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Microbiota and Biofertilizers, Vol 2

Ecofriendly Tools for Reclamation
of Degraded Soil Environs

 Springer

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Gowhar Hamid Dar • Rouf Ahmad Bhat
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Dedicated to COVID-19 frontline workers.

Foreword



The huge enslavement of modern farming and the additional artificial input of “chemical fertilizers” have caused numerous ecological tribulations associated with global warming and soil contamination. As a result, there is an essential requirement for doable agricultural practices on a comprehensive level. Accordingly, biofertilizers, including microbes, have been recommended as feasible, environmentally sound solutions for agricultural practices, which not only are natural and cost-effective but also preserve soil environs and important biota of agricultural land. Moreover, it enhances and sustains the nutrient status and quantity in soils in an ecofriendly manner. Microbial biofertilizers promote plant growth by escalating the proficient absorption of nutrients for the plants and by triggering an excellent disease-fighting mechanism.

The book is a coherent assembly of speckled topics relatable to ecofriendly tools for the reclamation of degraded soil environs. It contains 16 chapters from diverse geographic regions of the world. Chapter 1 deals with the subject of “Chemical Fertilizer Use and Their Impact on Soil Health”. The authors from India in this chapter have discussed the importance of soil as it carries out vital ecological functions that are imperative for the existence of life on earth, upkeep of biodiversity, and the continuation of sustainable agronomic practices. The chapter elaborates well how continuous chemical fertilizer usage can change the soil physicochemical characteristics, giving rise to issues as reduction of organic matter and humus content, distressed microbial communities, and inhibition of plant development.

Chapter 2 presents the valuable statistics about the “Microbial Bioremediation of Pesticides/Herbicides in Soil” by making use of microorganisms. Microbial remediation has been the most exceptional biotechnological tool for the revitalization of impaired environments. This chapter provides details about the categories of pesticides, their resources, environmental apprehensions, and the numerous microbes used for their bioremediation.

In Chap. 3, the latest techniques for cleaning polluted environments have been discussed in detail by the group of authors from India. They have emphasized that technological advancement, in addition to the growing population and over-exploitation of natural resources, has resulted in an upsurge in environmental pollution, and there is an immediate need to rectify the poor practices of humans by using cost-effective and ecofriendly techniques to regulate and terminate these pollutants. Techniques such as phytoremediation, rhizofiltration, and phytovolatilization have been stressed upon in this chapter.

Chapter 4 focuses on the “Role of Mushrooms in the Bioremediation of Soil”. It is a well-known fact that one of the prominent environmental issues the world is facing nowadays is soil pollution. Therefore, mycoremediation can be an appropriate, cost-efficacious, and environmentally friendly technique to clean up the contaminated soil. The authors have discussed how this technique makes use of fungi to degrade toxic pollutants proficiently and concretely.

The authors of Chap. 5 have presented how “Microbial Degradation of Organic Constituents for Sustainable Development”. Environmental contamination by organic compounds has resulted in a multitude of ecological threats, especially to agriculture. The problems are further escalating due to the recalcitrant nature of these organic constituents like hydrocarbons, pesticides, dyes, and heavy metals. The extermination of these toxic pollutants from the environment is vital for achieving sustainable development. This chapter has presented an overview of the role of microorganisms in the degradation of organic components to accomplish sustainable development.

In Chap. 6, titled “Traditional Farming Practices and Its Consequences”, the authors have discussed the traditional farming practices, based on the indigenous knowledge and experience developed over the centuries and has remained popular even now. They have mentioned various categories of traditional farming, which include agroforestry, intercropping, crop rotation, cover cropping, conventional organic composting, integrated crop-animal agriculture, shifting cultivation, and slash-and-burn farming. Furthermore, the authors conclude that there are many benefits involved in these practices such as improved soil fertility, carbon sequestration, resource utilization, biodiversity maintenance and sustainability, and environmental protection. Still, there are also specific negative implications associated with some practices, for example, slash-and-burn activities, in shifting agriculture.

The significance of “Soil Organic Matter and Its Impact on Soil Properties and Nutrient Status” has been discussed in Chap. 7. The authors have stressed the impacts of population explosion and an upsurge in food demand on the agriculture land depletion and how it is risking our future advancement. Moreover, the authors have discussed that to overcome this problematic situation, the soil organic matter plays a vibrant and pivotal role in the maintenance and recuperation of soil

properties as it has incredible ecological benefits such as regulating ecosystem productivity, soil health, and climate quality. This chapter reviews the influence of organic matter on the physical, chemical, and biological properties of soil.

The vital topic of “Sustainable Agricultural Practices” has been discussed in Chap. 8. The authors have emphasized that the rising need for non-toxic and nourishing food has caused organic agriculture to achieve great significance internationally and how food demand has moved from the chemically dependent farming to intensive organic agriculture. The role of organic agriculture is highlighted due to its capacity of sustaining soil health, increasing crop output, and providing disease-free crops.

Another group of authors from Pakistan have presented their views on the “Values of Composting” as an excellent tool for sustainable agriculture practice in Chap. 9. Composting is an ecofriendly technique, and it provides nourishment to plants on a long-term basis. The authors have discussed the various types of composts and their many benefits. This chapter also covers the role of composting on soil properties and its influence on soil and environmental pollution.

Chapter 10 has provided an introduction to “Microbiota and Biofertilizers”. The section discusses the repercussions of the extensive use of synthetic fertilizers on the environmental, plant, soil, and animal health. The chapter explores the use of microbes as fertilizers and their substantial role in nutrient cycling and nitrogen fixation as well as in enhancing and maintaining the content of soil nutrients. These microbes, as biofertilizers, are ecologically friendly, non-toxic, and best replacements for chemical fertilizers.

Chapter 11, titled “Fungi and Their Potential as Biofertilizers”, provides information on the use of fungi as an environmentally friendly alternative to man-made harmful chemical fertilizers. It has also been perceived in this chapter that fungi play a very central role in stimulating plant growth and output and for the improvement of soil fertility. How fungal biofertilizers influence plant growth and development has been discussed in detail by the authors. The chapter mainly focuses on the fungi that have the potential to be used as biofertilizers.

The title “*Bacillus thuringiensis* as a Biofertilizer and Plant Growth Promoter” is the topic of Chap. 12. According to the authors of this chapter, the utilization of beneficial microorganisms like *Bacillus thuringiensis* as biofertilizers is of paramount significance in agriculture. Its ability to check insect and pest populations and contribute to controlling plant diseases has been comprehensively elaborated. In addition, its plant growth-promoting abilities as biofertilizers are discussed in this chapter. The authors have stated that its use in agriculture can be a chance for curtailing the practice of using chemical fertilizers. This chapter further evaluates the role of phosphate-solubilizing *B. thuringiensis* as a biofertilizer and biostimulator.

The authors of Chap. 13 have presented valuable information on “Cyanobacteria as Sustainable Microbiome for Agricultural Industries” as an ecofriendly, effective, and economical alternative for synthetic fertilizers. They have discussed the importance of cyanobacteria as a potential stimulant of nitrogen fixation and plant growth-promoting hormones. Furthermore, the role of cyanobacteria for the remediation of pesticides, herbicides, and heavy metals has been studied significantly.

This chapter also emphasizes the utilization of these microbes as biofertilizers in the farming sector to improve crop production and increase crop output.

Chapter 14, “Intercropping: A Substitute but Identical of Biofertilizers”, has been presented by scientists from China and Pakistan. The authors have revealed that conventional cropping and the use of inorganic chemical-based pesticides and fertilizers are the main obstructions to sustainable agriculture. They have explored the practice of intercropping as an old but efficient and ecofriendly way to improve soil health and increase crop production. This chapter elucidates the mechanisms behind intercropping, which assist in nitrogen and phosphorus acquisition and prevent insect pest and disease frequency, and discusses how intercropping can be employed to reclaim degraded agricultural lands.

“Application of Phyllosphere Microbiota as Biofertilizers” is the theme of Chap. 15. The authors have discussed the influence of phyllosphere microbiota on the fitness and functions of the host plant, plant biogeography, and ecosystem working. This chapter focuses on the advantages of plant-microbe phyllosphere interactions that have a significant role in plant health and productivity as well as their contribution to achieving environmental sustainability.

The authors from Pakistan have presented their views on “Biofertilizers: A Viable Tool for Future Organic Agriculture” in Chap. 16. They have discussed the use of organic farming as a substitute nutrient management practice. Organic agriculture, as an ecofriendly, fiscally lucrative approach with pronounced market potential as well as a sustainable agricultural practice, has been particularized by the authors. They have also mentioned the significant obstacles in the implementation of organic farming.

I am convinced that the book titled *Microbiota and Biofertilizers, Vol. 2 – Ecofriendly Tools for Reclamation of Degraded Soil Environs* will serve as a milestone in conserving the soil health which is crucial in sustaining hundreds of the well-knit ecosystems of nature. Besides this book will serve as an inspiring force for teachers to make students and other stakeholders aware of the cutting-edge technologies that can be efficiently used for transforming agriculture under changing climatic scenario. Human population is increasing at an alarming pace, which gets aggravated with the shrinking of water and land resources. Human population is expected to increase from 7.6 billion (2020) to 9.4 billion in 2050. It is the need of the hour to sustain the crop production to feed the ever-increasing populace, for which technological interventions in the form of organic farming and biofertilizers are essentially required to improve and conserve “Mother Soil”. This book is a way forward in this direction. The editors must be highly praised and appreciated for their creditable hard work in bringing this book.



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Preface

Agriculture, the backbone of human sustenance, has been put under tremendous pressure by the ever-increasing human population. Although various modern agrotechniques boosted agricultural production, the excessive use of synthetic fertilizers, pesticides, and herbicides has proven to be extremely detrimental to agriculture as well as to the environmental quality. Besides this, some faulty agricultural practices like monoculture and poor irrigation further complicated the scenario of problems by eliminating the critical biota. The influx of nutrients into the waterbodies (eutrophication) and the formation of algal blooms further damage the water quality and reduce the fish stocks. Therefore, it becomes imperative to involve specific scientific approaches like the development of biofertilizers, which could be beneficial for sustainable agriculture practices.

The application of biofertilizers helps increase mineral and water uptake and root development in plants and also in nitrogen fixation. It liberates growth-promoting substances and minerals, which helps in the maintenance of soil fertility. Furthermore, biofertilizers act as antagonists and play a pivotal role in neutralizing the soil-borne plant pathogens, thus helping in the bio-control of diseases. The application of biofertilizers instead of synthetic fertilizers could be a promising technique to raise agricultural productivity without degrading the environmental quality.

The book is an articulate assemblage of assorted topics in the shape of chapters, pertinent to the reliability of “microbiota and biofertilizers” as ecofriendly tools for reclamation of degraded soil environs. The starting chapters provide an outline of the sources and detrimental impacts of synthetic chemical substances on soil and human health. A precious space has been given to the latest techniques for cleaning polluted environs, as well as for the bioremediation technologies involving various biota of the environment. Traditional and organic farming has been considered the backbone of this book. Furthermore, a valuable consigned space related to microbiota as biofertilizers is given at the end of the chapters. In general, the opening chapters of this book deal with the contamination and its impacts, followed by the ecofriendly strategies towards sustainable developments.

The book is an accurate, broad description of the involvement of biota and bio-fertilizers for pollution remediation and the sustainable strategies for healthy environs. Academicians, researchers, and students will find it a perfect bind about “microbiota and biofertilizers” as intrusion in agricultural practices for the reclamation of degraded soil environs and should sufficiently suffice the requirements of training, teaching, and research purposes.

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About the Book

This volume of the book series *Microbiota and Biofertilisers* provides clear information on the indiscriminate use of synthetic agrochemicals in conventional agriculture and their drastic impacts on the ecological balance aggravated by some critical environmental challenges like global warming, climate change, and environmental pollution. It focuses on the sustainable agriculture demands and alternatives to deal with both agricultural production and environmental pollution. Moreover, the book highlights the involvement of microbiota and organic amendments as emerging options which are cost-effective and provide overwhelming results at field level and at the same time maintain soil health without degrading the environmental quality, particularly on soil biota and biochemistry. In general, the introductory chapters of this volume focus on the environmental degradation and issues raised due to unplanned agricultural practices, followed by the ecofriendly approaches and alternatives replacing synthetic substances. The closing chapters provide an insight into the bioremediation with the help of the latest agricultural tools and techniques and research findings to keep a balance between the various components of the agricultural environment. Furthermore, the book is a detailed comprehensive interpretation of doable approaches from quiver to sustainable. Academicians, researchers, and students should find it as an absolute bind about microbial intrusion as biofertilizer for ecofriendly reclamation of degraded soil environs.

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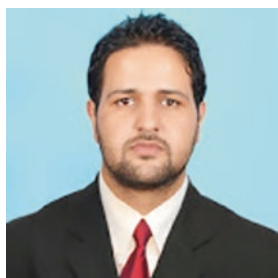
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Chapter 1

Chemical Fertilizers and Their Impact on Soil Health



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1.1 Introduction

Agriculture is facing considerable problems such as low soil organic carbon (SOC) stock, low fertilizer use efficiencies, and the imbalance between nutrient removal and addition to the soil. The whole scenario of agriculture is at a confluence where one has to rethink and improve agricultural packages and processes in meeting the dreams of millions of people. Improvement and maintenance of soil fertility and sustaining crop production are of worldwide importance. The management of soil health is necessary for securing sustainable agricultural production and sustenance of biodiversity. Modern agriculture mostly relies on various inputs, such as pesticides, chemical fertilizers, assured irrigation, improved seeds, and herbicides. Their employment in agriculture increases the production, but their improper utilization has unfavorable impact on environment quality and soil productivity which is a matter of concern (Dar and Bhat 2020). Chemical fertilizers are compounds which encompass the large nutrient concentration needed for growth of plant. In other words, these are man-made materials that supply the required nutrients to plants (Khanday et al. 2016; Dar et al. 2013; Bhat et al. 2018a, b; Mushtaq et al. 2018; Singh et al. 2018). Fertilizers and pesticides are unavoidable risks in agriculture. Nevertheless, they continue to be vital means for worldwide food safety, their negative impacts cannot be unnoticed particularly when sustainable agriculture is the

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Table 1.1 Chemical fertilizer usage in various countries

Country	Chemical fertilizers (kg/ha)
Netherlands	665.6
Egypt	624.8
Japan	373.2
China	301.5
Britain	287.5
Germany	205.4
France	180.1
USA	160.8
Italy	126.4
India	121.4
Greece	115.4
Indonesia	106.9
Turkey	100.4

global target. Chemical fertilizer has a vital role in increasing soil fertility and crop productivity (Hera 1996; Bhatti et al. 2017). Different countries use different amounts of fertilizers, for example, Turkey uses less chemical fertilizer per hectare than many developing and developed countries. The values for chemical fertilizer usage in different countries are shown in Table 1.1. Chemical fertilizer use in India has increased from 2.65 million tons (mt) of NPK in 1971–1972 to 28.12 mt in 2010–2011. Fertilizer usage in India is highly skewed (Chand and Pavithra 2015).

The fertilizer industry is a source of heavy metals and radionuclides. It consists of most of the metals such as cadmium (Cd), arsenic (As), mercury (Hg), nickel (Ni), lead (Pb), copper (Cu); natural radionuclide, such as U^{238} , Th^{232} , and Po^{210} (FAO 2009; Sönmez et al. 2007). Fertilization can be responsible for the accumulation of heavy metal in soil system. Through the soil, plants absorb fertilizers and these make their entry into the food chain. Large amounts of chemical fertilizers are used in greenhouses, aquacultures during the top season. The prolonged use of chemical fertilizer has declined the agricultural soil quality with the reduction in soil organic matter (SOM) content, and even an increase in environment pollution and soil acidity (Dinesh et al. 2010; Dar et al. 2016), that has become a serious concern (Chaudhry et al. 2009; Bhat et al. 2017a, b; Dervash et al. 2020; Mushtaq et al. 2020; Singh et al. 2020). Chemical fertilizers are detrimental to agriculture as salt is one of its major contents, which is detrimental for plants and soil. Chemical fertilizers lessen vital soil minerals and nutrients. They do not provide benefit in restoring soil fertility and its nutrients.

1.2 Types of Chemical Fertilizers

There are three kinds of chemical fertilizers available. These are nitrogenous fertilizer, phosphorus fertilizer, and potassium fertilizer. Chemical fertilizer consists of elements, such as nitrogen, potassium, and phosphorus. These are used for increasing productivity of land (Fig. 1.1).

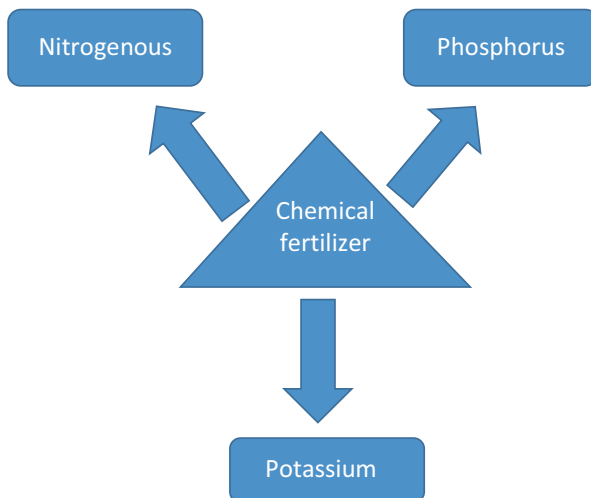


Fig. 1.1 Types of chemical fertilizers

1.3 Nitrogenous Fertilizer

In this type of fertilizer, nitrogen is present in the form of ammoniacal nitrogen, such as ammonium chloride, ammonium sulfate; amide nitrogen, such as urea; nitrate-nitrogen, such as calcium ammonium nitrate in which both ammoniacal and nitrate nitrogen are present. This fertilizer is used for meeting the nitrogen deficiency in the land. This is the most beneficial fertilizer for the plant. It supplies nutrients to both plants and land.

1.3.1 Characteristics

- (a) The power of ammonium sulfate is much greater than urea.
- (b) Nitric nitrogen is more effective when it is used during the start of a reproductive process of paddy plant.
- (c) After the ammonium nitrogen application, the paddy plant may take 30–35% of total nitrogen. But when fertilizers are used at 5–10 cm depth, the nutrient becomes more available.
- (d) The continuous use of acid-making fertilizers, such as ammonium sulfate, urea, nitrate, and ammonium chloride ought to be prevented in calcium-deficient soil and acid soil. At least, liming ought to be done 15 days prior to sowing of the crop.
- (e) It is water-soluble and flows quickly from the point of its application to all directions. Nitrogenous fertilizer application ought to be as per crop requirement.
- (f) Every nitrogenous fertilizer is equitably useful in the rainy season.

1.4 Phosphorus Fertilizer

In phosphorus fertilizers, phosphorus is present as available phosphate. This fertilizer is vital terrestrial fertilizer. Its requirement is less as compared to nitrogen fertilizer.

1.4.1 Characteristics

- (a) The combined use of phosphate and nitrogenous fertilizer boosts the uptake efficiency of the plant.
- (b) Phosphate fertilizers, such as rock phosphate, basic slag, are the most appropriate for acidic soils.
- (c) Phosphate compost such as superphosphate ought to be applied close to the crop root area or in the soil layer.
- (d) Super phosphate ought to be applied in the soil ranges from neutral to alkaline.
- (e) It ought to be placed deep in fruit trees, such as apple and citrus.

1.5 Potassium Fertilizer

The potassium requirement can be fulfilled with Muriate (potassium chloride) and sulfate of potash. Potassium sulfate is necessary for healthy plant growth. The formation of carbohydrate in the plants is possible with the help of potassium. It is divided into two subtypes, that is, potash in non-chloride and in chloride forms. Sulfate of potash and muriate of potash are the examples of potash in non-chloride nature and potash in chloride form, respectively.

1.5.1 Characteristics of Potassium Fertilizer

- (a) It is suitable for all crops.
- (b) It can be utilized for a wide range of soils.
- (c) It boosts power of resistance of plants.
- (d) Potassium sulfate is superior to potassium nitrate for a variety of crops such as fruit trees and potatoes.
- (e) It is soluble in water.
- (f) It dissociates to K^+ ions and gets consumed in the soil. It is later absorbed by the plant.

1.6 Advantages of Chemical Fertilizer

Chemical fertilizer is utilized to restore lands' fertility. The land loses its fertility because of the enormous number of crops cultivated during the year. Fertilizers are exceptionally fundamental for the development of the crop, quality parameters, yield, in any event, for soil well-being, when utilized appropriately. Fertilizer makes nutrient status better and the nature of the soil, by adding supplements which it needs. Some points of interest are as under:

1. It enhances power of resistance in plants.
2. Growing better crops.
3. Plant growth gets faster.
4. Plants obtain all the nutrients from chemical fertilizer in equal proportion.
5. Soil readily absorbs chemical fertilizer, as it is water-soluble.
6. No unnecessary ingredient is found in chemical fertilizer.
7. Plant growth and development become accurate.

1.7 Drawbacks of Chemical Fertilizer

The health of soil and plants will deteriorate by improper employment of chemical fertilizer. The primary drawbacks are as follows:

1. Some artificial fertilizer elements disturb the soil properties.
2. Artificial fertilizers become costly for small farmers.
3. It enhances the emission of nitrate.
4. Crop growth is not sufficient, so results in the decrease in crop production.
5. Often crops are damaged due to insufficient water supply, particularly in regions with less rainfall.
6. When N-fertilizer is applied in the field, only then bacteria already present can turn the nitrogen into nitrate.
7. It spoils the constitution of the soil due to lack of organic material.
8. Soil dwelling organisms, such as earthworms, which make the soil fertile, also are destroyed due to improper application.
9. The nitrogen fertilizer is toxic to both animals and humans.

1.8 Important Nutrients in Fertilizers

Chemical fertilizers are sources of the main macronutrients for plants, such as nitrogen, potassium, phosphorus, and a number of micronutrients and additives. Plants need sufficient amounts of nutrients in addition to three prime component elements,

Table 1.2 Important nutrients and their chemical forms

Element	Chemical forms of elements absorbed by plants
<i>Primary nutrients</i>	
Nitrogen (N)	NO_3^- , NH_4^+
Phosphorus (P)	H_2PO_4^- , HPO_4^{3-} , PO_4^{3-}
Potassium (K)	K^+
<i>Secondary nutrients</i>	
Magnesium (Mg)	Mg^{2+}
Calcium (Ca)	Ca^{2+}
Sulfur (S)	SO_4^{2-}
<i>Micronutrients</i>	
Boron (B)	BO_3^{3-}
Chlorine (Cl)	Cl^-
Copper (Cu)	Cu^+ , Cu^{2+}
Iron (Fe)	Fe^{3+}
Manganese (Mn)	Mn^{2+}
Molybdenum (Mo)	MoO_4^{2-}
Nickel (Ni)	Ni^{2+}
Zinc (Zn)	Zn^{2+}

oxygen, carbon, and hydrogen. The nutrients are classified as primary nutrients, secondary nutrients, and micronutrients (Table 1.2).

1.9 Primary Nutrients

Primary nutrients are those nutrients which are demanded by plants in adequate quantities for their development and growth. It comprises three elements, such as nitrogen (N), phosphorus (P) and potassium (K). They play an important role in plants.

- Nitrogen (N) is a basic element for various vital plant substances, such as chlorophyll, and thus help in plant growth.
- Phosphorus (P) is responsible for numerous important plant processes such as energy transfer, root growth, fruiting, and flowering. Plants need it in range of mild to high levels for proper development of fruits and seeds.
- Potassium (K) assists in activating various enzymes involved in different processes, such as respiration and photosynthesis. It also helps in movement of water, promotes fruiting and flowering.

1.10 Secondary Nutrients

The nutrients needed by plants in moderate quantities. It comprises calcium (Ca), magnesium (Mg), and sulfur (S).

- Calcium (Ca) is responsible for activation of certain plant enzymes, governs the transfer of certain nutrients into the plant. It plays an important part in the photosynthesis and structure of plants.
- Magnesium (Mg) is an important member of the chlorophyll molecule. It also plays a role as an efficient activator in various enzyme reactions.
- Sulfur (S) is an important constituent of a few vitamins and amino acids. It is necessary for function and growth of chloroplast and is essential for legumes to fix N_2 . It helps in transforming nitrate to amino acids and then to proteins.

1.11 Micronutrients

These are demanded by plants in minute quantities. These nutrients are equally essential as macronutrients. They are also known as trace elements, rare elements which are shown below:

- Copper (Cu) has a primary function in the photosynthesis process and in the developments of crops. It is also vital for the formation of lignin.
- Iron (Fe) acts as a cofactor of enzymes in plants and is important in the photosynthesis process.
- Manganese (Mn) is involved in chloroplast formation.
- Molybdenum (Mo) is an important cofactor in the formation of amino acid and also helps in nitrogen metabolism.
- Zinc (Zn) has an important role in a wide variety of enzymes. It has a vital function in the transcription of DNA.
- Boron (B) carries out various roles in a plant. It includes in cell division, pollen germination, fruiting and flowering, and active absorption of salt.
- Silicon (Si) enhances strength of cell walls, increases strength, productivity, and health of plant.
- Cobalt (Co) is necessary in nitrogen fixation.
- Vanadium (V) is needed, at very low concentration, by some plants. It may also be used as a substitute for molybdenum.

1.12 Soil Health Concept

The primary role of the soil is to produce sufficient food and secure human health. There is, increasingly, an understanding of the relation between human health and soil with respect to health enhancing elements such as nitrogen (N), phosphorus (P), and other elements, such as arsenic (As) and cadmium (Cd), which are detrimental to human health (Brevik and Burgess 2013). The idea of “health” also covers the soil. Soil, in the form of complex ecosystem, promotes an immense variety of life. The idea of soil health is easy to comprehend when the system is regarded as a whole. Soil quality (health) is characterized as a soil’s ability to perform within ecosystems and land use boundaries to preserve biological productivity and quality of environment, and to improve animal and plant health (Doran and Parkin 1994; Bhat et al. 2017a, b). While the basic concept is to maintain the soil so that it carries on various necessary functions without the deterioration of the soil itself or adversely impacting the environment. According to Kibblewhite et al. (2008), a healthy soil is able to support fiber and food production to a level, and with a quality, enough to satisfy human needs, and of sustaining certain functions which are vital to protect the human life, and conserve biodiversity. The fundamental idea is to sustain the quality of soil and prevent practices such as nutrient mining and erosion which deteriorate the soil. When the decaying rate of “inferior quality” or high C:N ratio organic materials and SOM increases, the health of soil is affected (Recous et al. 1995; Bhat et al. 2018a, b).

