation and Farmers Welfare states a total of 275.68 million tonnes (MT) of production of food grains in India in 2016–2017. The rural people spend over 85–90% of their earnings for meeting their basic needs particularly for food security. As over 40% of them are poor, they are unable to meet even their essential needs which prove the importance of agriculture not only for the country's economy but for mankind as well. Statistics from Central Statistics Office (2016–2017) place the

Table 11.1 Global annual organophosphate pesticide (OPP) consumption for agricultural purposes

Average annual consumption (tonnes)	Countries
0–50	Austria, Nigeria, Finland, Norway, Iceland, Ireland, Hungry, Slovakia, Tajikistan
50–300	Germany, Greece, Netherlands, UK, South Sudan, Zimbabwe, Nepal, Sri Lanka, Panama
300–1000	Poland, France, Portugal, Italy, Bangladesh, Sudan, Costa Rica
1000–3000	Argentina, Spain, Myanmar, Thailand, Malaysia, Japan
3000–20,000	India, USA, China, Ukraine, Iran

Source: Shi (2017) and Hertz-Picciotto Id et al. (2018)

Indian food/grocery market at sixth position in the world, with retail contributing to 70% of the sales, and 8.39% to GVA (gross value added) in agriculture (IBEF 2018).

Agriculture being such an important sector for country's growth, any factor that compromises the yields would have a considerable impact on the economy and livelihood. While bacterial and fungal pests are major contributors for yield loss, weeds are known to reduce yield of dry land crops by 37–79% (Bhan and Behera 2014). Severe infestation of pests, particularly in the early stage of crop establishment, ultimately accounts for a yield reduction of 40%. This is the leading factor that places major market emphasis on chemical inputs in agriculture with an aim to sustain yield dynamics. Application of pesticides is known in agriculture for several purposes such as protecting plants or plant products against all harmful organisms by the application of fungicides, insecticides, molluscicides, nematicides and rodenticides; preserving plant products using fumigants; destroying undesired plants or parts of plants, with the help of defoliants; and checking or preventing growth of weed plants through herbicides (Ortiz-hernández et al. 2013).

An earlier report from the Indian Labour Statistics (1994) shows fourfold increase in food grain production, from 50 million tonnes in 1948 to 198 million tonnes in less than five decades. Indian statistical report provided evidence showing massive increase in application of pesticides (51,755 tonnes) during the same period reflecting increased chemical load in the country (Aktar et al. 2009). Similar observations were made in many countries such as the UK (wheat yield) and the USA at a time when India too is registering increased chemical inputs in agriculture. Research by Webster et al. (1999) also supplied facts of heavy chemical inputs made in order to sustain agriculture outputs and to avoid great economic losses.

India is a country with vast regional differences in practice of agriculture systems and exhibits a change in pattern of application of pesticides. Being an agriculture-based economy, heavy input of chemical pesticides is registered from all major states of India. As per reports by the 37th Report of Standing Committee on Petroleum and Chemicals (2002), Indian states are divided into five categories based on levels of pesticide consumption (Fig. 11.1) in India, during the period 2000–2001. In the report, states of Uttar Pradesh (UP), Punjab and Haryana are leading states in pesticide utilization registering consumption around 5000MT annually (CPCB 2006). On the other hand, states like Goa, Meghalaya and Sikkim clock lowest pesticide consumption, viz. around 10MT (Fig. 11.1). A report by the Ministry of Agriculture and Farmers' Welfare 2011 on the amount of pesticides applied in different states of India during the period 2005–2010 still places UP, Punjab and Haryana amongst the top three, but with a phenomenal increase in the amount of pesticides applied (Fig. 11.2). As is evident from Fig. 11.2, UP showed an increase in application by eight times, Punjab by nearly six times and Haryana by four times in a period of less than 10 years, which is definitely alarming (Kumar et al. 2016). It is not only a serious indication of unstructured, unmonitored utilization of pesticides in far excess in Indian agriculture but also a grave contributor to risk of accumulation of these pesticide residues in the environment.

With respect to the usage and application pattern of pesticides, a difference is observed in India as compared to the rest of the world. In India application of pesti-

Category I
- Annual Consumption of 5,000 MT
- States : Haryana, Punjab , Uttar Pradesh

Category II
- Annual Consumption of 1000-5000 MT
- States: Andhra Pradesh, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Orissa and Tamil Nadu

Category III
- Annual Consumption of 100-1,000 MT
- States : Assam, Bihar and Himachal Pradesh

Category IV
- Annual Consumption of 10-100 MT
- States : Arunachal Pradesh, Jammu & Kashmir, Manipur, Mizoram, Nagaland, Tripura, Delhi and Pondicherry

Category V
- Annual Consumption of 10 MT
- States : Goa, Meghalaya, Sikkim and UT's of Andaman & Nicobar, Chandigarh, Dadra &Nagar Haveli, Daman & Diu and Lakshadweep

Fig. 11.1 Categorization of Indian states based on annual pesticide consumption during the period 2000–2001

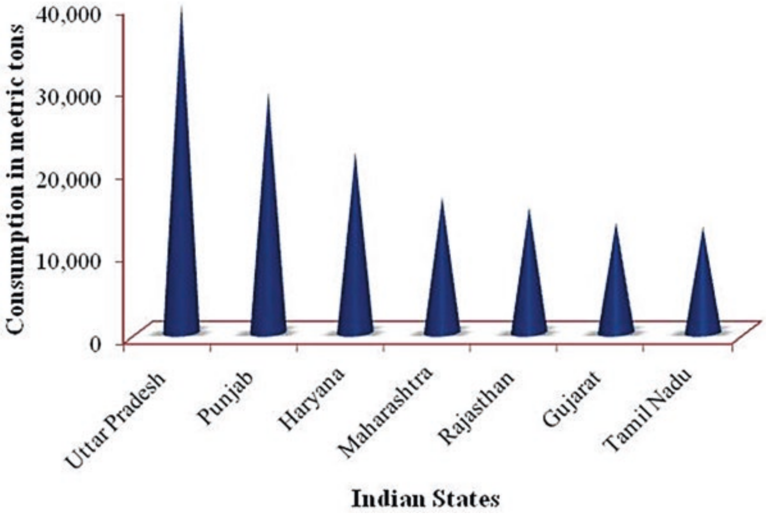

Fig. 11.2 Total pesticide consumption in metric tonnes in different states of India during the period 2005–2010

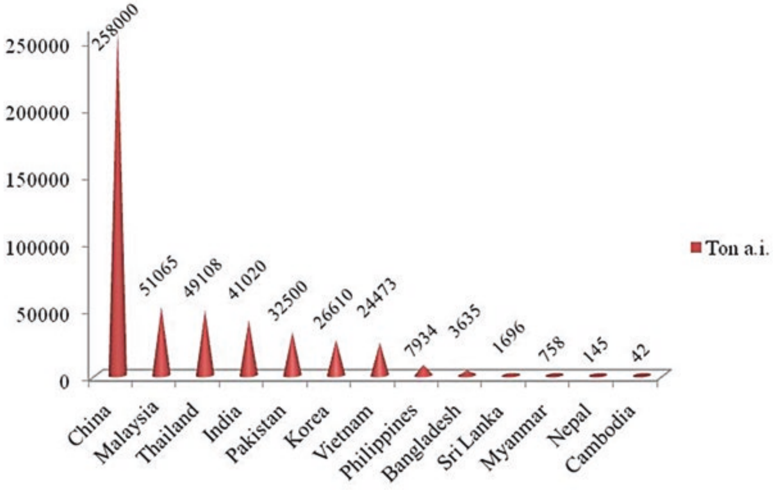

Fig. 11.3 Relative pesticide consumption in Asian countries

cides as insecticides was found to be maximum, accounting for more than 70% of the total domestic market, whereas at a global level, herbicides and fungicides have a significantly higher application (Abhilash and Singh 2009). Figure 11.3 denotes application of pesticides in India as compared to other Asian countries. While China shows highest consumption of pesticides amongst all Asian countries, Malaysia, a distant follower, though second in position, applies five times lesser amount as compared to China, and India comes as the fourth highest consumer of pesticides in Asian countries.

Pest control directly benefits plant yield, and this group of chemicals is largely named as plant protection products (PPP). Pesticides can be grouped into synthetic chemical families; pesticides with similar chemical structures have usually similar characteristics and mode of action. Insecticides include the following main chemical families: organochlorines (restricted application due to high toxicity), organophosphates, carbamates and pyrethroids. Typical chemical families of herbicides are phenoxy herbicides, benzoic acid herbicides, triazines and amides. Active ingredients of pesticides are often mixed with other compounds such as solvents, mineral clays, stickers, wetting agents or other adjuvant materials to improve their effectiveness, safety, handling and storage. Such a mixture is called ‘pesticide formulation’. This chapter emphasizes mainly upon organophosphate group of pesticides abbreviated as OPP. With an increasing application of chemicals in agriculture, several adverse effects impacting life forms in general and the environment in particular are occurring (Gevao et al. 2000). Pesticides can enter soil environments via several means including leaching into groundwater, chemical degradation, volatilization to air, binding to soil solid phases and transfer to organisms (Reid et al. 2000).

Use of pesticides not only degrades soil quality but also reaches the water table and enters aquatic environment. Because the fate of pesticides is often uncertain, it makes decontamination of pesticide polluted areas a very complex process. Low degree of biodegradability has classified them into persistent toxic substances. Extensive use of pesticides has resulted in serious environmental as well as health problems besides causing severe impact on microbial biodiversity as well. The effect of agricultural inputs on soil organisms can be measured either as changes in the amount of single organisms, organism groups or methodologically defined pools such as the microbial biomass or as changes in biological activity, e.g. soil respiration and enzyme activities (Bünemann et al. 2006; Lo 2010). An issue of concern here is the increasing persistence of certain organic pollutants in soil, which is also related to the hydrophobicity of these compounds. From a toxicological perspective, binding of xenobiotics to humus leads to (1) a decrease of material available to interact with biota, (2) a reduction in the toxicity of the compound and (3) immobilizing the compound, thereby reducing its leaching and transport properties (Carringer et al. 1975; Berry and Boyd 1985; Calderbank 1989). With more and more chemical toxicity in soils, the biodiversity and biological density of microbes, including plant helpful microbes, saprophytes and various other living organisms that contribute to nutrient cycling in soils, decrease. This results in reduced organic nutrients including a reduction in humic content in soils. In the absence of humus, the soil will be losing the benefits accorded by this important component. Hence it becomes imperative that, in order to address chemical toxicity in soils, one has to work on building soil microbial population, preferably with environmentally safe and helpful microbes (Arora 2018).

There is a need to encounter this problem with economically viable and sustainable solutions. Earlier techniques or technologies which were used to eliminate them from environment were landfills, recycling, pyrolysis, etc. However, these have adverse effects on the environment and may also lead to formation of toxic intermediates. Moreover, these methods have proved to be expensive and difficult to execute especially in case of pesticides. Bioremediation is the most promising technology which utilizes the ability of microorganisms to remove pollution from the environment and is eco-friendly, economical and also versatile (Ee et al. 2005; Uqab et al. 2016).

In this particular chapter, we focus on plant growth promoting microbes (PGPMs) as sustainable solution to remediate increasing pesticide residues in agriculture soil and provide healthy and toxin-free soils for agriculture.

11.2 Types of Pesticides

Based on the mode of action, pesticides can be classified into six classes, i.e. (a) direct contact pesticides that act only upon contact with the pest; (b) systemic pesticides that get into action only after being absorbed by the plant and subsequently when the specific plant part is consumed by the pest; (c) broad-spectrum pesticides, applied for the control of various pests and are non-selective in nature due to which they damage the beneficial soil organisms also; (d) selective pesticides that act against specific pests; (e) curative pesticides that can stop the damage once started, meaning they restrict the pathogen from colonizing the host and are found to be effective after 24–48 h from occurrence of infection; and finally (f) preventive pesticides, those that stop the damage before infection starts. These chemicals when sprayed over the host plant form a protective barrier and stop any infection from occurring.

The most popular and commonly used classification of pesticides is based on specific pests targeted by them. As per this system pesticides are majorly grouped in five classes:

1. Insecticides: Majorly applied for killing insects and are principally organochlorides, organophosphates and carbamates. This category of chemicals also includes certain insect repellents of natural origin, e.g. methyl parathion, dimethoate and chlorpyrifos.
2. Herbicides: Also known as weed killers, e.g. paraquat, glyphosate and propanil.
3. Fungicides: Principally target moulds or fungi and are popular as wood preservatives, e.g. mefenoxam and aldimorf.
4. Rodenticides: Applied to kill rodents such as mice, rats and other rodents, e.g. warfarin and bromadiolone.
5. Fumigants: Pesticides that exist in the form of a gas or a vapour and are applied as insecticides, rodenticides or fungicides, particularly in closed storage places – as they have potential to kill every living organism. They are extremely toxic, due to their physical properties, and show rapid environmental dissemination in addition to human or animal absorption, e.g. cyanide, aluminium phosphate and methyl bromide.

Other groups of pesticides including matricides, algaecides and acaricides are applied to kill moth, algae and ticks, e.g. copper sulphate, resin amine salts and formamides (WHO 2008).

Insecticides: This chapter focuses on one of the major groups of insecticide, namely, organophosphate pesticides, and their degradation. However before the OPP group is discussed in detail, a brief discussion about different insecticide groups, their application, origin and toxicity are presented here. There are two major kinds of insecticides: one, the synthetic group like organochlorides, organophosphate and carbamate and the other group derived from natural sources like pyrethroids, neonicotinoids and ryanoids.

Organochlorides: Also known as first-generation pesticides, as this group was introduced primarily to target insects. Amongst organochlorides, the best-known pesticide is DDT which was introduced in 1944 by Paul Muller. All the pesticides of this group are known for targeting the nervous system of insects, for instance, DDT kills insects by opening Na^+ channels in nerve cells of insects.

A huge application of organochlorides took place in the late 1960s in several sectors. Research studies across the world have reported harmful effects of this group on plants and animals including humans. Lately persistence of these pesticides in environment has been a major issue, and not only organochlorines but even several of their breakdown products and derivatives such as dioxins are much more harmful than the parent compound itself. These harmful chemicals are known to enter the food chain and affect life. In fact, pesticides from this group were also developed as chemical weapons, namely, lewisite, nitrogen mustards and sulphur mustards. As a result, usage of organochloride pesticides became restricted or banned in several countries including the USA, Europe, India, etc. (Zacharia and Tano 2011; Prieto Garcia et al. 2012).

Organophosphates**:** Also known as the second-generation pesticides, this group was introduced during World War II. The group consists of several chemicals comprising a huge numbers of contact insecticides. These pesticides affect the nervous system of insects by targeting acetyl cholinesterase and other cholinesterases leading to killing or disabling insects. The mode of action of OP insecticides are similar to some chemical warfare nerve agents, e.g. sarin, tabun, soman, etc. Reports on toxicity due to organophosphate have also increased over the time with their increasing application/utilization (Prieto Garcia et al. 2012; Gupta et al. 2012). This group is dealt with greater detail, further in this chapter.

Carbamates: Carbamates are derivatives of carbamic acid and are organic in nature. The carbamate group of pesticides differ from one another in the functional group, and these functional groups are interchangeable. For example, carbamate esters, also known as urethanes, and a compound of carbamic acid are structurally related and have interchangeable functional groups. The mode of action of carbamates was found to be similar to that of organophosphates, except for the fact that carbamates are less toxic and effective for short duration. Toxicity of carbamate insecticides in humans is due to their effect on human melatonin receptors (Prieto Garcia et al. 2012; Gupta 2006).

Pyrethroid pesticides: Largely applied in houses to control pests, pyrethroids mimic insecticidal activity of pyrethrum which is the biopesticide found in pyrethrins. This group shows non-persistent nature, and its mode of action in humans is through its impact on the sodium channels. These properties make it least toxic as compared to the above-mentioned pesticides (Ware and Whitacre 2004).

Neonicotinoids: These chemicals are synthetic analogues of the natural insecticide nicotine. The characteristic features of nicotine include maximum field persistence, lower acute mammalian toxicity, broad-spectrum systemic insecticidal activity and rapid action. Its mode of action involves the play of acetylcholine receptors as this group is agonist for the receptors. They are applied as sprays, drenches and seed and soil treatments. Commonly known pesticide amongst them is imidacloprid (Greenpeace 2015).

Ryanoids: Ryanoids are synthetic analogues of ryanodine extracted from native South American plant *Ryania speciosa*. This group of pesticides acts by blocking nerve transmission which occurs due to the binding of pesticide molecule to calcium channels present in cardiac and skeletal muscles. Only a single insecticide is registered in this group, i.e. Rynaxypyr, also known as chlorantraniliprole (Murugan et al. 2013).

11.3 Organophosphate Group of Pesticides (OPP)

The organophosphate group of pesticides are often abbreviated as OP pesticides/ OPPs. Despite a considerable number of efforts to substitute these chemicals with some alternative chemical tools that are less toxic and thus restrict their use, OPPs are still applied for several purposes. In fact, OPPs were the most extensively used insecticides during the twenty-first century. Chemically OPPs are known as phosphate ester/esters of phosphoric acid. OPPs constitute many insecticides, nerve agents and herbicides. Since World War II, different types of OPs were synthesized for various purposes and applied for different uses as shown in Fig. 11.4. OPP application is found in several sectors of day-to-day life such as agriculture, gardens, homes and veterinary practices. Other applications of OPP group of chemicals is their usage as solvents and plasticizers (Gupta 2006; Singh and Walker 2006; Abhilash and Singh 2009).

