Chapter 8 Bioremediation of Polluted Soil by Using Plant Growth–Promoting Rhizobacteria

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Abstract Soil pollution generally causes huge losses in the world's agricultural output, and therefore, soil pollution control is essential in agriculture crop production system. For soil pollution management, we usually reduce the use of chemical fertilizers, manures, and pesticide, reuse the domestic waste product materials such as glass containers, plastic bags, paper, and cloth, and recycle the materials such as some kinds of plastics and glass cane, but their indiscriminate use causes environmental problems and human health hazards. Moreover, the continuous use of those products without safe disposal leads to soil pollution. Thus, bioremediation of soil pollution is an alternate eco-friendly method for soil pollution management, in which plant growth–promoting rhizobacteria are used in alleviating the contaminated soil. Many rhizosphere microorganisms including Azotobacter spp., Pseudomonas aeruginosa, Glomus spp., Acaulospora spp., Scutellospora spp., Streptomyces spp., Klebsiella spp., Lysobacter spp., Rhizobium leguminosarum, Burkholderia spp., Diaphorobacter nitroreducens, Planomicrobium chinense, Promicromonospora spp., Mesorhizobium spp., Psychrobacillus psychrodurans, Pantoea spp., Arthrobacter spp., and Variovorax spp. have been found as plant growth–promoting rhizobacteria. These PGPR have been found to bioremediate the polluted soil by using various types of mechanisms such as through siderophore production, phosphate solubilization, biological nitrogen fixation, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing, signal interference and phytohormone production, exhibiting antifungal activity, production of volatile organic compounds, and induction of systemic resistance, promoting beneficial plant-–microbe symbioses. Thus, there are immense possibilities for identifying other growth-promoting rhizobacteria that could help in bioremediation of polluted soil as well as promote sustainable agriculture.

Keywords Bioremediation \cdot Soil pollution \cdot Plant growth–promoting rhizobacteria \cdot Siderophore production · Sustainable agriculture

8.1 Introduction

Soil is the most wondrous gift of nature to human society, it is a part of an ecosystem, it is the substance existing on the earth's surface, which grows and develops plant life (Terzaghi and Peck [1996\)](#page-23-0), it performs a wide range of functions (Jury and Roth [1990\)](#page-20-0) and renders a number of environmental services that connect it with the human society or in another word soil is essentially a natural body of mineral and organic constituents produced by solid material recycling, during a myriad of complex processes of solid crust modifications, which are closely related to the hydrological cycle (Mirsal [2008\)](#page-22-0). The soil is contaminated by several pollutants which are also known as soil pollutants, and this phenomenon are called as soil pollution, i.e., the occurrence of the chemical or other substances in the soil at a

concentration higher than normal causes adverse effects on non-targeted organism. Soil pollution often cannot be directly evaluated, constructing it a hidden hazard (Rodríguez-Eugenio et al. [2018\)](#page-22-0). The status of the World's Soil Resources Report (SWSR) identified soil pollution as one of the main soil threats affecting global soils and the ecosystems services provided by them. The main anthropogenic or manmade (Brookes [1995](#page-19-0)) sources of soil pollution are the chemicals used in or produced as byproducts of industrial activities (Vorobeichik et al. [2012\)](#page-23-0), domestic (Nyenje et al. [2013\)](#page-22-0), livestock (Zhang et al. [2012a](#page-23-0), [b](#page-23-0)), municipal wastes (Ali et al. [2014\)](#page-18-0), agrochemicals (Wimalawansa and Wimalawansa [2014\)](#page-23-0), and petroleum-derived products (Pinedo et al. [2013\)](#page-22-0). These chemicals are released to the environment accidentally (Kim et al. [2018;](#page-21-0) Awad et al. [2011\)](#page-18-0), for instance, from oil spills or leaching from landfills, or deliberately, as is the case with the use of fertilizers and pesticides, irrigation with untreated wastewater, or land application of sewage sludge. Soil pollution also results from atmospheric deposition from smelting (Zhang et al. [2012a](#page-23-0), [b;](#page-23-0) Gunawardena et al. [2013\)](#page-20-0), transportation (Wiłkomirski et al. [2011\)](#page-23-0), spray drift from pesticide applications, and incomplete combustion of many substances as well as radionuclide deposition from atmospheric weapons testing and nuclear accidents. Recently, new types of pollutants are developed such as pharmaceuticals, endocrine disruptors, hormones and toxins, among others, and biological pollutants, which include bacteria and viruses (Rodríguez-Eugenio et al. [2018](#page-22-0)) called micropollutants in soil. All these types of soil pollution need to be remediated by the development of a novel and science-based method, which includes a newly emerging method, i.e., bioremediation.