1.13 Positive Effects of Fertilizers

While fertilization does not have any direct useful effect on the activities of microbes in the soil, a gaining in microflora activity in soil, as well as diversity has been revealed as a secondary effect of increased SOC, elevated concentration of nutrients, such as NPK, and improved crop yields affecting rhizo deposition. A favorable stimulation of large number of soil parameters was observed, after the application of inorganic phosphorus fertilizers to paddy soil for 13 years. The number of cultivable microbes, population functional diversity, and microbial biomass increased in the soil without phosphate treatment. The application of N fertilizer, in combination with sufficient quantities of phosphorus, was found to be useful on microbial behavior, diversity, and rice crop production, while, on the other side, K showed no effect (Zhong and Cai 2007). Likewise, a positive effect has been observed in rice field after it is treated with NPK fertilizer for 39 years. This has improved C, N pools, which in turn resulted in positive effect on soil fertility and soil enzyme activities and microbial population (Bhattacharyya et al. 2013). In a few studies, it has been found that continuing application resulted in no significant alteration in the characteristics of soil microbes. In northeast China, black soils showed no significant difference in functional diversity and microbial biomass when exposed to varied

combinations of NPK fertilizers for prolonged periods (Kong et al. 2008). The functional variability is likely to increase with the increase in fertilization dose, that is, double or triple fertilizer therapy. Inorganic fertilizers can generate different consequences when applied alone or in association with organic inputs. For instance, no improvement in abundance of bacteria after incessant use of inorganic fertilizers in the rice field alone. Nevertheless, use of rice straw, coupled with chemical fertilizers, significantly increased abundance of bacteria with alteration in the composition of the bacterial population. Moreover, the bacterial phylogenetic classes also varied in their reaction to soil fertilization. Inorganic fertilizer primarily influenced π -proteobacteria and γ -proteobacteria, while rice straw incorporation affected β -proteobacteria and verrucomicrobia (Wu et al. 2011). Continuing fertilizer use in production of crops results in the accumulation of SOM (Geiseller and Scow 2014; Ladha et al. 2011) and soil health is enhanced by the addition of a large quantity of root biomass and litter to the soil.

1.14 Impacts of Chemical Fertilizers on Soil Health

1.14.1 Soil Quality

Soil quality has worsened due to increased application of chemical fertilizers, especially urea, muriate of potash, and single super phosphate (SSP), as a means to increase farm output. Since soils serve as the main reservoir of reactive nutrient types, their complete management is crucial to address worldwide food security challenges and reducing environmental nutrient losses that can affect the quality of water and air. There are various risks to soil health (Velthof et al. 2011): soil compaction, acidification, erosion, contamination, salinization, and decline in organic matter, which can affect P and N losses to water and air.

1.14.2 Physicochemical Properties of Soil

The management of soil nutrient is essential to preserve the quality of good soil and the constant productivity of nursery systems. Fertilization influences N and C quantity in soil by alteration of soil structure, organic N and C component of soil (Hai et al. 2010), and soil aggregation (Su 2007). Continuous utilization of chemical fertilizers has a distinct impact on the soil's biochemical properties, which results in the shift of microbial populations. Alteration in nitrogen (N) content, SOC, moisture, pH, and the availability of nutrients to microbes has been observed as a result of incessant use of fertilizer in different crops such as corn, wheat, and others (Bohme et al. 2005). Fertilizer application declined bulk density of soil, which may be due to increase in organic C of soil (Selvi et al. 2005). The higher SOC level can

be attributed to increased growth of root, resulting in more residues in soil, which may have enhanced SOC amount after decomposition (Kumpawat and Jat 2005). Sradnick et al. (2013) reported the difference in SOC amount and pH of soil, as a result of fertilization, which is a reason of variation in the profile of microorganisms of sandy soil.

1.14.3 Soil Enzyme Activity

The activities of various soil enzymes, such as alkaline phosphatases, dehydrogenase, proteases, and β -glucosidases, are essential indicators of soil fertility as well as microbial activity (Nannipieri et al. 1990). The long-term application of chemical fertilizer has no positive impact on microbial biomass and dehydrogenase activity, while organic fertilizers have positive influence on this. In addition, copper, a commonly found contaminant in soil because of excessive use of pesticides and fertilizers or irrigation, unfavorably affects the activity of soil dehydrogenase (Xie et al. 2009). Likewise, the organic nitrogen application stimulated different soil enzyme activities, such as saccharase and urease, in contrast to chemical fertilization. The significant decrease in enzyme activity, in inorganically treated soils than organic-treated soils continually cultivated with spring cereals, wheat, and clover, was observed (Balezentiene and Klimas 2009). The activities of various enzymes of soil, such as catalase, urease, invertase, arylsulfatase, and caseine protease and soil pH showed positive effect in organic-treated soils, but there is no considerable variation in functional activity of microorganisms of soils treated with organic and inorganic fertilizers (Lopes et al. 2011).

1.14.4 Soil Compaction

Soil compaction deals with the formation of thick, well-filled layers that occurs on cultivated ground; even more, the compressive forces are applied to compressible soil from wheels (Hamza and Anderson 2005). Soil compaction occurs by the lower use of organic fertilizer, heavy machinery use, constant use of chemical fertilizer and continual plowing at a consistent depth (Mari et al. 2008). In addition, compaction of soil causes problems, such as inadequate aeration, extreme soil strength, poor drainage, erosion, runoff, and soil degradations (Batey 2009). These changes lead to the decrease in permeability, hydraulic conductivity, and groundwater recharge (Blanco-Canqui et al. 2002; Mehmood et al. 2019). Soil compaction forms an essential part of land deterioration syndrome. It is a considerable threat facing agriculture which negatively impacts nearly all soil properties (Weisskopf et al. 2010). It alters soil structure by breaking aggregate units, decreasing the pore space size between the soil particles, lessening in soil volume and porosity resulting in an increase in the bulk density of soil.

1.14.5 Soil Acidification

Soil acidity is the concentration of H^+ in the solution. Acid soils are soils with pH levels below 7. The effect of constant and enormous application of N fertilizers, particularly as reduced N (NH_3 , NH_4^+), on the health of soil depends on the degree of its action on acidification (Table 1.3).

Nitrogen molecules which alter pH of the soil are ammonium ion, nitrate ion, and the urea molecule. The transformation of N from one form to the other and their uptake by plants influence the soil acidity. Acidification of soil, as a result of nitrogen fertilizer, relies on the kind of N added, soil buffering capacity, the net balance between proton-generating and consuming processes. Nitrogenous fertilizer, such as ammonium sulfate, binds to sulfuric acid in the soil with water. Due to the hydrogen ions emitted from the acid, it results in loss of soil nutrients replacing alkaline elements on the exchange sites. The free oxygen produced in the reaction oxidizes the organic matter, causing it to combust at low levels in the soil (Casiday and Frey 1998).

Ammonium-dependent fertilizers cause soil acidification as they produce two H^+ ions for each molecule of ammonia nitrified to nitrate. The degree of acidification relies on whether the nitrate is consumed by plant or is leached. When nitrate is absorbed by plant, the acidification is halved per molecule of ammonium compared to the nitrate leaching. It is because of the intake of one H^+ ion for each nitrate molecule taken up (Smiley and Cook 1973). Excess application of N fertilizers results in an increase in nitrate leaching and cations (Ca, Mg) to water bodies. The leached nitrate in the subsoil can lead to pyrite oxidation, which releases sulfate and other elements, such as arsenic (As), nickel (Ni), Copper (Cu), cobalt (Co), lead (Pb), manganese (Mn), and zinc (Zn).

Urea and anhydrous ammonia have a lesser acidification potential in comparison to ammonium-dependent products, as one H^+ ion is used in the transformation to ammonium. Nitrate-based fertilizers can raise pH of soil because one H^+ ion is absorbed by plant in the nitrate uptake.

Nitrogen-fixing bacteria and free-living fungi are very responsive to high levels of nitrogen and microbial community changes which influence various soil processes, such as nutrient cycling and mineralization of organic matter (Velthof et al. 2011). The nitrogen fixation process, that is, nitrification and denitrification, encourages nitrous oxide (N_2O) production, a greenhouse gas, in soil with less pH. Chemical

Table 1.3 pH and the degree of soil acidity

S. no.	Soil acidity	pH range
1.	Extremely acidic	<4.5
2.	Very strongly acidic	4.5–5.0
3.	Strongly acidic	5.1–5.5
4.	Moderately acidic	5.6–6.0
5.	Slightly acidic	6.1–6.5
6.	Neutral	6.6–7.3

fertilizer application causes an accumulation of acids, such as sulfuric acid and hydrochloric acid, to create a harmful impact on soil called soil friability. Such acids break down the soil crumbs that assist in holding the particles of rock together. The mixing of decomposed material such as dead leaves and humus with clay results in the formation of soil crumbs which help in improving air circulation in the soil and are important to soil drainage. Chemical fertilizers dissolve soil crumbs, which leads to a high compacted soil with decreased circulation of air and drainage (Tien and Chen 2012; Venkateshwarlu 1993).

Soil releases base cations, such as magnesium (Mg) and calcium (Ca), in the course of acidification process; the base cations may be decreased over time through continued addition of nitrogen and aluminum (Al^{3+}) released from minerals often reaching harmful stage, which cause nutrient deficiencies in plants. Indirectly, soil acidification results in decreased microbial N immobilization (Venterea et al. 2004). The acidification of soil can also influence the mineralization and decomposition of SOM, so that affects quality of SOM.

Soil acidity is influenced by phosphorus fertilizers through the gain or release of H^+ ions, depending on the type of P fertilizer used as well as soil pH. When phosphoric acid is added to the soil with pH less than 6.2, it releases one H^+ ion, and two H^+ ions will be released when the pH of soil is greater than 8.2. SSP, MAP, and TSP all can acidify the soil if the soil pH is above 7.2. They add phosphorus in the $H_2PO_4^-$ ion form. DAP, in the form of HPO_4^{2-} , is responsible for making acidic soils more alkaline. When the soil pH is greater than 7.2, it will not show any effect. In APP, phosphorus present in $P_2O_7^{4-}$ form undergoes hydrolysis and transforms to HPO_4^{2-} which is pH neutral. Thus, acidification as a result of APP is considered same as DAP.

1.14.6 Effect on Soil Biota

Soil microbial activity is a vital part of soil health. Bacteria, fungi, protozoa, algae, and viruses constitute soil microflora which form an important part of the agriculture ecosystem. These have many essential and basic roles in soil, such as soil fertility, nutrient cycling, enhancing productivity by increasing limited nutrient availability, and degradation of inorganic as well as organic matter (Fig. 1.2). Soil biodiversity is the cornerstone of global food security along with more aspects of agro-biodiversity, that is, plant and animal resources. The impact of fertilizer on microorganisms in the soil may be beneficial or harmful, and vary in duration relying on time-period considered, the amount, quality, and the way of application of fertilizer. More recently, it has been pointed out that diversity in microbial culture collection (MCC) is crucial in maintaining the health of soil (Mele and Crowley 2008; Shen et al. 2008) because of their role in the formation of soil structure, soil organic matter (SOM) decomposition, and the biogeochemical cycling of nutrients (Paul 2007).

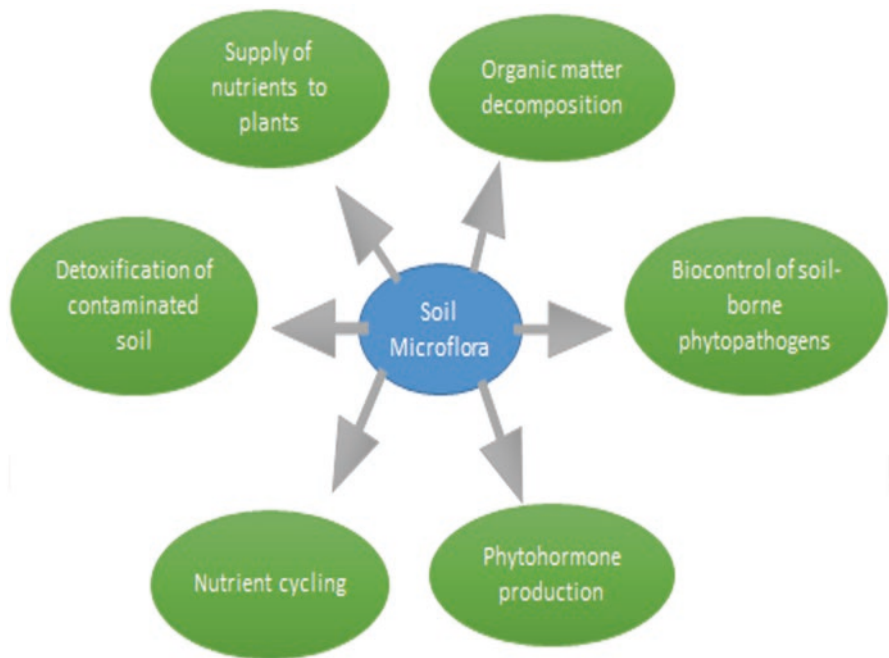


Fig. 1.2 Different functions of soil microorganisms

Agricultural activities, such as pesticide use, surplus inorganic fertilizer use, and tillage, influence soil microorganism by disturbing their habitats and functions (Kibblewhite et al. 2008). Fertilizers continue to remain in the soil for longer duration, so that they are confined to change soil microflora and thus affect soil health. The functional diversity of the soil microbial population is mainly controlled by the resource availability such as N, P, and C (Lupwayi et al. 2012). Therefore, there is an important co-relation between microbial communities and SOC and activities of microorganisms (Bohme et al. 2005). It clearly implies that the type as well as constituent of fertilizer used undoubtedly disrupt the structure of microbial community of the lands. Modification of soil with fertilizer greatly affects function and properties of soil, such as soil organic carbon (SOC), rhizodeposition, pH, soil nutrient content, enzyme activities of soil, moisture, and many others. All of these factors potentially result in an alteration in the soil microflora population dynamics. The constant use of pesticides and chemical fertilizers disturbs the functional as well as structural properties of soil microbial populations (Yang et al. 2000) and simultaneously forms a nutrient-imbalance in soils. Mycorrhizal fungi regularly reduced as a result of P fertilizer application, but the intensity of effect depends on the fungus species included and the amount of plant-available P.

The unlimited use of N fertilizers affects soil biota directly as well as indirectly and are caused by alteration in productivity, pH, SOM levels (Fig. 1.3). The disturbance of microorganisms of soil by application of the high ammonia fertilizer

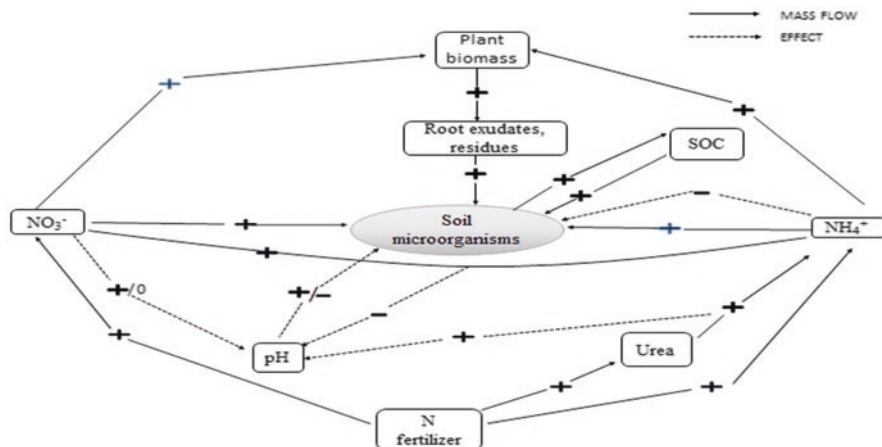


Fig. 1.3 A model showing direct and indirect effects of N fertilizer on microorganisms

concentration in bands is generally temporary, and only in the zone of application. Nitrogen fertilizer, such as urea, is transformed to anhydrous ammonia and carbon dioxide when it is consumed by bacteria. Ammonia is poisonous and is responsible for destroying life in the soil. The reaction takes place between urea and ammonia gas when urea is mixed with soil to form ammonium hydroxide with pH 11.6 (Anderson 2004). It is very caustic and creates serious burns. It forms an unsafe environment that destroys seedlings, seeds, plants, and soil dwelling organisms.

Potassium chloride associates with nitrates in the soil to form chlorine gas which destroys soil microorganisms. Excess potassium results in the deficiency of calcium in plants; some plants consume Ca, Mg, and K mostly in the percentage they are present in the soil. Excess potassium in the soil is responsible for the loss of soil structure (Anderson 2004). The decrease in soil air results in declined root respiration, and the generation of harmful compounds in plants. The reduction in soil air and inadequate calcium leads to reduction in the number of soil microbe and a subsequent reduction in the supply of organic matter/nutrients to plants (Pevear 1999).

Although total microbial counts are greater in fertilized soils relative to untreated soils, the impact is more definite in organic fertilizer treated soils than inorganic fertilizer treated soils, in the long run (Islam et al. 2009). In a few studies, an increase in organic C amount, microbial species and activities is observed in organic fertilizer-treated soils than inorganic ones in various crops, such as wheat, mustard, tobacco, and maize-wheat rotation (Chauhan et al. 2011; Yang et al. 2011). The structure of the bacterial population of inorganic fertilizer-treated soil is different from that of the untreated one (Sun et al. 2004). Furthermore, the population of bacteria, such as pseudomonas, a gram-negative bacteria, is affected by the constant use of chemical fertilizer, while the bacterial population in organic-treated soils remains similar in soils as that of untreated soil in crops, such as rice and wheat (Islam et al. 2009). In inorganic fertilizer-treated soils, the exposure of soil microflora to pesticides and heavy metals, such as cadmium and cypermethrin, is higher.

Additionally, lower cypermethrin dissipation rate is reported in soils. This indicates that, compared to organically treated soils, inorganic ones show more distinct effects of pollutants such as heavy metals (Xie et al. 2009). In agricultural environments treated with organic inputs, greater and more functionally diversified microbial communities were found as compared to inorganic ones in a wide range of crops (Tan et al. 2012). Fertilization has distinct effects on the composition of total agricultural soil bacteria. Chemical fertilization results in nutrient patches being produced, thus forming nutrient gradient in the soil that change the population of microbes (Wu et al. 2012). Li et al. (2013) investigated the effect of the N-gradient on nitrogen transition, microbial functional diversity, and soil microbial biomass due to chemical fertilizers such as ammonium sulfate or urea. The change with the N-gradient was observed in soil microbial biomass and microbial functional diversity. The magnitude of improvement, however, was governed by the type of inorganic fertilizer and concentration of nitrogen. Although functional diversity indices and microbial communities' average well color production (AWCD) decreased after ammonium sulfate treatment, the urea application resulted in the higher AWCD and Shannon indices. These have been found to differ with the soil layer depth. The impact of practices of soil management, can also differ with crop, especially in the form of fertilization.

1.15 Soil Organic Matter (SOM)

Different studies have reported that SOM alters with nitrogenous fertilizer inputs and cultivation; this is a problem which has become progressively contentious. SOM declines with cultivation where nitrogen fertilization is not performed, but may enhance with N fertilizer application. The use of nitrogen fertilizer influences SOM by way of two mechanisms:

1. It may help in increasing SOM by promoting growth of plant so that it increases the quantity of litter and root biomass added to the soil against the soil without nitrogen fertilization.
2. It can increase the loss of SOM by decay or microbial litter transformation (straw, leaves, etc.) and native types of organic carbon already present (Recous et al. 1995). Ammonium sulfate binds to sulfuric acid in the soil with water. Due to hydrogen ions emitted from the acid, it results in the loss of soil nutrients replacing alkaline elements on the cation exchange sites. The free oxygen produced in the reaction, causes the organic matter to combust at low levels, by oxidation of organic matter (Cassiday 1988).

1.16 Soil Salinity

Salts are a major content in chemical fertilizers and are believed to be destructive to agriculture, as salts are damaging to soil and plants. The dissolution of chemical fertilizer applied to the soil affects many soil properties, in particular the salinity. The salinity induced by N can adversely influence the nitrification process in soil, resulting in accumulation of NO_2 in the soil (Akhtar and Alam 2001). Upon applying phosphorus fertilizer, such as DAP, the concentration of soluble salts gets increased which causes salinity. Increased salinity causes inhibition of nitrification process, resulting in lesser transformation of ammonia (NH_4) to nitrate-nitrogen, therefore, an enormous amount of N was present in the form of NH_4 at the highest level of salinity (Irshad et al. 2018). K fertilizers usually have significant effects on salinity. The application of potassium fertilizer, typically based on sulfate or chloride salts, may lead to salinization of soil because of their large salt index (Pirhadi et al. 2018).

1.17 Effect on Plants

Soil fertility and vegetation are highly dependent upon a sufficient supply of essential minerals and nutrients. The excessive use of nutrients causes a soil nutrient supply imbalance, which lead, to deterioration of stable soil. Chemical fertilizers, however, help plants to grow quickly; but plants growing this way have little time to develop proper root growth. Plants are incapable of making their stems strong, or nutritious vegetables and fruits. In these circumstances, chances of survival are likely to be lower as they tend to be more susceptible to diseases and pests. Moreover, chemical fertilizers impede sufficient water intake for the plants, resulting in root burning or fertilizer burning.

1.18 Conclusion

The use of fertilizers today is viewed as a technology needed for agriculture. The long-term application of chemical fertilizers has a negative impact on the physico-chemical properties and soil biological properties. Chemical fertilizers disrupt the microorganisms in respect of their dominant soil species and structural and functional diversity. Soil reaction and electrical conductivity were influenced by inorganic fertilizer addition. NPK fertilizer application appears mainly to reduce the functions of various soil enzymes.

Chemical fertilizers should be applied in due time and in appropriate quantities. First, examination of the soil should be carried out carefully. Fertilizer should be given to the soil after that. The chemical content and structure of the soil should be

understood, and the most effective fertilizer should be picked. There should be processing of the most appropriate method. Otherwise, it will lead to loss of both finance and energy. Fertilizing ought to be performed in time, should not be at inappropriate times.

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Chapter 2

Microbial Bioremediation of Pesticides/ Herbicides in Soil



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2.1 Introduction

The term “pesticide” is characterized as a material or a combination of materials proposed to repel, devastate, or control several pests together with carriers of human or other faunal diseases, undesirable faunal/floral species, leading to destruction regardless meddling with the preparing, handling, stockpiling, or promoting of foodstuff, horticultural possessions, timber and timber items, or foodstuffs of animal, or might be directed to animals, for the management of bugs or other pests in or on their bodies (FAO and US EPA 2014). Pesticides are sorted primarily dependent on their combat targets or similarities in their chemical structure. Examples of the earlier are insecticides, acaricides, nematocides, fungicides, herbicides, or more rarely rodenticides, molluscicides, or plant growth regulators (Table 2.1), while the case of the latter is given in Tables 2.2–2.13. Herbicides are a class of pesticides that are utilized to execute weeds and other undesirable life forms, including insects, while fungicides are utilized to confine the development of molds and mildew. The use of disinfectants forbid the outbreak of bacteria and is also used to control mice and rodents (Fig. 2.1).