Structurally, OPs are esters of phosphoric acid along with different combinations of sulphur, oxygen, carbon or nitrogen attached to the parent structure. OP compounds possess certain differences and some similarities amongst themselves. Observing the structure of OP, we can find similarities within the group, which determine the anti-acetylcholinesterase (anti-AChE) activity which is the primary mode of its target attack. One common feature is the presence of phosphorus atom and its different linkages such as thiophosphoryl bond (P=S) or phosphoryl bond (~O). Table 11.2 shows the type of structural moiety of many of the OPPs. An interesting fact that emerges here is that some of the OPPs, though are lacking the

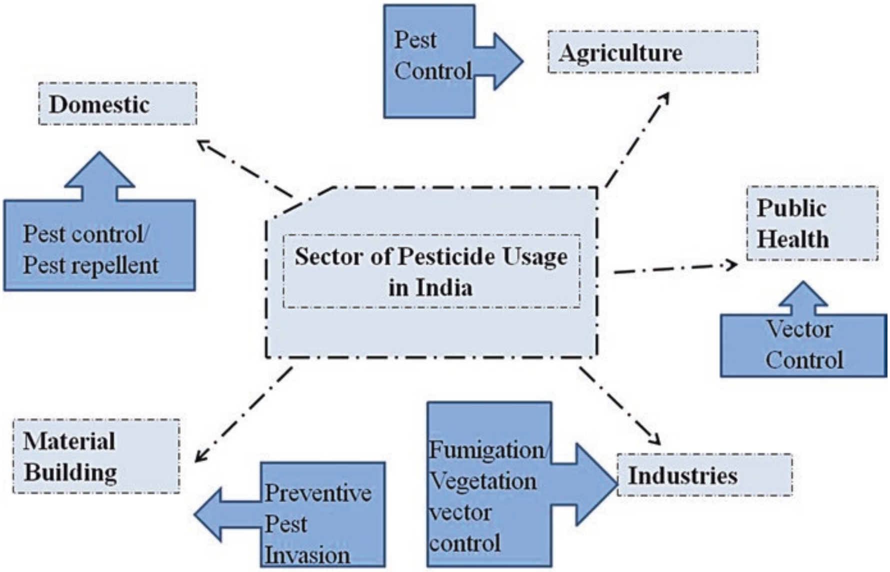

Fig. 11.4 Pesticide usage in different sectors of India

Table 11.2 Classification of different widely applied organophosphate pesticides

Type of OP	Name of OPP	PGPM found effective	WHO class of toxicity	Enzymes and genes
Phosphorodithioates	Phorate	*Azotobacter*	**Extremely hazardous LD_{50} < 5 mg/kg**	Aminopeptidase
				AMPP *Escherichia coli*
Phosphorotrithioates	Terbufos			
				A-OPH *Aspergillus niger (A-opd)*
Phosphates	Monocrotophos	*Pseudomonas mendocina, P. aeruginosa, Pseudomonas* spp.*, Bacillus* sp.*, B. megaterium, Arthrobacter atrocyaneus, Azospirillum lipoferum, Pseudomonas monteilii*	**Highly Hazardous LD_{50} 5–50 mg/kg**	
				Carboxyl amidase
				C-P lyase phosphatase
				C-P lyase *Pseudomonas* spp. *(opd)*
				HOCA *Pseudomonas monteilii (hocA)*
Phosphorothioates	Oxydemeton-methyl			
				Laccase
Phosphorothioates	Parathion/methyl parathion	*P. aeruginosa, Pseudomonas* sp.*, Bacillus* sp.*, Flavobacterium* sp.*, F. balustinium, P. stutzeri, P. diminuta, P. putida* sp.*, Xanthomonas, Agrobacterium radiobacter*		
				MPD – *Plesiomonas* sp. *M6*
				MPDB – *Burkholderia cepacia*
				MPH – *Pseudomonas* sp. *WBC-3*

Phosphoramidates	Fenamiphos			NS-OPH *Nocardioides simplex*
Phosphorodithioates	Phosphamidon			OPAA *Alteromonas* spp. (*opaA*)
Phosphoramidothioates	Acephate	*Pseudomonas* sp., *Flavobacterium* sp.	**Moderately hazardous LD_{50} 50–2000 mg/kg**	*OPAB-Mycobacterium sp.*
Phosphorodithioates	Anilophos			*OPDA A. radiobacter (opdA)*
Phosphorothioates	Chlorpyrifos	*Pseudomonas diminuta, Flavobacterium* sp., *Micrococcus* sp.		*OPDA ADPase (aryl dialkyl phosphatase)*
Phosphorothioates	Chlorpyriphos-methyl			*Nocardia sp.* *OPDB – Burkholderia* sp. *FDS-1*
Phosphorothioates	Diazinon	*Flavobacterium* sp.		OPH *Pseudomonas diminuta (opd)*
Phosphates	Dichlorvos	*Flavobacterium* sp.		
Phosphorodithioates	Dimethoate	*Pseudomonas* sp., *P. putida*, *P. aeruginosa, Acinetobacter* sp.		OPHC2 – *Pseudomonas pseudoalcaligens*
Phosphorodithioates	Edifenphos			Parathion hydrolase
Phosphorodithioates	Ethion, ethanox	*Azospirillum*		PEH *Burkholderia caryophylli*
Phosphorodithioates	Ethoprop			PEH Phosphonate ester hydrolase
Phosphorothioates	Fenitrothion	*Burkholderia* sp., *A. aurescenes*		PHoCA – Phosphotriesterase (hydrolysis of Corazon)
Phosphorothioates	Fenthion			

(continued)

Table 11.2 (continued)

Type of OP	Name of OPP	PGPM found effective	WHO class of toxicity	Enzymes and genes
Phosphoramidothioate	Iprobenfos			Phosphonatase *Bacillus cereus*
Phosphorodithioates	Malathion	*Pseudomonas* sp., *Brevibacillus* sp. *KB2*		P-OPH *Bacillus cereus (Phn)*
Phosphorothioates	Triazophos			P-OPH *Penicillium lilacinum*
Phosphonates	Trichlorfon			SC-OPH SC strain 67
Phosphorothioates	Profenofos			
Phosphorodithioates	Phenthoate			
Phosphorodithioates	Propetamphos			
Phosphorodithioates	Phosalone			
Phosphorothioates	Quinalphos			
Phosphorothioates	Pirimiphos-methyl		**Slightly hazardous** LD_{50} > 2000 mg/kg	
Phosphorodithioates	Temophos			

so-called common basic structure/feature, are still placed in OP group due to their anti-AChE activity. An important aspect to be noted about the OPPs is that to possess anti-AChE activity, analogous oxon must be desulphurated as negligible anti-AChE activity is observed with phosphorothioates (P – S). Some of the OPPs are classified as less toxic and less persistent as they do not possess anti-AChE activity but are reported to have low mammalian toxicity, e.g., merphos, glyphosate and gluphosinate (Singh and Walker 2006).

Hydrolysis reaction is reported as the first step in majority of cases of OPP breakdown. The structure of OPP has three substituent groups amongst which two are alkyl substituents and the third, a group known as leaving group, and it is this group that contributes to hydrolysis of OPP. The mode of action of OPP states that this group of pesticides possesses cholinesterase inhibition activity, thereby killing insects which are known universally. A schematic showing the mechanism through which OPPs target the nervous system is illustrated in Fig. 11.5. The figure shows an imbalance in signal transduction once ACE enzyme site is deformed by OPP. In mammals, OPP molecule undergoes metabolic transformation where the parent compound oxidizes to oxane molecule, and it is this oxane that causes toxicity to the cells. This activity would directly and indirectly affect non-target organisms in several ways. Recent studies stated possible undesirable effect on neurobehavioural development of the newborn and the foetuses even at very low exposure rate. Certain OPPs such as parathion and chlorpyrifos are no longer registered for use in different

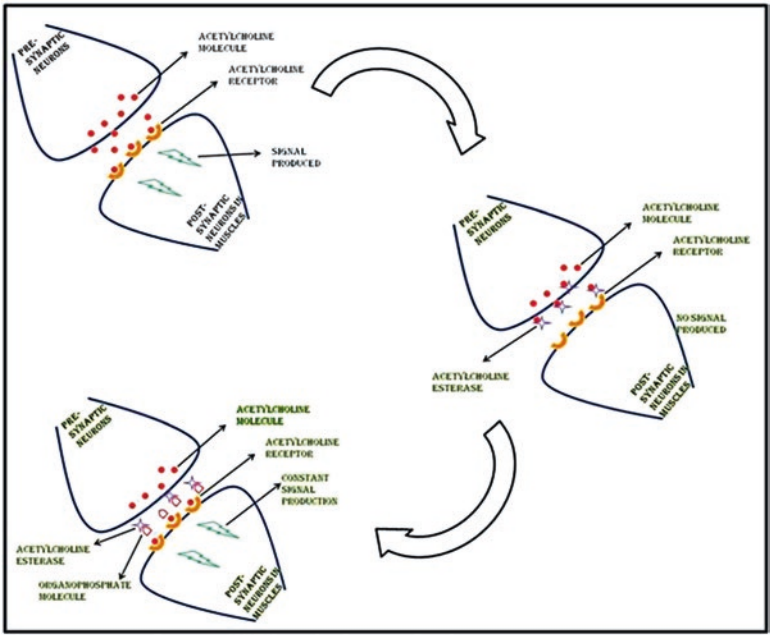

Fig. 11.5 Mechanism of action of organophosphate pesticides on the nervous system

sectors. The US Environmental Protection Agency (USEPA) listed some of the OPPs as highly dangerous and toxic for non-target organisms and has restricted its use (Sakata 2005; Bates and Campbell 2008; Gupta et al. 2012).

11.3.1 Classification and History

OPPs are divided into different classes based on several parameters such as their structure, extent of hazardous nature, mode of action, etc. Table 11.2 lists a variety of OPPs and their hazardous nature as per the categorization of WHO (2008). The history of pesticide application for control of pests in cultivated crops is known since the beginning of agriculture systems though the usage was very less. Prominent usage of pesticides in agriculture and household sectors came to being after incidence of pests and insects started affecting agriculture productivity, soil fertility and human health (Zacharia and Tano 2011). New chemicals were introduced generation after generation in order to overcome the drawbacks and toxic effects of the chemical compounds discovered earlier. In the 1860s synthetic pesticides were introduced to control pests like fungi, insects and bacteria. Some of these chemicals are highly toxic compounds of arsenic and fumigant (hydrogen cyanide); other compounds include Bordeaux mixture, etc. Followed by these were the first-generation pesticides that are technically the organochloride pesticides. Havoc caused by uncontrolled use of DDT is documented by hundreds of studies which resulted in banning its widespread application. This led to the entry of second-generation pesticides, i.e. organophosphate pesticides, introduced to replace the most toxic group of organochloride pesticides. German chemist Willy Lange along with his team researched and reported the cholinesterase toxicity of OPs in 1932. Large amount of OPs were synthesized during World War II. OPs were initially developed as highly toxic warfare agents. Parathion and malathion were amongst the first few OP pesticides to be introduced in the market at later times (Soltaninejad and Shadnia 2014).

Focusing on the history of OPP utilization, the intensive usage of these pesticides began during the Green Revolution that began in Mexico in 1944. Green Revolution was a worldwide agriculture movement. The mission of this movement was to boost the grain yield. Increasing population has placed huge demand for maximizing agricultural productivity from agricultural soils that were already stressed due to constant pest attack and low soil fertility. The term Green Revolution represents the enormous challenge of overcoming the limitation of yield reduction that was happening at that point in history. Since pest control became a major part of the revolution, application of pesticides was taken up on a massive scale in agriculture.

On the other hand, several vector-based diseases like malaria infected a large number of people, which created an urgent need to identify a chemical solution to this problem. This is how pesticides entered the household sector. Since OPs are known as efficient insecticides, they found larger application in household sectors as well (Farahata et al. 2016).

11.3.2 National and International Usage of Organophosphate Pesticides

Since pesticide production took place on a larger scale, as discussed in the above section, it is important to know which part of the world consume these pesticides and at what scale. A study by Abhilash and Singh (2009) states approximately 2 million tonnes of pesticide production per year worldwide. The study further states that the USA and Europe consume about 24% and 45% of total pesticide produced, respectively. Further on observing the type or class of chemical usage, it was seen that amongst the total consumption of pesticides, 80% are used as insecticides, 15% as herbicides, 1.46% as fungicides and less than 3% as others. Now further knowing the consumption per acre, it was reported that with the upsurge in chemical utilization approximately 160 metric tonnes of pesticide application in the year 1949–1950 increased gradually and became 64,000 metric tonnes in 2002–2003 (Sundari and Mishra 2013). A schematic showing the statistical data of mostly applied OPs in the country during the year 2005–2010 is given in Fig. 11.6. Multiple cases of chronic or acute poisoning were reported with each and every one of these

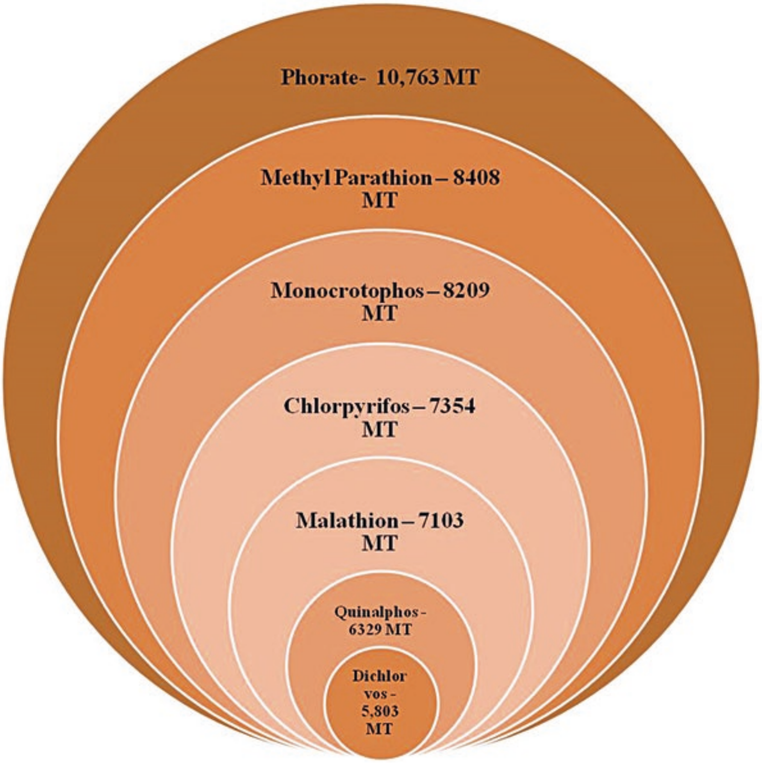

Fig. 11.6 Specific organophosphate pesticide used in India and its order of application

pesticides by researchers from laboratories in India and even from other parts of the world (Thompson et al. 1998; Gupta 2006; Goel and Agrawal 2007; Vijaya kumar et al. 2010; Kumar et al. 2016).

Almost 38–40% of all the pesticides applied worldwide are OPs. Despite such a large consumption of pesticides, it is estimated that crop losses vary between 10% and 30% due to pests alone. Study on pesticide usage in India revealed a total of over 5000 metric tonnes of pesticide production in the year 1958 alone, due to involvement in various sectors in the country. In the mid-1990s, 145 pesticides were found to be registered with the government, and a total of 85,000 metric tonnes of pesticide was produced. India is presently the second largest manufacturer of basic pesticides in Asia and ranks 12th globally. Field application of pesticides in India was found to be 0.5 kg/ha which is less as compared to certain countries like Korea and Japan that showed 6.6 and 12.0 kg/ha of pesticide application, respectively (Uqab et al. 2016). Currently, the consumption of pesticide is showing a slight decline due to shift of farmers towards biopesticides and other alternative methods (Aktar et al. 2009; Uqab et al. 2016). Based on scientific reviews, research and market analysis reports published recently (Department of Fertilizers, Ministry of Chemicals and Fertilizers report 2013; FICCI 2016), India seems to be rising to the challenge of incorporating bioinoculants into active agronomic practices. Analysis of consumption of PGPM-based biofertilizers in India during the past decade denotes a healthy employment of them in the South Zone (states, AP, Karnataka, Kerala, Tamil Nadu) posting a record consumption of 1.5 MMT between the years 2008 and 2016. Following these states is the West Zone (Chhattisgarh, Gujarat, Goa, MP, Maharashtra and Rajasthan), and last comes the North East Zone posting only 0.0046 MMT approximately. Figure 11.7 clearly presents the relative consumption levels of the five distinct zones of India, namely, SZ, South Zone (49%) > WZ, West Zone (28%) > NZ, North Zone (18%) > EZ, East Zone

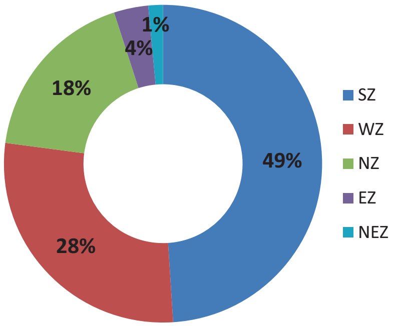

Fig. 11.7 Relative consumption of PGPM-based biofertilizers in India
Note:
SZ – South Zone states: Andhra Pradesh, Karnataka, Kerala, Pondicherry, Tamil Nadu
WZ – West Zone states: Gujarat, Goa, Madhya Pradesh, Maharashtra, Rajasthan
NZ – North Zone states: Delhi, Chandigarh, Haryana, Himachal Pradesh, Punjab, UP, Uttarakhand
EZ – East Zone states: Bihar, Jharkhand, Odisha, West Bengal
NEZ – North Zone states: Arunachal Pradesh, Assam, Mizoram, Nagaland, Sikkim, Tripura

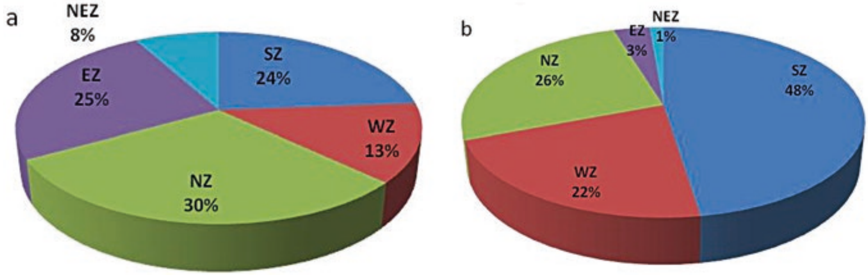

Fig. 11.8 Employment of chemical-based fertilizers vs. PGPM-based fertilizers across different states of India (Note: (a) consumption of chemical fertilizers, (b) consumption of biofertilizers)

(4%) > NEZ, North East Zone (1%). When one makes a comparison between the amounts of chemical fertilizers consumed in a specific zone as against the PGPM-based biofertilizers, in a single year, an interesting phenomenon appears (Fig. 11.8a, b). The North Zone uses a maximum share of chemical fertilizers (30%) and a near equivalent amount (26%) of PGPM-based fertilizers, whereas the South Zone uses relatively more share of biological inputs (48%) as against the share of chemical inputs (24%) at a national level. While the West Zone uses nearly double the share of chemical inputs (22%) vs. biological at 13%, EZ and NEZ can be termed the poorest performers in terms of incorporating biofertilizers. Both of them use eight times more of chemical inputs (EZ 25% and NEZ 8%) as compared to biological inputs (EZ 3% and NEZ 1%), respectively Mazumdar (2015).