Bioremediation is an ecofriendly and an efficient method, in which live microorganism and its products can be utilized for the alleviation of environment contamination (Ojuederie and Babalola [2017](#page-22-0)). These processes facilitate to crop reestablishment on treated soil. Microorganisms such as plant growth–promoting rhizobacteria (PGPR) and plants employ various mechanisms for the bioremediation of polluted soils (Chibuike and Obiora 2014), and it has been suggested to play a significant and vital role in alleviating the toxicity in different contaminated soils (Khan et al. [2009](#page-21-0); Jayabarath et al. [2009;](#page-20-0) Cardón et al. [2010;](#page-19-0) Cetin et al. [2011\)](#page-19-0). Use of PGPR strains with many properties, like metal resistance/reduction ability (Joseph et al. [2007](#page-20-0); Kumar et al. [2008;](#page-21-0) Wani and Khan [2010](#page-23-0)) and capacity to facilitate plant growth through variable mechanisms in contaminated soils (Khan et al. [2009\)](#page-21-0), is considered enormously important for the attainment of the bioremediation program.

8.2 Soil Pollution

Soil pollution includes disturbance of major ecosystem services provided by soil. It can also adversely affect the yield of plants due to toxic levels of contaminants. It can be defined as a chemical or a substance out of place and/or present at a higher than the normal concentration that has adverse effects on any non-targeted organism (Rodríguez-Eugenio et al. [2018\)](#page-22-0). The main anthropogenic sources of soil pollution

are the excessive use of chemicals in agricultural (S. Savci [2012](#page-22-0)), domestic waste (Nyenje et al. [2013\)](#page-22-0), livestock and municipal wastes (Ali et al. [2014\)](#page-18-0), agrochemicals (Wimalawansa and Wimalawansa [2014\)](#page-23-0), and petroleum-derived products (Pinedo et al. [2013\)](#page-22-0). Soil pollution also results from atmospheric deposition from smelting (Gunawardena et al. [2013\)](#page-20-0) and transportation (Begum et al. [2011](#page-19-0)). Generally, there are two types of soil pollution, which is natural and manmade soil pollution, which includes former factory sites, inadequate waste and wastewater disposal, uncontrolled landfills, excessive application of agrochemicals, spills of many types, etc. Soil pollution can be divided into six types based on the source of pollutant (Fig. 8.1).

Fig. 8.1 Types of soil pollution based on sources of soil pollution

8.3 Impact of Soil Pollution

Soil pollution adversely affects the plants, animals, and humans health (Lu et al. [2015\)](#page-21-0). Those persons who directly or indirectly inhaled or ingested the soil pollutant may lose the general health or face health problem in the form of diseases such as high lead blood levels in children, arthralgia, osteomalacia, and excessive cadmium in urine (Zhang et al. [2012a,](#page-23-0) [b\)](#page-23-0). However, children are very sensitive to exposure to soil pollutants or contaminants, whenever they come in close contact with the contaminated soil by playing in the ground; then the pollutant may affect those children, and due to this, they suffer from asthma or allergenic-related problems (Heinzerling et al. [2016\)](#page-20-0) as well as adults also affected. Humans living near the polluted soil are facing health-related problems such as migraines, nausea, fatigue, skin disorders, and even miscarriages, and those people who are exposed to soil contamination for a longer period of time are suffering from cancer, leukemia, reproductive disorders, kidney and liver damage, and central nervous system failure (Mishra et al. [2015](#page-22-0)). Soil pollution is considered a big problem globally with respect to decreasing soil fertility and productivity, so the microbial activity including PGPR helps to cope up with such kind of situation; for example some PGPR have the ability to grow in the polluted soil by utilizing various kinds of pollutants or form the energy through the degradation of the pollutants present in the soil, so the application of such kind of PGPR in a timely manner in the soil helps to alleviate soil pollution by the process of bioremediation (Pilon-Smits [2005](#page-22-0)).