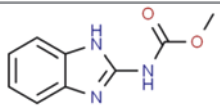
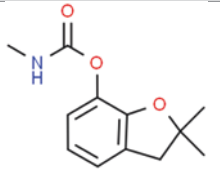
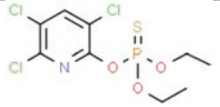
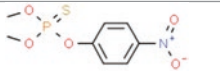
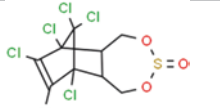
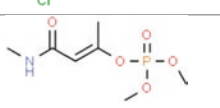
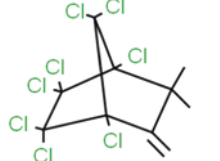
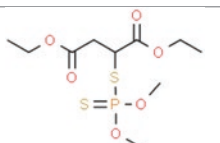
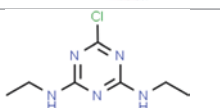
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Table 2.1 Characterization of pesticides based on their target

S. No	Pesticides	Chemical nature	Target hosts	Chemical structure
1.	Carbendazim	Fungicides	<i>Pericularia oryzae</i>	
2	Carbofuran	Nematodes	<i>Hershieminella oryzae</i>	
3	Chloropyrifos	Insecticide	Yellow stem borer	
4	Methyl parathion	Insecticide	Stem and leaf borer	
5	Endosulfan	Insecticide	Fruit and leaf borer	
6	Monocrotophos	Insecticide	Brown plant hopper	
7	Toxaphene	Insecticide	Fruit borer	
8	Malathion	Insecticides	Leaf hoppers	
9	Simazine	Herbicides	Grass plants	

(continued)

Table 2.1 (continued)

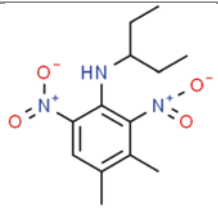
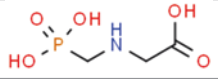

S. No	Pesticides	Chemical nature	Target hosts	Chemical structure
10	Pendimethalin	Herbicides	Broad leaves, Grass	
11	Glyphosate	Herbicides	Cyanodondoctylon	
12	Phorate	Nematodes	<i>Hershieminella oryzae</i>	

Table 2.2 Characterization of pesticides based on their chemical nature

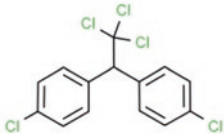
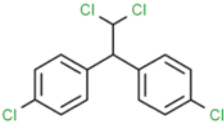
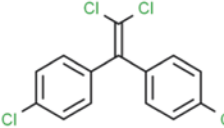
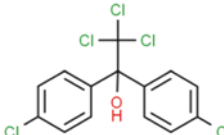
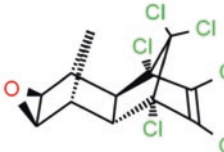
S. no	Chemical group	Chemical names
1	Organochlorines	BHC, Chlordane, Chloro propylate, Chlorobenziate, DDT, DDD, Dicofol, Dieldrin, Eldrin, Endosulfan., Heptachlor, Isobenzan, Isodrin, Lindane, Methoxychloro Aldrin, Toxaphene
2	Organophosphates	Abate, Caumphos, Dimefox, Dicrotophos, Dimethoate, Dichlorovas, Demethon-S-methyl, Dipterex, Fenitrothion, Fenthion, Malathion, Mipafos, Methyl Parathion, Oxydemeton-methyl, Phorate, Phosphamidon, Ronnel, Trichlorofan
3	Carbamates	<i>Methyl</i> Aminocarb, Aldicarb, Carbaryl, Carbanolate, Carbofuran, Dimetilan, Isolan, Propoxur, Pyrolan <i>Thio</i> Butylate, Cycloate, Diallyate, Pebulate, Trillate, Thiourea, Vernolate <i>Dithio</i> Dithane M-45, Thiram, Ferbam, Amoban, Naban, Zineb, Maneb, Ziram Polyran
4	Pyrethroids	Allethrin, Alphamethrin, Bifenthrin, Cypermethrin, Cyclethrin, Dimethrin, Decamethrin, Furethrin, Fenvalerate, Pyrethrin, Tetramethrin
5	Phenyl amides	<i>Carbanilates</i> Barban, Bromuron, Carbetamide, Chlororprofan, Prophan, Phenyl Urea, Fenuron, Monuron, Diuron, Flumeturon, Chloroxuron, Neburon <i>Acylanalide</i> Propanil, Solan, Dicryl, Karsil, Propachlor, Alachlor, Butachlor <i>Toluidines</i> Trifluralin, Dipropanil, Benefin, Oryzalin, Isopropanil, Nitralin <i>Acetamide</i> Diphenamid

(continued)

Table 2.2 (continued)

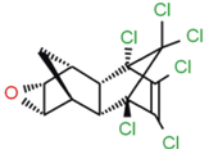
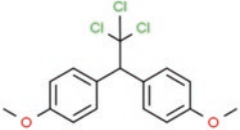
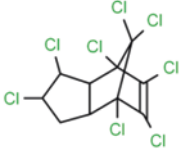
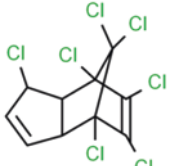
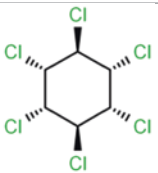
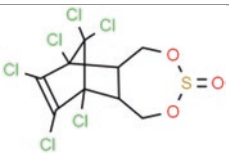
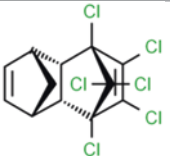
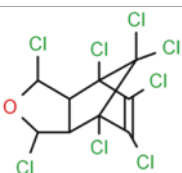
S. no	Chemical group	Chemical names
6	Phenoxy alkonates	2,4-D(2,4 Dichloro phenoxy acetic acid) 2,4 5 T(2,4 5 Trichloro Phenoxy acetic acid) Dichloroprop, Mecoprop, Erbin, Sesone
7	Trazines	Atrazine, Simazine, Ametryn, Atraton, Chlorazine, Cynazine, Cyprazine, Metribuzin, Propazine, Turbutryn, Simetryn
8	Benzoic acid	Dicamba, Dichlorobenil, Chloroambin, Tricamba, Neptalan, Bromoxynil
9	Phthalimide	Captan, Diflolan, Folpet
10	Dipyrids	Paraquat, Diaquat
11	Others	Pentachlorophenol, Floroacetate, Phenyl mercuric acetate, Ethyl mercuric Phosphate, Methyl mercuric chloride, Sodium arsenate, Calcium arsenate, Lead arsenate, Cacodylic acid, Aluminium phosphide, Zinc phosphide

Table 2.3 Organochlorine pesticides, their chemical structures, and use

S. no	Chemical name	Chemical structures	Use
1	Dichlorodiphenyltrichloroethane (DDT) (C ₁₄ H ₉ Cl ₅)		Acaricide and insecticide
2	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) (C ₁₄ H ₁₀ Cl ₄)		Insecticide
3	Dichloro diphenyl dichloroethane (DDE) (C ₁₄ H ₈ Cl ₄)		Insecticide
4	Dicofol (C ₁₄ H ₉ Cl ₅ O)		Acaricide
5	Endrin (C ₁₂ H ₈ Cl ₆ O)		Insecticide

(continued)

Table 2.3 (continued)

S. no	Chemical name	Chemical structures	Use
6	Dieldrin (C ₁₂ H ₈ Cl ₆ O)		Insecticide
7	Methoxychlor (C ₁₆ H ₁₅ Cl ₃ O ₂)		Insecticide
8	Chlordane (C ₁₀ H ₆ Cl ₈)		Insecticide
9	Heptachlor (C ₁₀ H ₅ Cl ₇)		Insecticide
10	Lindane (C ₆ H ₆ Cl ₆)		Acaricide, insecticide, and rodenticide
11	Endosulfan (C ₉ H ₆ Cl ₆ O ₃ S)		Insecticide
12	Isodrin (C ₁₂ H ₈ Cl ₆)		Insecticide
13	Isobenzan (C ₉ H ₄ Cl ₈ O)		Insecticide

(continued)

Table 2.3 (continued)

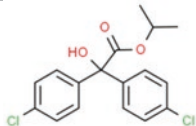
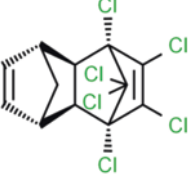

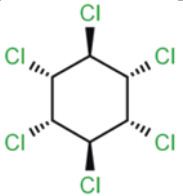
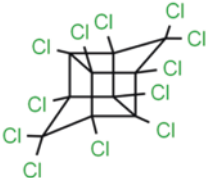
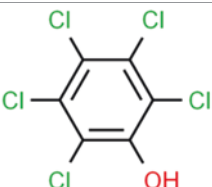
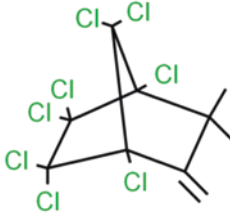
S. no	Chemical name	Chemical structures	Use
14	Chlorpropylate ($C_{17}H_{16}Cl_2O_3$)		Insecticide and acaricide
15	Aldrin ($C_{12}H_8Cl_6$)		Insecticide
16	1,4- dichlorobenzene ($C_6H_4Cl_2$)		Insecticide
17	Benzene hexachloride (BHC) ($C_6H_6Cl_6$)		Acaricide, insecticide, and rodenticide
18	Mirex ($C_{10}Cl_{12}$)		Insecticide
19	Pentachlorophenol (C_6HCl_5O)		Fungicide, herbicide, and insecticide
20	Toxaphene (Camphechlor) ($C_{10}H_{10}Cl_8$)		Acaricide and insecticide

Table 2.4 Organophosphates pesticides, their chemical structures, and use

S. no	Chemical name	Chemical structures	Use
1	Dimefox ($C_4H_{12}FN_2OP$)		Insecticide
2	Mipafox ($C_6H_{16}FN_2OP$)		Insecticide
3	Methyl Parathion ($C_8H_{10}NO_5PS$)		Insecticide
4	Ronnel ($C_8H_8Cl_3O_3PS$)		Insecticide
5	Fenitrothion ($C_9H_{12}NO_5PS$)		Insecticide
6	Dicrotophos ($C_8H_{16}NO_5P$)		Insecticide
7	Phorate ($C_7H_{17}O_2PS_3$)		Insecticide and acaricide
8	Fenthion ($C_{10}H_{15}O_3PS_2$)		Insecticide
9	Caumaphos ($C_{14}H_{16}ClO_5PS$)		Insecticide
10	Abate ($C_{16}H_{20}O_6P_2S_3$)		Larvicide
11	Dichlorvos ($C_4H_7Cl_2O_4P$)		Insecticide

(continued)

Table 2.4 (continued)

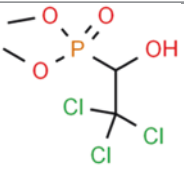
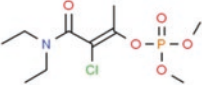
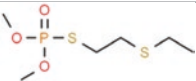
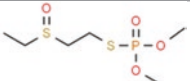
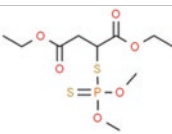
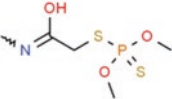
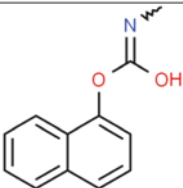
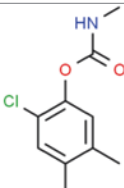
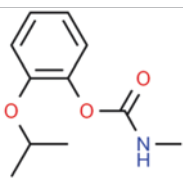
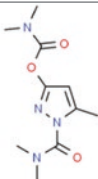
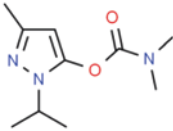
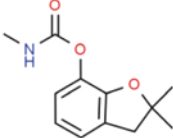
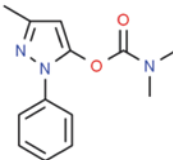
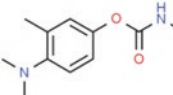
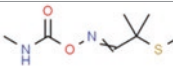
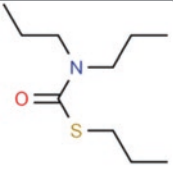
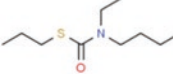
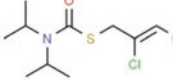
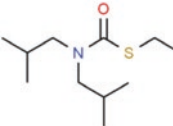
S. no	Chemical name	Chemical structures	Use
12	Dipterex (C ₄ H ₈ Cl ₃ O ₄ P)		Insecticide
13	Phosphamidon (C ₁₀ H ₁₉ ClNO ₅ P)		Insecticide
14	Demethon-S-methyl (C ₆ H ₁₅ O ₃ PS ₂)		Insecticide and acaricide
15	Oxydemeton-methyl (C ₆ H ₁₅ O ₄ PS ₂)		Insecticide
16	Malathion (C ₁₀ H ₁₉ O ₆ PS ₂)		Insecticide
17	Dimethoate (C ₅ H ₁₂ NO ₃ PS ₂)		Insecticide and acaricide

Table 2.5 Carbamate pesticides, their chemical structures, and use

	S. no	Chemical name	Chemical structures	Use
<i>Methyl</i>	1	Carbaryl (C ₁₂ H ₁₁ NO ₂)		Insecticide
	2	Carbanolate (C ₁₀ H ₁₂ ClNO ₂)		Insecticide
	3	Propoxur (C ₁₁ H ₁₅ NO ₃)		Insecticide

(continued)

Table 2.5 (continued)

	S. no	Chemical name	Chemical structures	Use
	5	Dimetilan (C ₁₀ H ₁₆ N ₄ O ₃)		Insecticide
	6	Isolan (C ₁₀ H ₁₇ N ₃ O ₂)		Aphicides and insecticides
	7	Carbofuran (C ₁₂ H ₁₅ NO ₃)		Insecticide
	8	Pyrolan (C ₁₃ H ₁₅ N ₃ O ₂)		Insecticide
	9	Aminocarb (C ₁₁ H ₁₆ N ₂ O ₂)		Insecticide
	10	Aldicarb (C ₇ H ₁₄ N ₂ O ₂ S)		Insecticide
<i>Thio</i>	1	Vernolate (C ₁₀ H ₂₁ NOS)		Herbicides
	2	Pebulate (C ₁₀ H ₂₁ NOS)		Herbicides
	3	Diallate (C ₁₀ H ₁₇ C ₁₂ NOS)		Herbicide
	4	Butylate (C ₁₁ H ₂₃ NOS)		Herbicide

(continued)

Table 2.5 (continued)

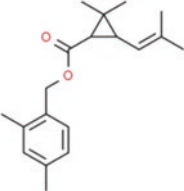
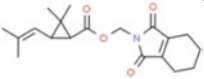
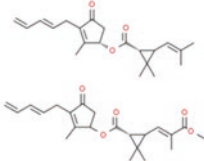
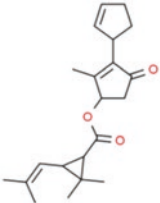
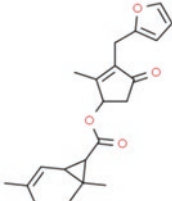
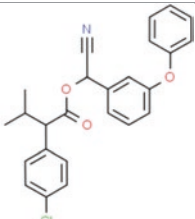
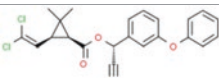
S. no	Chemical name	Chemical structures	Use	
5	Cycloate (C ₁₁ H ₂₁ NOS)		Herbicide	
6	Triallate (C ₁₀ H ₁₆ Cl ₂ NOS)		Herbicide	
7	Thiourea (CH ₄ N ₂ S)		Herbicide	
<i>Dithio</i>	1	Thiram (C ₆ H ₁₂ N ₂ S ₄)		Fungicide
	2	Ferbam (C ₉ H ₁₈ FeN ₃ S ₆)		Fungicide
	3	Zineb (C ₄ H ₆ N ₂ S ₄ Zn)		Fungicide
	4	Maneb (C ₄ H ₆ MnN ₂ S ₄)		Fungicide
	5	Ziram (C ₆ H ₁₂ N ₂ S ₄ Zn)		Fungicide
	6	Dithane M- 45 (C ₈ H ₁₂ MnN ₄ S ₈ Zn)		Fungicide

Table 2.6 Pyrethroid pesticides, their chemical structures, and use

S. no	Chemical name	Chemical structures	Use
1	Allethrin (C ₁₉ H ₂₆ O ₃)		Insecticide
2	Bifenthrin (C ₂₃ H ₂₂ ClF ₃ O ₂)		Insecticide

(continued)

Table 2.6 (continued)

S. no	Chemical name	Chemical structures	Use
3	Dimethrin (C ₁₉ H ₂₆ O ₂)		Insecticide
4	Tetramethrin (C ₁₉ H ₂₅ NO ₄)		Insecticide
5	Pyrethrin (C ₄₃ H ₅₆ O ₈)		Insecticide
6	Cyfluthrin (C ₂₁ H ₂₈ O ₃)		Insecticide
7	Fenrethrin (C ₂₁ H ₂₆ O ₄)		Insecticide
8	Fenvalerate (C ₂₅ H ₂₂ ClNO ₃)		Insecticide
9	Alphamethrin (C ₂₂ H ₁₉ Cl ₂ NO ₃)		Insecticide and acaricide

(continued)

Table 2.6 (continued)

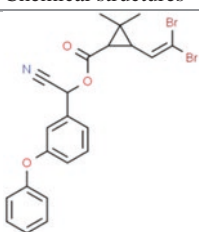
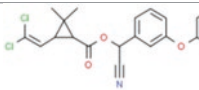
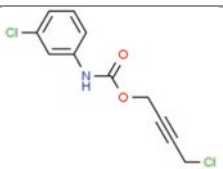
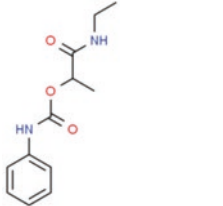
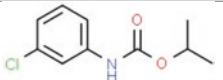
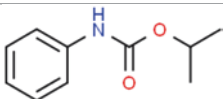
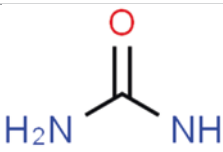
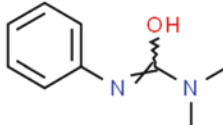
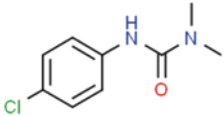
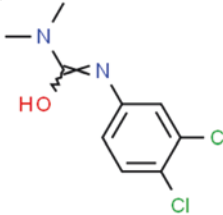
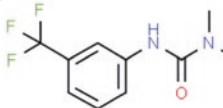
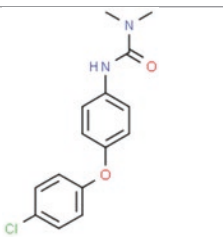
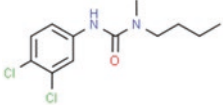
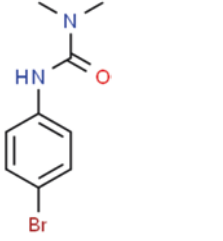
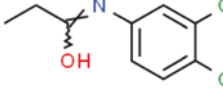
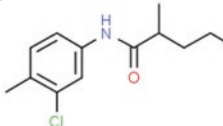
S. no	Chemical name	Chemical structures	Use
10	Decamethrin (C ₂₂ H ₁₉ Br ₂ NO ₃)		Insecticide
11	Cypermethrin (C ₂₂ H ₁₉ Cl ₂ NO ₃)		Insecticide and acaricide

Table 2.7 Phenyl amide pesticides, their chemical structures, and use

	S. no	Chemical name	Chemical structures	Use
<i>Carbanilates</i>	1	Barban (C ₁₁ H ₉ Cl ₂ NO ₂)		Herbicide
	2	Carbetamide (C ₁₂ H ₁₆ N ₂ O ₃)		Herbicide
	3	Chlorpropham (C ₁₀ H ₁₂ ClNO ₂)		Herbicide
	4	Propham		Herbicide
	6	Urea CH ₄ N ₂ O		Fungicide and herbicide
	7	Fenuron (C ₉ H ₁₂ N ₂ O)		Herbicide

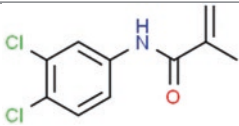
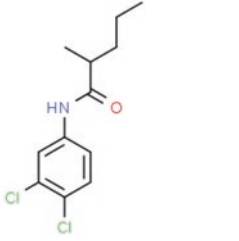
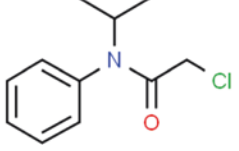
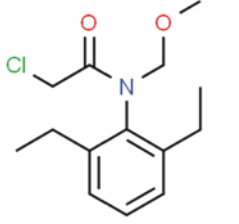
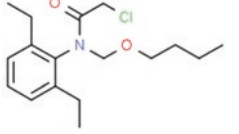
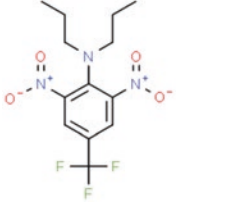
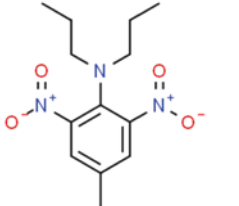
(continued)

Table 2.7 (continued)

	S. no	Chemical name (Chemical formula)	Chemical structures	Use
	8	Monuron (C ₉ H ₁₁ ClN ₂ O)		Herbicide
	9	Diuron (C ₉ H ₁₀ Cl ₂ N ₂ O)		Herbicide
	10	Flumeturon (C ₁₀ H ₁₁ F ₃ N ₂ O)		Herbicide
	11	Chloroxuron (C ₁₅ H ₁₅ ClN ₂ O ₂)		Herbicide
	12	Neburon C ₁₂ H ₁₆ Cl ₂ N ₂ O		Herbicide
	13	Bromuron (C ₉ H ₁₁ BrN ₂ O)		Herbicide
<i>Acylalanide</i>	1	Propanil (C ₉ H ₉ Cl ₂ NO)		Herbicide
	2	Solan (C ₁₃ H ₁₈ ClNO)		Herbicide

(continued)

Table 2.7 (continued)

	S. no	Chemical name	Chemical structures	Use
	3	Dicryl (C ₁₀ H ₉ Cl ₂ NO)		Herbicide
	4	Karsil (C ₁₂ H ₁₅ Cl ₂ NO)		Herbicide
	5	Propachlor (C ₁₁ H ₁₄ ClNO)		Herbicide
	6	Alachlor (C ₁₄ H ₂₀ ClNO ₂)		Herbicide
	7	Butachlor (C ₁₇ H ₂₆ ClNO ₂)		Herbicide
<i>Toluidines</i>	1	Trifluralin (C ₁₃ H ₁₆ F ₃ N ₃ O ₄)		Herbicide
	2	Dipropalin (C ₁₃ H ₁₉ N ₃ O ₄)		Herbicide

(continued)

Table 2.7 (continued)

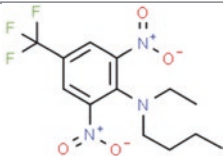
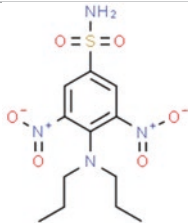
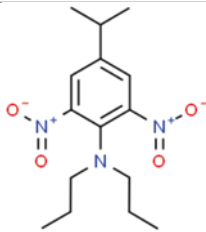
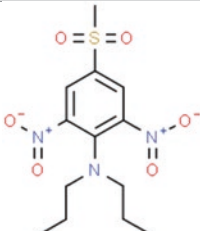
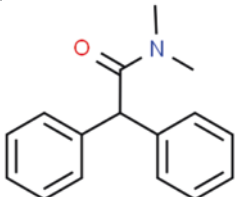
	S. no	Chemical name ($C_{13}H_{16}F_3N_3O_4$)	Chemical structures	Use
	3	Benefin ($C_{13}H_{16}F_3N_3O_4$)		Herbicide
	4	Oryzalin ($C_{12}H_{18}N_4O_6S$)		Herbicide
	5	Isopropalin ($C_{15}H_{23}N_3O_4$)		Herbicide
	6	Nitralin ($C_{13}H_{19}N_3O_6S$)		Herbicide
Acetamide	1	Diphenamid ($C_{16}H_{17}NO$)		Herbicide

Table 2.8 Phenoxy alkonnate pesticides, their chemical structures, and use

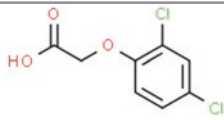
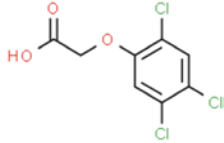
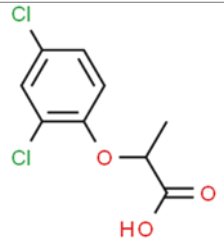
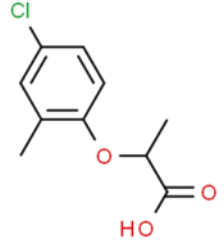
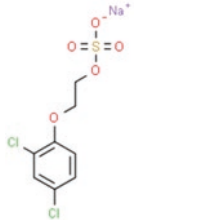
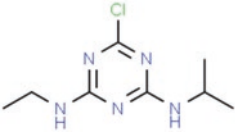
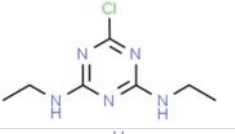
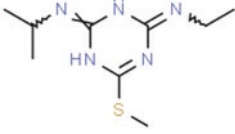
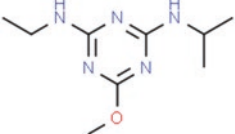
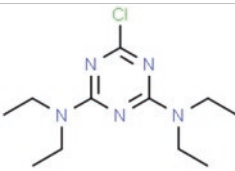
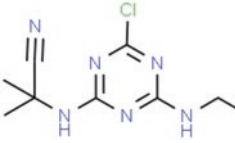
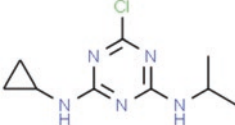
S. no	Chemical name	Chemical structures	Use
1	2,4-Dichloro phenoxy acetic acid ($C_8H_6Cl_2O_3$)		Herbicides
2	2,4, 5-Trichloro Phenoxy acetic acid ($C_8H_5Cl_3O_3$)		Herbicides
3	Dichloroprop ($C_9H_8Cl_2O_3$)		Herbicides
4.	Mecoprop ($C_{10}H_{11}ClO_3$)		Herbicides
5.	Sesone ($C_8H_7Cl_2NaO_5S$)		Herbicides

Table 2.9 Trazine pesticides, their chemical structures, and use

S. no	Chemical name	Chemical structures	Use
1	Atrazine (C ₈ H ₁₄ ClN ₅)		Herbicide
2	Simazine (C ₇ H ₁₂ ClN ₅)		Herbicide
3	Ametryn (C ₉ H ₁₇ N ₅ S)		Herbicide
4	Atraton (C ₉ H ₁₇ N ₅ O)		Herbicide
5	Chlorazine (C ₁₁ H ₂₀ ClN ₅)		Pesticide
6	Cynazine (C ₉ H ₁₃ ClN ₆)		Herbicide
7	Cyprazine (C ₉ H ₁₄ ClN ₅)		Pesticide

(continued)

Table 2.9 (continued)

S. no	Chemical name	Chemical structures	Use
8	Metribuzin (C ₈ H ₁₄ N ₄ OS)		Herbicide
9	Propazine (C ₉ H ₁₆ ClN ₅)		Herbicide
10	Terbutryn (C ₁₀ H ₁₉ N ₅ S)		Herbicide
11	Simetryn (C ₈ H ₁₅ N ₅ S)		Herbicide

Table 2.10 Benzoic acid pesticides, their chemical structures, and use

S. no	Chemical name	Chemical structures	Use
1	Dicamba (C ₈ H ₆ Cl ₂ O ₃)		Herbicide
2	Dichlobenil (C ₇ H ₃ Cl ₂ N)		Herbicide

(continued)

Table 2.10 (continued)

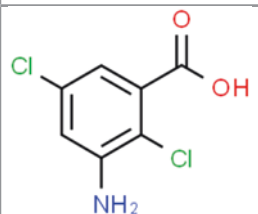
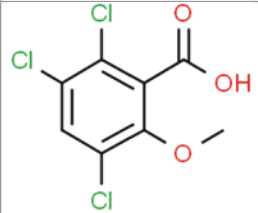
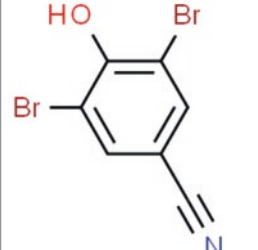
S. no	Chemical name	Chemical structures	Use
3	Chloramben ($C_7H_5Cl_2NO_2$)		Herbicide
4	Tricamba ($C_8H_5Cl_3O_3$)		Herbicide
6	Bromoxynil ($C_7H_3Br_2NO$)		Herbicide

Table 2.11 Phthalimide pesticides, their chemical structures, and use

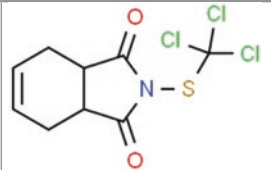
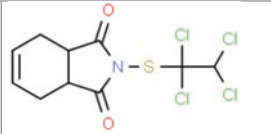
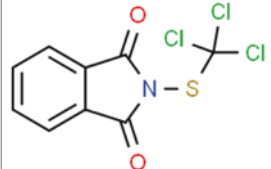
S. no	Chemical name	Chemical structures	Use
1	Captan ($C_9H_8Cl_3NO_2S$)		Fungicide
2	Diflotan ($C_{10}H_9Cl_4NO_2S$)		Fungicide
3	Folpet ($C_9H_4Cl_3NO_2S$)		Fungicide