11.4 OPP Accumulation Risks to Environment and Biota

One of the primary reasons for large-scale adaptation of OPP was the perception about their low persistence in environment unlike their predecessors (organochlorides). However, excessive and unmanaged application of these pesticides over the decades is ultimately reflecting in edible food products risking accumulation in food chain. Studies on toxic effects of OP pesticides emerged during the late 1990s. Chemicals sprayed to the agricultural land have different means of entry in the environment. It is important to understand the ways of chemical entry, as this will help to understand firstly and, most importantly, the source of chemical release, secondly the rate of exposure and thirdly the effect on non-target organisms due to exposure. Pesticides enter the system by any of the following means, namely, emission, volatilization, drift, leak/spill, drain and sedimentation. From soil, these chemicals either leach into the ground water or evaporate/volatilize in air and also can enter flowing water bodies. These chemicals then deposit as sediments in water bodies affecting the aquatic life and non-target organisms. Humans and other terrestrial animals may come under the effect of pesticides even by coming in contact through air (Aktar et al. 2009; Agrawal et al. 2010). Pesticide persistence depends on its chemical and physical properties and on climatic conditions of the place. Studies have reported the presence of some majorly applied OPPs like malathion, chlorpyrifos, ethion, monocrotophos and dimethoate above the minimum residue level (MRL) registered for their usage. Researchers reported residues of above-mentioned OPPs in different regions of the countries such as wheat and sugarcane fields of Haryana, lake waters of Bijapur Karnataka, tea leaves from Southern India and winter vegetables grown in Haryana. Other studies have observed OPP residues reaching near MRL range in soils from tea fields and water samples near tea gardens of West Bengal; in seasonal vegetables grown in Northern India and vegetables from farm fields of Andhra Pradesh. OPP pollution was reported in many riverine ecosystems including the rivers Ganga and Yamuna and approximately 50 more water bodies distributed across different Indian states (Agrawal et al. 2010; Alam et al. 2015; Kumar et al. 2016).

The means of OPP exposure in humans are through inhalation, ingestion or dermal contact. Different toxic effects reported ranged from mild discomfort to nicotinic manifestations, neuromuscular paralysis and even death in case of extreme poisoning where affected individuals die due to difficulty in breathing. Symptoms of OP toxicity are generally classified under two broad headings, i.e. acute and chronic symptoms. Squeal of convulsions, anoxia, respiratory failure and cardiac arrhythmias are few symptoms occurring in acute cholinergic syndrome. Drowsiness, confusion, lethargy, anxiety, emotional ability, depression, fatigue and irritability are grouped amongst some chronic OP effects (Agrawal et al. 2010; Kumar et al. 2016). A report of Vijaya kumar et al. (2010) provided several supportive cases where more than 80% of hospitalization cases of pesticide poisoning were due to OPPs. A report by the Poison Information Centre in NIOH, Ahmedabad, names organophosphate pesticides as the most hazardous group as compared to all other current chemical inputs in agriculture. The National Institute of Occupational Health (NIOH) provides a report showing 73% of poisoning is due to OPP. Developing countries like India were affected largely due to low awareness rate about chemical toxicity and poisoning (Singh and Sharma 2000; Singh and Khurana 2009; Vijaya kumar et al. 2010). Studies by multiple authors reviewed in the publication of Agarwal et al. (2010) caution on possible dose-dependent DNA damage, disruption of endocrine system, cancerous/tumorigenic properties and adverse affects on immunity and reproductive biology. Profound impact of OPP was seen on respiratory tract, cardiovascular system and nervous systems. All the above information strongly indicates that the life-threatening effects of OPP should not be neglected. Remediation of OPP residues into simpler and safer metabolic products and preventing the passage of OPP from soil to edible parts of plants and subsequently to the higher life forms are the need of the hour.

Many abiotic stressors including higher concentrations of OPP induce many adverse reactions in plants that affect its productivity. Plant's innate stress tolerance mechanisms may be overrun by such stressors particularly beyond threshold concentrations (Sundari and Kotiyal 2016). Under such conditions plants require the support of efficient options that not only possess the capability of OPP breakdown in soil but also are harmless to the non-target organisms. Further, if they can also aid in supporting plant growth, it would be a definite advantage. Based on the scientific literature available, such a value-added two-way benefit system which not only degrades OPP and remediates the landforms but also aids in enhancing plant growth can be none other than the PGPM.

11.5 Why PGPM Can Be a Solution?

PGPM are group of microorganisms that are able to confer growth, development and yield benefits to host plants without causing any adverse impact on their metabolic efficiencies. Species of *Rhizobium, Pseudomonas, Bacillus, Azotobacter, Azospirillum, Clostridium, Frankia, Burkholderia, Flavobacterium, Microbacter, Acinetobacter and Arthrobacter,* arbuscular mycorrhizal fungi and ectomycorrhizal fungi are some of the most prominent members of PGPMs (bacteria and fungi) (Sundari and Mishra 2013). PGPMs influence plants directly by increasing the nutrient availability for plants by nitrogen fixation and phosphate and potassium solubilization or by producing plant growth-promoting substances such as plant growth regulators, siderophores, etc. On the other hand PGPMs also produce biomolecules that help plants to develop tolerance during biotic and abiotic stresses which may be considered as an indirect mechanism of support (Gouda et al. 2018). Some of the PGPM-generated biomolecules (e.g. siderophores) may have multiple beneficial roles (both direct and indirect) as they not only sequester iron for plants but they can also be used for biocontrol of fungal phytopathogens (Sasirekha and Srividya 2016). Besides providing such direct and/or indirect benefits to plants, PGPMs also improve the physicochemical conditions of the soil. Soil is not just a physical support system for plants but also serves as the reservoir from where plants draw/derive all nutrients to support their physiological and metabolic requirements. Therefore, healthy soil is a must for better crop yield. PGPMs produce gluelike sticky substances in the form of polysaccharides which bind soil particles together, influence soil structure by providing means that help in the formation of soil aggregates and make soil structurally stable, which further improves water holding capacity and water infiltration supporting the crop growth (Delgado and Gomez 2016). Certain species of PGPMs belonging to the genera *Pseudomonas, Flavobacterium, Brevibacillus, Bacillus, Micrococcus, Azospirillum, Agrobacterium,* etc. are reported to secrete enzymes that are capable of degrading OPP by utilizing as carbon or as nitrogen/energy sources (Yongliang et al. 2013; Vejan et al. 2016). Other biomolecules produced by PGPM that may have an important role in bioremediation are emulsifiers and biosurfactants. These molecules not only help in elimination of plant pathogens but also modify the solubility of different complex molecules in the soil solution and thus increase the bioavailability and associated processing/metabolic involvement of such nutrients. Biosurfactants can be widely applied for improving the soil quality (Sachdev and Cameotra 2013). Figure 11.9 explains the principal contributions of PGPM grouped into three main categories, namely,

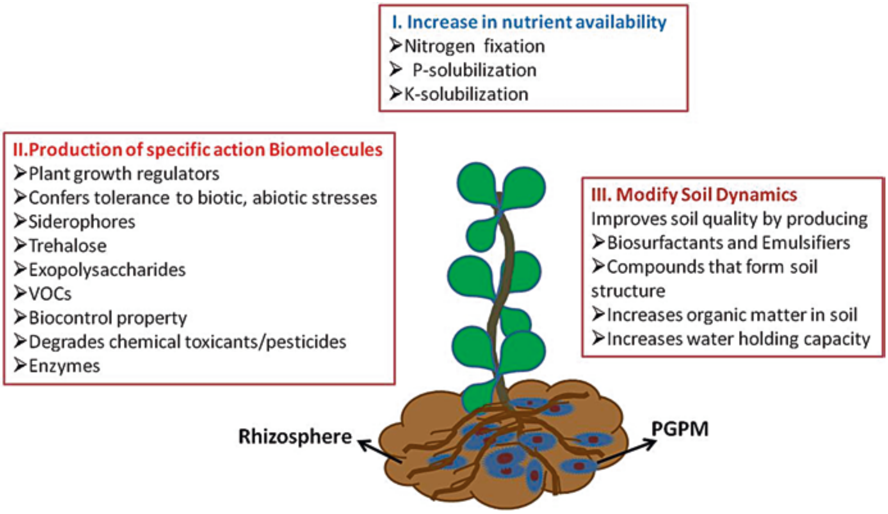

Fig. 11.9 Multiple roles of PGPM in plant growth promotion

increase in nutrient availability, production of specific action biomolecules to improve plant growth and health and modifying soil dynamics.

11.5.1 Increase in Nutrient Availability

Rhizosphere-competent PGPMs facilitate the nutrient acquisition in plants. They enrich the nutrient content and bioavailability of several micro- and macronutrients through mechanisms such as biological nitrogen fixation (BNF) and solubilization of phosphorus, potassium and other complex substrates.

Nitrogen Fixation: Nitrogen is the key component of nucleic acids, proteins and chlorophyll. Around 75% of total nitrogen fixed is contributed by BNF. BNF is a well-acclaimed, environmentally friendly alternative to chemical fertilizers (Gopalakrishnan et al. 2015; Gouda et al. 2018). PGPMs identified to have a role in nitrogen fixation are classified into two categories: free living and symbiotic. *Azotobacter*, *Beijerinckia*, *Nostoc* and *Clostridium* are free-living nitrogen fixers, while *Rhizobium* and *Frankia* are symbiotic in nature and associated with leguminous plants and *Azospirillum* is associated with cereals. Field experiments have shown that *Azospirillum* inoculation led to statistically significant increases in crop yields varying from 5% to 30% (Souza et al. 2014).

Phosphate Solubilization: Among the nutrients phosphorus is only next in importance to nitrogen having a significant role in limiting crop productivity and growth (if defficient). Phosphorus makes up about 0.2% of plants' dry weight (Bagyaraj et al. 2000). Although phosphorus is abundant in many soils, it is one of the least available nutrients in the soil. Due to their highly reactive nature, phosphatic anions may be immobilized through precipitation with cations. Additionally, a large population of phosphatic fertilizers added to the soil are converted to insoluble forms and become unavailable to the plants (Naik et al. 2008). They solubilize the fixed P, which ultimately becomes available to the plants (Krishnaraj and Goldstein 2001). Potential phosphate-solubilizing bacterial representatives are *Pseudomonas* sp., *Agrobacterium* sp., *Bacillus* sp., *Bacillus megaterium* var *phosphaticum, Bacillus circulans, Azotobacter* sp., *Burkholderia* sp., *Enterobacteriaceae* and *Pseudomonas striata*. Amongst fungi, the inevitable role of mycorrhizal fungi in offering phosphorus nutrition to innumerable plant hosts is indisputable (Smith et al. 2011; Alori et al. 2017).

Potassium solubilization: Potassium is the third most vital macronutrient for plants; it regulates several metabolic processes such as photosynthesis, opening and closing of stomata, osmoregulation and protein synthesis. Enzymes responsible for synthesis of starch are also activated by potassium. More than 90% of potassium in soil is found in insoluble forms and thus is available in very low concentrations to plants (Parmar and Sindhu 2013). Some of the key potassium solubilizers are *Pseudomonas, Burkholderia, Acidithiobacillus ferrooxidans, Bacillus mucilaginosus, B. edaphicus, B. circulans, Paenibacillus* sp. and *Aspergillus* (Shanware et al. 2014). Zhang and Kong (2014) isolated potassium-solubilizing bacteria from tobacco rhizospheric soil; these potassium solubilizers were identified as *Klebsiella* and *Pantoea*, thus acting as plant growth promoting rhizobacteria (PGPR) enhanced the plant growth.

11.5.2 Production of Specific Action Biomolecules to Improve Plant Growth and Health

PGPM produce several biomolecules that enhance the plant growth and development. Biomolecules like plant growth regulators, siderophores, trehalose, exopolysaccharides, volatile organic compounds (VOCs) and antibiotics help plants to overcome the adverse effects of biotic and abiotic stresses.

Plant Growth Regulators: There are two sources of phytohormones available for the plants, endogenous production by plant tissues and exogenous production by PGPM. PGPM capable of producing exogenous phytohormones are called phytostimulators (Vejan et al. 2016). Under growth limiting environmental conditions, plant requires to adjust/reorganize levels of their endogenous phytohormones so as to lessen the negative effects of the environmental stressors. Under such circumstances, PGPM become a potential source of plant growth regulators. Application of PGPM allows plants to survive under abiotic and biotic stress conditions. They produce growth stimulating phytohormones such as indole-3-acetic acid (IAA), gibberellic acid (GA3), zeatin, ethylene, abscisic acid, etc. (Verma et al. 2016). IAA is one of the most active auxins produced by a majority of PGPMs. IAA affects plant cell division, extension and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; and affects photosynthesis, pigment formation, biosynthesis of various metabolites and resistance to stressful conditions (Ahemad and Kibret 2014). GA3 helps in shoot elongation. Vejan et al. (2016) did an extensive review on contribution of PGPMs as phytostimulators where the authors have quoted multiple examples including increase in various growth characteristics of tomato plants inoculated with the gibberellin-producing *Sphingomonas* sp. LK11 strain while *Bacillus* sp. increased gibberellin synthesis in pepper. Cytokinins stimulate cell division in plants, vascular cambium sensitivity and vascular differentiation and induce the proliferation of root hairs. However, they inhibit lateral root formation and primary root elongation. Liu et al. (2013) showed that the oriental *Thuja* seedlings inoculated with cytokinin-producing *B. subtilis* strains were more resistant to stress due to drought. Ethylene is another plant hormone known to regulate many processes such as the ripening of fruits and abscission of leaves. At higher concentrations, ethylene induces defoliation and other cellular processes that lead to the inhibition of root and stem growth together with premature senescence, all of which lead to poorer crop performance. Plants synthesize 1-aminocyclopropane-1-carboxylate (ACC), which is the precursor for ethylene, in response to exposure to various types of environmental stress, such as cold, drought, flooding, infections with pathogens and the presence of heavy metals. High levels of ethylene, produced under stress conditions, can halt certain processes such as root elongation or nitrogen fixation in legumes and cause premature senescence. PGPM produce ACC deaminase that acts upon the substrate ACC (a precursor for ethylene), thus influencing the release of ethylene in the rhizosphere (Vejan et al. 2016). A limited availability of ethylene would further regulate the associated phenomena such as premature senescence, defoliation and other processes related to host growth.

Siderophores: Iron is an essential micronutrient required for various metabolic processes of plants and associated microbes. However, bioavailability of iron in soil is very limited, as ferric ions (Fe^{+3}) are only sparingly soluble in soil solution and tend to get accumulated as iron oxides and hydroxides (Rout and Sahoo 2015). In the rhizosphere, there is intense competition for iron between microorganisms and plants. PGPM produce small molecular siderophores under Fe-limiting conditions that have a very high affinity towards iron. Siderophores are amongst the strongest soluble Fe^{3+} binding agents known. Presently, there are over 500 known siderophores, out of which chemical structures of 270 were determined (Sajeed Ali and Vidhale 2013). The primary function of siderophores is to chelate the ferric ion and make it available for the plants and microbes. Siderophores have potential application in agriculture as they can contribute to improving available iron to plants and other soil biota, thus increasing soil fertility while enhancing growth. *Pseudomonas fluorescens,* a known PGPM, was shown to produce extracellular water-soluble, yellow green siderophores of pyoverdine type in succinate medium. Siderophores were also reported to help in increasing root length, shoot length and number of leaves in leguminous plants like *Lens culinaris* and *Phaseolus lunatus* when grown under iron limiting conditions with siderophore supplements (Parmar and Chakraborty 2016). Some studies have thus recommended siderophore supplementations to be used in combination with other biofertilizers to increase crop productivity.