8.4 Bioremediation

Bioremediation includes the use of living organisms and their products, to remove contaminants from soil (USEPA [2012](#page-23-0); Leung [2004\)](#page-21-0) or to transform high toxic into less toxic forms (Memon and Schröder [2009](#page-22-0)). Certain microorganisms are involved in bioremediation of polluted environment. Maximum bioremediation processes utilize native microbial species including plant growth–promoting rhizobacteria (PGPR) (Khan et al. [2009](#page-21-0)), fungi (Zaidi et al. [2011\)](#page-23-0), actinomycetes (El-Syed et al. [2011\)](#page-19-0), algae (Huq et al. [2007](#page-20-0)), or plants (Marchand et al. [2010](#page-21-0)) which can be helpful in reclamation of the soil at optimum level.

According to Zaidi et al. [\(2012](#page-23-0)), bioremediation can be divided into two categories, which is in situ and ex situ bioremediation. In situ bioremediation includes the utilization of microorganism for the treatment of the hazardous chemicals in the soil and surface or subsurface waters while ex situ bioremediation requires diggings of contaminated soil or pumping of groundwater to facilitate microbial degradation; it has some disadvantages. So, in situ bioremediation method is considered more superior than ex situ bioremediation because it does not need digging of the contaminated soil as well as low-cost technology of contaminated soil bioremediation.

Fig. 8.2 Techniques of bioremediation for treatment of contaminated soil environment

8.5 Techniques of Bioremediation Treatment

Rajendran and Gunasekaran [\(2019](#page-22-0)) described eight categories of bioremediation treatment of contaminated soil environment (Fig. 8.2).

8.5.1 Bioaugmentation

Bioaugmentation technique is an in situ process of bioremediation of contaminated soil. In this process, the contaminated soil is treated with the microbial culture, which has immense properties of remediation of the soil through the various biological mechanisms. The microbial activity totally depends on the congenial environmental condition (Zaidi et al. [2012](#page-23-0); Vidali [2001\)](#page-23-0).

8.5.2 Biomineralization/Biocrystallization

In this technique, microbes generate the ligands which cause the precipitation of heavy metals as biomass-bound crystalline deposits.

8.5.3 Biostimulation

Biostimulation technique includes the stimulation of the indigenous microbes present in the contaminated soil by employing the necessary nutrients required. Necessary nutrients may supply through the mineral application as well in the form of manure, compost, etc.

8.5.4 Bioattenuation

This technique includes monitoring the process of natural degradation to ensure the decrease of the contaminant with time at the relevant sampling point is done.

8.5.5 Bioventing

It is an in situ bioremediation technique which is a relatively passive technique. In this method oxygen is supllied to the soil in order to stimulate aerobic soil microbial growth and degradation activity. It works for simple hydrocarbons and can be used where the contamination is deep under the surface (Vidali [2001\)](#page-23-0). The monitoring difficulty is there (Zaidi et al. [2012\)](#page-23-0).

8.5.6 Biofilters

Biofilters technique includes the use of microbial stripping columns to treat air emissions. The microbes generally break the toxic substances into a non-toxic compound e.g., carbon dioxide $(CO₂)$, water $(H₂O)$, and salts.

8.5.7 Bioreactors

This process involves the use of a container/reactor for the treatment of the liquid or slurries. The advantage of the bioreactors is rapid degradation kinetics, optimized environmental parameters, enhanced mass transfer, and effective use of inoculants and surfactants. It is a relatively expensive technique that limits its use in bioremediation program, e.g., slurry reactor and aqueous reactor (Zaidi et al. [2012;](#page-23-0) Vidali [2001\)](#page-23-0).