Table 2.12 Dipyrids pesticides, their chemical structures, and use

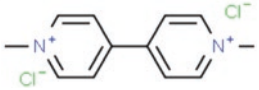
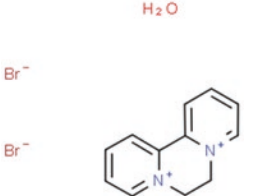
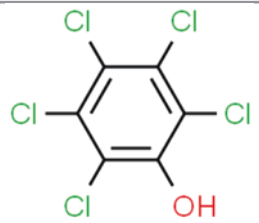
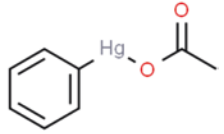
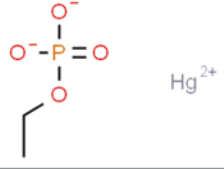
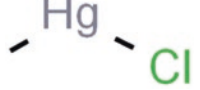
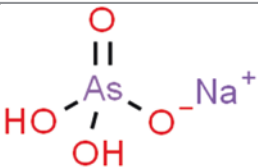
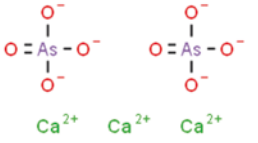
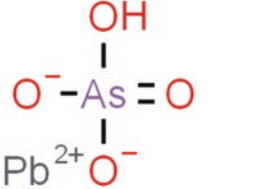
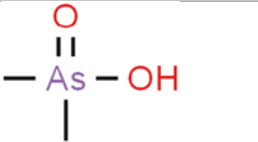

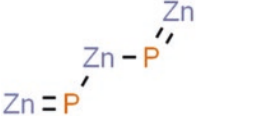
S. no	Chemical name	Chemical structures	Use
1	Paraquat (C ₁₂ H ₁₄ Cl ₂ N ₂)		Herbicide
2	Diaquat (C ₁₂ H ₁₄ Br ₂ N ₂ O)		Herbicide

Table 2.13 Other pesticides, their chemical structures, and use

S. sno	Chemical name	Chemical structures	Use
1	Pentachlorophenol (C ₆ HCl ₅ O)		Pesticide
3	Phenyl mercuric acetate (C ₈ H ₈ HgO ₂)		Herbicide and fungicide
4	Ethyl mercuric Phosphate (C ₂ H ₅ HgO ₄ P)		Pesticide
5	Methyl mercuric chloride (CH ₃ ClHg)		Pesticide

(continued)

Table 2.13 (continued)

S. sno	Chemical name	Chemical structures	Use
6	Sodium arsenate (H_2AsNaO_4)		Insecticides and herbicides
7	Calcium arsenate ($As_2Ca_3O_8$)		Germicide
8	Lead arsenate ($HAsO_4Pb$)		Insecticides
9	Cacodylic acid ($C_2H_7AsO_2$)		Herbicides
10	Aluminium phosphide (AIP)		Rodenticide and insecticide
11	Zinc phosphide (P_2Zn_3)		Rodenticide

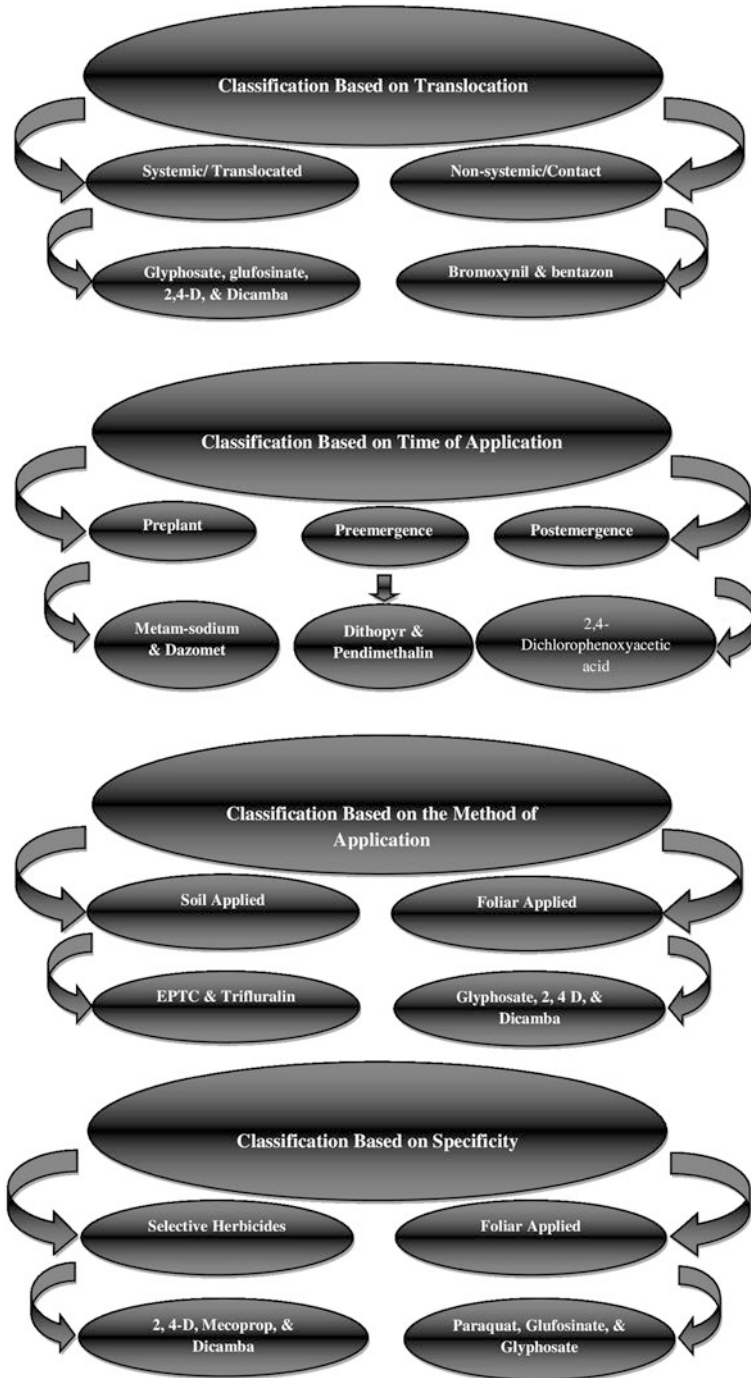


Fig. 2.1 Classification of herbicides

2.2 Merits of Pesticide Use

The primary, as well as secondary, assets are being provided by pesticides, the primary asset is evident by their direct usage, for example, the effect of killing caterpillars feeding on the harvest brings the prime advantage of increasing yields and improved quality of cabbage. The secondary assets are the consequence of the primary benefits and they are for longer periods. It follows that for secondary gain, it is consequently more complicated to set up cause and effect, however, in any case, they provide strong justifications for pesticide use. For instance, the increased yield of cabbage may provide supplementary income that possibly will be utilized towards education of children as well as their health care, which ultimately leads towards an improved, healthier, and better-educated community. In Table 2.14, we have provided the detailed results including primary and secondary advantages, and their connections. Around the world, 40% of the agronomical manufacture has vanished because of diseases occurring in plants, unwanted plants and pests on the whole. On the other hands, crops would have been in more loss if there would have been no pesticides. Additionally, these crop-sparing resources do not just shield the harvests from diseases inflicted by pests, they likewise improve the crop yields impressively. In their investigation, Webster et al. (1999) confirmed that “widespread economic losses” might be tolerated with no use of pesticides and quantified the considerable boost in yield and financial margin that result from pesticide use. Warren (1998) likewise drew awareness to the notable boost in yield of crops in the USA in the twentieth century. Furthermore, in presence of light, majority of the pesticides undergo chemical transformation to deliver metabolites that are rather non-toxic to both humans and the habitat (Kole et al. 1999; Bhat et al. 2017a, b; Bhat et al. 2018a, b; Mushtaq et al. 2018).

The handling of a broad array of human and flock disease vectors thus diminishing the quantity of contaminated persons and deaths, followed by the avoidance of global sickness spread is among critical obvious rewards of wide pesticide use. The killing of vectors is the best technique to battle them. Many insect-borne ailments, such as encephalitis, Rocky Mountain, typhoid fever, yellow fever, typhus, bubonic plague, ticks, and rodents, have been managed by the successful utilization of pesticides. As per the WHO, without the use of chemical control techniques, existence of life will be unsafe for an enormous extent of humanity (Anon 2004).

In the annihilation of various living beings that adversely affect human behavior, communications, and the tools of daily life, pesticides assume an important role. In various specified sectors of human operation, pesticides are used to handle undesirable species, such as preventing accelerated corrosion of metallic constructions, maintaining the turf on game pitches, golf courses and cricket grounds, serving an immensely common activity that provides hygienic air and exercise for a huge number of people around the globe (Maksymiv 2015; Bhat et al. 2017a, b).

Table 2.14 The primary and secondary benefits of pesticides

Primary advantages	Secondary advantages
1. Plant disease vectors and Controlling pests	Community advantages
(a) Improved crop/ flock quality (b) Reduced energy use for weeding (c) Reduced soil interruption (d) Invasive species prohibited	(a) Nutrition and fitness improved (b) Food security (c) Life likelihood increased (d) Reduced protection costs
2. Nuisances and organisms Controlling disease vectors	National benefits
(a) Human lives rescued (b) Human disorder reduced (c) Animal distress reduced (d) Increased stock quality	(a) National agricultural wealth (b) Increased export interest (c) Reduced soil erosion/ moisture loss
3. Prevent or manage of organisms that damage other human actions and structures	Global benefits
(a) Tree/bush/leaf hazards stopped (b) Recreational sod protected (c) Frame structures protected	(a) Less demands on uncropped land (b) Scanty pest introductions outside (c) Global tourism revenue

2.3 Risks Associated with Pesticide Use

Certainly, the broad utilization of pesticides and chemical fertilizers boost agricultural productivity. Nonetheless, from the last 20 years, the pesticide utilization throughout the globe has been growing and their occurrence grew to be inescapable in freshwater and marine environments. As indicated by United States Environmental Protection Agency (2012), the pesticide utilization had been lifted from two billion kg to two and half billion kg between year 2000 and 2007 all over (Staley et al. 2015). In systems of croplands throughout the globe, utilization of pesticides was up to 328,854.2 tonnes in 2007, in which commitments of herbicides was used to be most (200,487.66 tonnes), trailed by insecticides (78,471.42 tonnes), fungicides (19,958.05 tonnes), pesticides (18,597.27 tonnes), rodenticides (11,339.8 tonnes) and other pesticides (18,597.27 tonnes), respectively. In the Indian context, the utilization of herbicides, fungicides, and insecticides was 20,618.83, 13,055.44, and 6334.98 tonnes, respectively in 2010 (Roser and Ritchie 2017). The normal application of pesticides (kg ha^{-1}) per zone of cropland has been expanded much since the 1990s in many countries, such as Columbia (1990–3.64, 2014–20.79) chased by China, (1990–0 but in 2014 accomplished to 14.82), while in India the utilization rate was 0.44 in 1990 which reduced to 0.24 (kg ha^{-1}) further in 2010 (Roser and Ritchie 2017).

Pesticides have dire effects on non-target species and have an effect on plant and animal biodiversity, including both terrestrial and aquatic communities. Over 95% of herbicides and 98% of splashed insecticides arrive at a goal besides their objective species, together with non-target species, atmosphere, H₂O and soil (Miller 2004). Unscientific and unseemly use may likewise decrease the populace of useful organisms existing within the soil. The chemical fertilizers and pesticides entering the soil in critical amounts pose an immediate threat to the biological aspects of soil biota, health hazards, and environmental contamination (Martin et al. 2011; Dar et al. 2013; Dar and Bhat 2020). They cause abiding alterations to the flora present within the soil (Aleem et al. 2003; Bhat et al. 2018a; Bhat et al. 2018b; Singh et al. 2020a, b), hazardous impact on fertility of soil and yield of crops, having strong negative impact on nitrogen-fixing microorganisms (Sachin 2009), intervene with process of ammonification, negative impact on fungal existence associated with plant roots and nodulation in legumes (Reinhardt et al. 2008). The detailed study proved that target pests get hold of only about 0.1% of the tested pesticides, whereas remaining 99.9% is targeted into the soils, therefore manipulating the biota inside the soil and soil health parameters, such as enzyme activities, diversity of microbes (Singh et al. 2018). Hill reaction or attack against the PS-II is found in herbicides such as acylanilides, cyclic urea, phenyl carbamates, triazinones, triazines and urea, while others such as paraquat and diquat, are negative in PS-I. The mode of action of pesticides may vary among species of microorganisms and are known to inhabit cellular processes such as photosynthesis, respiration, and chemical reactions concerned with biosynthetic pathways, cell division, process of growth, molecular composition, etc. (DeLorenzo et al. 2001).

Pesticides can produce grave troubles for individual well-being; for example, genomic mutations, and may additionally cause fatality (Morillo and Villaverde 2017). Generally, there are four main ways through which pesticides can go into the individual body: oral, dermal, respiratory pathways, and through the eyes. The main path of human contact to pesticides is through water, food chain, soil, air, plants, and animals (Anderson and Meade 2014; Mehmood et al. 2019). Among the several groups of pesticides, insecticides are treated as the most lethal, followed by fungicides and herbicides, as far as their toxicity is concerned.

Water-soluble pesticides get easily dissolved in H₂O and come into groundwater, lakes, and rivers thus causing harm to non-targeted species. Conversely, the pesticides which are soluble in fats invade the animal bodies by a procedure recognized as “bioamplification” and get retained in the oily tissues of animals, consequently resulting in the perseverance of pesticides for prolonged durations in food chains (Daley et al. 2009; Dar et al. 2020).

2.4 Microbial Bioremediation

The remediation techniques which are used to overcome the polluted H₂O and soil by these pesticide sprays can be biological, physical, and chemical, or a grouping of a few or all of these processes (Pascal-Lorber and Laurent 2011). By tradition, the pollution caused by the pesticides has been reduced utilizing various physical and chemical methods whereby soils are later shifted to specific landfills, where the substances may likewise be burned or neutralized nearby (McGuinness and Dowling 2009). The frequently utilized chemical and physical strategies are not only costly but their side-products are also dangerous to the surroundings (Gaur et al. 2014). Moreover, when evaluation of soils is done by this mode, the disturbance caused in ecosystems is serious and restoration possibly will take generations (Dijkgraaf and Vollebergh 2004). As these strategies are so expensive and comprehensive, they found advantage in only small areas with high pollution; however, their use on extensive areas with limited pollution is unpracticable (Jin et al. 2006; Dar et al. 2016).

Data has indicated that there are new convenient remediation strategies for the end of these ecological pollutants and, among them, bioremediation is the most convenient and naturally accepted management that utilizes life forms or their outputs to diminish or kill the unfavorable impacts of toxic waste in the surroundings (Quintella et al. 2019; Mushtaq et al. 2020). The procedure is improved either during bioaugmentation, which includes the addition of extrinsic micro-biota into polluted soil or during biostimulation, where the inventory of supplements administered into the soil is changed, which ultimately quickens the breakdown ability of native microorganisms (Imam et al. 2019). A huge variety of micro-biota, for example, fungi, bacteria, algae, and genetically modified organisms have been examined in detail for terrestrial and aquatic habitat bioremediation (Alegbeleye et al. 2017; Khanday et al. 2016).

A few ecological parameters, for example, amount of moisture, degree of heat or coolness, and pH, alter the development of micro-biota. Control and maintenance of these frameworks can extend the pace of breakdown process significantly (Guarino et al. 2017). There are several fungal species (*Lecanicillium muscarium*, *Pestalotiopsis palmarum*, *Pleurotus eryngii*, *Phanerochacte chryosporium*, *Trametes versicolor*, etc.), algal species (*Anabaena*, *Chlorella*, *Chlamydomonas reinhardtii*, *Cladophora*, *Monoraphidium braunii*, *Oscillatoria*, *Phaeodactylum tri-cornutum*, *Spirulina* sp., *Scenedesmus*, etc.) and bacterial species (*Acinetobacter radioresistens*, *Enterobacter hormaechei*, *Pseudomonas aeruginosa*, *Rhodococcus erythropolis*, etc.), which are having a distinct catabolic route for the breakdown of pollutants at the site of contamination. There are also examples of some species of microorganisms which degrade contaminants solely under in vitro conditions (Abtahi et al. 2020; Singh et al. 2020a, b; Bwapwa et al. 2017). Microbial bioremediation of various pesticides and herbicides is summarized in Table 2.15.

Table 2.15 Microbial bioremediation of various Pesticides and herbicides

Phenoxyacetic acid (C ₈ H ₈ O ₃) herbicide	Degrading strain	References
2,4-D (C ₈ H ₆ Cl ₂ O ₃)	<i>Bacterium globiforme</i>	Audus (1951)
	<i>Flavobacterium aquatile</i>	Jensen and Petersen (1952)
	<i>Clavibacter michiganese</i> ATZ1	Johnson et al. (1967)
	<i>Pseudomonas</i> sp. N.C.I.B.9340	Gaunt and Evans (1971)
	<i>Aspergillus niger</i>	Loos et al. (1967a)
	<i>Arthrobacter</i> sp.	Loos et al. (1967b)
	<i>Pseudomonas</i> sp. HV3	Kilpi et al. (1983)
	<i>Cupriavidus necator</i> JMP 134	Don et al. (1985)
	<i>Flavobacterium</i> sp. 50001	Chaudhry and Huang (1988)
	<i>Sphingomonas herbicidovorans</i> MH	Zipper et al. (1998)
	<i>Xanthobacter</i> sp. CP	Ditzelmüller et al. (1989)
	<i>Pseudomonas</i> sp. EST4002	Ausmees and Kheřnaru (1990)
	<i>Azotobacter chroococcum</i>	Balajee and Mahadevan (1990), Chinalia et al. (2007)
	<i>Alcaligenes eutrophus</i> JMP134	Haugland et al. (1990)
	<i>Alcaligenes eltrophui</i> , <i>A. faecali</i> , <i>A. paradoxus</i> 2811P, <i>Sphingomonas paucimobil</i> , <i>Pseudomonas picketti</i> , <i>P. solanacearum</i> ,	Ka et al. (1994)
	<i>Comamonas testosteroni</i> JH5	Arensdorf and Focht (1995)
	<i>Pseudomonas aeruginosa</i> PA0lc	Filer and Harker (1997)
	<i>Pseudomonas cepacia</i> P166	Noh et al. (2000)
	<i>Halomonas</i> sp. EF43	Kleinstauber et al. (2001)
	<i>Bradyrhizobium</i> sp. HW13	Kitagawa et al. (2002)
<i>Pseudomonas putida</i>	Khalil (2003)	
<i>Aeromonas hydrophila</i> IBRB-36 4CPA	Markusheva et al. (2004)	

(continued)

Table 2.15 (continued)

Phenoxyacetic acid (C ₈ H ₈ O ₃) herbicide	Degrading strain	References
	<i>Achromobacter xylosoxidans</i> subsp. <i>denitrificans</i> EST4002	Vedler et al. (2004)
	<i>Sphingomonas</i> sp. TFD44	Thiel et al. (2005)
	<i>Corynebacterium</i> sp. SOGU16	Igbinosa et al. (2007a, b)
	<i>Achromobacter</i> sp. SOGU11	Igbinosa et al. (2007a, b)
	<i>Penicillium</i> sp.	Silva et al. (2007)
	<i>Sphingomonas agrestis</i>	Shimojo et al. (2009)
	<i>Delftia acidovorans</i>	González et al. (2012)
	<i>Burkholderia</i> , <i>Dyella</i> , <i>Mycobacterium</i> , <i>Microbacterium</i>	González-Cuna et al. (2016)
	<i>Achromobacter</i> sp. QXH	Quan et al. (2015)
	<i>Burkholderia cepacia</i> (DS-1), <i>Pseudomonas</i> sp. (DS-2) and <i>Sphingomonas paucimobilis</i> (DS-3)	Cycoñ et al. (2011)
	<i>Cupriavidus campinensis</i> BJ71	Han et al. (2015)
	<i>Cupriavidus necator</i> strain N-1	Zabaloy and Gómez (2014)
	<i>Pseudomonas</i> sp.; <i>Stenotrophomonas species</i> , <i>Alphaproteobacteria</i> , <i>Betaproteobacteria</i> , <i>Gammaproteobacteria</i>	Han et al. (2014)
	<i>Achromobacter anxifer</i> LZ35	Xia et al. (2017)
	<i>Corynebacterium humireducens</i> MFC-5	Wu et al. (2013)
	<i>Cupriavidus</i> sp. CY-1	Chang et al. (2015)
	<i>Novosphingobium</i> strain DY4	Dai et al. (2015)
	<i>Delftia acidovorans</i>	
MCPA (C ₉ H ₉ ClO ₃)	<i>Pseudomonas</i> sp. HV3	Kilpi et al. (1983)
	<i>Flavobacterium</i> sp. 50001	Chaudhry and Huang (1988)
	<i>Alcaligenes eutrophus</i> JMP134	Pieper et al. (1988)
	<i>Flavobacterium</i> sp. MH	Horvath et al. (1990)
	<i>Sphingomonas herbicidovorans</i> MH	Kohler (1999)
	<i>Alcaligenes denitrificans</i> Strain	Marriott et al. (2000)

(continued)

Table 2.15 (continued)

Phenoxyacetic acid (C ₈ H ₈ O ₃) herbicide	Degrading strain	References
	<i>Enterobacter</i> sp. SE08	Tan et al. (2013)
	<i>Sphingomonas</i> sp. ERG5	Nielsen et al. (2013)
Quinclorac (C ₁₀ H ₅ Cl ₂ NO ₂)	<i>Burkholderia cepacia</i> WZ1	Lü et al. (2003)
	<i>Bordetella</i> sp. HN36	Xu et al. (2012)
	<i>Alcaligenes</i> sp. J3	Dong et al. (2013)
	<i>Pantoea</i> sp. QC06	Fan et al. (2013)
	<i>Bacillus megaterium</i> Q3	Liu et al. (2014)
Endosulfan (C ₉ H ₆ Cl ₆ O ₃ S)	<i>Bacillus</i> , <i>Staphylococcus</i>	Abatenh et al. (2017)
Chlorpyrifos (C ₉ H ₁₁ Cl ₃ NO ₃ PS)	<i>Enterobacter</i> strain B-14, <i>Providencia stuartii</i> strain MS09, <i>Enterobacter</i> , <i>Aeromonas</i> sp.,	Singh et al. (2004), Rani et al. (2008), Abatenh et al. (2017), Chen et al. (2012)
Ridomil MZ 68 (C ₂₃ H ₃₃ MnN ₅ O ₄ S ₈ Zn), Malation (C ₁₀ H ₁₉ O ₆ PS ₂)	<i>Pseudomonas putida</i> , <i>Acinetobacter</i> sp., <i>Arthrobacter</i> sp.	Abatenh et al. (2017)
Chlorpyrifos (C ₉ H ₁₁ Cl ₃ NO ₃ PS) & methyl parathion (C ₈ H ₁₀ NO ₅ PS)	<i>Acinetobacter</i> sp., <i>Pseudomonas</i> sp., <i>Enterobacter</i> sp. and <i>Photobacterium</i> sp.	Abatenh et al. (2017)
s-Triazine (C ₃ H ₃ N ₃)	<i>Pseudomonas</i> sp. strain MHP41, <i>Pseudomonas</i> sp. strain YAYA6, <i>Pseudomonas</i> sp. strain ADP, <i>Arthrobacter</i> sp. strain AD1, β-proteobacterium strain CDB21, <i>Nocardioideis</i> sp, <i>Chelatobacter heintzii</i> , <i>Aminobacter aminovorans</i> , <i>Stenotrophomonas maltophilia</i> (Gram-negative species), <i>Arthrobacter crystallopoietes</i> (Gram-positive genus), <i>Rhodococcus</i> strain TE1, <i>Pseudomonas putida</i> , <i>P. fluorescens</i> , and <i>P. stutzeri</i> , <i>Streptomyces</i> sp. PS1/5,	Hernández et al. (2008), Yanze-Kontchou and Gschwind (1994), Mandelbaum et al. (1993), Cai et al. (2003), Iwasaki et al. (2007), Topp et al. (2000), Rousseaux et al. (2001), Behki et al. (1993), Behki and Khan (1986), Fadullon et al. (1998)
Pentachlorophenol (C ₆ HCl ₅ O)	<i>Arthrobacter</i> strains KC-3 and ATCC 33790	Chu and Kirsch (1972), Reiner et al. (1978)
Metamitron (3-methyl-4-amino-6--phenyl-1,2,4-triazin-5-one) (C ₁₀ H ₉ N ₃ O)	<i>Arthrobacter</i> sp. DSM 20389	Engelhardt et al. (1982)
N-phenyl carbamate (IPC) (C ₇ H ₆ NO ₂ ⁻)	<i>Arthrobacter oxydans</i> P52	Pohlentz et al. (1992)

(continued)

Table 2.15 (continued)