Pyoverdine siderophores produced by *Pseudomonas* were found to control the wilt diseases of potato caused by *Fusarium oxysporum.* They were also observed to suppress the growth of phytopathogens in peanuts and maize (Ahmed and Holmström 2014). Siderophores produced by *B. subtilis* had a significant role in the biocontrol of *F. oxysporum* that causes *Fusarium* wilt of pepper (Yu et al. 2011). These studies strongly support the role of siderophores as probable biocontrol agents. Their biocontrol activity could be because of preferential Fe allocation to hosts and creating an iron starvation in pathogens. Besides iron sequestration, siderophores are extremely effective in solubilizing and increasing the mobility of a wide range of metals such as cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn) and the actinides (Johnstone and Nolan 2016). Due to their metal binding properties, siderophores have become an environmentally friendly and cost-effective tool in the remediation of metal pollutants. Siderophore-producing isolate of *P. aeruginosa* was found to increase the concentrations of chromium (Cr) and Pb in maize by mobilizing them. *P. aeruginosa* ZGKD3 isolated from soil contaminated by gangue pile of coal area exhibited high tolerance to Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Mn^{2+} (Shi 2016).

Trehalose: Trehalose is a non-reducing disaccharide, i.e. an α-1, 1-glucoside, consisting of two molecules of α-glucose and is widely distributed in nature. It is found in bacteria, yeast, fungi, plants, insects and invertebrates. Trehalose is one of the most effective osmoprotectant against many stresses such as drought, salinity and high temperature (Müller et al. 1999). Out of the three known trehalose biosynthetic pathways in bacteria, the best characterized pathway includes condensation of UDP-glucose and glucose-6-phosphate by the enzyme trehalose-6-phosphate synthase (TPS) to form trehalose-6-phosphate, which is subsequently dephosphorylated to trehalose by trehalose-6-phosphate phosphatase (TPP) (Ponnu et al. 2011). By producing trehalose, PGPM assists plants to tolerate abiotic stress. *A. brasilense* genetically engineered with a trehalose biosynthetic gene from yeast could increase not only the productivity but also stress tolerance in maize (Rodríguez-Salazar et al. 2009).

Exopolysaccharides (EPSs): EPS are high-molecular-weight polymers that are composed of sugar residues and are secreted by microorganisms into the surrounding environment. They are produced in response to biotic and abiotic stress factors. The main function of EPS is to aid in protection against environmental pressures. EPS have unique water holding and cementing properties. They help in the formation and stabilization of soil aggregates along with regulation of nutrients and water flow across plant roots through biofilm formation. Co-inoculation of *Phaseolus vulgaris* L. with *Rhizopus tropici* and *Paenibacillus polymyxa* has shown increased plant growth, N content and nodulation under conditions of drought stress. EPS also facilitates interaction between the host plant and bacteria. The EPS-producing *Pseudomonas* strain GAP-P45 acts as a PGPR and can alleviate the effect of drought stress in sunflower plants (Sandhya and Ali 2015). It was proposed that EPS-producing PGPMs form a blanket salt-tolerant cover surrounding the root. As the microbial EPS matrix can trap cations like Na^+, it would mitigate salinity stress by reducing the content of Na^+ available for plant uptake.

Volatile Organic Compounds or VOCs: VOCs produced by PGPR are low-molecular-weight, gaseous, metabolic compounds emitted from bacterial cells under normal conditions and are active even at low concentrations (Santoro 2015). Some examples of VOCs are 2,3-butanediol acetoin, 2-pentylfuran, e13-tetradecadien-1-ol, 2-butanone, 2-methyl-n-1-tridecene, e3-pentanol and 2-butanon (Tahir et al. 2017). VOCs produced by PGPM aid in enhancing plant growth, control plant pathogens and also induce systemic resistance. Compared to other biomolecules produced by PGPM, VOCs have a unique advantage, i.e. VOCs don't need any physical contact with pathogen or plant parts. Besides inducing tolerance to salinity and drought stress, VOCs also improve sulphur and iron nutrition in plants. Salt-stressed *Arabidopsis* plants treated with GB03 VOCs showed greater biomass production and less Na^+ accumulation compared to salt-stressed plants without VOC treatment (Zhang et al. 2010). Several fungal and bacterial isolates were known to produce a common VOC, namely, DMDS (dimethyl disulphide). For instance DMDS from *Bacillus* sp. B55 was observed to cause growth reduction particularly under sulphur deprivation (Meldau et al. 2013).

PGPM improve the plant growth and health by acting as biocontrol agents and by giving tolerance to various environmental stresses. PGPM can suppress the growth of a wide spectrum of bacteria and fungi. It can be through one of the many ways like antibiosis, outcompeting the pathogen in host rhizosphere, providing systemic resistance to plants through a multitude of biomolecules that they produce and hyperparasitism. Biocontrol ability of several microorganisms has been studied till date. Some of the commonly mentioned PGPM with biocontrol activity are species of *Pseudomonas, Enterobacter, Bacillus, Variovorax, Klebsiella* and *Paenibacillus* and isolates of *Trichoderma harzianum, Trichoderma viride, Trichoderma asperellum, Chaetomium globosum, Chaetomium cupreum, Coniothyrium minitans, Burkholderia cepacia, Burkholderia tropicalis* and *Xanthomonas maltophilia* (Sundari and Mishra 2013). Kumar and Verma (2018) reviewed the biocontrol potential of several PGPR such as *Pseudomonas putida, Pseudomonas fluorescens, Azotobacter chroococcum* and *Bacillus subtilis* as biocontrol agents for *Cucumber mosaic cucumovirus* (CMV), *Bacillus amyloliquefaciens* HK34 for the biocontrol of root diseases (*Phytophthora cactorum*) and *Bacillus* sp. for control of bacterial leaf blight caused by *Xanthomonas oryzae*. Sivasakthi et al. (2014) provided proof of potential biocontrol ability in *Pseudomonas* and *Bacillus* sp. to control various crop diseases.

Examples of PGPM that help plants tolerate various abiotic stresses include *Azospirillum lipoferum*, *A. brasilense*, *Bacillus* spp., *Pseudomonas putida H-2–3*, *Rhizobium leguminosarum*, *R. phaseoli, Mesorhizobium ciceri,* etc. The main mechanisms employed by these microbes in mediating drought stress tolerance are increased accumulation of bioactive molecules such as soluble sugars (trehalose), free amino acids and proline. PGPM also act by lowering the level of abscisic acid and salicylic acid that have an interconnecting role in controlling ABA-dependent or ABA-independent pathways of stress control in host plants. PGPM also modulate level of ROS and enzymatic (superoxide dismutase) and non-enzymatic (flavonoids) antioxidants that play a significant role in stress-related oxygen metabolism and associated cell death. Thus associations with PGPM community can maintain proper growth and survival of plants under drought/abiotic stress conditions (Ahemad and Kibret 2014; Sundari 2014; Kumar et al. 2018). Bano and Fatima (2009) studied the ability of *Rhizobium* and *Pseudomonas* species to facilitate plant growth in saline soils and overcome salinity stress by increasing root and shoot dry weight, increasing ACC deaminase activity and lowering ethylene content.

Degradative Enzymes: Microbes are known to produce a wide range of enzymes, in context to degradation of diverse range of organic substrates. Pesticides are one such example of complex organic substances that can be targeted by microbial enzymes bringing about their hydrolysis or metabolic breakdown through a series of enzyme-catalysed reactions ultimately releasing simpler and safer end products. Biotransformation of toxic pesticides via microbes is principally carried out by the action of different enzymes produced either in response to stress (pesticide) stimulus or by innate metabolic enzymes. The role of PGPMs such as *Pseudomonas*, *Azospirillum*, *Flavobacterium*, *Bacillus*, *Burkholderia*, *Arthrobacter*, *Micrococcus* and *Brevibacillus* in degradation of chemical pollutants/crop pesticides (malathion, parathion, monocrotophos, dimethoate) is discussed further in the next section. These microorganisms have evolved pesticide degradation pathways. They have opd genes that code OPP hydrolyzing enzymes that either degrade OPPs or convert them into safer compounds that ultimately help the plant to overcome pesticide stress (Sundari and Mishra 2013; Yongliang et al. 2013; Kumar et al. 2016). Several studies presented evidence depicting faster rate of pesticide degradation through application of enzymes as compared to chemical-assisted degradation (Aislabie and Lloyd-Jones 1995; Sakata 2005; Yongliang et al. 2013). More importantly, remediation of pesticides through microbial enzyme route is biologically safe and also an economical means to reduce the adverse impact of pesticides on the environment. Amongst several enzymes produced by microbes, enzymes belonging to the group of esterases, phosphatases, hydrolases and oxygenases were found to play a major role in pesticide degradation by targeting specific bonds such as P-O, P-C, P-S, P-N, etc., present in the organic pollutants including pesticides (Table 11.2).

The specific nature of these enzymes makes them a perfect candidate to deal with the pesticide pollution leading to their degradation to less toxic metabolites. Table 11.2 refers to several in vitro and in vivo studies pertaining to application of PGPM for breakdown of OPP as reported in the literature (Horne et al. 2002a; b; Singh and Walker 2006; Sundari and Mishra 2013; Barman et al. 2017). Continuous addition of toxic chemicals such as pesticides in the environment over a prolonged period of time has made the microbial species inhabiting in such areas to gain resistance towards these toxic chemicals. Resistance of bacterial species towards such environmental pollutants must have resulted in some specific changes at genetic level, i.e. gene alteration/gene modification/gene silencing or any such phenomena. These altered genes coding for some broad specificity proteins are what we know as degradative enzymes aiding in the breaking down of pesticides (Bansal 2011; Shahgholi and Ahangar 2014; Uqab et al. 2016). Some examples of widely reported

PGPM involved in pesticide tolerance/degradation and genes responsible in coding of particular degradative enzymes are listed in Table 11.2.

OPP Hydrolase: OPP hydrolases are identified both in bacteria and fungi and were reported to be involved in the degradation of majority of OPP applied globally (Singh and Walker 2006; Karigar and Rao 2011, Sundari and Mishra 2013, Yongliang et al. 2013). OPP hydrolases are amongst the most researched enzymes in relation to biotransformation of OPPs and were studied from different PGPMs stating their effectiveness in the breakdown of a wide variety of pesticides. This was possible because OPP hydrolases have low substrate specificity, targeting a wide variety of bonds and hydrolysing them, dissipating the polymers and releasing the monomer units that are more amenable as substrates to other degradative enzymes. The unique nature of OP hydrolase with multiple substrate preferences is the reason why these enzymes have gained more attention as compared to other degradative enzymes. One such example is the OP hydrolase produced by *Pseudomonas diminuta* exhibiting affinity towards a broad range of substrates. In many instances, they form the first step of degradation of complex pesticide compounds. Table 11.2 presents the source of origin and structure details of these OP hydrolase enzymes released by multiple representative groups (Kanekar et al. 2004; Gupta 2006; Singh and Walker 2006; Karigar and Rao 2011; Kumar et al. 2016).

Initial work on pesticide degradation was concentrated on identifying the presence of OP hydrolase enzyme and genes coding the enzymes particularly from bacterial species. Hydrolysis of parathion and dimethoate by OPP hydrolases was reported in a study by Munnecke et al. (1976). In subsequent studies OPP hydrolase proteins were isolated, purified and studied for substrate binding efficiency (Singh and Walker 2006; Yongliang et al. 2013). Later the emphasis was found to have shifted to fungal species as these were showing better hydrolase activity. One such study was done by Ortiz-Hernández (2013) where fungal hydrolase transformed multiple pesticides, viz. parathion, methyl parathion, paraoxon and coumaphos to less toxic forms. Phosphotriesterases reported in bacteria also belong to the hydrolase group of enzymes involved in OPP degradation (Karigar and Rao 2011). Structural studies such as nuclear magnetic resonance (NMR) and X-ray crystallography done on OPP hydrolase showed it as a dimeric metalloenzyme along with 2 metal equivalents of zinc per monomer. Hydrolases such as carboxylesterases and phosphotriesterases were reported in the degradation of major OPPs like dimethoate, malathion and parathion. Phosphotriesterases were found to exhibit improved activity by many folds upon purification and also revealed sensitivity to specific reaction temperature and pH, respectively. However, OPP hydrolases with a broad range of pH stability are also known, though extensive work was not available on this. Rabie (1995) reported hydrolases from *Penicillium* sp., facilitating monocrotophos degradation around a wide pH range (6–9 pH).

Oxygenases: Oxygenases are the second most widely reported degradation enzymes for remediation of OPP (Singh and Walker 2006; Karigar and Rao 2011). Oxygenases belong to the oxidoreductase group of enzymes. This class of enzymes reduce the substrate (OPP) by transfer of molecular oxygen. On the basis of number of oxygen atoms required to bring about reduction of the substrate, oxygenases are further divided into two groups, i.e. monooxygenase and dioxygenase. These enzymes function by utilizing FAD/NADH/NADPH as a substrate. There are several reports on degradation of OPP by mono- and dioxygenase group of enzymes. This group of enzymes not only degrades OP pesticides but also breaks down a wide variety of insecticides, herbicides and fungicides.

Monooxygenases incorporate a single oxygen molecule in the substrate and brings about its reduction. They are further divided into two classes based on the type of cofactors required, i.e. flavin-dependent monooxygenases and P450 monooxygenases. In flavin-dependent oxygenases, flavin is the prosthetic group which requires NADP and NADPH as coenzyme, while P450 oxygenase is known as heme-containing enzyme. P450 oxygenase is known to play a major role in pesticide biotransformation as compared to flavin-dependent oxygenases (Aislabie and Lloyd-Jones 1995; Brar et al. 2006; Singh and Walker 2006; Karigar and Rao 2011; Yongliang et al. 2013).

The dioxygenase group of enzymes act by introducing two molecular oxygen atoms to the substrate and catalyse oxygenation of a wide range of substances. Dioxygenases are known to be multicomponent systems belonging to non-heme iron oxygenases with prime application in environmental biotransformation of toxic compounds. This group of enzymes consists of one or two electron transport proteins. They are majorly reported in soil bacteria and transform complex toxic compounds to simpler ones (Uqab et al. 2016).

Above biomolecules produced by PGPM either directly enhance the plant growth and development or support the host plant indirectly by decreasing the inhibitory effects of various pathogens on plant growth.

11.5.3 Modifying Soil Dynamics

In the rhizosphere, plant roots, soil and microbial community influence each other. Microorganisms present in soil are interconnected with the physical (soil porosity, aeration, bulk density, water holding capacity) and chemical (pH, electrical conductivity, macro- and micronutrient content) properties of soil. Besides producing biomolecules that increase the plant growth and development and/or improve plant health, PGPM also produce some biomolecules that improve the soil quality. Microbes decompose complex organic matter in soil, thus improving available nutrient content of soils. In a research study, Pereira et al. (2013) evaluated microbiological properties such as microbial biomass carbon (MBC), basal respiration (CO_2-C), metabolic quotient (qCO_2), dehydrogenase enzyme activity (DHA) as well as the physical (moisture, bulk density, macroporosity and total porosity) and chemical [pH, organic carbon (org-C), P, Ca, K, Mg, Al, H^+, Al, C, N and S contents] properties of soil in two seasons. They showed a positive correlation between physicochemical and microbiological properties of soil. Microbes even assist in the production of polysaccharides, with a role in binding soil particles into aggregates and better structured soils. Increased aggregation tends to improve soil health with more efficient drainage, water holding capacity and water infiltration. The role of PGPM in colonizing the plant roots and improving soil quality by releasing exogenous biomolecules to mix in the soil has been revealed even in the recent publication of de Souza et al. (2015). Additionally mineralization activity of microbes also increases the nutrient content of soil and makes it more suitable for crop production (Delgado and Gomez 2016). Soil organic matter or pH affects the activity of many microorganisms in soils which in turn perform actively in carbon and nutrient cycling. PGPM are called rhizoremediators by Sharma (2017) as they are used in degradation of herbicides, pesticides and other hazardous organic compounds and/or pollutants in the soil which could be helpful in plant growth by increasing soil fertility.

11.6 Current Status of Pesticide's Market and Degradation Research

Market Scenario: The presence of patented pesticide products is predominantly found in developed countries like the USA, Canada, Australia, etc. Majority (approx 70%) of Indian pesticide market contains generic products. India has a decent presence in patented pesticide products (nearly 30%) and has a huge body of research going on in this area. An encouraging factor for Indian generic pesticide sector is that agrochemicals worth 4.1 billion US dollars are expected to go off-patent by the year 2020. Indian generic research can explore possibilities of developing generics from such off-patented agrochemicals (FICCI report, 2016). As per an estimate future projection for Indian pesticide market is approx 2.5 L million for the year 2018. The same report proposes an estimated demand of approx 21 thousand million rupees for biopesticide market in India for the year 2018 (KEN research 2013). Amongst Indian pesticides available in the market, microbial biopesticides are more prominent as compared to botanical biopesticides. Within microbial biopesticides, biofungicides take the major share as compared to bioinsecticides. Presently, leading organisms available in the market as biopesticides are *T. viride, T. harzianum, B. subtilis, Bacillus pumilus, P. fluorescence, Fusarium proliferatum, Ampelomyces quisqualis* and *Paecilomyces fumosoroseus.* There is hardly any information about biopesticides that can serve as herbicides, weedicides, rhodenticides, etc. (KEN research 2013). Thus concentrated efforts have to be made to capture more market share developing more PGPM-based biocontrol agents.