8.5.8 Composting

It is a type of ex situ and cost-efficient bioremediation program. It is the process of the aerobic and thermophilic treatment in which contaminated soil is mixed with a bulking agent. The development of a rich microbial population and the elevated temperature are a characteristic of composting (Vidali [2001](#page-23-0)). The extended treatment time is the limitation of the composting (Zaidi et al. [2012\)](#page-23-0).

8.5.9 Land Farming

It is a simple type of ex situ and cost-efficient bioremediation technique in which contaminated soil is excavated and spread over a prepared bed and intermittently plowed until contaminants are degraded (Vidali [2001\)](#page-23-0) or it is a solid-phase treatment system for contaminated soil or maybe in constructed soil treatment cell. The space requirement is the limitation of land farming (Zaidi et al. [2012](#page-23-0)).

8.6 Plant Growth-Promoting Rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria living in the soil in association with plant roots and are known to enhance the plant growth through a variety of direct and indirect mechanisms (Asad [2017](#page-18-0)) (Fig. [8.3](#page-8-0)). Direct mechanisms include nitrogen fixation, phosphate solubilization, potassium solubilization, phytostimulation, siderophore production which limits the Fe activity (Bhattacharyya and Jha [2012](#page-19-0)), heavy mineral uptake by plants (Ma et al. [2011\)](#page-21-0), etc. while indirect mechanisms include antibiotics production, chitinase and glucanase activity, induced systemic resistance against plant diseases which is termed as systemic resistance, exopolysaccharide production, phytoremediation (Nadeem et al. [2014\)](#page-22-0), etc. The PGPR facilitate plant growth under stressful environmental conditions by producing some key enzymes such as ACC-deaminase, chitinase, and rhizobitoxine exopolysaccharides.

Fig. 8.3 Mechanism of action of plant growth-promoting rhizobacteria (PGPR) in bioremediation of polluted soil

8.7 Role of Plant Growth–Promoting Rhizobacteria (PGPR) in Bioremediation of Polluted Soil

Plant growth–promoting rhizobacteria (PGPR) are the rhizosphere bacteria that can facilitate the plant growth under polluted environment by various mechanisms or they can help in bioremediation of polluted soil (Patel et al. [2016\)](#page-22-0) which can improve the plant growth by siderophore production (Sayyed et al. [2013](#page-22-0)), phosphate solubilization (Ahemad and Khan [2010\)](#page-18-0), biological nitrogen fixation (Yadegari et al. [2010\)](#page-23-0), production of 1-aminocyclopropane-1-carboxylate deaminase (ACC) (Gontia-Mishra et al. [2017\)](#page-20-0), quorum sensing (Podile et al. [2014\)](#page-22-0) signal interference and phytohormone production (Cassán et al. [2014](#page-19-0)), exhibiting antifungal activity (Ingle and Deshmukh [2010;](#page-20-0) Shobha and Kumudini [2012\)](#page-22-0), production of volatile organic compounds (Santoro et al. [2015\)](#page-22-0), induction of systemic resistance (Annapurna et al. [2013](#page-18-0)), promoting beneficial plant–microbe symbioses (Bhattacharyya and Jha [2012\)](#page-19-0), it could detoxify the contaminated environment sequestration of the metal ions inside the cell (Antony et al. [2011](#page-18-0)), biotransformation—transformation of toxic metal to less toxic forms (Cheung and Gu [2007;](#page-19-0) Shukla et al. [2009](#page-23-0)), adsorption/desorption of metals, etc. (Mamaril et al. [1997;](#page-21-0)

Fig. 8.4 Schematic representation of different mechanism followed by plant growth-promoting rhizobacteria (PGPR) during bioremediation of polluted soil

Johnson et al. [2007](#page-20-0)) (Fig. 8.4). It is considered extremely important for the success of the bioremediation program. Some examples of plant growth–promoting rhizobacteria and target pollutant with their mechanism to improve plant growth under polluted environment are listed in Table [8.1](#page-10-0).