Phenoxyacetic acid (C ₈ H ₈ O ₃) herbicide	Degrading strain	References
Isopropyl <i>N</i> -(3-chlorophenyl) carbamate (CIPC) (C ₁₀ H ₁₂ ClNO ₂)	<i>Pseudomonas striata</i> Chester, <i>Flavobacterium</i> sp., <i>Agrobacterium</i> sp., and <i>Achromobacter</i> sp.	Kaufman (1967)
Dalapon (C ₃ H ₄ Cl ₂ O ₂)	<i>Arthrobacter</i> sp.	Burge (1969)
Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl <i>N</i> -methylcarbamate) (C ₁₀ H ₁₂ O ₂)	<i>Arthrobacter</i> sp.	Ramanand et al. (1991)
Tetrachlorvinphos (TCV) (C ₁₀ H ₉ Cl ₄ O ₄ P)	<i>Stenotrophomonas malthophilia</i> , <i>Proteus vulgaris</i> , <i>Vibrio metschnikovii</i> , <i>Serratia ficaria</i> , <i>Serratia</i> spp. & <i>Yersinia enterocolitica</i>	Ortiz-Hernández and Sánchez-Salinas (2010)
Atrazine (C ₈ H ₁₄ ClN ₅)	<i>Pseudomonas</i> strain YAYA6	Yanze-Kontchou and Gschwind (1994)
2,4,5-Trichlorophenoxyacetic acid (C ₈ H ₅ Cl ₃ O ₃)	<i>Brevibacterium</i> sp	Horvath (1970)
Thiobencarb (C ₁₂ H ₁₆ ClNOS)	<i>Corynebacterium</i> sp.	Miwa et al. (1988)
Pentachlorophenol (C ₆ HCl ₅ O)	<i>Mycobacterium chlorophenicum</i> PCP-1	Briglia et al. (1994)
Fluoranthene (C ₁₆ H ₁₀)	<i>Alcaligenes denitrificans</i>	Tewari et al. (2012)
Carbofuran (C ₁₂ H ₁₅ NO ₃), Parathion (C ₁₀ H ₁₄ NO ₅ PS), <i>S</i> -ethyl dipropylthiocarbamate (C ₉ H ₁₉ NOS), Pentachlorophenol (C ₆ HCl ₅ O), Glyphosate (C ₃ H ₈ NO ₅ P)	<i>Arthrobacter</i> sp.	Tewari et al. (2012)
Urea herbicides, Parathion (C ₁₀ H ₁₄ NO ₅ PS)	<i>Bacillus sphaericus</i>	Tewari et al. (2012)
Cyclohexylamine (C ₆ H ₁₃ N)	<i>Brevibacterium oxydans</i> DH35A	Tewari et al. (2012)
1,2,4,5-TeCB (C ₆ H ₂ Cl ₄)	<i>Burkholderia</i> sp. P514	Tewari et al. (2012)
Quinoline (C ₉ H ₇ N), Glyphosate (C ₃ H ₈ NO ₅ P)	<i>Clostridium</i>	Tewari et al. (2012)
Acetonitril (C ₂ H ₃ N)	<i>Corynebacterium nitrophilus</i>	Tewari et al. (2012)
Trichloroethylene (TCE) (C ₂ HCl ₃)	<i>Dehalococcoides ethanogenes</i>	Tewari et al. (2012)
Pentachlorophenol (C ₆ HCl ₅ O), Parathion (C ₁₀ H ₁₄ NO ₅ PS)	<i>Flavobacterium</i> sp.	Tewari et al. (2012)
Aromatic compounds	<i>Geobacter</i> sp.	Tewari et al. (2012)
3 Hydrobenzoate (C ₇ H ₅ O ₃ ⁻) & 4 Hydrobenzoate (C ₇ H ₆ O ₃)	<i>Klebsiella pneumoniae</i>	Tewari et al. (2012)
Trichloroethylene (C ₂ HCl ₃)	<i>Methylococcus capsulatus</i>	Tewari et al. (2012)
1,1,1-Trichloroethane	<i>Nitrosomonas europaea</i>	Tewari et al. (2012)
Quinoline (C ₉ H ₇ N)	<i>Nocardia</i>	Tewari et al. (2012)

(continued)

Table 2.15 (continued)

Phenoxyacetic acid (C ₈ H ₈ O ₃) herbicide	Degrading strain	References
Parathion (C ₁₀ H ₁₄ NO ₅ PS), Methyl parathion (C ₈ H ₁₀ NO ₃ PS)	<i>Pseudomonas stutzeri</i>	Tewari et al. (2012)
2,4,5-T (C ₈ H ₅ Cl ₃ O ₃), Diazinon (C ₁₂ H ₂₁ N ₂ O ₃ PS)	<i>Pseudomonas capaciea</i> , <i>Pseudomonas</i> sp.	Tewari et al. (2012)
Pentachlorophenol (PCP) (C ₆ HCl ₅ O)	<i>Rhodococcus chlorophenolicus</i>	Tewari et al. (2012)
Carbaryl (C ₁₂ H ₁₁ NO ₂)	<i>Agrobacterium</i> sp., <i>Bacillus</i> sp.	Zhao et al. (2005), Hamada et al. (2015)
Butachlor (C ₁₇ H ₂₆ ClNO ₂)	<i>Anabaena</i> sp.	Agrawal et al. (2015)
Cyhalothrin (C ₂₃ H ₁₉ ClF ₃ NO ₃), cypermethrin (C ₂₂ H ₁₉ Cl ₂ NO ₃), HCH (C ₆ H ₆ Cl ₆)	<i>Arthrobacter</i> sp., <i>Bacillus</i> sp.	Wang et al. (2015), Pankaj et al. (2016)
Ethion (C ₉ H ₂₂ O ₄ P ₂ S ₄), cyanophos (C ₉ H ₁₀ NO ₃ PS)	<i>Azospirillum</i> sp.	Foster et al. (2004), Romeh (2014)
Acibenzolar-S-methyl (C ₈ H ₆ N ₂ OS ₂), Metribuzin (C ₈ H ₁₄ N ₄ OS), napropamide (C ₁₇ H ₂₁ NO ₂), propamocarb hydrochloride (C ₉ H ₂₁ ClN ₂ O ₂), thiamethoxam (C ₈ H ₁₀ ClN ₅ O ₃ S)	<i>Bacillus</i> sp.	Myresiotis et al. (2012)
Bifenthrin (C ₂₃ H ₂₂ ClF ₃ O ₂)	<i>Bacillus</i> sp.	Chen et al. (2012)
Allethrin (C ₁₉ H ₂₆ O ₃), beta-cyfluthrin (C ₂₂ H ₁₈ Cl ₂ FNO ₃), cyper- methrin (C ₂₂ H ₁₉ Cl ₂ NO ₃), flumethrin (C ₂₈ H ₂₂ Cl ₂ FNO ₃), permethrin (C ₂₁ H ₂₀ Cl ₂ O ₃)	<i>Acidomonas</i> sp., <i>Aspergillus niger</i> , <i>Pseudomonas</i> sp., <i>Pseudomonas stutzeri</i> , <i>Serratia</i> sp.	Grant and Betts (2004), Liang et al. (2005), Paingankar et al. (2005), Saikia et al. (2005)
Gramoxone (C ₁₂ H ₁₄ Cl ₂ N ₂)	<i>Pseudomonas putida</i>	Kopytko et al. (2002)
Trifluralin (C ₁₃ H ₁₆ F ₃ N ₃ O ₄)	<i>Bacillus</i> sp., <i>Herbaspirillum</i> sp., <i>Klebsiella</i> sp., <i>Pseudomonas</i> sp.	Bellinaso et al. (2003)
Linuron (C ₉ H ₁₀ Cl ₂ N ₂ O ₂)	<i>Variovorax</i> sp.	Horemans et al. (2013)
Azoxystrobin (C ₂₂ H ₁₇ N ₃ O ₅)	<i>Rhodanobacter</i> sp.	Howell et al. (2014)

2.5 Factors Affecting Microorganism Bioremediation of Pesticide

Degradation of pesticide residues by microbes is limited by numerous components which are separated into internal and external ecological components, in which the impact of internal components commenced from the pesticide structure and the microbes. As reported by Hugo et al. (2014), transformation and deterioration of

pesticides were influenced by microorganism species, adaptability, and metabolic activity. Numerous trials have indicated that the responses of various species of microorganisms or similar types of various strains to the toxic metal or similar organic substrate had been diverse, and the micro biota in the soil is having a solid capability to acclimatize to the surroundings and to be cultivated. With the aid of modified procedure, the novel blends enable these organisms to provide the related enzyme machinery or create a fresh enzyme machinery to degenerate them. Utilitarian distinctiveness and variations in degradation had been the most critical factors (Hussain et al. 2009; Baxter and Cummings 2006).

The intrinsic factors of pesticides, for example, their molecular mass, configuration, the quantity and nature of constituents, constituent distinctiveness, and position influenced the speed and effectivity of microbic breakdown of pesticides (Chaw and Stoklas 2013; Mahro et al. 2001). Luan et al. (2006) noted that the large-sized molecules were least biodegradable as compared to low mass molecules. The large sized molecules have been found to be resistant to biodegradation in comparison to the simpler ones, which were efficiently broken down.

Humidity, temperature, pH, salinity, oxygen, carbon dioxide, substrate concentration, surfactant, redox conditions, wetness, content of organic residues, content of nutrients, and nature and quantity of clay influence the microorganism action and chemical dissemination in soils (Sartoros et al. 2015). Soil water influences the dampness accessible to microorganisms as well as the reduction oxidation state in soil that prompt diverse biochemical responses. Schroll et al. (2006) assessed the impact of soil water oxygen consuming microbial mineralization of preferred pesticides (isoproturon, glyphosphate, and benzoic-ethyl) in various types of soils. The workers examined a direct relationship ($p < 0.0001$) between growing moisture in the soil and accelerated relative mineralization of pesticides. The most ideal mineralization by pesticides was achieved in a soil having Ψ value of -0.015 MPa. Additional increase in water diminished the pesticide mineralization as excess water limits oxygen diffusion and accessibility and can make the surroundings anoxic. Further rise in H_2O reduced the pesticide mineralization because surplus H_2O restricts O_2 dispersion, and availability causes the environment to become anoxic.

The temperature and pH are the main elements influencing the microbial assisted breakdown of pesticides in soil (Arshad et al. 2007; Bhatti et al. 2017; Dervash et al. 2020). The proper temperature not just influences the degree of chemical reactions, but it directly affects the proteins involved in various physiological process and permeability of plasma membranes (Alberty 2006; Guillot et al. 2000; Mastronicolis et al. 1998). A temperature scale between 15 to 40 °C is viewed as agreeable for the deterioration of pesticides by microorganisms meant for their breakdown (Hong et al. 2007). It has been revealed by Singh et al. (2006) that the bacterial population had been capable of deteriorating fenamiphos and chlorpyrifos between 15 and 35 °C; however, their breakdown capacity was forcefully diminished at 5 or 50 °C. Siddique et al. (2002) examined that an incubation temperature of 30 °C was best for the successful breakdown of alpha and gamma -HCH isomers.

2.6 Conclusion

Pesticide contamination creates a widespread risk to the surroundings and general well-being. In soil, different species of microorganisms exist with enormous actions equipped for deteriorating various groups of pesticides. This inherent potential of microbial organisms could be used for both on-site and off-site bioremediation purposes. Stimulation and expression of microbial genes preferred pesticide-deteriorating enzymes in original microflora have magnified the viable utilization of bioremediation in the detoxification of polluted environments. Conditions at every polluted location fluctuate, thus all location-specific variables need to be studied, and on the basis of the most appropriate accessible skill, a choice needs to be made. The organization of pilot-scale remediation investigation by various research teams vows to extend our insight into the application and limitation of this novel strategy. In order to widen the utilization and effective use of remediation, in-depth study is required for better and clear understanding of the effectiveness of microbial biota under various natural environments. This would probably assist in effective planning of the designed frameworks for remediation of polluted locations. In order to accomplish this objective, attempts need to be directed to extend the study concerning the interaction of soil and microbial organisms to interpret successfully the bench- and pilot-scale results to field scale.

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Chapter 3

Pollution Cleaning Up Techniques



Baba Uqab, Jeelani Gousia, Syeed Mudasir, and Shah Ishfaq

3.1 Introduction

The relentless and inexorable extraction of natural resources, mostly from the nineteenth century, is doing irrevocable harm to the Earth's ecological balance. The intense and unsustainable development work gave rise to an unstoppable beast "Environmental Pollution". The word pollution became much familiar with the industrial revolution (Wu et al. 2018). Development of scientific and industrial technology has benefited the society in numerous ways, but has also produced many undesirable and toxic pollutants. A number of contaminants, such as heavy metals, pesticides, aerosols, sulfur oxides, and nitrogen oxides (SOX and NOX), now abundantly present in the atmosphere, are directly correlated with the industries. The whole world bears the brunt of the rapid and unplanned build-up. Pollution of all kinds has serious consequences for all spheres of life (Dar et al. 2016; Wu et al. 2018; Mushtaq et al. 2018). The haphazard use of chemicals to solve any agricultural or industrial problem contributes to the nasty aftermath of the existence in virtually all environments of widespread and unnecessary harmful pollutants (Lajayer et al. 2019; Bhatti et al. 2017; Bhat et al. 2018a, b). This poses a serious threat to the biosphere either in the form of diversity loss or degradation.

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Various pollutants have direct impact on human life, which is a serious concern and calls for immediate attention (Wu et al. 2018; Bhat et al. 2017a, b; Dar and Bhat 2020).

Heavy metal pollution is one of the worst issues that seriously affect the world's environment (Wong et al. 2006). Heavy metals are the metals or metalloids with densities greater than 5 g/cm^3 . At present, the increased concentration of heavy metals in an environment can be due to the unnecessary use of pesticides (Zhang et al. 2011; Bhat et al. 2017a, b, 2018a, b; Mushtaq et al. 2020). Because of its unfavorable effects, soil containing heavy metal above the permissible limits is one of the major concerns. Even at a very low concentration, heavy metals are known to be one of the major pollutants in the soil.

Heavy metals act as major soil contaminants due to their widespread occurrence, their toxicity (acute and chronic), and their nature (persistence, non-biodegradability, and non-thermo-degradability). The reason for accumulation of heavy metals to toxic levels in soils is mostly due to their non-biodegradable and persistent nature (Dar et al. 2013; Uqab et al. 2016; Zojaji et al. 2014; Rezaei et al. 2014; Khanday et al. 2016; Singh et al. 2018).

Pesticides are the substances used for pest control. Pesticides are the xenobiotics that accumulate in the environment and require the adoption of new techniques in order to eliminate them from the environment. Techniques such as pyrolysis, landfilling, and recycling are some of the earlier techniques used for remediation of xenobiotics, but these also affect the environment adversely due to formation of intermediate toxic products (Debarati et al. 2005; Dervash et al. 2020) and proved difficult to execute due to their expensiveness (Jain et al. 2005).

Biotechnology provides an appropriate solution for the management of these deteriorated ecosystems. Environmental biotechnology investigators have extensively studied and managed many contaminations, including chlorinated solvents, hydrocarbons, PAHs, heavy metals, among others. Bioremediation is one such technology associated with reclamation of sites polluted with contaminants through bacteria, fungi, and plants which immobilize and detoxify toxic chemicals from the environment (Kvesitadze et al. 2006; Morel et al. 2002; Gadd 2001; Baker and Herson 1994). Alternative to incineration and catalytic destruction, bioremediation is a cost-effective technique and natural process. Bioremediation involves the combination of areas such as microbiology, biochemistry, molecular biology, chemical and environmental engineering, and analytical chemistry (Uqab et al. 2016; Tang et al. 2007). Microbes that are native to the degraded site are allowed to grow and perform the restoration activities (Agarwal 1998). Sometimes nutrients are added to promote the growth of microbes or the ways, such as terminal electron receptor and temperature and moisture control are applied to enhance the microbial growth (Hess et al. 1997). For the body metabolism, microbes are in need of nutrients or the energy source and the same is present in the contaminants in degraded environment (Uqab et al. 2016; Tang et al. 2007; Singh et al. 2020).

3.2 Agricultural Pollution

Agricultural practices in the world are considered incomplete without the use of fertilizers, pesticides, insecticides, and other chemicals as these are directly related to agriculture production (Aelion et al. 2008). The excessive and indispensable use of such chemicals has resulted in heavy metal accumulation in the soils above permissible limits (Table 3.1). Majority of the pesticides are mostly organic compounds and not many are inorganic compounds that contain metals such as As, Hg, Cu, and Zn, among others (Arao et al. 2010) and therefore, contribute largely toward the heavy metal contamination of soil.

Besides these sources, farmyard manures and coal ash metal processing units are also sources of heavy metal contamination in the soils around the globe (Bhat et al. 2019).

3.3 Bioremediation Via Microbes

Microorganisms that are used for bioremediation are known as Bio-remediators. Microbial tendency to inhabit and adapt to the extreme conditions have made these tiny creatures ideal for the remediation of xenobiotics as they can survive in aerobic as well as anaerobic conditions. The basic requirement for their survival is energy and carbon (Vidali 2001). The key needs for the bioremediation process are microorganisms that are either indigenous or foreign in nature (Prescott et al. 2002). The process of bioremediation depends upon the chemical composition of the polluting agent and the type of microbe used. The selection of microorganism should be performed carefully as they have a limited range of chemical pollution in which they live (Dubey 2004; Prescott et al. 2002). There is diversity of pollutants present in the degraded environment and to deal with these pollutants diverse microorganisms are needed (Watanabe et al. 2001). Microbes that have shown promising results in dealing with the xenobiotics and have degrading potential includes variety of species from *Pseudomonas*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Azotobacter*, *Rhodococcus*, *Mycobacterium*, and *Xanthomonas*. These microorganisms have potential to deal with toxic chemicals such as PCBs, benzene, anthracene, aromatics, polycyclic aromatic, long chain alkanes, phenols, halogenated hydrocarbons, cycloparaffins, formaldehyde, and biphenyls (Uqab et al. 2016).

About 70 microbial agents were reported to have the capability to degrade the petroleum compounds (US Congress, 1991) and, subsequently, an equal number was added to the list in the recent decades (Gjorgieva 2018). The contact between bacteria and the chemical is a prior requirement for degradation of contaminants, which is not easily achievable due to nonuniform distribution of microbes in soil. However, certain bacteria show chemo-tactic response, that is, sensing the contaminant and move toward it.

Table 3.1 Heavy metal concentration around globe in soil (1000 tonnes/annum) along with their few sources

Source	Arsenic	Cadmium	Chromium	Copper	Mercury	Nickel	Lead	Zinc	References
Agriculture and food waste	0 ~ 0.6	0 ~ 0.3	4.5 ~ 90	3 ~ 38	0 ~ 1.5	6 ~ 45	1.5 ~ 27	12 ~ 150	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Municipal wastes	0.09 ~ 0.7	0.88 ~ 7.5	6.6 ~ 33	13 ~ 40	0 ~ 0.26	2.2 ~ 10	18 ~ 62	22 ~ 97	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Commodity impurities	36 ~ 41	0.78 ~ 1.6	305 ~ 610	395 ~ 790	0.55 ~ 0.82	6.5 ~ 32	195 ~ 390	310 ~ 620	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Logging and timber industry wastes	0 ~ 3.3	0 ~ 2.2	2.2 ~ 18	3.3 ~ 52	0 ~ 2.2	2.2 ~ 23	6.6 ~ 8.2	13 ~ 65	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Organic wastes	0 ~ 0.25	0 ~ 0.01	0.1 ~ 0.48	0.04 ~ 0.61	–	0.17 ~ 3.2	0.02 ~ 1.6	0.13 ~ 2.1	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Municipal sludge	0.01 ~ 0.24	0.02 ~ 0.34	1.4 ~ 11	4.9 ~ 21	0.01 ~ 0.8	5.0 ~ 22	2.8 ~ 9.7	18 ~ 57	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Marl	0.04 ~ 0.5	0 ~ 0.11	0.04 ~ 0.19	0.15 ~ 2.0	0 ~ 0.02	0.22 ~ 3.5	0.45 ~ 2.6	0.15 ~ 3.5	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Atmospheric deposition	8.4 ~ 18	2.2 ~ 8.4	5.1 ~ 38	14 ~ 36	0.63 ~ 4.3	11 ~ 37	202 ~ 263	49 ~ 135	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)
Fertilizer	0 ~ 0.02	0.03 ~ 0.25	0.03 ~ 0.38	0.05 ~ 0.58	–	0.20 ~ 3.5	0.42 ~ 2.3	0.25 ~ 1.1	Bhat et al. (2019); Uqab et al. (2016); Chao et al. (2014)

3.4 Microbial Processes Concerned with Bioremediation

Biological or microbial processes for elimination and remediation of toxic metals is considered to be economical due to certain features such as selective metal binding, high capacity to bind metal, and effective absorption methods. Microbial transformation of heavy metals mostly affects solubility, bioavailability, and mobility (Francis 1997) and the mechanisms associated with these processes include chelation of these elements by metabolites, oxidation – reduction in metals, that affects the solubility, change in pH, biosorption, biotransformation, immobilization, biomethylation, and biodegradation.

A number of microorganisms have shown promising results in association and subsequent transformation of metals (Poole and Gadd 1989). Metal resistance is an inherent property of most microorganisms but these microorganisms have evolved/enhanced this property of metal resistance due to constant exposure to the heavy metal-contaminated environments (Banjerdki et al. 2003). Different microbially mediated heavy metal transformations have been identified (Roane and Pepper 2000), many of which can immobilize metals in the environment. The processes of metal resistance include complexation, precipitation, and solubilization, leading to microbial metal transformation (Table 3.2).

Table 3.2 Mechanisms of resistance shown by microbes utilizing the heavy metals

Mechanisms of resistance	Microorganisms	Heavy metals	References
1. Intracellular and extracellular sequestration 2. Efflux reduction	1. <i>Gloeotheca magna</i> 2. <i>Staphylococcus aureus</i> 3. <i>Fusarium oxysporum</i> 4. <i>Pseudomonas sp.</i> 5. <i>Azotoformans sp.</i>	Cd	1. Ahmad et al. (2002) 2. Nair et al. (2007) 3. Uqab et al. (2016) 4. Hesse et al. (2018)
1. Intra and extracellular sequestration	1. <i>Saccharomyces cerevisiae</i>	Cu	1. Culotta et al. (1994) 2. Kacholi and Sahu (2018)
1. Extracellular sequestration and reduction	1. <i>Streptomyces sp.</i>	Cr	1. Amoroso et al. (2001) 2. Liloyd (2003)
1. Efflux	1. <i>Ralstonia metallidurans</i>	Zn	1. Nies (2003) 2. Uqab et al. (2016)
1. Volatilization	1. <i>Clostridium glycolicum</i>	Hg	1. Meyer et al. (2007) 2. Stanković et al. (2018)
1. Biosurfactant 2. Extracellular sequestration	1. <i>Pseudomonas aeruginosa</i>	Pb	1. Nair et al. (2007) 2. Uqab et al. (2016)
1. Reduction 2. Efflux 3. Intracellular sequestration	1. <i>Lactobacillus sp.</i>	Ar	1. Nair and Pradeep (2002) 2. Stanković et al. (2018)

3.4.1 Metal Microbe Mechanism of Interaction

The approach implemented to bioremediate polluted lands depends entirely on the association of metals with microbes (Tabak et al. 2005). Forces such as electrostatic force, Van-der Waals force, redox reactions, covalent bonding, and extracellular precipitation promote the metal binding to the cell surface in ionic form. In fact, contact may be a mixture of all these mechanisms as well. Bacterial cell wall tends to maintain metal cations through mineral nucleation after adsorption by groups that are negatively charged, such as carboxyl, hydroxyl, and phosphoryl (Wase and Forster 1997). Material biosorption, such as U, Zn, Pb, Cd, Ni, Cu, Hg, Cs, Th, Au, Ag, Sn, and Mn, varies significantly with both the material and microorganism. In nature, microbes immobilize heavy metals through the process of cell accumulation or extra-cellular precipitation (Maier et al. 2009). Through generating surfactants, such as rhamnolipids, *Pseudomonas aeruginosa* shows a high sensitivity for some metals such as Pb and Cd. Similarly, other bacteria, including *Thiobacillus ferroxidans* and *Leptospirillum ferroxidans*, can oxidize iron and sulfur (Sand et al. 1992). Eubacteria and archaeobacteria also conserve energy by reducing metals such as, Mn, Fe, Co, As, and Se. A strain of *Alcaligenes faecalis* oxidizes AsO^{2-} to AsO_4^{3-} and *Pseudomonas fluorescens* LB 300 and *Enterobacter cloacae* reduces CrO_4^{2-} to $Cr(OH)_3$ enzymatically. The non-enzymatic detoxification involves the secretion of inorganic metabolic products, such as carbonate, sulfide, and phosphate ions precipitating toxic metals (Maier et al. 2009).

Factors such as humidity, pH, and temperature influence microbial development. Although microbes have been isolated from extreme environments, but maximum growth for most of them is obtained over a narrow range as optimal conditions are rarely achieved. For example, additives such as lime can be added to elevate pH if soil is acidic; similarly temperature maintenance is done using plastic covers in late spring, winter, and autumn to boost solar warming. The other imperative requirements for most favorable microbial growth are availability of water supply and oxygen, which are obtained by irrigation and tillage or sparge air, respectively. Sometimes hydrogen peroxide can also be used as an aerator of soil. However, soil structures control the delivery of air, water, and nutrients; these can be improved by using gypsum or organic matter. It is necessary to maintain the soil structures as low permeability can affect the movement of nutrients, water, and oxygen and hence are rendered not appropriate for in situ clean up (Tabak et al. 2005; Mehmood et al. 2019; Dar et al. 2020).

3.5 Bioremediation Strategies

Stimulation of the indigenous microbes for their activity is the primary and most important requisite for the bioremediation process and activation of the indigenous microbes is carried out by nutrient addition and regulation of redox condition and pH control (Table 3.3).

Table 3.3 Developmental methods applied in bioremediation

Technique	Examples	Applications
Microfiltration	Microfiltration membranes are used at a constant pressure	Mostly used for waste water treatment
Electrodialysis	Uses cation-anion exchange membrane pairs	Efficiently removes dissolved solids
Precipitation	Non-directed physicochemical complex action reaction between contaminant and charged particles	Heavy metal removal
Ex situ	Land forming Composting Bio-piles	Surface application Application of organic material to natural soils Agriculture to municipal wastes
In situ	Bio-sparging Bioventing Bioaugmentation	Biodegradative capacities of indigenous microorganisms Distribution of pollutants
Bioreactors	Slurry reactors Aqueous reactor	Decreases toxic concentration of contaminants

3.6 Phytoremediation

Phyto-remediation is an environmental clean-up treatment of plants and their associated microbes (Salt et al. 1998; Raskin et al. 1994). It involves detoxification organic and inorganic contaminants using plants and their rhizospheric microbiota. Chemical pollutants found in the environment are of xenobiotic origin, emitted primarily from leaks, combat operations, forestry, mining, and wood processing, etc. Such organic pollutants are remedied by sequestration, degradation, or volatilization according to their plant properties. Some of the successfully phytoremediated organic pollutants are most common groundwater pollutant-TCE (Newman et al. 1997), herbicides such as atrazine (Burke 2003), explosives such as TNT (Hughes et al. 1997), PCBs, PAHs, and MTBE (Davis et al. 2003). Phytoremediation is a modern method used by plants to remove toxins from water and soils (Bhadra et al. 1999).

The biotechnological interventions, such as genetically modified plants along with the rhizospheric microbiota, have proved promising in decontamination of soil and water (Uqab et al. 2016). Pollutants such as metals, hydrocarbon products, fungicides, pesticides, antibiotics are removed by phytoremediation. These pollutants are sequestered in the cell walls and chelated to inactive forms in the soil or complex and store them in vacuoles and produce small proteins such as metallothioneins and phytochelatin unavailable to the metabolically responsive cytoplasm. The volatilization of highly toxic chemicals, such as mercury and methyl mercury, can be acquired by the process of remediation through plants. In addition, plants have acquired the tendency to extract micronutrients from the atmosphere even at lower concentrations. The remediation techniques through plants involve; phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization.