Impact of Pesticides on Plant and Soils: Unregulated use of pesticides leading to increased soil pollution has caused serious concern as researchers are releasing study after study establishing the adverse effect of pesticides on soil, plants and even processed plant/food products. Kumar et al. (2016) reviewed the contamination of different plant products with residues of organophosphates reporting below to moderate MRL in cauliflower and above MRL values in leafy, root, modified stem and some fruity vegetables, namely, banana, guava, grapes and oranges.

Pesticide chlorpyrifos was found above MRL in butter and ghee samples collected from Haryana, India. Honey samples collected from Himachal Pradesh (India) contained residues of malathion, dimethoate and quinalphos pesticides above MRL. Malathion and chlorpyrifos were the most common OPP found in the packaged water of Delhi and NCR region, India (Agarwal et al. 2010). The severity of OPP toxicity on host plant was studied by Zhigacheva et al. (2007), where they checked that plant growth regulator melaphen not only gets accumulated but also alters the structural characteristics of plant and animal membranes. This alteration was attributed to changes in micro-viscosity of annular lipids, which are directly linked to protein-lipid interactions. OPP also deteriorate the soil quality by compromising the soil microbial activity. Raju and Venkateswarlu (2013) studied the impact of two pesticides, namely, buprofezin and acephate, on soil microorganisms at concentrations ranging from 2.5 to 10 μg g − 1 and found that enzymes cellulases, amylases and invertases are adversely affected. Study by Filimon (2015) highlights the impact of pesticides cypermethrin and thiamethoxam on metabolic processes of soil microflora. These studies present an evidence of residual pesticide in plants and plant products beyond detectable limits. This not only warrants for judicious use of pesticides but also implements environmentally friendly methods for decontaminating the soils from pesticide residues.

Pesticide Degradation Research: Multiple studies published in the past decade on microbial degradation of OPP worldwide are a reflection of intense research interest in the field. Microbial (bacterial and fungal) degradation of some of the most widely used OPPs, viz. chlorpyrifos (Chen et al. 2012; Zhang and Kong 2014; Deng et al. 2015; Akbar and Sultan 2016), monocrotophos (Jain et al. 2013), diazinon (Zhang and Kong 2014; Deng et al. 2015), dimethoate (Li et al. 2010; Ishag et al. 2016), parathion (Deng et al. 2015), methyl parathion (Wang et al. 2012) and phorate (Jariyal et al. 2018), has been studied. Primary research in the field included isolation of potent pesticide degrading organisms from contaminated sites. Organophosphate degrading bacteria and fungi were isolated from India, Saudi Arabia, Poland, Japan, China and Mexican soils, industrial sludge of China, agricultural wastewater and natural lake water (Li et al. 2010; Chen et al. 2012; Zhang and Kong 2014; Akbar and Sultan 2016; Kumar et al. 2018). Most of the studies targeted degradation of a single pesticide (concentrations ranging between 50 and 1000 ppm) (Pakala et al. 2007; Li et al. 2010; Srinivasulu et al. 2017). Several in vitro studies identified and quantified residual pesticides and degradation products, using Fourier-transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS) techniques (Neti and Zakkula 2013; Buvaneswari et al. 2018). Several degradation intermediates such as catechol, methylamine, trimethyl phosphorothioate, methyl phosphoric esters, diethyl thiophosphate, ethyl thiophosphate and end products valeric acid, acetic acid, phosphoric acid and other organic acids were reported (Malghani et al. 2009; Bhadbhade et al. 2002). Molecular techniques applied to study the microbial degradation of OPPs include analysis of pesticide degradation genes, designing gene-specific primers to identify candidate microbes, cloning and expression of

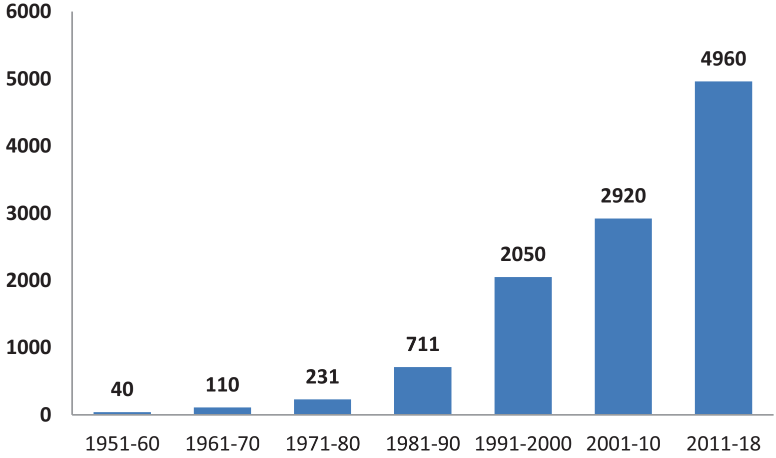

Fig. 11.10 Publications on PGPM in the past six decades (Source: Google trends as per publication in Google Scholar)

potential pesticide degrading genes (viz. Opd A, opd, mpd) in rhizo-competent bacteria (Ortíz-Castro et al. 2009). Sun et al. (2018) used recombinant DNA technology to transfer key pesticide degrading gene *TnopdA* of *Sphingobium* sp. YW16 and evaluated its role in the degradation of monocrotophos. Iyer et al. (2013) extensively reviewed and compared key organophosphate degradation genes and bioremediation applications. In-depth study on organophosphate degrading enzymes (monocrotophos hydrolase), its kinetics as well as protein profiling was done (Jain et al. 2013).

Pesticide Degradation by PGPM: Research on PGPM dates back to more than six decades, and the past two decades however have seen a phenomenal increase in this field as it is evident from the articles published in national/international scientific journals (Fig. 11.10). Researchers have addressed growth-promoting characteristics of PGPM and provided experimental proof through in vitro greenhouse studies about the multiple growth supporting capabilities of these organisms. Starting with few bacterial examples to start with, now we have many bacterial and fungal genera contributing to plant growth, and hence the term PGPM, including members from both bacteria and fungi, is more applicable in the current context. Work on PGPM's ability towards pesticide degradation have started much later. In fact a bird's eye view of published literature indicates awakening of the scientific community in exploring pesticide degradation ability of PGPM as recently as 2006. The current decade has witnessed a huge allocation of PGPM research on pesticide degradation (Fig. 11.11). In most of the studies, the potential of PGPM to degrade pesticides is studied either at a lab scale at very low concentrations of pesticides or at greenhouse level with a very narrow choice of pesticides vs. PGPM. The main objective of the authors is not only to appraise the readers on various capabilities of PGPM but also

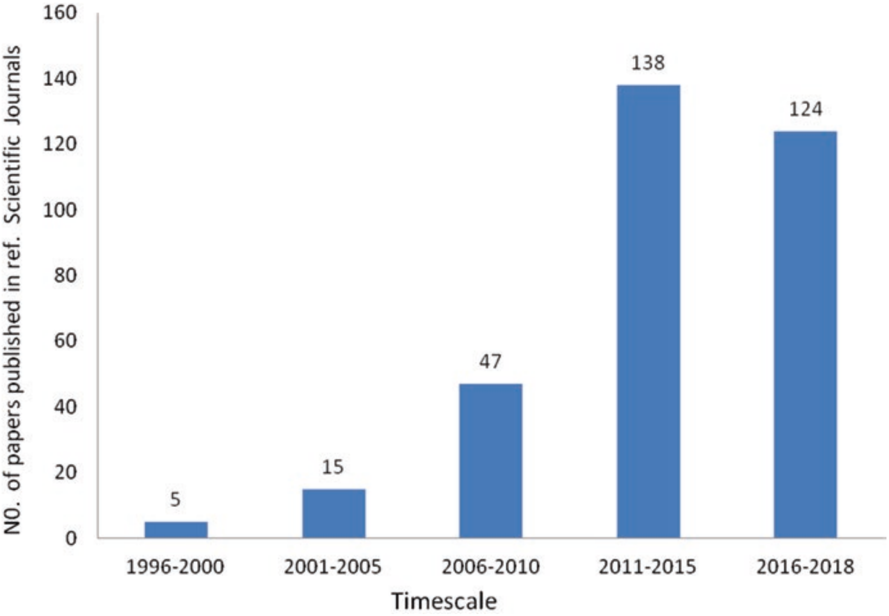

Fig. 11.11 Publications on pesticide degradation in the past two decades (Source: Google trends as per publication in Google Scholar)

to put before them the untapped potential of PGPM to develop them into dedicated bioinoculants to address the problem of residual pesticides. It is evident that PGPMs such as *Pseudomonas*, *Arthrobacter*, *Flavobacterium*, *Bacillus*, *Micrococcus*, *Azospirillum*, *Burkholderia* and *Brevibacillus* have the ability to target complex pesticide compounds. Recently, Sahoo and Chaudhuri (2019) reported the ability of an *Ochrobactrum* strain in degrading lindane (organochlorine pesticide) with simultaneous capabilities of producing plant growth-promoting factors. Future prospects of PGPM research would be in developing PGPM formulations that can degrade the OPP, help in soil building and curtail their entry into the edible parts of plants. It is now required to effectively employ them or any such potential PGPM to carry out large-scale, scientifically planned greenhouse studies, establish their potential in soil remediation and progress towards technology development.

11.7 Future Scope and Prospects

Decoding the mechanism and pathway of OPP degradation is an important area to be explored. Though metabolic pathway of some of the pesticides, e.g. methyl parathion, is available (Meta Cyc https://metacyc.org/), degradation pathways of all OPPs are not yet deciphered. Advances in computation biology facilitated prediction of degradation pathways for certain organic pollutants. This provides a wider ground to envisage the ultimate fate of xenobiotic compounds as modulated by rhizosphere microbes and their functional enzymes (Arora and Bae 2014; Jeffries et al. 2018). Most of the research concerning pesticide degradation focused on two principle components, i.e. pesticide and microbes for degradation. However these degradation studies have to be further extended in order to know the actual impact of microbial degradation on soil and plant health. Pesticide degradation by microbes has to be studied in conjunction with different crop plants and rhizosphere conditions. Establishing the beneficial role of these degrading microbes would aid in developing multipotent bioinoculant for agricultural application. Microbial formulations that can help crops to cope up with pesticide stress while also increasing growth and yield in crop plants would be of great advantage. In this context PGPM can play a pivotal role. Research pertaining to consortia development and establishing their tolerance towards a variety of pesticides across different concentration ranges can also be a challenging area with potential applicability.

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Chapter 12
Influence of Zeolite Support on Integrated Biodegradation and Photodegradation of Molasses Wastewater for Organic Load Reduction and Colour Removal

Seth Apollo, Benton Otieno, Maurice Onyango, and Aoyi Ochieng

Abstract Molasses wastewater (MWW) is characterised by a biorecalcitrant dark brown colour caused by melanoidin pigments and a high organic load. Anaerobic digestion (AD), mostly applied as a first step treatment method for MWW, can effectively remove the high organic load, but with minimal colour mitigation. Photodegradation is among the preferred advanced treatment methods to complement AD for the colour abatement. This study looked into the effect of employing zeolite as a biomass and catalyst support in AD and photodegradation, respectively. Zeolite was applied in a fixed bed bioreactor for biodegradation, while a composite of TiO_2/zeolite catalyst was prepared and used in a fluidised bed photoreactor. The zeolite and TiO_2 were characterised using scanning electron microscopy (SEM) and Brunauer-Emmett-Teller (BET) where it was found that the zeolite surface area and pore volume were almost twice that of TiO_2. Introduction of zeolite improved chemical oxygen demand (COD) reduction by 20% during AD with a fivefold increase in biogas production. However, a 40% increase in colour was recorded during AD due to a possible re-polymerisation of the recalcitrant colour-causing melanoidins. The photodegradation process was effective in colour remediation with 99% colour removed on employing the TiO_2/zeolite composite catalyst, as compared to 44% removed when TiO_2 was solely used. Degradation of melanoidins into simpler products was responsible for the colour disappearance. Zeolite due to its large specific surface area and good adsorption properties was found suitable for application as a biomass and catalyst support in AD and photodegradation, respectively.

Keywords Anaerobic digestion · Colour removal · Melanoidins · Photodegradation · Titanium dioxide · Zeolite

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12.1 Introduction

Alcohol distilleries are some of the major polluting industries as about 88% of the raw material used ends up as waste mainly in the form of wastewater. Two very important raw materials for distilleries are cane and beet sugar molasses with cane molasses having the highest application (Shivajirao 2012). Most cane molasses-based distilleries produce about 15 litres of spentwash effluent (wastewater) per litre of ethanol produced. In 2008, approximately 79 billion litres of ethanol was produced globally resulting in the generation of 2.4×10^{12} litres of distillery effluent (Rajagopal et al. 2014). During distillation, the viscous molasses is diluted with clean water and subjected to fermentation, thereby producing alcohol (bioethanol) with the aid of added yeast. Thereafter, the resulting fermentation broth is filtered and finally distilled to concentrate the bioethanol. After the distillation process, the final effluent discharged as waste is the molasses wastewater (MWW) (Chavan et al. 2006; Arimi et al. 2014; Mabuza et al. 2017). MWW is characterised by a high organic load and a characteristic dark brown colour caused by biorecalcitrant melanoidin pigments. When discharged into aquatic systems without proper treatment, MWW can cause eutrophication and hinder the photosynthetic activity of aquatic plants (Navgire et al. 2012; Otieno et al. 2016; Wekneh et al. 2019).

Anaerobic digestion (AD), a conventional treatment method, has been long established for the remediation of MWW. The high biodegradable organic content of MWW, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of 40,000–50,000 mg/L and 80,000–100,000 mg/L, respectively, makes AD beneficial for the reduction of the high organic load with the additional benefit of producing useful energy in the form of methane gas (Satyawali and Balakrishnan 2008; Chavan et al. 2006). Whereas AD can remove the high organic load, it cannot effectively remove the characteristic dark brown colour of MWW that is majorly caused by the biorecalcitrant melanoidin pigments (Chandra et al. 2008). Melanoidins are heterogeneous, nitrogen-containing brown pigments formed during Maillard reaction (a non-enzymatic reaction involving carbohydrates and amino compounds). Melanoidins are polymeric and aromatic in nature with a high molecular weight (> 5.0 kDa) making them anti-oxidative and thus biorecalcitrant (Liang et al. 2009; Liu et al. 2013; Otieno et al. 2016). Given the inability of the AD to abate colour, the introduction of post-treatment has been considered (Apollo et al. 2016). Moreover, to improve the digester performance during the AD, system operating parameters which facilitate the growth of microorganism consortia should always be monitored to prevent reactor failure. One major cause of bioreactor failure is a microbial washout, which can be overcome through the introduction of an inert solid material in the reactor such as zeolite to retain the microbes. Zeolite due to its high porosity can provide a large specific surface area for microbe attachment, thus enabling high retention of biomass concentration in the reactor (Milan et al. 2010).

Photocatalytic degradation can mineralise complex organic compounds into simple products such as CO_2 and H_2O, hence suitable for introduction as a post-treatment to the AD process for the elimination of biorecalcitrant melanoidins (Otieno et al.

2017). Several semiconductor oxides including titanium dioxide (TiO_2), zinc oxide (ZnO), indium oxide (In_2O_3), tin dioxide (SnO_2) and iron (III) oxide (Fe_2O_3) have been used as photocatalysts for the degradation of organic pollutants (Kaan et al. 2012; Segneanu et al. 2013; Mabuza et al. 2017). Of all the semiconductors, TiO_2 photocatalyst has been widely applied because of its several desirable properties including high activity, robustness against photocorrosion, chemical stability, low cost, negligible volume change upon cycling and low toxicity (Song et al. 2014; Gao et al. 2015). In the photodegradation process, the need for a large catalyst surface area has led to the application of nanosised TiO_2. However, there are drawbacks in using the photocatalysts in powdered or nanopowdered form, such as aggregation of the powder especially at high concentration and the costly post-treatment separation of the nanopowder from wastewater (Asiltürk and Şener 2012). Moreover, solid semiconductor catalysts such as TiO_2 have a low surface area to contact the organic pollutants (Li et al. 2016). To overcome these challenges, the nanosised TiO_2 can be supported onto a support material such as zeolite for enhanced adsorption of substrate and ease of separation after treatment (Durgakumari et al. 2002). Pre-concentration of organic compounds on the catalyst surface by an adsorbent employed as a support material can lead to a reduction in energy requirements since TiO_2 quantum yields increase linearly with concentration (Haque et al. 2005).

The aim of this study was to apply natural South African zeolite as biomass support in a bioreactor and as an adsorbent support to TiO_2 in a photoreactor. Anaerobic digestion was applied for the reduction of the high organic load of molasses wastewater followed by photodegradation post-treatment for colour removal. Of special interest was the monitoring of the performance-enhancing effect of zeolite support in the two processes and the determination of possible reaction pathway during photodegradation leading to the colour disappearance.

12.1.1 Methodology

12.1.1.1 Materials and Equipment

Major materials that were used include zeolite (obtained from Pratley mining, South Africa), titanium dioxide (technical grade, 99% purity) obtained from Sigma Aldrich, inoculum (provided by a local plant treating municipal wastewater), anaerobic sludge (from Heineken distillery in Midvaal, South Africa) and cow dung for bioreactor start-up and commercial molasses (obtained from local stores) for preparing MWW. Anaerobic digestion was carried out in a laboratory-scale fixed bed digester with a working volume of 0.5 L shown in Fig. 12.1, while photodegradation was carried out in a photocatalytic reactor (Fig. 12.1 b) with a capacity of 0.45 L and a 15 W UVC lamp. The radial length of the annular space of the photoreactor was minimised to ensure effective and uniform distribution of the irradiated UV light within the photoreactor. A peristaltic pump was used to feed the photoreactor and to recirculate substrate during treatment.