8.8 New Emerging Technologies of Bioremediation

In recent years, there are several new technologies that gained much attention to overcome the negative impact of the contaminants in the soil, leading to improvement in reliability, cost efficiency, and speed of bioremediation (Rayu et al. [2012\)](#page-22-0). The old method of bioremediation which is microbes based is considered slower due to environmental conditions such as soil structure and moisture. New emerging tools based on advanced engineering technology of bioremediation provide much reliability to improve the performance of the bioremediation process. This new technique ranges from mere monitoring and advancement of inherent bioremediation to novel ideas of genetically engineering the functional genes for bioremediation application. Some of the new important tools are as follows:

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Table 8.1 (continued) Table 8.1 (continued)

8.8.1 Metagenomics

Metagenomics include phylogenetic analysis of soil microbial flora (Daniel [2005](#page-19-0)) for creating soil-based metagenomics library. It promises a continuous source of pollutant-degrading genes for increased efficiency and utility of transgenic (microbes and plants) technologies for direct use in bioremediation program (Daniel [2005\)](#page-19-0). This technology also facilitates the mass production of the degrading enzymes from uncultivable bacteria for improvement of enzymatic remediation technology. By this technique, we can produce a marketable product based on bioremediation gene/enzyme product from uncultivable microbes (Rayu et al. [2012\)](#page-22-0). For example, thermostable pyrethroid hydrolyzing enzyme could be used in the detoxification of pyrethroids (Fan et al. [2012\)](#page-20-0), a novel gene responsible for the degradation of 3,5,6-trichloro-2-pyridinol; a persistent and toxic metabolite of the insecticide chlorpyrifos was isolated (Math et al. [2010\)](#page-22-0) from cow rumen and gene products for remediation including biphenyl-degrading genes (Sul et al. [2009](#page-23-0)).

8.8.2 Metabolic Engineering

Metabolic engineering includes the improvement of cellular activities by manipulations of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology (Nielsen [2001\)](#page-22-0). By this technique, we can combine analysis of the metabolic pathway and other pathways that can help to improve cellular properties by designing and implementing rational genetic modifications (Koffas et al. [1999](#page-21-0)). This type of metabolic pathway analysis is rapidly becoming one of the significant features of bioremediation, e.g., Pseudomonas putida degrades chloro- as well as methylo-aromatics; the combination of tod and tol pathways in P. putida can increase biodegradation rate of benzene, toluene, and p-xylene (Rayu et al. [2012](#page-22-0)).

8.8.3 Protein/Enzyme Engineering

Improving the stability, substrate specificity, and kinetic properties of proteins/ enzymes can be engineered (Dombkowski et al. [2014](#page-19-0)). It can be done to fine-tune enzymes for desired substrate specificities and stereo-selectivity. This method helps to modify the active site volume and topology of cytochrome P450cam enhanced the catalytic activity of the enzyme (Kumar [2010;](#page-21-0) Holloway et al. [1998\)](#page-20-0). Another modification is the incorporation of multiple binding sites within a single peptide, for binding of the co-factors and other small molecules, can enhance the catalytic power of the enzyme; this is found to bioremediate the metal wastes (Pazirandeh et al. [1998](#page-22-0)).

8.9 Factor Affecting the Bioremediation

The bioremediation of the polluted environment is a complex process which is influenced by certain factors such as microbial factors including growth until critical biomass is reached, mutation and horizontal gene transfer, enzyme induction, enrichment of the capable microbial populations, and production of toxic metabolites; environmental factors include depletion of preferential substrates, lack of nutrients, inhibitory environmental conditions viz soil, temperature (Chitara et al. 2017), pH, $O₂$ and nutrients; substrate factor includes too low concentration of contaminants, chemical structure of contaminants, toxicity of contaminants, and solubility of contaminants; biological aerobic vs anaerobic process factor includes oxidation/reduction potential, availability of e-accepters, and microbial population present in the site; growth substrate vs co-metabolism factor includes type of contaminants, concentration, alternate carbon source present, and microbial interaction (competition, succession, and predation); physico-chemical bioavailability of pollutants include equilibrium sorption, irreversible sorption, and incorporation into humic matters, and some of the mass transfer limitations are O_2 diffusion and solubility, diffusion of nutrients, and solubility/miscibility in/with water (Boopathy [2000\)](#page-19-0). The microorganisms are cosmopolitan in nature which can be isolated from everywhere such as at subzero temperatures, extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream (Boopathy [2000](#page-19-0)). The microbes utilize the energy source and carbon source and other biological systems. These microbes can be used to remediate environmental hazards. Joshi [\(2018](#page-20-0)) divided the microbes into two groups viz. aerobic and anaerobic groups as follows:

8.9.1 Aerobic

This group includes those microbes which exist in the presence of oxygen (Rayu et al. [2012](#page-22-0)), e.g., Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium. These bacteria are helpful in bioremediation of polluted soil and are reported to degrade pesticide and hydrocarbon both as well as alkenes compounds.

8.9.2 Anaerobic

This group includes those microbes which exist in the absence of oxygen (Rayu et al. [2012\)](#page-22-0); for example ligninolytic fungi such as the white-rot fungus Phanaerochaete chrysosporium have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants, such as Acromobacter, Alcaligenes, Arthrobacter, Bacillus, Acinetobacter, Corneybacterium, Flavobacterium, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Vibrio, Rhodococcus, and Sphingomonas species (Gupta et al. [2001](#page-20-0); Kim et al. [2007;](#page-21-0) Jayashree et al. [2012\)](#page-20-0); these bacteria are helpful to use in the bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform.

8.10 Advantages of Bioremediation

According to Vidali ([2001\)](#page-23-0), the advantages of bioremediation of the polluted soil are as follows:

- It is a natural process so it is perceived by the public as an acceptable waste treatment process for contaminated material such as soil.
- It conserves the natural properties of soil.
- It utilizes energy from sunlight for performing its activity.
- It helps in increasing microbial biomass in the rhizosphere.
- It is useful for the complete destruction of a wide variety of contaminants.
- The end products of treatment are usually harmless which are usually CO_2 , H_2O , and cell biomass.
- It is a low-cost application or less expensive than other technologies.

8.11 Limitations

Plant growth–promoting rhizobacteria play a significant role in bioremediation of polluted soil program. The success of these programs solely depends upon the activity of PGPR and those need optimum environmental conditions for their growth and colonization. But recently, the climate change influences the environment; due to this, the PGPR performance disturbs or gets changed (Compant et al. [2010\)](#page-19-0). Therefore, climate change may also affect the microbial population present in the soil surface, subsurface, and plant-associated communities (Drigo et al. [2009\)](#page-19-0). Climate change affects all the metabolic process, i.e., crop or plant physiology and metabolism are affected; for example, in plants the production of amino acid (tryptophan) decrease, which also results in the decrease in the production of IAA, which disturbs the [vegetative growth](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/vegetative-growth) and root proliferation of the plant (Kravchenko) et al. [2004](#page-21-0)). The high temperature may also hamper the growth of plant and physiology together, they are likely to lead to changes in the configuration, abundance, or activity of plant-associated microbial communities. Consequently, population of microorganisms known for their valuable effects on plant health or growth might also be reduced, in terms of exhibiting their desirable properties and [colonization](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/colonisation) capacity under certain environmental conditions (Compant et al. [2010\)](#page-19-0).

8.12 Future Prospects

For the past few decades, the researchers are giving more attention to the management of soil pollution caused by various chemicals or other substances only. The bioremediation of polluted soil serves as one of the best ways to manage the polluted soil. This approach utilizes the plant growth–promoting rhizobacteria (PGPR) whose activity is influenced by climate change. So, the success rate of PGPR is highly associated with climate, so it is important to understand the plant growth patterns along with its surrounding environment before the application of PGPR especially for a particular given set of conditions. Therefore, it is needed to identify a specific PGPR strain for a particular region for ensuring their better performance and effectively facilitate the bioremediation of polluted soil under changing climate conditions.

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