3.7 Phytoextraction

Phytoextraction involves the uptake of pollutants by the roots of the plants and sequestering them in their biological system, which are later available at the harvesting of associated plant. This method is most effective for soil, sediment, and sludge treatment of contaminants (Raskin et al. 1994). Plants are capable of absorbing huge amounts of heavy metals through roots and store it into the upper ground parts of the plant, resulting in formation of large biomass (Macek et al. 2000). Plants used for phytoextraction are called “hyperaccumulators”. These are the plants which achieve greater than one shoot-to-root metal-concentration ratio. A plant can grow in hazardous conditions to be an effective hyperaccumulator, need minimal maintenance and yield high biomass, but few plants fulfill such criteria completely (Meenambigai et al. 2016). Hyperaccumulator plant species have tendency to bind metals such as Cd, Zn, Co, Mn, Ni, and Pb upto concentrations 100–1000 times more than the nonaccumulator plants (Endo et al. 2002). About 400 plant species, belonging to 45 plant families, are known to be hyperaccumulators. Among them, most of the plants bioaccumulate Ni, about 30 are capable of binding Co, Cu, and Zn, and few are efficient in Mn and Cd accumulation (Baker 1999).

3.8 Phytostabilization

This is the approach used for sediment, soil, and sludge remediation. The primary process of this approach requires the processing of certain chemicals by plants that immobilize the pollutants rather than degrade them and thus avoid their transfer to groundwater or their exposure to the food chain (Verma et al. 2006). The phytostabilization method is straightforward as it happens by the removal of precipitation, sorption, complexation, or metal valence, and because of these properties, this technique is commonly used to handle metals, such as arsenic, cadmium, chromium, copper, and zinc contaminants (Raskin et al. 1994).

3.9 Rhizofiltration

Rhizofiltration is the deliberate use of plants belonging to both terrestrial and aquatic ecosystems to consume, collect, and store pollutants in their roots from contaminated aqueous sources (Verma et al. 2006). The terrestrial plants are much preferred in order of preference over aquatic plants, since their root system, which is long and fibrous, increases the area of roots and removes potentially toxic metals effectively (Newman and Reynolds 2004). This is also called as Hydroponic

Systems for Treating Water Streams. Rhizofiltration is used primarily for remediating soil, groundwater, and low-contaminant wastewater. It can also be used for the metals which are primarily held in the roots, such as Cd, Cu, Pb, Zn, Ni, and Cr.

3.10 Phytovolatilization

The approach mainly uses plants to absorb pollutants from waste water as well as soil and convert them into volatilized compounds and finally transpire them into the atmosphere, the process known as phytovolatilization (Vazquez et al. 2006). It is used primarily for soils contaminated with mercury. In this process, growing trees and other plants will take up the pollutants with water and transfer them to the leaves through the xylem vessels, turn them into non-toxic forms and eventually volatilize them into the atmosphere (Newman and Reynolds 2004).

3.11 Aquatic Plant Species Studied for Phytoremediation

Various aquatic plants have been reported to remediate pollutants, namely, *Azolla pinnata*, *Lemna minor* accumulate Cu and Cr (Jain et al. 1990), *Pistia stratiotes* accumulates Ag, Cd, Cr, Cu, Hg, Pb and Zn (Odjegba and Fasidi 2004), *Lemna gibba* biosorbs As (Mkandvire and Dude 2005), and *Myriophyllum heterophyllum* and *Potamogeton crispus* accumulate Cd (Sivaci et al. 2008). Table 3.4 provides a list of some aquatic plants investigated for their phytoremediation potential (hydroponics) in water media.

3.12 Pesticide Degradation by Bacteria

Bacteria that remediate the pesticides belong to genera such as *Pseudomonas*, *Burkholderia*, *Azotobacter*, *Flavobacterium*, *Arthobacter*, and *Bacterium raoultella* sp. (Glazer and Nikaido 2007). The complete biological degradation of pesticides involves the oxidation resulting carbon dioxide and water as products that provide energy to microbes. In case the native bacterial diversity is incapable of bioremediation of pesticides, external addition of capable microbiota is added to the soil. Bacterial degradation of pesticides depends on the enzyme systems as well as conditions such as pH, temperature, and nutrients. Certain pesticides are easily degraded, where some are recalcitrant due to presence of anions in the compound. The pesticides such as organophosphorus compounds, the Neonicotinoids are degraded by the *Pseudomonas* species.

Table 3.4 Plants investigated for phytoremediation potential on water medium (hydroponics)

S. No.	Plant species	Researcher	Heavy metals	Result
1.	1. <i>Ceratophyllum demersum</i> 2. <i>Myriophyllum spicatum</i>	El-Khatib et al. (2014)	(Lead)	Plants accumulated large concentrations of Pb and thus demonstrated promise for usage in aquatic bodies with low Pb contamination as phytoremediator organisms
2.	1. <i>Ceratophyllum demersum</i>	AlUbaidy and Rasheed (2015)	(Cadmium)	<i>C. Demersum</i> has a high potential in ecosystem eradication of cadmium
3.	1. <i>Utricularia gibba</i>	Augustynowicz et al. (2015)	(Chromium)	<i>U. Gibba</i> may be successful for chromate removal on a short time scale
4.	<i>Ceratophyllum demersum</i> , <i>Myriophyllum spicatum</i> , <i>Eichhornia crassipes</i> , <i>Lemna gibba</i> , <i>Phragmites australis</i> , and <i>Typha domingensis</i>	Kamel (2013)	(Cadmium, copper, cobalt, nickel, lead, and zinc)	The surveyed native aquatic plant species displayed higher rates of aggregation of heavy metals which have the ability to be used in phytoremediation

3.12.1 Role of Fungi

The fungi render the pesticides into non-toxic substances by incurring minor structural changes in pesticides and later release them into the surrounding soil, where it is further degraded (Gavrilescu 2005).

3.12.2 Role of Enzymes

Enzymes play a vital role in the degradation of any xenobiotics and possess the capability of regenerating toxins at a significant pace and are likely to regenerate the degraded atmosphere (Rao et al. 2010). Enzymes also lead to the destruction of pesticide compounds in the target organism by intrinsic detoxification pathways and established metabolic tolerance, as well as in broader environments. *P. putida* theoretical oxygen necessity (TOD) enzyme is a subset of a much broader enzyme family of application of biologically-related reactions in biocatalysis (Watanabe and others 2001). In particular, fungal enzymes, oxidoreductases, lacquers, and peroxidases have a popular role in the removal of pollutants of polyaromatic hydrocarbons (PAHs) in natural, marine, or terrestrial water (Balaji et al. 2014). The enzymes play an important role in the biodegradation of any compounds in xenobiotics. The compounds of organophosphorus have been analyzed in depth and so much of the

literature is available that explains the enzymes that kill the OP. The first bacterium to kill OP compounds was extracted from a Philippine soil sample in 1973 and has been known as *Flavobacterium* sp. ATCC 27551-ATCC (Suthersan 1999). Several bacteria have since been isolated, including a few fungi and cyanobacteria, which can use OP compounds as a source of carbon, nitrogen, or phosphorus.

3.13 Conclusion

Pollution has no doubt caused severe impact on every part of the earth. Pesticide and heavy metal-polluted fields have gained significant concern because it affects human health and the natural environment. Bioremediation has enormous potential for soil remediation which is affected by pesticides. Microorganisms present in soils are capable of removing pesticides from the environment. Biopesticides and enzymatic-contaminated environment degradation represent the most important strategy for the removal of contaminants and the degradation of persistent chemical substances by metabolic enzymes. Bioremediation is therefore a very positive approach that can definitely solve the problem of soil contamination. This technique has consistently demonstrated the ability for destroying not only pesticides but also other organic compounds. Knowing the microbial populations and their reaction to the natural environment and contamination, improving the awareness of microbial genetics to enhance their capacity to degrade the contaminants, and performing field trials of new bioremediation techniques are the need of the hour. Bioremediation paves the way for greener pastures. Its technique provides a reliable and cost-effective way to treat contaminated groundwater and land, irrespective of which type of bioremediation is being used. The positives of this tool have out-classified the weaknesses and the obvious example of this is that this methodology is in increasing demand. This thereby demonstrates that bioremediation is a form of management. So the time has come to use this environmentally friendly technology for a better and safer world.

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Chapter 4

Role of Mushrooms in the Bioremediation of Soil



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4.1 Introduction

The soil has much importance for human health and the environment. Presently, the world is facing a global soil crisis (Zhu and Meharg 2015; Bhatti et al. 2017). The whole biodiversity depends on this resource as it is a fundamental part of our planet due to water resources going through it and this soil becomes a substrate for flora and fauna (Montanarella and Vargas 2012). The turnover of organic matter in soil and the transformation of nutrients are an essential part of soil quality. The soil has potential to improve or hinder the success of revegetation on retired agricultural land, but the enrichment of soil by decomposition of nitrogen requires longer years since they were revegetated (Bourne et al. 2008). There are many shreds of evidence supporting that the productivity of terrestrial ecosystems and their multifunctionality are determined by the biodiversity of soil and composition of a biological community (Wagg et al. 2014; van der Heijden et al. 2008). Industrial development continues to increase and release chemicals into the soil with the consequent accumulation of heavy metals in agricultural soils causing growing public concern about food security worldwide (Wong et al. 2002). These heavy metals are non-biodegradable and persistent, which can pose long-term environmental and health

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implications (Zhao et al. 2011; Dar and Bhat 2020). Heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), silver (Ag), and copper (Cu) are non-essential for growth and development of plant, but unnecessarily absorbed by them and accumulated in toxic forms, and consumption of these plants represents a possible risk to human health and wildlife. Various researchers showed that the use of contaminated water with heavy metals for irrigation increases these contents in the soil above the permissible limit. The uptake of heavy metals by plants depends upon the soil type, plant growth stages, and plant species (Abbas et al. 2011; Dar et al. 2013; Bhat et al. 2017a, b). Major sources of pollutants are industrial effluents, sewage sludge, inadequate use of fertilizers, pesticides, and insecticides, etc. (Varjani 2017; Bagul et al. 2015; Bhat et al. 2018a, b). All these pollutants can be divided into two major groups: (i) organic and (ii) inorganic, which can cause an adverse effect on flora, fauna, and human health (Varjani et al. 2015; Dervash et al. 2020; Mushtaq et al. 2020; Singh et al. 2020). Soil pollution has harmful consequences for the ecosystem. It is a necessary and challenging job to remediate the polluted soil. Biological approaches, mainly mycoremediation, are used as a cleanup technology, which has value on the conversion of pollutants into a usable form.

Mycoremediation is derived from the ancient Greek term “mukes” meaning fungus and “medium” meaning restoring balance. It is the use of fungi to degrade or remediate pollutants (Esterhuizen-Londt et al. 2016; Bhat et al. 2017a, b; Singh et al. 2018). It is the usage of fungi-based technology to decontaminate the environment from pollution. It is an eco-friendly method for decontamination of soil by the fungi present in aquatic sediments, terrestrial habitats, and water surfaces and plays a significant role in natural remediation of heavy metals (Dugal and Gangawane 2012; Dar et al. 2016; Mehmood et al. 2019).

It is a cheaper remediation process that does not require any expensive equipment. For these reasons, its use is in small-scale applications, namely, mycofiltration of domestic wastewater (Molla and Fakhru'l-Razi 2012) and to help with the decomposition process of a compost toilet. This process is a very cheap, effective, and environmentally beneficial way for the removal of toxins from the polluted environment. The organic pollutants include heavy metals, dyes, chemicals and wastewater, petroleum fuels, polycyclic aromatic hydrocarbon, pharmaceuticals, pesticides and herbicides, inland, freshwater, and marine environments (Deshmukh et al. 2016; Mushtaq et al. 2018). The by-products of the remediation are valuable enzymes such as laccase (Strong and Burgess 2007). This enzymatic machinery for the degradation of pollutants can be applied to a wide variety of pollutants (Kulshreshtha et al. 2013; Purnomo et al. 2013). It is described that edible or medicinal mushrooms make the remediation process even more profitable (Kulshreshtha et al. 2014). Mushrooms are not only used for bioremediation but can grow its sporocarp as a source of protein. They can degrade waste by the secretion of hydrolyzing and oxidizing enzymes (Zhu et al. 2013; Bhat et al. 2018a, b). The extracellular enzymes produced by mushrooms are cellulases, pectinases, peroxidases, ligninases (lignin peroxidase, manganese dependent peroxidase, and laccase), oxidases, and xylanases (Kulshreshtha et al. 2014; Khanday et al. 2016). These enzymes can oxidize recalcitrant pollutants *in vitro*. These enzymes also degrade non-polymeric,

recalcitrant pollutants, namely, nitrotoluenes (Kulshreshtha et al. 2014), PAHs (Kulshreshtha et al. 2014), organic and synthetic dyes, and pentachlorophenol, under in vitro conditions (Kulshreshtha et al. 2014). These macrofungi are also able to degrade plastics (Kulshreshtha et al. 2014). It is a complex process as the influence of other biochemical systems and interactions of ligninolytic enzymes with cytochrome P450 monooxygenase system, hydroxyl radicals and the level of H_2O_2 , which are produced by the mushroom. Mushrooms are future vegetables, so the researchers have a keen interest in the cultivation of mushrooms and waste remediation. Various workers have studied and examined the enzymatic role in the waste degradation process (Zhu et al. 2013; Akinyele et al. 2011; Novotný et al. 2004). Mycoremediation degrades hazardous materials into nonpoisonous substances. It is a biological tool to degrade, transform or immobilize environmental contaminants.

Bioremediation is the process of living organisms, such as fungi, bacteria, and green plants, to breakdown hydrocarbon and organic contaminants from soil (Atlas and Bartha 1992). Bioremediation is the use of plants to degrade environmental pollutants or to prevent pollution (Varjani 2017). Among these, the fungi are important in the decomposition, transformation, and nutrient cycling (Archana and Jaitly 2015). These organisms enhance biodegradation of contaminated soil. The synthetic organic compounds called xenobiotics do not occur naturally in the biosphere and some of them are not easily degraded by microbes (Sullia 2004). Several classes of chemicals are targeted by the United States Environmental Agency (USEPA) as priority toxic pollutants, namely, polycyclic aromatic hydrocarbons (PAHs), pentachlorophenols, polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane, benzene, toluene, ethylbenzene xylene, and trinitrotoluene. Loske et al. (1990) reported that the main contaminants in polluted soils are polycyclic aromatic hydrocarbons (PAHs), i.e., residues from the processing of oil, tar, coal, and comparable substances, polychlorinated biphenyls (PCBs), used as cooling agents in transformers, and dioxins, which are by-products of chemical manufacturing and are found in fly-ashes from combustion processes. These chemicals are a significant threat to the earth system. So it is an absolute requirement to promote sustainable development for low environmental impact. Due to the magnitude of this problem and the lack of a reasonable solution, a rapid, cost-effective, ecologically responsible method of clean up is greatly needed (Hamman 2004). Mycoremediation has a versatile role in environmental protection. It is an eco-friendly, cheaper, and easy way that can overcome the pollution of environment and convert the same into harmless forms (Perelo 2010). Mushrooms are disintegrators and the degradation is dependent upon the occurrence of nutrients present in the soil (Rhodes 2012). Some mushroom species, such as *Pleurotus ostreatus*, *P. tuber-regium*, *P. pulmonarius*, *P. eryngii*, *Lentinula edodes*, *Lentinus squarrosulus*, *L. tigrinus*, *Agaricus bisporus*, *Nematolana prowardii*, *Irex lacteus*, and *Stropharia coronilla*, are suitable for interacting with intractable substrates (fats, chitin, and keratin, and degrade their starches, hemicellulases, celluloses, pectins) and other sugar polymers. Flack (1902) developed a method for improving the digestibility of lignocellulosic material by white-rot fungi, such as *Pleurotus* sp. It has the ability to show rapid growth on

cotton stacks (Kerem et al. 1992). This activity can also be applied to bioremediation of various pollutants having structural similarities to lignin.

4.2 Mushroom as a Significant Tool for Mycoremediation

The extensive research on mycoremediation shows that this method is to treat soil without the formation of dangerous metabolites. They were recognized to degrade various compounds (Abdel-Shafy and Mansour 2016). Various published reports emphasize the role of mushroom in the remediation of pollutants by the process of biodegradation, biosorption, and bioconversion (Kulshreshtha et al. 2013; Kumhomkul and Panich-Pat 2013; Lamrood and Ralegankar 2013; Akinyele et al. 2012), as given in Table 4.1. Mushrooms are major decomposers of cellulose, hemicelluloses, and lignin (Pletsch et al. 1999; Christian et al. 2005). They can store and release different elements and ions and also accumulate toxic elements (Annible et al. 2006). Edible and medicinal mushrooms are natural environment remediators

Table 4.1 List of mushrooms/fungi with their roles in the biodegradation of pollutants/waste

Mushroom species	Waste pollutants	References
<i>Pleurotus ostreatus</i>	Green polyethylene (GP) starch based plastic polymer degradation and oxodegradable plastic degradation; convert passion fruit waste into β glucoside	Da Luz et al. (2013, 2015) and Zilly et al. (2012)
<i>Pleurotus platypus</i>	Copper, zinc, iron, cadmium, nickel, lead	Lamrood and Ralengenker (2014)
<i>Pleurotus pulmonarius</i> , <i>Pleurotus</i> sp., <i>Polyporus</i> sp.	Crude oil/petroleum hydrocarbons	Njoku et al. (2017) and Kristanti et al. (2011)
<i>Pleurotus sajor</i>	Heavy metals	Jibran and Milsee Mol (2011)
<i>P. ostreatus</i> var. <i>florida</i>	Grow on solid sludges and effluent of cardboard and handmade paper industries	Kulshreshtha et al. (2013)
<i>Lentinula edodes</i>	Degradation of Eucalyptus waste	Tsujiyama et al (2013)
<i>Lentinula tigrinus</i>	Characterization of the production of ligninolytic enzymes and wheat straw into fruiting body	Lechner and Papinutti (2006)
<i>Coriolus versicolor</i>	Mushroom species possess the ability to degrade PAH with enzymes MnP and LiP	Jang et al. (2009)
<i>Trametes versicolor</i>	Act as biocatalysts for decolorization of different industrial dyes and waste	Amaral et al. (2004)

(continued)

Table 4.1 (continued)

Mushroom species	Waste pollutants	References
<i>Auricularia</i> sp. <i>Schizophyllum</i> , <i>Polyporus</i> sp.	Green dye, namely, malachite was degraded	Rajput et al. (2011)
<i>Ganoderma lucidum</i> , <i>Volvariella volvacea</i> , <i>Coriolopsis strumosa</i> , <i>Daedalea tenuis</i> , <i>Lenzites malacanis</i> , <i>Phellinus xeranticus</i> , <i>Trametes lactenia</i> , <i>Agaricus bisporus</i> , <i>Lactarius piperatus</i>	CU, CD, PB, CO, CU II	Nagy et al. (2013) and Das (2005)
<i>Agaricus macrosporus</i> , <i>Beauveria bassiana</i>	Cd, Hg, Cu, Zn, Pb, Cr, Cd	Gola et al. (2018) and García et al. (2005)
<i>Flammulina velutipes</i>	Copper ions from aqueous waste	Luo et al. (2013)
<i>Fomes fasciatus</i>	Copper ions	Sutherland and Venkobachar (2013)
<i>Agaricus bisporus</i> , <i>Calocybe indica</i>	Copper, zinc, iron, cadmium, nickel, lead	Lamrood and Ralengenker (2014)
<i>L. edodes</i>	Grow on agricultural waste such as oakwood sawdust, wheat straw corn cobs	Israilides and Philippoussis (2003)
<i>Macrocybe titans</i> , <i>Grifola frondosa</i> , <i>G. lucidum</i>	Convert passion fruit waste into β glucoside	Zilly et al. (2012)
<i>Pleurotus</i> sp., <i>Lentinus connatus</i>	Convert sorghum stalk, paddy straw, and banana pseudostem waste into mushroom	Rani et al. (2008)
<i>V. volvacea</i>	Enzymatic activities were measured during fermentation of agroindustrial wastes, such as cassava, sugar beet pulp, wheat bran, and apple pomace	Akinyele et al. (2012)

(Pletsch et al. 1999). These remediators stimulate organisms with nutrients and other chemicals for decontamination. It is a biotechnological method to clean up the contaminated sites (Thomas et al. 2009). This method has broader potential to perform and clean the polluted soil. Native mushrooms and their inoculum are applied to the contaminated soils to remove and degrade contaminants (Thomas et al. 2009). Mushroom taxa, such as *Agaricus*, *Amanita*, *Cortinarius*, *Boletus*, *Leccinum*, *Suillus*, and *Phellinus* are applicable for the mobilization of various toxic metals in the soil (Ali et al. 2017). The fungal hyphae have the ability to degrade heavy metals as well as xenobiotic compounds. This property makes them more advantageous and beneficial than bacteria. The moulds, yeasts, and filamentous fungi are removing the wastes (Thakur et al. 2015). Fungal spores and their hyphae can be used for their regeneration.

4.3 Remediation Through Mushrooms

Mushrooms use three effective methods to reclaim and ameliorate polluted lands. These methods are (a) biodegradation, (b) bioconversion, and (c) biosorption.

4.3.1 *Biodegradation*

The breakdown of organic matter by organisms, such as bacteria and fungi, is called biodegradation (Vert et al. 2012). This biodegradation includes biodeterioration, fragmentation, and assimilation. Biodeterioration is a surface-level degradation that modifies the mechanical, physical, and chemical properties of the material (Lucas et al. 2008). It is influenced by abiotic factors which allow degrading by weakening the material's structure. These factors are light, temperature, and chemicals in the environment. This process is the first stage of biodegradation. Biodeterioration is the undesirable action of living organisms on materials, such as the breakdown of stone, corrosion of metals by organisms (Hueck 1966). Mushrooms accumulate toxic metals with the hyphal network. They have the ability to absorb these metals from the soil (Mejstrik and Lepsova 1992). Mushrooms are being utilized as myco-remediation agents due to their characters concerning the uptake potential of heavy metal. Mushrooms degrade the polycyclic aromatic hydrocarbons (Hammel et al. 1991). It converts compounds to simple and inorganic forms. They can produce extracellular enzymes that degrade PAHs, Plastic, organic and synthetic dyes, 2, 4-dichlorophenol, crude oil, malachite green, radioactive cellulosic-based waste (Nyanhongo et al. 2007; Da Luz et al. 2013).

4.3.2 *Bioconversion*

It is biotransformation that is the conversion of organic materials, namely, plant or animal waste, into usable products by biological agents, such as mushrooms. This process is based on utilizing sugar from cellulose and hemicellulose to form their metabolites that are very essential for the growth and survival of mushrooms. The sources of lignocellulosic wastes are pulp and paper, agriculture, and food industries. Lignolytic enzymes catalyze lignin compounds and fall into peroxidases and oxidases. Peroxidases use hydrogen peroxide (H_2O_2) or organic hydroperoxides ($R-OOH$) as co-substrates. Some peroxidases contain heme proteins with substrate spectrum, including organic and inorganic compounds (Dunford 1999). They form free radicals, such as phenoxyl and aryl cation radicals, reactive cations (e.g., Mn^{3+}), or anions (e.g., OCl^-), which destroy the lignin and humic substances (Hofrichter and Ullrich 2010). Phenol oxidases are biocatalysts and the representatives are laccase and tyrosinase, that consist of copper in their active sites, which are produced

by various mushrooms, such as *Agaricus*, and *Pleurotus* (Mikolasch and Schauer 2009). According to Reddy and Mathew (2001), white-rot fungi consists of peroxidases, laccases, etc., that are involved in the formation of free radicals that cleave the carbon-carbon and carbon-oxygen bonds of the lignin xenobiotic by a free radical mechanism. Cellulolytic enzymes are responsible for cellulolytic and xylanolytic activities. Fungal cellulolytic enzymes include cellulases, hemicellulases, pectinases, chitinases, amylases, proteases, phytases, mannosases, and xylanases. By using agricultural wastes as substrate, fungi produce cellulases, mannanases, and pectinases which can degrade cellulosic materials.

Wild mushrooms are a source of enzymes and secondary metabolites. Mushrooms use enzymes to biodegrade and biotransform the lignin of wood. The enzymes degrade lignocellulosic material for the production of ethanol using yeast, (Conceição et al. 2018). The waste, consisting of lignocellulose, can be used for mushroom cultivation and helps in solving pollution problems. The cultivation of *Pleurotus citrinopileatus*, *Lentinula edodes*, *Pleurotus eous*, *Lentinus connatus*, etc., can be carried out on various agro wastes. These grown mushrooms biotransform the agro biomass into valuable commercial substances, such as enzymes, carbohydrates, proteins, and various secondary metabolites (Brienzo et al. 2007; Rani et al. 2008; Kulshreshtha et al. 2014). Wild mushrooms, such as *Lactarius* sp., *Lentinula boryana*, and *Pycnoporus* sp., produce enzymes such as laccase (Khaund and Joshi 2014; Gavrilesco 2004). *Tricholoma saponaceum* can hydrolyze fibrinogen and fibrin by an enzyme Metaloendo-peptidases (Kim and Kim 2001).

4.3.3 Biosorption

It is the removal of metals or contaminants by mushrooms from aqueous solution. Various species of mushrooms remove pollutants by biosorption. *Lentinus tuberegium* biosorbs heavy metals from contaminated soil (Oyetayo et al. 2012). *Pleurotus platypus*, *Agaricus bisporus*, *Calocybe indica*, etc., are biosorbent agents for the removal of Cu, Zn, Fe, Cd, Pb, and Ni from aqueous solution (Lamrood and Ralegankar 2013). Accumulation of metal contents in fruiting bodies is affected by factors, namely, mycelium age, substrate composition, and the life span of fructification. It was observed by Thomet et al. (1999) that maximum metal concentrations in the pileus, but not in basidiospores, lower contents in cap and the very low in the stipe.