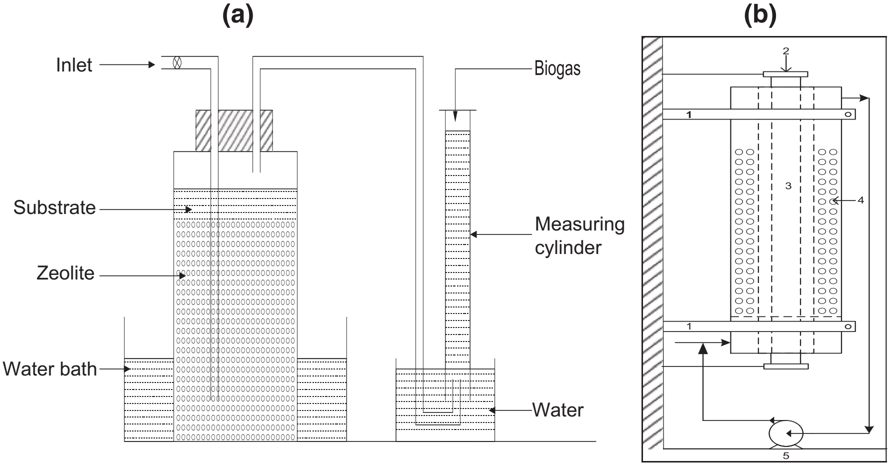

Fig. 12.1 (**a**) Laboratory-scale fixed bed anaerobic reactor and (**b**) fluidised bed photoreactor (1) clamp, (2) lamp holder, (3) 15 W UVC lamp, (4) substrate and catalyst and (5) peristaltic pump

12.1.1.2 Inoculum Preparation

Seed inoculum was obtained from the bottom sludge of a secondary digester in a local municipal wastewater treatment works (Sebokeng Water Works, South Africa). The seed inoculum was mixed with sludge obtained from an anaerobic digester treating distillery wastewater (Heineken distillery in Midvaal, South Africa) and thereafter diluted with fresh cow dung. Preparation of inoculum using sludge from different sources ensured that it (inoculum) contained a wide range of bacterial classes which could digest a wide variety of organic substances. The inoculum was prepared by gradually adding the prepared MWW to the seed inoculum to acclimatise the microorganisms.

12.1.1.3 Anaerobic Digestion Experiments

The commercial molasses was dissolved in distilled water obtaining MWW with desired initial concentrations. The wastewater and inoculum were mixed at a ratio of 7:3. It was then dosed with various nutrient supplements ($KHPO_4$ (20 ppm), $MgSO_4.7H_2O$ (5 ppm), $NiSO_4$ (10 ppm), NH_4NO_3 (20 ppm) $FeSO_4$ (5 ppm) and $Ca(HCO_3)_2$ (20 ppm), and the pH adjusted to values between 6.8 and 7.2. The mixture was then transferred to the bioreactor which was packed with zeolite for biomass support. The reactor was purged with nitrogen before incubation in a water bath at 37 °C. A control experiment was carried out in similar conditions but without adding zeolite to the reactor. The Mariotte water displacement method was used for biogas collection and determination of the amount of gas produced within a specified period. The AD process was evaluated by a reduction in colour and COD.

12.1.1.4 Photocatalyst Preparation and Photodegradation Experiments

The natural zeolite was first crushed in a mortar with a pestle and then sieved to obtain a particle size of around 2 mm before being washed with deionised water to remove dissolved contaminants and fine particles. Finally, the zeolite was dried at 105 °C for 24 h and stored in a desiccator for use. The TiO_2 supported onto zeolite catalyst (TiO_2/zeolite, 75% TiO_2 composition by weight) was prepared following a solid-state dispersion (SSD) method as reported by Durgakumari et al. (2002). The obtained molasses was dissolved in distilled water with stirring to form samples with initial COD concentrations ranging from 1 g/L to 20 g/L. An appropriate mass of the TiO_2/zeolite catalyst was weighed, then mixed with the substrate at 1 g/L composite catalyst loading (corresponding to 0.75 g TiO_2/L) and stirred in the dark for 1 hour before being transferred into the photoreactor for irradiation. During the photodegradation process, samples were withdrawn at given time intervals and filtered through 0.45 μm pore size filters to remove any suspended catalyst particles before being subjected to analyses.

12.1.1.5 Catalyst Characterisation and Chemical Analyses

The morphologies of the catalysts were studied using a scanning electron microscopy (SEM) with an integrated energy dispersive X-ray spectroscopy (EDX) (FEI Nova Nano 230). Nitrogen adsorption was employed in determining the specific surface areas of the prepared catalysts based on the Brunauer-Emmett-Teller (BET) equation (ASAP 2020 V3.00 H). The extent of organic load reduction was determined from COD, while total organic carbon (TOC, Teledyne Tekmar) reduction was used in determine the extent of mineralisation. For the COD analysis, closed reflux method using dichromate solution as the oxidant and Nanocolor colourimeter were used for analysis. Colour analyses were done using UV-vis spectrophotometer (DR 2800, HACH) at the maximum absorption wavelength of 475 nm (Navgire et al. 2012). Fourier transform infrared (FTIR) spectroscopy (PerkinElmer FT-IR/NIR) and ion chromatography (IC, 882 Compact) were used to monitor change in functional groups and determination of major products formed, respectively, during the photodegradation process.

12.1.2 Results and Discussion

12.1.2.1 Catalyst Characterisation

The SEM analysis of TiO_2, zeolite and TiO_2/zeolite in Fig. 12.2 shows that the zeolite particles had much bigger sizes as compared to TiO_2 particles. Upon mixing the two while grinding with pestle and mortar in the presence of ethanol, the zeolite particles were further reduced in size providing a large surface area for the uniform

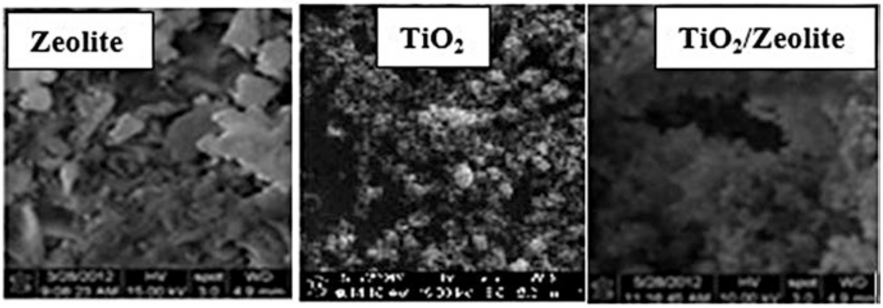

Fig. 12.2 SEM images of zeolite, TiO_2 and TiO_2/zeolite

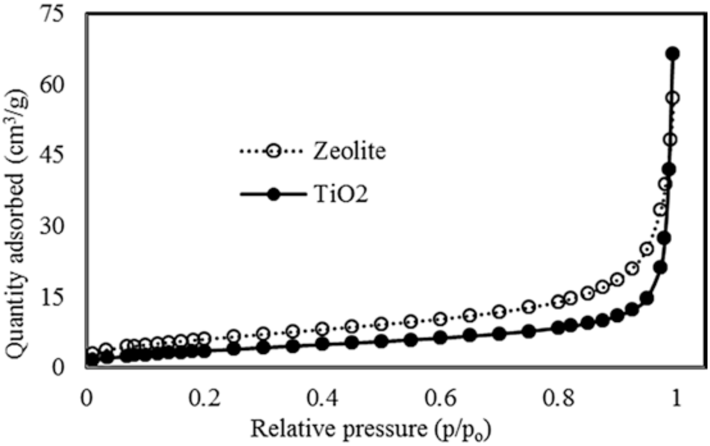

Fig. 12.3 Nitrogen adsorption isotherm of zeolite and TiO_2

Table 12.1 Surface properties of zeolite and TiO_2

Property	Zeolite	TiO_2
BET surface area (m^2/g)	22.4417	13.8481
Pore volume (cm^3/g)	0.051474	0.032730

attachment of TiO_2 particles as shown with the TiO_2/zeolite SEM image. The surface area of zeolite and TiO_2 was investigated using the nitrogen adsorption method. For the zeolite and TiO_2 adsorption isotherms shown in Fig. 12.3, type II hysteresis loop was exhibited indicating non-uniform pore sizes (Yang et al. 2010). The specific surface areas of 21.3309 m^2/g and 13.8481 m^2/g were obtained for zeolite and TiO_2, respectively, from BET. The zeolite despite having a much bigger particle size as compared to TiO_2 still had a larger specific surface area, hence can be applied as a support for the catalyst. This can be explained by the information on pore volume given in Table 12.1, which shows that zeolite had a larger pore volume as compared to TiO_2. The large pore volume and the surface area provide for effective adsorption

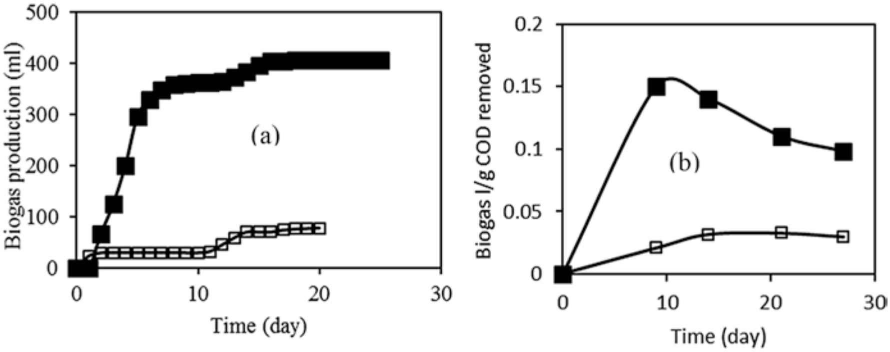

Fig. 12.4 (**a**) Cumulative biogas production and (**b**) biogas yield during AD of MWW (20 g/L initial COD) with zeolite (■) and without zeolite (□) in the bioreactor

of the substrate and enough surface for biomass support during photodegradation and AD, respectively.

12.1.2.2 Biodegradation Studies

It was observed that a higher biogas production was achieved in the zeolite-packed bed reactor during the anaerobic digestion of MWW with 20 g/L initial COD concentration (Fig. 12.4a). This confirms that zeolite performs well in microorganism immobilisation, thereby improving the AD process. It was also observed that zeolite improved biogas production by fivefold as well as decreasing the lag phase for the pollutant. Similar observations were reported by Umaña et al. (2008) when treating dairy manure using lab-scale fixed bed anaerobic digester packed with zeolite. The reactors produced 40% more biogas than those without zeolite. Nikolaeva et al. (2009) also reported that kinetic constant to produce methane was 29.4% higher in a zeolite-packed reactor than in one without zeolite.

Figure 12.4b shows the biogas yield (biogas l/g COD removed) was also high in packed bed reactor compared to that of the reactor without zeolite The yield increased with time for the packed bed up to the tenth day after which it gradually started to reduce. This observation can be attributed to the depletion of the substrate after the tenth day. However, it remained constant for the digester without zeolite. At the beginning of the digestion, there was sufficient substrate which microorganisms could convert to biogas but after 10 days of degradation, the substrate concentration reduced remarkably leading to low biogas yield in the packed bed digester.

The results for the final COD removal in Fig. 12.5a show that the packed bed (with zeolite) reactor performed better than the unpacked bed (no zeolite) reactor. The packed bed achieved 84% COD removal, while the unpacked bed had 63% removal. This was attributed to the high surface area provided by zeolite for microbial growth. Colour removal during the anaerobic digestion process in the packed bed reactor was also monitored, and the results are shown in Fig. 12.5b. A general

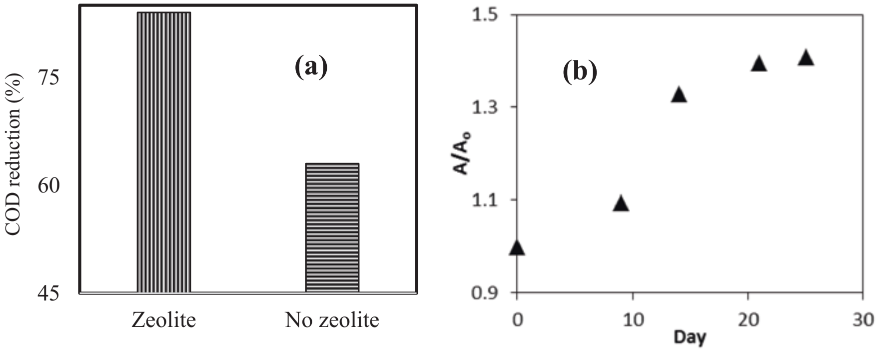

Fig. 12.5 (**a**) COD removal in the fixed bed anaerobic reactor after 27 days of anaerobic digestion of MWW (20 g/L initial COD) and (**b**) colour increase in the packed bed (with zeolite) in the same period

increment in colour intensity was observed instead of an expected reduction. Melanoidin compounds which are responsible for colour in the MWW have been reported to resist biodegradation due to their high resistance to microbial attack (Georgiou et al. 2016). The observed colour increase can be explained by re-polymerisation of the melanoidin compounds during biological treatment processes (Mohana et al. 2009). Moreover, the anaerobic digestion carried out at 37 °C would favour the re-polymerisation of the melanoidin compounds. At 37 °C conditions are similar to those leading to melanoidin formation during the heating of sugars, where the non-enzymatic chemical reaction between the carbonyl group of reducing sugars and the amino group of peptides, amino acids or proteins occurs (Ojijo et al. 2010). Since high molecular weight (HMW) melanoidins have been found to contribute more to the colour than the low molecular weight (LMW) component, the re-polymerisation which leads to the formation of HMW melanoidins will, therefore, lead to an increase in colour intensity as was observed (Arimi et al. 2014).

12.1.2.3 Photodegradation Studies

Photodegradation of MWW with 2 g/L initial COD (close to the COD of the AD effluent from the packed bed bioreactor) was carried out while monitoring colour and COD reductions (Fig. 12.6). Figure 12.6a shows that there was very little colour removal by UV photolysis alone. This is because UV photolysis produced minimal hydroxyl radicals as compared to the photocatalysed processes. On employing TiO_2 to catalyse the photodegradation process, a higher colour removal of 45% was achieved. In the presence of TiO_2, absorption of light by the catalyst causes charge separation resulting from the electron (e^-) promotion from the valence band (VB) to the conduction band (CB) of the absorbing semiconductor catalyst and the generation of a hole (h^+) in the VB (Akpan and Hameed 2009). The promoted electrons in the conduction band can reduce oxygen to $O_2^{\bullet -}$, while the positive holes oxidise

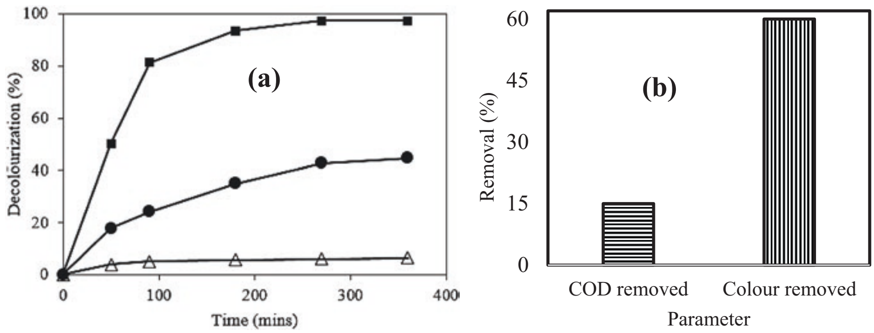

Fig. 12.6 (**a**) Photodegradation of MWW (2 g/L initial COD, pH 4) using UV/TiO_2/zeolite (■), UV/TiO_2 (●) and UV photolysis (Δ) and (**b**) effect of photodegradation on COD and colour removal after 60 minutes of irradiation

H_2O forming hydroxyl radicals ($HO^{\bullet}$). The hydroxyl radicals and the holes then react with the colour-causing melanoidins leading to their partial or complete mineralisation. The holes and electrons generated upon irradiation of TiO_2 are thus more effective in the generating the desired highly oxidative hydroxyl radicals than in the case of direct UV photolysis.

The TiO_2/zeolite combination doubled the colour removal by the TiO_2, possibly due to the created synergistic effect caused by the zeolite support material. Apart from providing an increased surface area for melanoidin degradation by minimising the aggregation of the TiO_2 catalysts, the zeolite support adsorbed melanoidin compounds followed by their transportation to the catalyst for immediate oxidation. The TiO_2/zeolite material composed of a catalyst and an adsorbent was, therefore, able to utilise adsorption and catalytic technologies, and hence highest colour removal was observed. The first 100 min for all the systems recorded very high reaction rate as depicted by rapid and high colour removals. This could be due to the significant number of hydroxyl radicals generated during this period. Also, the substrate was readily available; therefore, time could have been the limiting factor in the reaction. The reaction profile then levels off after about 180 min suggesting that either the hydroxyl radicals or the substrate had been depleted. In fact, from the high colour removal of 97% attained, it can be deduced that it was a substrate that got depleted. However, in the direct photolysis systems, lower reaction rates were observed. This may be due to the low generation of hydroxyl radicals, and this implies that generation of the radicals was the limiting factor. The high colour removal achieved with TiO_2/zeolite for the of MWW (2 g/L initial COD concentration) is an indication that photocatalytic degradation is effective for colour removal even at higher initial concentration.