4.4 White-Rot Fungi Degradation System

Approximately 30% of the literature on fungal bioremediation is concerned with white-rot fungi (Rhodes 2014; Singh 2006). Four groups of white-rot fungi include *Phanerochaete*, *Trametes*, *Bjerkandera*, and *Pleurotus* that has the potential for

bioremediation (Hestbjerg et al. 2003). This group of fungi can degrade naturally occurring polymer, lignin, a component of wood that is similar in molecular structure to petroleum hydrocarbons (Varjani 2017; Kshirsagar 2013; Hattaka 1994; Shah et al. 1992). These white-rot fungi can degrade toxic or insoluble compounds more efficiently than another group of fungi. Several white-rot fungi, such as *Lentinula edodes*, *Pleurotic ostreatus*, *Bjerkandera adusta*, *Trametes versicolor*, and *Irpex lacteus*, are well-known fungi that are able to degrade insoluble compounds (Siddiquee et al. 2015; Adenipekun and Lawal 2012; Hamman 2004; Shah et al. 1992; Bumpus et al. 1985). These have lignin degradation systems, such as manganese peroxidase (MnP), lignin peroxidase (LiP), H₂O₂ producing enzymes (Kirk and Farrell 1987) and laccase. All these enzymes are stimulated by nutrient limitation (Aust et al. 2004). These extracellular lignin modifying enzymes (LMEs) have very low substrate specificity, therefore mineralize highly recalcitrant organ pollutants which are similar to lignin (Veignie 2004; Pointing 2001; Cajthaml et al. 2002). Lignin peroxidase is a glycosylated heme protein that has a higher redox potential as compared to most peroxidases and oxidizes chemicals, which include some non-phenolic aromatic compounds (Reddy and Mathew 2001). Manganese peroxidase (MnP) also requires hydrogen peroxide for oxidation. The Mn³⁺ state of the enzyme mediates the oxidation of phenolic substrates (Mester and Tien 2000). Laccase is a multicopper oxidase enzyme that can catalyze the oxidation of dihydroxy and diamino aromatic compounds (Aust et al. 2004). The main reactions that are catalyzed by enzymes are demethoxylation, depolymerization, hydroxylation, decarboxylation, and aromatic ring opening resulting in oxygen activation, creating radicals that perpetuate oxidation of the organ pollutants (Reddy and Mathew 2001). The details of enzymes producing fungal taxa and mechanism of action are given in Table 4.2. White-rot fungi decontaminate the polluted sites by removing coal tar, creosote, and crude oil (Loske et al. 1990). Isikhuemhen et al. (2000) showed in his experiment that *Lentinus squarrosulus* has maximum lignocellulolytic enzyme activities and are a good producer of exopolysaccharides. *Lentinus squarrosulus* is ideal for application in industrial pretreatment and biodelignification of lignocellulosic biomass. *Elfvigia applanata* successfully applies for the bioconversion of bisphenol. The sophisticated symbiotic system of white-rot fungi of the genera *Termitomyces* with fungus-growing termites is also an attractive example which is a cooperation between termites and fungi that accomplishes the efficient decomposition of lignin and complete bio recycling of plant litter.

Brown rot fungi produce compounds which are able to penetrate the cell wall and help in degradation reactions. Their compounds depolymerize polysaccharides of wood and also break the glycosidic bonds of polysaccharides by penetration spores (Koenigs 1972). They have the ability to produce hydrogen peroxide (Koenigs 1974). The hydrogen peroxide produced by this group of fungi oxidizes the two valance transition metals (Fe²⁺, Mn²⁺, Co²⁺).

Another group of fungi, called soft rot fungi, also helps in the degradation of cell walls of wood by forming chains that follow the orientation of the S2 elementary fibrils causing soft rot. They also produce cellulolytic enzymes. Their lignin-degrading ability is variable as there is a limitation in the functioning of peroxidases and oxidases (Cragg et al. 2015).

Table 4.2 Fungal taxa, their enzymes, along with their mechanism of action

Fungal taxa	Enzymes	Action of enzymes	References
<i>Ascomycota</i>	Caldariomyces fumago haemethiolate chloroperoxidase	H ₂ O ₂ -dependent halogenation of organic compounds in the presence of halides; H ₂ O ₂ -dependent one-electron oxidations of phenols and anilines in the absence of halides, and H ₂ O ₂ -dependent peroxygenation	Hofrichter et al. (2010)
<i>Basidiomycota</i>	Lignin peroxidases	H ₂ O ₂ -dependent one-electron oxidation of aromatic compounds	Hofrichter et al. (2010)
	Versatile peroxidases	H ₂ O ₂ -dependent one-electron oxidation of Mn ²⁺ to Mn ³⁺ and oxidizes organic compounds.	Hofrichter et al. (2010) and Ruiz-Dueñas et al. (2009)
	Coprinopsis cinerea peroxidase	H ₂ O ₂ -dependent one-electron oxidation of aromatic compounds	Hofrichter et al. (2010) and Ikehata et al. (2005)
	Dye decolonizing peroxidases	H ₂ O ₂ -dependent one-electron oxidation of organic compounds, additional hydrolyzing activity	Hofrichter et al. (2010)
	Reductive dehalogenases	Two-component system with membrane-bound glutathione S-transferase and a soluble glutathione conjugate reductase that releases reductively dechlorinated compounds	Nakamiya et al. (2005) and Jensen et al. (2001)
	Haemethiolate peroxygenases	H ₂ O ₂ -dependent peroxygenation of aromatic aliphatic, and heterocyclic compounds, leading to aromatic and alkylic carbon hydroxylation, double-bond epoxidation, ether cleavage, sulfoxidation or N-oxidation reactions (depending on the substrate), H ₂ O ₂ -dependent one-electron abstractions from phenols, and H ₂ O ₂ -dependent bromination of organic substrate	Hofrichter et al. (2010)

(continued)

Table 4.2 (continued)

Fungal taxa	Enzymes	Action of enzymes	References
<i>Ascomycota</i> , <i>Basidiomycota</i> , <i>Mucoromycotina</i> and <i>Chytridiomycota</i>	Cytochrome P450 monooxygenases	Incorporation of a single atom from O ₂ into a substrate molecule, with concomitant reduction of the other atom to H ₂ O	Kasai et al. (2010), Subramanian and Yadav (2009), Ullrich and Hofrichter (2007), and Yadav et al. (2006)
<i>Ascomycota</i> and <i>Basidiomycota</i>	Phenol 2- monooxygenases	Incorporation of a single atom from O ₂ into a substrate molecule, with concomitant reduction of the other atom to H ₂ O	Hofrichter et al. (2010) and Ullrich and Hofrichter (2007)
	Laccases	O ₂ -dependent one-electron oxidation of organic compounds	Baldrian (2010) and Majeau et al. (2010)
<i>Ascomycota</i> , <i>Basidiomycota</i> , and <i>Mucoromycotina</i>	Nitroreductases	NADPH-dependent reduction of nitroaromatics to hydroxylamino and amino(nitro) compounds and of nitro functional groups of N-containing heterocycles	Crocker et al. (2006), Bhushan et al. (2002), Fournier et al. (2004), Esteve- Núñez et al. (2001), and Scheibner et al. (1997)
	Tyrosinases	O ₂ -dependent hydroxylation of monophenols to o-diphenols (cresolase activity) oxidation of o-diphenols to catechols	Ullrich and Hofrichter (2007) and Halaouli et al. (2006)
	Miscellaneous transferases	Formation of glucoside, glucuronide, xyloside, sulfate, or methyl conjugates from hydroxylated compounds	Kasai et al. (2010)

4.5 Mycoremediation of Solid Wastes

The process of construction and demolition result in the spread of waste debris into the environment. The agricultural solid wastes from agricultural industry are used for mushroom cultivation. In agricultural, paper-pulp industries contaminate the environment with lignocellulosic waste. Mushrooms use agricultural wastes as a substrate for growing and production of enzymes. Therefore, it is a good idea to solve environmental problems. These wastes can be dealt with efficiently by employing lignocellulolytic microorganisms for composting the wastes, such as *Pleurotus ostreatus*, *Polyporus ostriformis*, and *Phanerochaete chrysosporium*. Crop residues such as straw, leaves, and trash can also be degraded by these fungi. Cellulases are hydrolytic enzymes that hydrolyze these agricultural wastes. Several

filamentous fungi, also produce cellulases. The cellulases are endo1,4 β -glucanase, cellobiohydrolase, and β -glucosidase. These enzymes enhance the potential for hydrolysis and saccharification of biomasses of willow, rice straw, etc., by application of two fungi *Armillaria gemina* and *Pholiota adiposa*. *P. pulmonarius* engenders pectinase and laccase utilizing orange waste as substrate (Inácio et al. 2015). Orange wastes contain pectin which is one of the most abundant carbohydrates. *P. pulmonarius* can be induced to produce pectinase and laccase by the orange waste. Utilizing orange waste as a solid substrate system, hydrolytic and oxidative enzymes are being engendered by *Pleurotus pulmonarius* and *Pleurotus ostreatus* (Alexandrino et al. 2007).

In recent years, scientists have paid keen attention towards the decomposition of leather wastes. It comprises collagen, keratin, elastin, albumins, and globulins. Their degradation can be possible only due to the action of fungi that are very rich in enzymes. To make the treatment of leather wastes cost-effective and appropriate, fungal mycelia, mycelia pellets, immobilized fungi, or their enzymes are being used (Christopher et al. 2016). Industrial development is the main cause of toxic pollution. Paper and pulp production resulted in contamination of soil. Solid wastes from pulp and paper industries are lime mud, lime slaker grits, green liquor dregs, boiler and furnace ash, scrubber sludges, and wood processing residuals.

Thus essential steps are needed to be taken to completely degrade resultant pollutants which cannot be completely degraded by conventional wastewater treatment methods. Mycoremediation of industrial wastes has gained interest among scientists in recent years. This technology is treating the environmental and health problems associated with these wastes and pollutants (Kulshreshtha et al. 2012). Pulp and paper industry effluents contain lignin, tannic acid, resin, cellulose, and hemicellulose, which are difficult to degrade (Kumar et al. 2012).

Unfortunately, electronic waste has become the fastest-growing segment of Indian waste pour out. Several fungi such as *Penicillium simplicissimum*, *P. bilaiae*, *Saccharomyces cerevisiae*, and *Yarrowia lipolytica* have been found to grow in the presence of electronic scrap (Dave et al. 2016). These fungi act as complexing agents and help in the extraction of Cu, Cd, Sn, Al, Ni, Pb, Zn, etc. Arbuscular mycorrhizal fungi can revegetate degraded lands, namely, coal mines or waste sites (Gaur and Adholeya 2004). These mycorrhizal fungi assist plants to grow and degrade unfertile soil of coal mine areas.

4.6 Xenobiotic Organic Compounds (XOCs) and Mycoremediation

Intensive agriculture has resulted in increased release of xenobiotic compounds to the environment. Xenobiotics is derived from the Greek words “Xenos” means foreigner and “bios” means life. XOCs are the unnatural synthetic organic compounds which are not easily biodegradable (Sullia 2004), such as petroleum hydrocarbons,

halogenated organic compounds, dyes, polycyclic aromatic hydrocarbons (PAHs), pesticides, and heavy metals. Contamination of soil by XOCs is a worldwide problem. Mycoremediation includes the process, such as myco-enzymes, myco-degradation, and myco-sorption, and is the application of enzymes, namely, manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase is a common and preferred technology for the removal of XOCs (Noman et al. 2019). The main contaminants of polluted soils are petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and halogenated organic compounds (Loske et al. 1990).

4.6.1 Petroleum Hydrocarbons

These are complex compounds which are differentiating into saturates, aromatics, asphaltenes, and resins. Various hydrocarbon compounds based on petroleum sources, namely, diesel, kerosene, petrol, and lubricating oils, are described as total petroleum hydrocarbons. There are several ways that lead petroleum hydrocarbon to contaminate soil. The presence of these components is toxic (Scott 2003) and cause mutations and deaths (Alvarez and Vogel 1991). The prime reason for their toxicity is a low boiling point, with carbon ranging from C10 to C19. Bioremediation is an effective way to treat petroleum-contaminated soils and their applications (Gerhardt et al. 2009; Phillips et al. 2008, 2009; Euliss et al. 2008; Newman and Reynolds 2004).

4.6.2 Polycyclic Aromatic Hydrocarbons (PAHs)

These are the released varieties of environmental activities, namely, incomplete combustion of fossil fuels, accidental disposal of petroleum products, coal gasification, and liquefaction. Incomplete combustion of carbon materials forms PAHs. The man-made sources of PAHs include residential heating, coal-tar pitch and asphalt production, coke and aluminum production, and many more activities related to petroleum refineries and motor vehicle exhaust (Abdel-Shafy and Mansour 2016; Peng et al. 2008). PAHs are hydrophobic and so can be easily accumulated in fatty tissue (Varjani 2017). The harmful PAHs are with more than four rings which are mutagenic and carcinogenic (Varjani et al. 2015; Steffen et al. 2007). According to Fernandez-Luqueño et al. (2011) more than 50 fungal groups can degrade various PAHs. Moreover, they have a mechanism to attack specific PAHs (Abdel-Shafy and Mansour 2016). Oyster mushroom, *Pleurotus ostreatus* can degrade 80–95% of PAHs present in soil (Steffen et al. 2007). *Stropharia rugosoannulata* is the most efficient species of mushroom for the removal of PAHs (anthracene, pyrene, and benzo (a) pyrene).

4.6.3 *Halogenated Organic Compounds*

These include pentachlorophenol (PCP), trichloroethene (TCE), 2, 4-dichlorophenoxyacetic acid (2, 4-D), polychlorinated biphenyl (PCB), and dioxins. These halogen substituents contribute harmful effects of the compounds, increasing their toxicity, mutagenicity, and other detrimental capacities. These halogen substituents increase the hydrophobicity of the compounds, increasing their tendency to bioaccumulate in food chains and to sorb to the soil.

4.6.4 *Synthetic Dyes*

These are increasingly employed in various industries, namely, textile, paper, cosmetic, pharmaceutical, and food industries. These are commonly used in printing industries, color photography, and petroleum products. The dyes with azo, anthraquinone, tri-phenylmethane, and heterocyclic polymeric structures, the largest and most versatile class of dyes, the azo dyes, constitute more than half of the yearly manufactured synthetic dyes (Bonugli-Santos et al. 2015; Diwaniyan et al. 2010). The release of such dye-containing effluent results in impaired primary production and interferes dilution of gasses and affects human health (Baughman and Weber 1994; Ciullini et al. 2008; Rodriguez et al. 2015). It is necessary to control dyes for their possible toxicity and carcinogenicity.

4.6.5 *Synthetic Pesticides*

Mycoremediation is an eco-friendly approach for degradation of pesticides. These were known since DDT was discovered (Tessier 1982). Pesticides contaminate global ecosystems due to their toxicity and persistence in the environment. They can be grouped into insecticides, herbicides, and fungicides. Besides agricultural applications, large amounts of pesticides are used in reducing diseases, namely, malaria, and typhus fever. Large quantities of pesticides are used annually to increase the production through controlling harmful effects caused by the target organisms around the world (Liu and Xiong 2001). Spray of these chemicals also hits no target vegetation and that contaminate the soil. Their misuse results in destruction of soil along with microfauna and flora (Edwards 1986). The influences of pesticides include hormonal disruption, immunosuppression, mutagenicity, reproductive abnormalities, diminished intelligence, and various other health issues (Gupta 2004). The chlorinated insecticides, including DDT, aldrin, dieldrin, heptachlor, endrin, chlordane, and endosulfan, are of major environmental concern. Degradation of DDT by ectomycorrhizal fungal species, namely, *Gomphidius viscidus*, *Boletus edulis*, *Laccaria bicolor*, and *Leccinum scabrum*, has been reported (Huang et al.

2007). Various fungi, such as *Agrocybe semiorbicularis*, *Flammulina velutipes*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, and *Phanerochaete velutina*, degrade herbicides, such as diuron, atrazine, and terbuthylazine (Bending et al. 2001). *Pleurotus pulmonarius* degrades atrazine and produces the N-dealkylated metabolites deethylatrazine, deethyl- deisopropylatrazine, deisopropylatrazine, and 2-chloro-4-ethylamino-6-(1-hydroxyisopropyl) amino- 1,3,5-triazine (Masaphy et al. 1993).

4.6.6 Heavy Metals

These refer to metallic elements having a specific mass $>5 \text{ gcm}^{-3}$ and are able to form sulfides. The sources of heavy metals are the anthropogenic activities, namely, industrial production, mining, steel and iron industry, agriculture, transportation, chemical industry, as well as domestic activities that release heavy metals into soils, and ultimately to the biosphere (Jantschi et al. 2008; Pantelica et al. 2008; Abbas et al. 2011). The fungi tolerate metals by various mechanisms that help them survive and grow even in metal-contaminated sites (Gadd 2007). To survive under metal-stressed environment, organisms have evolved several mechanisms. Mushrooms are useful to decontaminate soil from heavy metals as they consist of good content of cell wall materials that offer excellent metal-binding features (Mann 1990; Muraleedharan et al. 1991). The mushroom cell walls are cation exchanger because of negative charge (Fomina et al. 2007). Mushrooms, such as *Pleurotus*, have proven to be effective in the removal of lead (Gazem and Nazareth 2013), cadmium, nickel, mercury, arsenic (Joshi et al. 2011), iron (Taştan et al. 2016), and zinc (Vaseem et al. 2017) in marine environment, wastewater, and on land. Mushrooms reduce the toxicity of metallic contaminants through pH change, biosorption, and bioaccumulation. The bioaccumulation of heavy metals and pollutants, such as mercury (Hg), iron (Fe), zinc (Zn), lead (Pb), copper (Cu), nickel (Ni), and cadmium (Cd), by edible mushroom, namely, *Pleurotus squarrosullus*, *Volvariella volvacea*, *Schizophyllum commune*, and *Auricularia auricular* (Udochukwu et al. 2014).

4.7 Role of Mycorrhizae in Remediation of Soil

All types of mycorrhizal fungi have paramountcy for planet earth. They perform a vital role in the maintenance of a pristine ecumenical ecosystem. Their mechanism helps sustainable applications in agriculture, conservation, and recuperation, concretely in the exhaustion of natural resources. The state of mycorrhizal fungi in an agroecosystem is not confined to the supply of nutrients to the plant. One consequential concern is the agricultural soil, its health, and structure (Willis 2018). The soils having mycorrhizae decreases the drought conditions (Ortiz et al. 2015; Jayne and Quigley 2014). Agricultural exercises condense and change mycorrhizal diversity (Verbruggen et al. 2013; Verbruggen and Toby Kiers 2010; Williams et al. 2017).

Mycorrhizal fungi growing as symbionts with plant roots can degrade organic pollutants in soil (Robertson et al. 2007). Several types of mycorrhizae, such as ectomycorrhizae, increase the tolerance against heavy metals. The fungi capture phosphorus (P), nitrogen (N), and other nutrients from the soil and exchanges them with the plant partner derived carbon (C) compounds that feed on fungal metabolism. Various classifications of mycorrhizal fungi are ectomycorrhizas (ECM), arbuscular mycorrhizae (AM), ericoid mycorrhizas (ERM), arbutoid mycorrhizae (ARM), ectendomycorrhizae, orchid mycorrhizae, and monotropoid mycorrhizae. Mycotrophic plants grow on heavily polluted soils due to the accumulation of metals in extrametrical hyphae and extrahyphal slime. Various types of mycorrhiza, such as ECM, ERM, and VAM, increase tolerance to heavy metals. This leads to the immobilization of metals in roots and decreases uptake to shoots. ECM fungi act as useful bioindicators of pollution (Haselwandter et al. 1988). In conclusion, the majority of mycorrhizal fungi appear to be able to degrade a range of contaminants. Mycorrhizal fungi prevent heavy metals from traveling to plant through roots (Rajkumar et al. 2012) and store them in vacuoles. Some mushrooms increase their tolerance against toxicity. They modify the response of the plant against heavy metals at transcription and translation (Ferrol et al. 2016). Mycorrhizae help in the colonization of barren soil. They remain functional under extreme conditions, i.e., after a forest fire. After obtaining minerals and nutrients, released during forest fire before they are leached out of the soil. It increases the ability of regeneration after forest fires (Buchholz and Motto 1981). Mycorrhizal remediation ensures the removal of pollutants from soil and improves the structure of the soil. In addition to that, they detoxify the organic and inorganic toxic substances (Chibuike 2013).

The natural role of mycorrhizosphere organisms declined due to the excessive use of inorganic fertilizers, herbicides, and pesticides (Sturz et al. 1997). Intensive agricultural practices continue stepping up agricultural production regionally and globally along contamination of soil, its fertility, ecosystem balance, and soil biodiversity which can be observed superficially. Nowadays, an appreciation of soil microorganisms has been given in the literature for their role in agricultural sustainability and ecosystem management. The mycorrhizal fungi have beneficial effects on crop production and soil quality (Bethlenfalvay and Schüepp 1994). Artificial mycorrhizae inoculum production should be carefully practiced as soil chemical fertilizer for soil fertility management. An appropriate selection of host/fungus combinations would allow a reduction in the number of inputs without losses in productivity.

4.8 Disadvantages of Mycoremediation

Plenty of research is yet going on in the area of mycoremediation and consequently mycoremediation can be studied to be in the trial phase. Further explained procedures in the field of biodegradation by fungi are required. The traditional remediation technologies are still conquering the faith of the processing units. They can

manage sizably large amplitudes of wastes at a time and are thought as fast processes. Mycoremediation, in this case, would be too slow, and the space required for treatment or storage of materials could be restrictive. The cessation users are always in search of technologies which are 100% efficient, whereas the biological systems cannot promise that caliber of efficiency. The efficiency level of biological systems is never 100% efficient, which is arduous for some end-users to understand. The natural systems always face problems with the competitive natural habitat in a particular area and are withal affected by seasonal variations in extreme habitats. Though we are in the orchestration of commercialization of the technology by fungi, we very strongly ken that the research is still in the experimental phase and, additionally, we are not certain of the time it will take. More work is needed in the field of fungal bioremediation to apply the technology to more astronomically immense-scale projects. We expect to extemporize ways and betokens to eliminate the shortcomings that cause obstructions in the potential of mycoremediation. An immensely large licit issue additionally predominates as many of the fungi and their products and sundry technologies involving them are controlled by one or other scientists. Thus utilization of the mushrooms in bioremediation may pose several licit difficulties.

4.9 Conclusion and Future Aspects

The purpose of this chapter is to provide knowledge about major environmental pollutants and the use of fungi to treat these contaminants present in the environment. Bioremediation is a powerful cleanup technology for hazardous waste pollutants from soil, such as heavy metal. Although there are various sources of bioremediation, only biological treatment is not enough to decontaminate the soil. Only those biological agents should be cultured in the laboratory that require minimal requirement and can be treated pollutants of soil. A detailed study of pollutant region is much needed to finalize the priority area that needs effective removal of the pollutants. Mycoremediation is a necessary bio-tool for the conservation of natural resources and environmental management. Biosorption, a process of bioremediation, is efficient for the remediation of heavy metals from contaminated soil. It could be mediated by both living and dead biomass. Mushrooms are highly preferred biosorbents for heavy metals as they can be grown easily and produce an enormous quantity of biomass. This method has many advantages such as separation of metals over a wide range of pH/temperature and rapid kinetics of adsorption and desorption. Mushrooms play an important function in the renewal of the environment. Toxic metals, synthetic compounds, and chemicals can be transformed/degraded by various mushrooms. Molecular biology studies in future might enhance the biodegradative capabilities of fungi. There is a need for genetic manipulation – different strains of fungi capable of remediating a wide variety of pollutants on multiple heavy metal-contaminated sites under stress conditions. Cumbersomely heavy metal contamination of soil is one of the top ten concerned events cognate to

environmental issues throughout the world (Yang and Sun 2009). Besides sundry challenges, mycoremediation is a most preferred method for xenobiotics, trichloroethylene, petroleum, BTEX (benzene, toluene, ethylbenzene, and xylene), explosives, and cumbersomely heavy metal compounds. The use of genetic engineering methods for bioremediation has much importance and interest. Genetically engineered organisms with enzymatic abilities can degrade any environmental pollutant. Many species of mushrooms are biosensors for metal pollution sites. The biodegradation capacity of mushroom is essential to compensate for the depletion of microbial communities due to soil contamination. Even more, the bioremediation potential of different groups of fungi from different ecosystems appears to be interconnected, engendering communities that favor the survival of its members and enhancing the detoxification activities of the different ecosystems. Wunch et al. (1999) for the first time studied that the fungi have the facility to degrade anthropogenic compounds. They reported the degradation of benzo(α) pyrene by *Marasmiellus troyanus* in liquid culture. Since this time, researchers have been given the task of finding more evidence to solve this quandary (Singh et al. 2015; Rhodes 2014; Anastasi et al. 2013).

The application of this technology is severe; adept and trained personnel with appropriate cognizance of the subject are required to do the work of fungal remedy. Moreover, there is a purpose to research the ability of mushroom and additionally to employ other species of mushrooms for the degradation of pollutants. The main drawback of physicochemical approaches has been mainly owed to the high cost, low wages inhibited multifariousness, and intrusion by other wastewater ingredients and the approach of the excess engendered. The biotic action of the contaminated environment is an economically and ecologically alluring alternative to the present physicochemical methods of practice. This chapter compiles the recent developments, conceptions on the mycoremediation of hydrocarbon, and cumbersomely heavy metal-contaminated environment that is an environmental affair in jeopardous places using fungi.

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Chapter 5

Microbial Degradation of Organic Constituents for Sustainable Development



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5.1 Introduction

A dramatic increase in the range of chemically synthesized products such as pesticides, plastics, hydrocarbons, dyes, soaps, detergents, and other useful substances have been found in the last few decades. These pollutants impart an immense effect on the environment and are considered as potential pollutants because most of these compounds are resistant to biodegradation. To clean up these pollutants from the environment is a global problem and that too real. These pollutants directly or indirectly affect all forms of life on the planet (Sajna and Pandey 2015; Souza et al. 2014).

The various compounds, including halogenated organic pollutants such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), poly brominated diphenyl ethers (PBDEs), decarbromodiphenyl ethane (DBDPE), and dechlorane plus (DP) have been of great concern due to their perseverance, bioaccumulation, and potential toxicity to animals and humans. PCBs were primarily used as dielectric and coolant fluids (Xing et al. 2005). The rest are used as flame retardants in textiles, thermoplastics, electronics, polyurethane, foams, and building materials (Covaci et al. 2011; Alaei et al. 2003; Bhat et al. 2018a; Qadri and Bhat 2020). Petroleum hydrocarbons are the important source of energy and a raw material for numbers of industries. Hydrocarbon contaminants are recalcitrant substances and are therefore considered as priority pollutants. Industrial effluents, municipal runoffs, offshore and onshore petroleum activities, as well as accidental spills, cause (petroleum) hydrocarbon pollution.