The effect of photodegradation on COD removal as compared to colour removal is shown in Fig. 12.6b. It was observed that after 60 min of irradiation, the colour removed (60%) was higher than the COD removed (15%). The observation is attributable to the fact that the HMW colour-causing compounds (melanoidin) present in the wastewater easily react with the generated hydroxyl radical, leading to the formation of stable LMW colourless intermediate or final organic compounds such as carboxylic acids, which are not easily mineralised into inorganic products. Whereas the colour rapidly disappears, the stable compounds formed still impart COD, and hence low COD removal is observed. In this study, photodegradation was found not to be an appropriate technology for COD mitigation for molasses wastewater because of the low COD removal. However, it is an efficient technology for colour removal since high efficiencies can be obtained. The high colour removal associated with low COD reduction is an indication that photodegradation did not result in complete mineralisation but rather in degradation of complex compounds to simpler ones.

12.1.2.4 Photodecolourisation Process

To further monitor the photodegradation process, the surface of the catalyst was analysed for the presence of organic compounds before and after photodegradation of MWW samples of different initial COD concentrations (0.1 g/L and 2 g/L), as shown in Fig. 12.7. Given the low COD removal of 15% after 60 min of degradation of the MWW with 2 g/L initial COD, the catalyst surface was expected to still contain adsorbed organic compounds. This was confirmed by the FTIR spectra of the catalyst surface before, after 30 min and 60 min of photodegradation (Fig. 12.7a). The broad and strong peak observed at 3300 cm^{-1} after 30 min of degradation, did not disappear or reduce even after 60 min of irradiation, signifying the high stability of the organic compounds responsible for COD to UV photodegradation. For the MWW wastewater with initial COD concentration of 0.1 g/L, however (Fig. 12.7b), the peaks obtained after 30 min were less intense than those observed for the 2 g/L COD MWW due to the partial mineralisation of the organic substrate. After irradiation for 60 min, the peak intensities were further reduced, and upon an additional 60 min of continued irradiation, the characteristic broad OH peak appearing in the range 2500–3300 cm^{-1} was completely diminished signifying complete mineralisa-

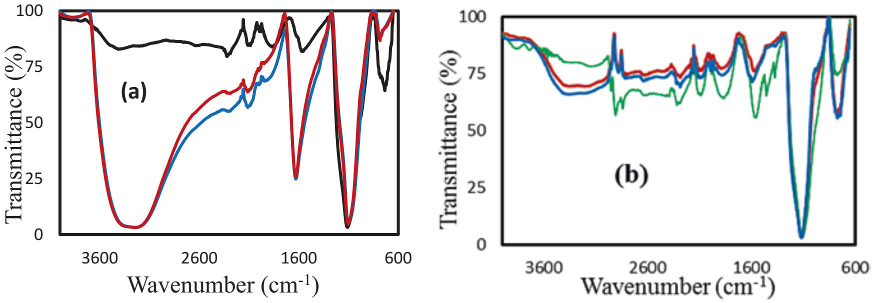

Fig. 12.7 FTR spectra TiO_2/zeolite surface during photodegradation of MWW with different initial COD concentrations, (**a**) 2 g/L and (**b**) 0.1 g/L at varying time intervals, before use (—), after 30 (—), 60 (—) and 120 (—) minutes of irradiation

tion. The complete mineralisation of the MWW with 0.1 g/L initial COD was also confirmed by TOC analysis which showed 93% reduction in TOC after the 120 min of irradiation.

The complete mineralisation at a low initial concentration of 0.1 g/L COD of MWW was attributable to the low number of organic compounds present at this concentration. Complete mineralisation of the organic compounds present in MWW is therefore only achievable at low initial COD concentration. Due to the high energy-intensive UVC lamp, prolonged periods of light irradiation with the aim of achieving complete organic mineralisation especially at high initial organic loading (COD) will result in a costly process. This further signifies the advantage of employing photocatalysis in tandem with anaerobic biodegradation, where the high organic load (COD) is first removed by the low-cost AD before a rapid removal of the bio-recalcitrant colour through UVC photodegradation, at a short period of irradiation, thus a reduced cost as well.

12.1.2.5 Decolourisation Mechanism

During the photodegradation process, the formation of major inorganic products was determined from IC. After 60 min of degradation of MWW with initial 2 g/L COD concentration, 0.6 mg/L of nitrate (NO_3) and 1.3 mg/L of ammonium (NH_4^+) were formed (Fig. 12.8). Colour-causing non-biodegradable melanoidin compounds constitute an approximated 2% of the MWW with 1–2 g/L total nitrogen (TN) content (Tsioptsias et al. 2015). Melanoidins like other coloured organic compounds such as dyes contain chromophores and auxochromes. The chromophores have

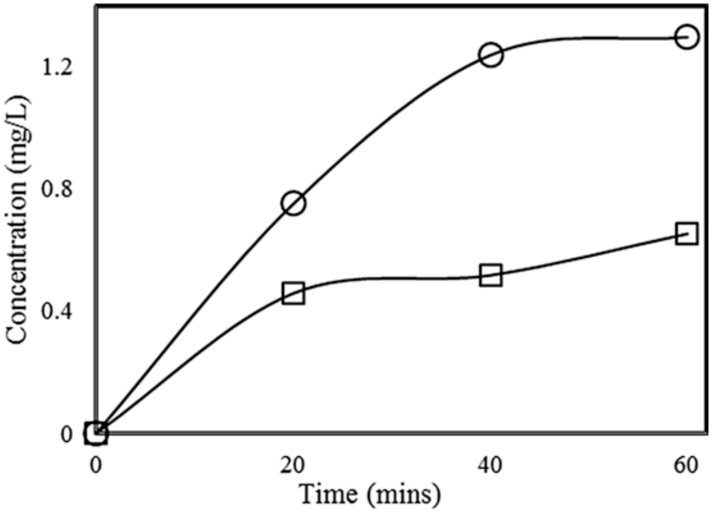

Fig. 12.8 NO_3^- (□) and NH_4^+ (○) formation during photodegradation of MWW (initial COD concentration 2 g/L, pH 4) using TiO_2/zeolite

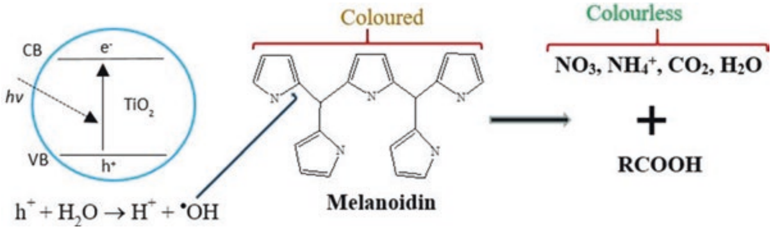

Fig. 12.9 Schematic representation of MWW decolourisation during photodegradation

delocalised electron systems consisting of conjugated double bonds, while the auxochromes contain electron-withdrawing or donating systems. Alteration of the overall energy of the electron systems can intensify the colour of the chromophoric compounds, which include -C=O-, -C=C- and -C=N- (Panthi et al. 2015). During the photodegradation of MWW, the hydroxyl radicals attack the chromophoric C-N bonds leading to the colour disappearance and formation of NO_3^- and NH_4^+ (Chandra et al. 2008). Formation of more $NH4^+$ as compared to NO_3 indicated a reductive pathway for melanoidin degradation. A schematic representation of the processes leading to colour disappearance is shown in Fig. 12.9. In the process, the generated highly reactive hydroxyl radicals attack the melanoidin compounds leading to the formation of smaller organic compounds, stable intermediates and inorganic compounds such as NO_3, NH_2, H_2O and CO_2.

12.2 Conclusion

Anaerobic digestion can effectively reduce COD load of molasses wastewater (MWW), but with an increase in colour intensity due to re-polymerisation of melanoidin pigments during the AD process. The colour can be effectively removed by subjecting the anaerobically digested effluent to photodegradation where the biorecalcitrant colour-causing organic compounds are degraded into simple inorganic compounds. Natural zeolite due to its large specific surface area and high porosity can be effectively used as a biomass support in a bioreactor and as a catalyst support in a photocatalytic process for MWW treatment. The zeolite provides large surface area for biomass support and prevents microorganism washout during anaerobic digestion leading to improved biodegradation. During photodegradation using TiO_2/zeolite, the zeolite support adsorbs colour-causing melanoidin organic substrates and concentrates them near TiO_2 for immediate degradation leading to rapid colour removal. Thus, photodegradation using TiO_2/zeolite can be applied to complement AD for effective COD and colour removal when treating MWW.

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Chapter 13

An Overview on the Microbial Degradation of Linear Alkylbenzene Sulfonate (LAS) Surfactants

Anushree Suresh and Jayanthi Abraham

Abstract Linear alkylbenzene sulfonate (LAS) is one of the synthetic surfactants which has created havoc in wastewater treatment. Aerobic or conventional wastewater treatment was unable to remove the toxic compounds formed by synthetic surfactants. This book chapter focuses on the microbial degradation and the most effective way for the elimination of this toxic chemical accumulating in the environment. By using genetic engineering technique, LAS compounds can be degraded by microorganisms and hydrolyzed into simpler compounds. *Bacillus* sp. and *Cladosporium resinae* are extensively studied organisms for degrading LAS compounds. Enzyme-mediated degradation has also been explored. Monooxygenases, lipase, 4-sulfophenylacetate esterase, and 4-sulfoacetophenone Baeyer-Villiger monooxygenase are some of the enzymes which help in degradation of xenobiotic compounds to simpler compounds. Co-metabolic transformation results in slight modification in the structure of xenobiotic molecules, leading to incomplete or even complete degradation.

Keywords Linear alkylbenzene sulfonates (LAS) · Microbial remediation · Wastewater treatment · Co-metabolism

13.1 Introduction

Linear alkylbenzene sulfonate (LAS) is one of the world's mostly consumed synthetic surfactants, containing different sulfonated alkylbenzene salts, and is extensively used in disinfectant detergents used in household as well as in other industrial applications. Linear alkylbenzene sulfonic acid compound (LABSA) is an organic detergent manufactured commercially by sulfonating the linear alkylbenzene (LAB) compound (IHS market 2015). LAS is a biodegradable alternative surfactant for

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Table 13.1 Some of the microorganisms involved in the degradation of alkylbenzene sulfonate compounds

Organism	Source	References
Aeromonas caviae, Pseudomonas alcaliphila, Vibrio sp.	Río de la Plata (Argentina) and adjacent waters	Peressutti et al. (2008)
Pseudomonas nitroreducens (L9), *Pseudomonas aeruginosa*	Detergent contaminated domestic laundry premises of Meenachil River (Kottayam, Kerala)	
Pantoea agglomerans, Serratia odorifera	Wastewater treatment plan	Khleifat (2006)
Bacterioplankton	Coastal seawater from the Mediterranean sea	Vives et al. (2000)
Aeromonas caviae, Enterobacter cloacae, Klebsiella ozaenae,, Pasteurella multocida,Acinetobacter calcoaceticus, Alcaligenes denitrificans, Moraxella osloensis, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas stutzeri, Corynebacterium bovis, Micrococcus luteus, Micrococcus varians, Staphylococcus epidermidis, Staphylococcus sp.	Surface water of Chao Phraya River (Thailand)	Vives et al. (2000)
Phylum Bacteroidetes, Niastella sp., *Terrimonas* sp.	Soil microbiota	Peressutti et al. (2008)
Mycobacterium, Rhodococcus	Soil microbiota	Chilima et al. (2006)
Pseudomonas aeruginosa, Bacillus subtilis, Bacillus agglomerans, Bacillus cereus, Bacillus alvei	Soil surface	Chilima et al. (2006)
Alcaligenes odorans, Bacillus subtilis, Burkholderia cepacia, Citrobacter freundii, Escherichia coli, Micrococcus albus, Micrococcus luteus, Pseudomonas stutzeri, Staphylococcus bovis, Proteus vulgaris, Pseudomonas putida	Okun and Osere streams, North Central Nigeria	Knaebel et al. (1990)

branched alkylbenzene sulfonate (BAS) by replacing them in disinfectant detergents used throughout in the world (Table 13.1).

Approximately 83–87 percent of LAS is used in disinfectant detergents, such as laundry liquids, dishwashing liquids, laundry powders, and other household cleaners. LAS compounds are also used as emulsifiers (e.g., for agricultural use like pesticides, herbicides, and emulsion polymerization) and as lubricating agents in various fields like institutional, industrial, and commercial cleaners. For personal care products, only a small amount of LAS compounds have been used.

Of late, demand for LAS has stabilized or declined significantly in the developed regions, whereas, an increase of 3.0–5.0% in some less-developed regions and countries, such as in the Middle East, India, Africa, and China, as well as in Southeast Asia, was observed. As a result, rapid growth of LAS demand in the Asia Pacific region was seen, and it is expected that by the year 2019, these regions will account for global demand of more than 50%. In 2014, demand in developed regions and countries reached 50% of total world demand. The growth of LAS compounds around the globe is expected to increase by an average of 2.7% per year from 2014–2019. Consumption of LAS will be affected in a positive manner for countries like India, China, and Africa, where powder detergents are preferred over liquid detergents.

LAS faces tight competition with existing commonly used surfactants in detergents used in household. The existing surfactants have properties like better compatibility with enzymes and good hard water tolerance and contain more chemical constituents in comparison to LAS compounds. Historically, LAS is cost-effective and possesses many other favorable properties compared with competing surfactants. During the year 2007–2011, LAS compounds' price ranked more closely to those of the other competitive toxic surfactants. Hence a more stable pattern of consumption was practiced in most of the developing countries. In late 2014 and early 2015, low crude oil prices caused LAS compounds to become more demanding. Production of LABSA/LAS has a huge impact by the supply situation for competing common surfactant products such as alcohol ether sulfates (AES). In developing countries, LAS competes with liquid detergents, soaps, and other common surfactants sold in the market.

13.2 Effects on Humans

There has been substantial data recorded for mammalian toxicity due to usage of LAS detergents. The recorded data shows that LAS exhibits a condition of acute toxicity. Oral LD50 value of LAS for rats ranges from dosage varying 1080 to 1980 mg/kg bw. The oral and dermal acute toxicity data for LAS compounds generally indicates it to be less hazardous. Acute inhalation toxicity data value indicates that LAS compound is discreetly toxic, with mortality rate occurring at respirable particle concentrations of 310 mg/m^3 (MMAD = 2.5 microns). In a series of in vivo studies on rabbits, LAS was not found to cause any irritation in the skin or eyes at low concentrations (0.5–2.5%). The eye irritation can be diminished with rinsing after 30 s of exposure. In a low volume eye test (LVET) using concentration of 35%, LAS solution on rabbits experienced moderate irritation that was completely reversible post 35 days.

13.3 Environmental Fate of LAS

13.3.1 Fate of the Compound

The vital source of LAS buildup in the environment is by improper disposal of laundry and spilling of cleaning solutions into municipal or on-site waste treatment systems.

Before the practice of LAS, branched surfactants were used in household detergents which biodegraded slowly, thereby leaving foaming residue condition in many wastewater treatment plants. Conversion of formulation of existing detergent formulations to LAS in the mid-1960s eliminated the major tricky situation of foam production. It was observed that LAS undergoes rapid and efficient biodegradation in the environment. Biodegradation is the most appropriate biological process for LAS surfactant degradation. Photodegradation, chemical degradation, and hydrolysis were not efficient enough in the process of removal of LAS surfactants from the environment.

LAS compounds readily undergo primary and secondary biodegradation post wide variety of wastewater treatment processes. The well-known biodegradation pathway of LAS degradation by photooxidation involves the carbon atoms of the alkyl side chain being oxidized to form intermediate compounds which are less toxic, such as carboxylate sulfophenyl compounds. This is followed by the aromatic ring cleavage in the compound and thereby mineralization to by-products such as inorganic sulfate, water, and carbon dioxide. The biodegradation pathways for LAS have been reported, and the mechanism of biodegradation of LAS using enzymes has been summarized (Swisher 1987) in this chapter (Table 13.2).

Table 13.2 Some of the enzymes involved in the degradation of LAS compounds

Organism	Enzyme	References
Comamonas testosteroni KF-1.	4-Sulfophenylacetate esterase and 4-sulfoacetophenone Baeyer-Villiger monooxygenase	Knepper and Berna (2003)
Pseudomonas nitroreducens (L9), *Pseudomonas aeruginosa*	Microbial oxidoreductases	Schleheck et al. (2012)
Pantoea agglomerans, Serratia odorifera	Monooxygenases	Schleheck et al. (2012)
Aspergillus sp. and *Cladosporium* sp.	Microbial laccase	Vives et al. (2000)
Aeromonas caviae, Enterobacter cloacae, Klebsiella ozaenae, Phanerochaete chrysosporium, Acinobacter calcoaceticus, Pseudomonas putida, Pseudomonas stutzeri, Corynebacterium bovis, Micrococcus luteus, Micrococcus varians, Staphylococcus epidermidis	Lignin peroxidase and manganese peroxidase	Schleheck et al. (2012)
Phylum Bacteroidetes, Candida rugosa	Lipase	

Very few amounts of LAS and its intermediate compounds found in effluents in municipal wastewater treatment plant are not easily biodegradable in the environment and persist for a longer period of time. A recent study at Mississippi River by a survey named United States Geological Survey (USGS) found that LAS contamination in the environment was at higher concentration and contains toxic compounds in the effluents. LAS was detected at low concentration in treatment plants, with the highest level being found at approximately 0.005 mg/L which is below the level of point discharges (Tabor et al. 1993). LAS biodegradation rates are comparable in concentration whether it is attached to sediment or dissolved in the aqueous phase. This conclusion, regarding the fate of LAS, was based on the studies conducted in laboratory (Larson et al. 1993).

LAS is readily soluble in water. The partition coefficient of water over solids was greatly favored by the type of sediment, soil, or sludge. Sorption process depends on the alkyl chain length and position of phenyl isomer. As mentioned earlier, sorption increases with the length of alkyl chain, which is quite evident from the enrichment of longer alkyl chain of LAS homolog compounds in wastewater sludges compared to other compounds found in municipal wastewater facilities (McAvoy et al. 1993). The external isomers, 2-sulfophenyl isomer, have coefficient of adsorption approximately twice as that for the internal isomer compounds, 5-sulfophenyl isomers (Hand and Williams 1987). The external isomers are not augmented on wastewater sludge, which indicates that biodegradation rather than absorption process is the major removal mechanism for these isomers. Carboxylate sulfophenyl isomers are biodegraded to metabolites which are water soluble and, therefore, do not appear separately in water onto sediments or soil.

13.3.2 Treatability of Compound

A study conducted in the USA with existing 50 US sewage treatment plants showed LAS compound removal percentage of approximately 98.5% in lagoon treatment systems, 99.3% in activated sludge, and 98.0% in oxidation ditches (McAvoy et al. 1993). Physical and chemical treatment of LAS was found to be inefficient. Rotating biological contactor plants help in removing 96% of LAS, while trickling filter systems remove approximately 77% of the LAS. This result was less effective, in case of trickling filter. Another study showed complete removal of LAS compounds by introducing various techniques that are on-site treatment plants, such as septic tank systems.

A significant amount of sewage sludge is disposed onto land as soil conditioner and fertilizer. Biodegradation of LAS with half-life value ranging from 5 to 33 days was usually found in sewage sludge (Berna et al. 1989). LAS and its breakdown intermediates are biodegraded in sludge-amended soil treatment processes. It was also reported that LAS showed no drastic effect on growth of plants like radish, potatoe, bean, and brinjal in soils modified with sewage (Figge and Schoberl 1989).

13.3.3 Exposure

In a recent study of 50 site monitoring systems in the aquatic environment focusing on low dilution (worst case) streams, the receiving water concentrations of LAS were approximately 0.044 mg/L and the length of LAS alkyl chain averaged to 11.9 carbon units (McAvoy et al. 1993). Samples were collected from the downstream of wastewater treatment plant at a point where complete mixing of sediments occurs. The concentration of LAS found in the environment is usually in the range equal to or less than 0.01–0.33 mg/L. These results were compared to more typical values that are observed in the environment when samples are collected along the river systems without focusing on low dilution (current) immediately below outfalls. The recently completed USGS (United States Geological Survey) monitoring study found that the concentration of LAS was rarely at concentrations greater than 0.005 mg/L in the Mississippi River (Tabor et al. 1993).

13.4 Environmental Effects

13.4.1 Terrestrial

LAS-containing detergent compounds biodegrade rapidly under aerobic units employed in wastewater treatment plant (Bema et al. 1989), whereas it is a slow process in anaerobic digesters. Concentrations of LAS compounds found in anaerobically digested sludge plant from several countries have reported the concentration ranging from 0.1% to 3% (Painter and Zabel 1988). Usually post-treated sludges from wastewater treatment plant are disposed off at landfills and agricultural lands or incinerated.

Researchers have studied the fate of LAS compounds in sludge contaminated soils in about 51 fields on 24 farms in the UK. The LAS concentrations in 42 sites showed concentration of less than 1 mg/kg post-biodegradation process. The biodegradation rate of LAS compounds that persisted in the fields had half-lives for 7–22 days (Waters et al. 1989; Holt et al. 1989).

Additional studies were performed on vineyards and vegetable soils (Berna et al. 1989) on a test plot area where LAS compounds had been repeatedly added over 10 years (De Henau 1986; Giger et al. 1989) resulting in fast and efficient biodegradability of LAS compounds.

13.4.2 Aquatic

Approximately 400 toxicity tests were performed and studied with LAS compounds on a wide range of invertebrates, freshwater and saltwater fishes, and algae (BKH Consulting Engineers 1993). Acute toxicity of LAS on fish and invertebrates was found to be within 0.5–20 mg/L range (Kimerle 1989). The LAS toxicity has a greater impact on algae with 90% of the algal species having an EC value between 0.1 and 100 mg/L (Kimerle 1989). The toxicity of LAS compounds depends upon the alkyl chain length. LAS chronic toxicity is mainly due to the phenyl isomer content present.

13.5 Environmental Concerns and Biodegradability of LAS

LAS is one of the major anthropogenic sources of organic compounds found in primary sludge in municipal wastewater treatment plants. These can be adsorbed onto suspended solids ranging from 30% to 70% (Berna et al. 1989) and avoiding the aerobic treatment process. These compounds are found in surface water supplies in a concentration of lower than pico g/L (Tabor et al. 1993) and in drinking water in the concentration of 0.001–0.008 mg/L (in different countries). It is able to augment the solubility of LAS compounds in water, which are otherwise insoluble in other matrices; hence, the resistance to mass transfer gets reduced (Tabor et al. 1993). High mobility of LAS due to water solubility activity and its polarity makes it a hazardous contaminant in ground and surface water supplies (Reemtsma 1996). LAS can be degraded in activated sludge plant system using a consortium of various aerobic microorganisms (Van Ginkel 1996) by up to 99.5% and its intermediates by up to 99.1% (Trehy et al. 1990). On the other hand, it has been reported that the intermediates produced by biological treatments are 10–100 times less toxic than the parent compounds. The solid residence time (SRT) of mixed culture is very important to preserve adequate surfactants' degrading microorganism in the wastewater treatment plant (Van Ginkel 1996). Moreover, it was also seen that in the activated sludge procedure, the effluent LAS compound concentration was a function of influent concentration when hydraulic residence time (HRT) was observed less than 10 h (Kaiser et al. 1997). LAS compound residues and metabolites enter the receiving water through the effluent if the HRT value is not chosen properly. In some of the developing countries sludge effluent is discharged directly or indirectly into rivers due to improper or inadequate wastewater treatment systems. Another source of LAS entering into the environment is by using the sludge resulting from wastewater treatment plants on to agricultural lands. Consequently, the terrestrial and aquatic organisms are vulnerable to surfactants. LAS has shown toxic effect on nitrogen-fixing bacteria such as *Nitrosomonas* and *Nitrosopria* (Brandt et al. 2001). The inhibitory effect of LAS was observed to be more on growth rate and viability of the organisms than that on their metabolic activity. LAS also showed inhibitory effect on anaerobic biological treatment process (Morales et al. 2001; Gavala and Ahring 2002). It has been reported that acidogenic and methanogenic microorganisms are sensitive to LAS-containing compounds although after acclimatization there could be a decrease in the inhibitory effect (Morales et al. 2001). Rittmann et al. (2001) reported that LAS in the concentrations of 1/3/10 mg/L is biodegradable under aerobic activated sludge treatment, following growth kinetics of Monod's equation over the ranges of 0.1–20 mg/L (Rittmann et al. 2001). Moreover, it has been reported that biodegradation of LAS can follow first-order kinetics (Huang et al. 2000). However, mixed culture of microorganisms' growth rate has shown to inhibit at the concentration of 95 mg/L. The minimum concentration of LAS toxicity is not yet known (Patterson et al. 2002). Acute toxicity LC50 against different microorganisms differs from one species to another, for example, saline water species are more sensitive to LAS compounds than freshwater. LAS biodegradation follows two phases: first is primary degradation, wherein oxidation of alkyl chain takes place resulting in the formation of sulfophenyl(di)carboxylates, and, second, ultimate biodegradation, wherein breakage of phenyl ring along with removal of sulfonate groups takes place. Finally, LAS is converted into by-products such as inorganic salts, CO_2, H_2O, and biomass. The bacteria that are capable of breaking the ring are extremophiles (Patterson et al. 2002). It was also investigated that ultimate biodegradation of LAS cannot be achieved at high concentration. Although the inhibitory effect of LAS compound increases with the concentration of LAS and inhibits the process of primary degradation. LAS is intractable at very high concentration (3000 mg/dm^3) (Patterson et al. 2002). This can be due to the effect of chemical toxicity and cell membrane destruction caused by high LAS concentration (Patterson et al. 2002). It has been reported by the World Health Organization (WHO) that ultimate biodegradation of LAS is possible only under suitable conditions. Hydrophobic and hydrophilic parts of LAS can react with polar and non-polar structures such as proteins and peptides. This binding can modify the structure and charge of proteins and peptides; furthermore, it can modify their biological functions (Cserhati et al. 2002). Therefore, LAS can accumulate in living organisms, as it can inhibit in the hepatic liposomes of rat and inhibit the activity of some of the enzymes (Cserhati et al. 2002). LAS can also damage human skin and irritate eyes. It has been established that longer the alkylbenzene chain, the more severe the skin irritancy potential (Cserhati et al. 2002). LAS and its intermediates resulting from biological treatments are not estrogenic (Cserhati et al. 2002).

13.6 Degradation of LAS by Using Microorganisms

Degradation using microorganisms has been the most effective way for the elimination of toxic or harmful chemicals accumulated in the environment. These organisms are easily available in the environmental niche and can be normally cultured or grown in the laboratory for degradation purposes. They can be used for degradation of different types of compounds such as organic and inorganic wastes. Some of the microbes/enzymes found in the environment are not metabolically capable or equipped to hydrolyze the complex compounds to simpler forms. In order to break down these complex compounds, genetic engineering is applied. Scientists modify the organism by inserting the gene which can be made responsible for the degradation of compounds to break down the xenobiotic compounds (Arora et al. 2018; Arora 2018). One such genetically modified and most studied organism is

Pseudomonas species for the bioremediation of xenobiotic compounds. *Pseudomonas* contain TOL plasmids which is capable of degrading toluene, p-xylene, and m-xylene. To track down the respective gene involved in bioremediation, a bioluminescence gene is inserted into the gene of *Pseudomonas*.

According to the previous records, degradation of LAS has been studied using microorganisms such as *Bacillus* sp. and *Cladosporium resinae.* These two organisms oxidize the side chain present in the xenobiotic compound and also in the aromatic ring of dodecylbenzene sulfonate. Mineralization of these organisms takes place and follows different pathways for degradation process. *Bacillus* sp. degrade the sulfonyl groups to sulfite, and on utilizing acetyl-CoA, alkyl chain is cleaved by an unknown sequence. These above mentioned procedures result in the formation of p-hydroxyphenylalkanoates which is the precursor for the catabolism of dodecylbenzene sulfonate by *Bacillus* sp. and thus helping in the aromatic degradation moiety (Willets and Cain 1974). Fungus, *C. resinae*, isolated from the airplanes' fuel tanks was also capable of alkyl chain degradation and the change in polar moiety by using similar metabolic pathway. Here, the fungus helps in cleaving the aromatic C-S bond, thereby releasing sulfates as by-product instead of the intermediate compounds, p-hydroxyphenylalkanoates, phenylalkanoates (Willets 1973).

13.7 Enzymatic Biodegradation of LAS Surfactants

On continuous exposure of the microorganisms to xenobiotic compounds, they undergo mutation followed by acclimatization of the toxic compounds and later degradation. Mutations can modify the genes of microorganisms thereby altering the active sites of enzymes, increasing their affinity towards xenobiotic compounds. Some of the mutations result in the formation of new metabolic pathways for degradation of xenobiotic compounds. Use of more than one microorganisms can be done for degradation as they can result in breakage in different parts through various mechanisms, thereby leading to degradation of the compound through a co-metabolic activity (Rajkumari et al. 2019). These gene modifications of the organisms capable of degrading compounds have been carried out and also produce good level of accuracy in degradation.

Commercially, LAS is made up of 20 mixtures of congeners of secondary 4-sulfophenylalkanes of which 18 are chiral (Knepper and Berna 2003). A combination of congener and enantiomer compounds is completely degraded by the complex heterotrophic aerobic bacterial communities within two steps (Dong et al. 2004; Schleheck et al. 2004; Van Ginkel 1996). First step involves the utilization of alkyl chains present in LAS for growth through the attainment of acetyl coenzyme A by the process of beta oxidation of the alkyl group, thereby releasing complex mixture of 50 small chains containing 4-sulfophenyl carboxylates. It was primarily observed in the organism, *Parvibaculum lavamentivorans*, to yield (R,S)-3-(-4-sulfophenyl)butyrate (3-C4-SPC) as the major metabolite.

Second step involves the degradation of SPC's (R,S)-3-(-4-sulfophenyl)butyrate by heterotrophic bacteria into CO_2, sulfate, cell material, and water (Schleheck et al. 2004).

13.8 Co-metabolism of Environmental Pollutants by Microorganisms

Co-metabolic transformation of LAS can result in a minor modification of the parent molecule, or it may lead to incomplete or complete degradation. The by-products of partial biodegradation or co-metabolic conversion of any xenobiotic compounds are less harmful when compared to parent compound. In natural environmental condition, the products of bioconversion processes are further transformed or degraded with the help of other microorganisms, eventually leading to complete degradation (Rajkumari et al. 2019). Co-metabolic processes and biodegradation of toxic compounds using microbial consortium are thought to be of huge ecological importance. However, persistent xenobiotic compounds and metabolic dead-end products will accumulate in the environment, become part of the soil humus, or enter the food chain leading to biomagnification (Fig. 13.1).

Degradation of the surfactant, alkylbenzene sulfonate (ABS), also appears to occur via a co-metabolic reaction series (Horvath 1972). The microbial degradation

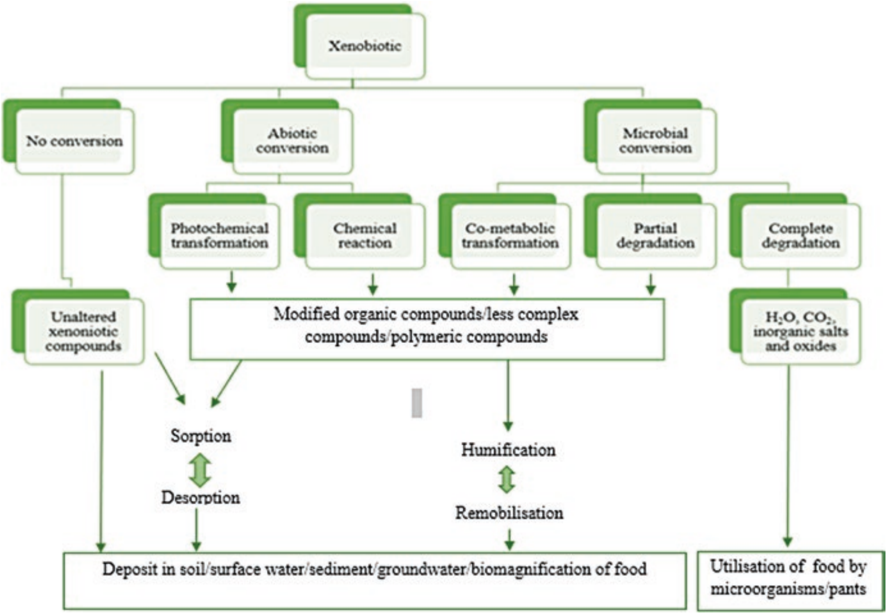

Fig. 13.1 Schematic representation of biodegradation of xenobiotic compounds

of ABS represents a unique combination of co-metabolism of a portion of the molecule and complete metabolism of the remainder of the molecule. Initial oxidation of the surfactant apparently required an expenditure of energy since co-metabolism of ABS by *Pseudomonas* did not occur in the absence of an energy source (glucose). Co-metabolism of ABS by this organism yielded isopropanol from side chain oxidation and catechol from aromatic ring oxidation. Isopropanol, once formed, was capable of supporting growth of the isolate, indicating complete metabolism of this substance. Desulfonation of ABS occurred by the process of co-metabolism and involved a coupled reaction between phenol, an intermediate product of ABS co-metabolism, and a sulfonated alkylbenzene intermediate. Catechol, the end product of aromatic ring co-metabolism, accumulated in stoichiometric amounts and appeared to result from oxidation of phenol via the coupled reaction.

Evidence for the co-metabolic degradation of environmental pollutants by naturally occurring microbial populations is now available and indicates the importance of this phenomenon in the ecosystem. Pure culture studies conducted had established the occurrence of co-metabolism in the degradation of ABS, indicating the possibility of this degradative mechanism under mixed culture conditions. Data obtained from pure culture studies can be extended and used for simulating under natural conditions, thereby establishing the applicability to the situation in the biosphere. Degradation of ABS was shown to occur in an aquarium containing lake water and ABS, to the extent of 90% in 40 days. The addition of an energy source (glucose) to this system resulted in a 100% decrease of the surfactant concentration that too in 20 days. Although the participation of co-metabolism was not clearly understood, two possibilities can be worked out; (Horvath 1972) one, the enhancement of the rate of degradation of ABS by the addition of energy source suggests a co-metabolic process in this system and two, the inability of to isolate from this system a microorganism which could utilize ABS as sole carbon and energy source indicates that co-metabolism is responsible for the decomposition of ABS.

13.9 Conclusion

Even though a connection between bioavailability in anaerobic reactors and biodegradation has been established, very little data is available concerning anaerobic LAS biodegradation. From the above mentioned co-metabolic and enzyme-mediated pathways, degradation of LAS compounds can be converted to simpler eco-compatible metabolites. To assess the feasibility of bioremediation technique of LAS compounds by using microorganisms, further research is required on the diversity, and biochemical and molecular mechanisms so as to carry out the degradation at efficient level and at large scale in the ecosystems. Proper degradation of these surfactants should be done in order to eliminate the risk of ecotoxicological effect of the partially degraded compounds, and this can be only achieved in an eco-friendly and sustainable manner by utilization of microorganisms.

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