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In addition to the above, a wide spectrum of hazardous pollutants with different structures originates from human activities and cause global threat to environment continuously. The three main sources of organic pollutants are classified as: industrial wastes, agricultural wastes, and military wastes; as already mentioned, the important organic contaminants include petroleum products, polycyclic aromatic hydrocarbons, dioxins, organophosphates, carbonates, plastics, and related compounds (Connell et al. 2006). Modern agronomy depends on the four main aspects, namely, water, fertilizers, seeds, and pesticides. Pesticides are considered as essential part of modern agronomy. About 35–45% is lost due to weeds, insects, pests and diseases, while during storage, 35% of crop produced is lost. Heavy metals are other potential anthropogenic pollutants.

Urban and industrial wastes are frequently disposed directly into pits dug into the ground, which leads to pollution of the earth and, sometimes, into the nearby ground waters. Organic contaminants can be grouped into following classes, namely, (a) oxygen, nitrogen, and phosphorous compounds, (b) hydrocarbons, and (c) metallic substances. These substances may encompass elements such as carbon, oxygen, and sometimes chlorine as well. Hydrocarbons are lipophilic, least soluble in water, and persistent in the environment. The source of hydrocarbon is already discussed as from industries and motor vehicles, etc. The other group which contains oxygen, nitrogen, and phosphorous compounds is very diverse, but constitutes the components having comparatively higher solubility in water but low fat solubility and rather low perseverance in the environment (Connell et al. 2006). The third group is the organometallic group which is said to be of least importance in view of environmental properties. It may consist of substances that result from the amalgamation of metals such as lead and tin with carbon-based compounds (Connell et al. 2006; Dar et al. 2016; Bhat et al. 2017; Dar and Bhat 2020; Dervash et al. 2020).

5.2 Some Persistent Organic Pollutants (POPs)

A diverse group of chemicals have created a particular havoc to the environment, due to their fat solubility, bio magnification, bioaccumulation, and persistence in the ecosystem. These substances are termed as organic pollutants and are now widely distributed up to the global scale. They have been observed to remain for a longer period of time in the environment and can accumulate, and thereby show biomagnification from one tropic level to another in a food chain (Eskenazi et al. 2009). Persistent organic contaminants include two types of important compounds:

- (a) Polycyclic aromatic hydrocarbons (PAHs): These are the class of hydrophobic organic contaminants (HOCs) broadly established in air, soil, and sediments. They are mainly released from industries, are highly toxic, persistent, and can accumulate in fish as well as human beings via consuming aquatic foods (Connell et al. 2006; Dar et al. 2013; Bhat et al. 2017a, b).
- (b) Polychlorinated biphenyls (PCBs) or halogenated hydrocarbons. Halogenated ones contain the organochlorines such as DDT and PCBs. The dioxins produced

at large scale are released to the environment. They do not easily undergo microbial degradation. The biphenyls being highly chlorinated tend to accumulate up to higher extent than less chlorinated PCBs. PCBs disrupt the endocrine system and can cause cancer. Thus, PCBs being the highly toxic and mutagenic components, are therefore of increasing concern (Seeger et al. 2010; Bhat et al. 2018a, b).

Pesticides are chemical substances utilized for prevention, destruction, and mitigation of pest in agricultural fields. Long-term application of pesticides can lead to contamination of pesticide residues in the soil and causes a serious problem, as most of the used pesticides cannot undergo degradation easily.

Dyes are chemical compounds used in textile industries, printing, paper, color photography, cosmetics, pharmaceuticals, and various other industries and factories (Raffi et al. 1997). There are large numbers of dyes present with different uses. The most commonly used are basic dyes, acidic dyes, mordant dyes, azoic dyes, vat dyes, reactive dyes, etc. Among all the dyes, azoic dyes are a large class of the synthetic dye and highly important for commercial purposes (Verma and Madamwar 2003; Mushtaq et al. 2018). They have the complex structure due to which they are poorly degradable and contaminate the soil as well as water bodies (Mehmood et al. 2019).

Heavy metals are another class of contaminants which can persist in the soil for extended period and create threat to both flora as well as fauna because of their bioaccumulation and non-biodegradability in nature (Gautam et al. 2014; Wai et al. 2012; Singh et al. 2018). Heavy metals such as lead, aluminium, cadmium, gold, mercury, and silver have no biological role and are very toxic to living beings. These metals cannot be degraded, but can be converted from complex form to simple and stable form with the help of essential soil microbes.

There is an immediate need to remediate these pollutants from the surroundings. However, several physiochemical strategies have been used to detoxify these persistent pollutants, but these methods are costly and also give rise to secondary pollutants (Zhang et al. 2004). Microbes or their by-products are used in degradation and detoxification of contaminants from soil, and can successfully increase plant growth and development (Mushtaq et al. 2020). They can significantly remove, immobilize, and transform into less toxic forms the pollutants from the environment and also reduce their harmful impacts on plants as well animals. This is a cheap, natural, sustainable and eco-friendly approach to clean up the environment (USEPA 2012). This book chapter aims to provide a general overview about the role of microbes in the degradation of organic substances for sustainable development.

5.3 Role of Microbes in the Degradation of Organic Substances

In the past few decades, wide research has resulted in isolation of different microorganisms having the capability of degrading a huge number of organic compounds. They are used for eradicating the various types of pollutants from surroundings and

diminishing their hazardous effects on the environment (EPA 2016). Microbes possess the enzyme system with the help of which they can eradicate, degrade, or immobilize the pollutants from the soil either naturally or artificially (Uqab et al. 2016).

5.4 Microbial Degradation of Persistent Organic Pollutants

PAHs are highly carcinogenic organic pollutants with complex structures. They are difficult to degrade and impose a threat on the environment. However, microbes show the property of degrading these organic contaminants and convert them into less toxic forms. Microorganisms, such as bacteria, fungi, and other microbes are involved in the biodegradation of PAHs and are cost-effective and natural processes (Anwar et al. 2016; Khanday et al. 2016). Bacteria which can degrade the hydrocarbons are called as hydrocarbon degrading bacteria. Bacteria degrade these substances both, aerobically (*Pseudomonas* sp., *Brevibacillus* sp.) as well as anaerobically. However, anaerobic biodegradation is of much importance (Wiedemeier et al. 1995). *Streptococcus*, *Klebsiella*, *Bacillus*, *Shigella*, *Staphylococcus*, *Alcaligenes*, *Escherichia*, *Corynebacterium*, *Acinetobacter*, and *Enterobacter* are a few well-known bacteria involved in biodegradation of hydrocarbons (Kafilzadeh et al. 2011). Teng et al. (2010) observed that microbes, such as *Sphingomonas*, *Beijerinckia*, and *Rhodococcus*, can degrade anthracene with the help of dihydriol, a first oxygenated intermediate component. Jin et al. (2016) reported that the *Pseudomonas* strain can degrade aromatic hydrocarbon, such as pyrene, efficiently. Microbes possess varied genes related to the degradation of PAHs and usually follow different mechanisms for the degradation of these compounds. In some mechanisms, nutrients such as carbon, nitrogen, and phosphorous are added to soil contaminated with hydrocarbons. These nutrients stimulate the microbes that are involved in biodegradation processes. Application of nutrients to hydrocarbon contaminant soils boost the growth of microbes and enhance the degradation process as these sites are usually polluted and did not contain enough nutrient for microbial growth.

A single bacterium is less potent to degrade the large number of organic substances present in polluted soil as it alone does not have all the enzymatic properties of degradation. It was proved that the microbial consortium which contains the number of microbes that live together symbiotically and has the most potent biodegradation capability because, in consortium, different microbes have different functions that enhance the biodegradation process of a mixture of organic compounds (Fritsche and Hofrichter 2005). Few bacteria have the property of co-metabolism, which means that microbes at polluted sites convert or degrade the organic compounds into the simple compounds which are easily degradable without growing and deriving energy in polluted sites. After that, the coexisting bacteria which actually grow these sites utilize these degraded substances as an energy as well as growth substrate. Beam and Perry (1974) reported that *Mycobacterium vaccae* proliferate on propane while cometabolizing cyclohexane and converts it into cyclohexanol via oxidation and the cyclohexanol then generated can be used by different microbes.

Table 5.1 Some examples of microbial strains involved in degradation of polyaromatic hydrocarbons

Microbes	Target substrate	References
<i>Pseudomonas</i> sp. PB2	Degrades pyrene, chrysene, and naphthalene	Nwinyi et al. (2016)
<i>Thalassospira</i> sp. TSL5-1	Pyrene degradation	Zhou et al. (2016)
<i>Pseudomonas oleovorans</i>	Tetrahydrofuran	Zhou et al. (2011)
<i>Acinetobacter</i> sp. WSD	Phenanthrene and fluorine degradation	Shao et al. (2015)
<i>Rhodococcus</i> sp. PI8	Degradation of various high molecular weight poly hydrocarbons	Isaac et al. (2015)
<i>Arhodomonas</i> rozel	Toluene benzene	Dalvi et al. (2012)
<i>Acinetobacter venetianus</i>	n-alkanes	Di Cello et al. (1997)
<i>Burkholderia cepacia</i> F297	Naphthalene degradation	Grifoll et al. (1995)
<i>Stappia aggregata</i>	Phenanthrene and pyrene	Cui et al. (2008)
<i>Oceanobacter kriegii</i>	Petroleum	Teramoto et al. (2009)
<i>Bordetella avium</i>	Naphthalene	Abo-State et al. (2018)

Different microbes degrade the different persistent organic compounds in environments. *Pseudomonas putida* is potent to degrade benzene and toluene, while *Pseudomonas oleovorans* are capable of degrading tetrahydrofuran, xylene, and ethylbenzene (Zhou et al. 2011). An aromatic hydrocarbon, alkylbenzoate, was found to be degraded potentially by *Pseudomonas putida* (Kaldalu et al. 2000). It has been reported that *Virgibacillus salarius* bacteria utilize benzene, toluene, and ethylbenzene as a carbon source, catechol 2, 3-dioxygenase, and chlorocatechol 1,2-dioxygenase enzymes present in this bacterium help in the degradation of HCs (Solanki and Kothari 2012). Examples of microbial stains verified for the degradation of hydrocarbons are listed in Table 5.1.

5.5 Microbial Degradation of Pesticides

Pesticides are broadly consumed to stop the growth of pests in agricultural products. In India, about 11–15% of grains produced annually are lost due to pests. To control such losses of crop productivity, pesticides are widely applied which plays an important role in crop protection. However, the long-term use of pesticides causes a severe threat to soil, water bodies, atmosphere, food stuffs, and threatens human health as well (Fenner et al. 2013; Bhatti et al. 2017; Dar et al. 2020). However, researchers have conducted studies of bacteria that could either degrade the persistent pesticides completely or convert them into degradable form (Akbar and Sultan

2016; Jabeen et al. 2015). From the last few decades, various researches cultured as well as screened numerous microbes from different locations to degrade the microbes. Recent reports regarding pesticide degrading bacteria include *Klebsiella*, *acinetobacter*, *flavobacterium*, *alcaligenes*, and *bacillus* are able to degrade endosulfans potentially (Kafilzadeh et al. 2015). Jayabarath et al. (2010) reported that, *Streptoverticillium album*, *Nocardia vaccine*, *Nocardia farcinia*, *Streptomyces alanosinicus*, *Micromonospora chalcea*, *Nocardia amarae*, and *Streptomyces atratus candegrade* can also degrade pesticides very well.

Microbes can degrade the pesticides more easily as most of the microbes used the pesticides as their nutrients and converted them into CO₂ and H₂O via enzymatic reactions. Microbes have different enzymatic activities and, when absorbed, these pesticides go through various biochemical and physiological activities and change or degrade these substances into simple molecules with least toxicity (Tang 2018; Chen et al. 2011). Certain microbes have the ability to degrade the pesticides through a series of specific enzymes. For example, Atrazine is said to be the only carbon source of ADP strain of *Pseudomonas*, and this bacterium degrades the atrazine with the activity of three enzymes, namely, AtzA converts atrazine into hydroxyl atrazine, a non-toxic compound via hydrolysis dechlorination reaction and AtzA is a vital enzyme for atrazine's biological degradation. After that, another enzyme, AtaB, carries the dehydrochlorination reaction of hydroxyl atrazine and generates N-isopropyl cyanuric amide, and then AtzC converts the N-isopropyl cyanuric amide into cyanuric amide and isopropylamine. These two constituents of atrazine i.e cyanuric amide and isopropylamine are then finally converted into NH₃ and CO₂ (Czarnecki et al. 2017; Wackett et al. 2002). Kanade et al. (2012) observed that *Bacillus*, *Stenotropomonas*, and *Staphylococcus*, obtained from cultivated as well as non-cultivated soils, are able to decompose DDT efficiently.

5.6 Bacterial Degradations of Azo Dyes

Bacterial strains have been recognized that can capably degrade azo dyes. Bacteria usually degrade azo dyes via biosorption and enzymatic degradation pathways (Wu et al. 2012). Some bacteria degrade azo dyes aerobically and anaerobically (Dos Santos et al. 2007), but microbial degradation or decolorization of azo dyes are more effective under anaerobic environment (Lodato et al. 2007). Microbes degrade azo dyes by producing enzymes, for example, peroxidase, laccase, hydrogenase, and reductase. Wide arrays of microbes are capable of degrading the azo dyes, including bacteria, fungi, yeast, and actinomycetes (Olukanni et al. 2006; Wesenberg et al. 2003). Some examples of bacterial strains which degrade azo dyes either aerobically or anaerobically extensively are *Escherichia coli*, *Pseudomonas* sp., *Corneybaterium* sp., *Bacillus subtilis*, *Clostridium* sp., *Enterococcus* sp., *Rhabdobacter* sp., *Acinetobacter* sp., *Lactobacillus*, *Xenophilus* sp., *Staphylococcus* sp., *Micrococcus dermacoccus* sp., *Geobacillus*, *Rhizobium*, *Proteus* sp., *Morganella* sp., *Alcaligenes* sp., *Klebsiella* sp., and *Aeromonas* sp. (Lin and Leu 2008; Vijaykumar

Table 5.2 Microbial degradation of azo dyes

Microorganism	Target substance	References
<i>Enterobacter agglomerans</i>	Methyl Red decomposition	Keharia and Madamwar (2003)
<i>Bacillus subtilis</i>	Acid Blue 113	Gurulakshmi et al. (2008)
<i>Bacillus fusiformis</i> kmk 5	Acid Orange and Disperse Blue	Kolekar et al. (2008)
<i>Sphingomonas</i> sp. BN6	Acid azo dyes and Direct azo dyes	Russ et al. (2000)
<i>Rhizobium radiobacter</i>	Reactive Red 141	Telke et al. (2008)
<i>Brevibacillus laterosporus</i>	Direct Brown MR, Golden Yellow, and Remazol Red	Gomare et al. (2009)
<i>Proteus mirabilis</i>	Reactive Blue 13	Olukanni et al. (2010)
<i>Sphingobacterium</i> sp.	Orange 3R, Direct Blue GLL	Tamboli et al. (2011)
<i>Bacillus</i> sp. ADR	Methyl Red, Reactive Orange	Telke et al. (2011)
<i>Gloeocapsa pleurocapsoides</i> and <i>Chroococcus minutus</i>	Direct Blue-15	Parikh and Madamwar (2005)
<i>Pseudomonas aeruginosa</i> and <i>Bacillus circulans</i>	Reactive Black 5	Dafale et al. (2008)
<i>Providencia</i> sp. SDS	Remazol Black, Congo Red, and Methyl Orange Remazol Black	Phugare et al. (2011)

et al. 2007). Coughlin et al. (2002) reported that azo dyes are the only source of carbon and nitrogen to few aerobic bacteria. Various microbes usually degrade azo dyes by secreting different enzymes, such as peroxidases, phenolic oxidases, laccases, and lignin peroxidases.

Besides bacterial degradation, fungi also play an important role in the degradation of dyes (Bumpus 2004). Abundant research exists about the capability of fungi to oxidize the phenolic, soluble, as well non-soluble dyes (Libra et al. 2003; Kapdan and Kargi 2002). Some examples of fungi involved in the degradation of dyes are ligninolytic fungi, *Aspergillus flavus*, *Penicillium gastrivous*, *Aspergillus niger*, *Rhizopus oryzae*, *Coriolus versicolor*, *Trametes versicolor*, *Phanerochaete chrysosporium*, *Fungalia trogii*, *Pleurotus ostreatus*, *Pycnoporus sanguineus*, and *Rigidoporus lignosus* (Bumpus 2004; Wesenberg et al. 2003; Fu and Viraraghavan 2001). It was observed that the White-rot fungi degrade aromatic dyes by secreting different enzymes (Harazono and Nakamura 2005; Madhavi et al. 2007). Degradation of the dyes by different types of microorganisms is listed in Table 5.2.

5.7 Bioremediation of Heavy Metals

Microbes cannot degrade heavy metals completely but can convert them from one oxidation state to another, but are very efficient in bioremediation of heavy metals (Garbisu and Alkorta 2001). Bioremediation is an eco-friendly technique which

Table 5.3 Bacteria used in bioremediation of heavy metals

Microbial strain	Heavy metals	References
<i>B. subtilis</i> BR151 (pTOO24)	Arsenic	Liu et al. (2011)
<i>Pseudomonas</i> sp. (K-62)	Mercury	Kiyono et al. (2009)
<i>Achromobacter</i> sp. (AO22)	Arsenic	Ng et al. (2009)
<i>E. coli</i>	Arsenic	Singh et al. (2008)
<i>Methylococcus capsulatus</i>	Chromium(IV)	Hasin et al. (2010)
<i>Pseudomonas putida</i> (06909)	Cadmium	Wu et al. (2006)
<i>P. fluorescens</i> (4F39)	Nickel	Lopez et al. (2002)
<i>Staphylococcus</i> sp.	Chromium	Sharma and Adholeya (2012)
<i>Cellulosimicrobium</i> sp. (KX710177)	Lead	Bharagava and Mishra (2018)
<i>Bacillus firmus</i>	Lead	Salehizadeh and Shojaosadati (2003)
<i>Enterobacter cloacae</i>	Copper	Jafari et al. (2015)
<i>Vibrio parahaemolyticus</i> (PG02)	Mercury	Jafari et al. (2015)
<i>Bacillus licheniformis</i>	Mercury	Saranya et al. (2017)
<i>Bacillus firmus</i>	Zinc	Salehizadeh and Shojaosadati (2003)
<i>Gemella</i> sp.	Copper	Marzan et al. (2017)

employs microbes, plants, or enzymes for the transformation of heavy metals, as well as other organic contaminants into CO₂, H₂O and other by-products which are less harmful than their parent substances (Chakraborty et al. 2012). Microbes usually detoxify heavy metals by various mechanisms, such as biosorption, extrusion, biotransformation, bioaccumulation, bioleaching, biomineralization, and phytoremediation (Ojuederie and Babalola 2017). Microbes involved in the removal of heavy metals from the soil generally use these heavy metals as an energy source for their growth and development. In a bioaccumulation process, microbes hold and concentrate heavy metals in their body and the microbes having this property are said to be strong agents of decontamination. Akhter et al. (2017) identified and isolated three stains of *Bacillus*, namely, NC7401, AVPI2, and PDPC01 from the root area of *Tagetes minuta* and put forth that these microbial strains have strong potential to solubilize and accumulate nickel, cadmium, and chromium and can biosorb these metals efficiently. Some microbial strains involved in bioremediation of heavy metals are listed in Table 5.3 below.

5.7.1 Biosorption

Biosorption by microbes is simply the sequestration of positively charged heavy metal ions by the ions present on the microbial cell surface (Malik 2004). Microorganisms can accrue HMs either by adsorption or absorption, in which adsorption is a surface phenomenon and absorption takes place all over the microbe's body. Microbes can adsorb a large amount of heavy metals very quickly. He and Tebo (1998) reported that at pH 7.2, *Bacillus* can adsorb up to 60% of Cu ions

within 1 minute. While absorption is a slower process and less efficient. Microbial adsorption involves complexation of heavy metals on their cell surfaces from which these metals get absorbed into the cell (Danis et al. 2008). Bacterial cell surface contains ions having nitrogen, phosphorous, sulfur, and oxygen as functional groups which can bind easily with the heavy metal ions and form coordination atoms and permits the metal ions to pass through the cell membranes (Sarret et al. 1998).

5.7.2 Bioleaching

In this process, microbes secrete organic acids that can dissolve the heavy metals present in soil. This process is energy- and nutrient-dependent. Microbes can efficiently leach the metals in presence of nutrients and energy sources. Marchenko et al. (2015) observed that *Citrobacter*, in presence of glucose and nutrients, could generate free inorganic phosphate that can form insoluble metal phosphate coating which can trap abundant quantity of toxic metals. Some microbes took part in redox reactions and can alter the valence of certain heavy metals, thus altering their property, and make them immobile as well as reduce their toxicity. It was reported *Coryne bacterium* can reduce the Cr^{6+} into Cr^{3+} , and *B. licheniformis* R08 can reduce Pb^{2+} Pb^0 (Goyal et al. 2003).

5.7.3 Bioaccumulation

It is a metabolically active process of microbes. In this process, microbes use their importer complexes that act as translocators and uptake heavy metals through lipid bilayer into their intracellular space. When these heavy metals reach inside the cell, they are sequestered by proteins as well protein ligands (Mishra and Malik 2013).

5.8 Conclusion

Increased industrialization has caused harmful effects on soil health due to the generation of polluted as well as non-degradable substances. Microbial degradation is an eco-friendly approach for removing the toxic substances from the environment. Microbes have the capability of detoxifying the contaminants from the soil as well as from water bodies and convert them into easily degradable forms. This chapter briefly overviewed the role of microbes to sustain and decontaminate the environment and demonstrates that microbes are potent agents for the degradation of hydrocarbons, pesticides, dyes, heavy metals, and other industrial effluents. Moreover, imminent information about microbes and their function in degradation processes should be researched.

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Chapter 6

Traditional Farming Practices and Its Consequences



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6.1 Introduction

Traditional farming is as a primitive method of farming, which is still being used by half of the world's farming population (Shakeel 2018). It involves the application of indigenous knowledge, traditional tools, natural resources, organic fertilizers, and cultural beliefs of the farmers (Shakeel 2018). The production of a variety of household crops and livestock was made possible through this farming (Alam et al. 2014). Traditional farming evolved over foremost thousands of years of agriculture as a sustainable system and in equilibrium with the surrounding ecosystems (Alam et al. 2014). The information has been used over the generations by the producers to produce the materials of their necessities.

With the passing years, the human population increased at a tremendous pace and food security became a major challenge. Although production multiplied several

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folds because of Green Revolution, but it had its own implications. The excessive use of fossil fuels, natural resources, agrochemicals, and machinery exposed the ecological integrity of agroecosystems to a greater risk (Singh and Singh 2017).

Therefore, with the growing environmental issues, such as climate change, population explosion, and natural resource degradation and biodiversity loss, sustainable food production is the need of the hour. Traditional farming is receiving attention worldwide for being a source of sustainable food production in times of global environmental crises (Singh and Singh 2017). The preservation of indigenous knowledge of traditional farming is advantageous in maintaining the biodiversity (Peroni 2017; Alam et al. 2014; Acquah 2002; Burton 1998), enhancing food-security, and protecting natural resources (Peroni 2017). However, there are certain negative implications as well, which is also a matter of concern.

6.2 Background

Going back to history, the development of agriculture can be classified into three phases, which includes traditional stage, green revolution era, and post-green revolution phase (Kunju 2013). Agriculture transformed gradually through these phases with the passing time, and change in demand and technology. Technology employed for farming operations has been crude in traditional agriculture, which gradually modernized through the modern eras. The period of traditional agriculture can be divided into two phases, which includes the early phase and the pre-modern phase. The time from which the agriculture spread to the beginning of the second half of the nineteenth century marks the early phase of traditional era, after which the pre-modern phase starts (Kunju 2013).

Traditional farming systems gave importance to the agricultural practice which involved sustainable interaction between plants, animals, soil, water, and food, leading to retained soil fertility, pest control, and mixed crop production (Kunju 2013). The inputs used in this system are mainly organic in nature and produced mainly on the farm itself, making it an integrated system of farming (Shiva 1996). Livestock rearing was being combined with agriculture by the farmers at that time and mixed or rotational cropping systems were followed (Shanin 1976). The practice of recycling farm wastes was a common practice, which would help in maintaining the fertility of the soil (Kunju 2013; Dar et al. 2013). Selection of good varieties and maintaining diversity marked the fundamentals of farming. Human and livestock have been used for harvesting and thrashing operations (Barker et al. 1985). The techniques developed have been practiced over the generations and have remained popular in many parts of the Asian subcontinent.

The informal experimentation of judging varieties and making selective choices by the farmers led to the evolution of agriculture (Swaminathan 1993). Knowledge was set by practices and not by scientific factors (Ludden 1996). Traditional farmers passed on the indigenous knowledge about farming practices and related aspects across generations verbally and through practice.

6.3 Traditional Farming Practices

Prominent traditional agricultural practices include agroforestry, intercropping, crop rotation, cover cropping, traditional organic composting, integrated crop-animal farming, shifting cultivation, and slash-and-burn farming (Singh and Singh 2017).

6.3.1 Agroforestry

Agroforestry, an age-old practice dating back to the beginning of farming and animal husbandry (Oelbermann et al. 2004), involves the practice of planting trees along with crops (Patel et al. 2019; Albrecht and Kandji 2003). It has a potential for mitigation of the effects of climate change, adaptation, food security, and crop productivity (Coulibaly et al. 2017; Mbow et al. 2014). In addition to this, the practice plays a role in improving soil quality, water retention, carbon sequestration, agrobiodiversity, and ultimately farmers' income (Zomer et al. 2016; Abbas et al. 2017; Paul et al. 2017). The system is used worldwide as a land-use management system (Pandey 2002). Agroforestry has a high significance in drought prone areas owing to the fact that the deep roots of tree explore a larger soil volume for water and nutrients (Verchot et al. 2007). A system of agroforestry, known as silvopastoral system, is beneficial for livestock, in which leguminous fodder grasses are grown with trees (Reis et al. 2010; Isaac et al. 2005). This makes nutritious green fodder available for farm animals, which is required for their health and productivity.

6.3.2 Intercropping

The practice of cultivating more than one crop species on the same field is referred to as intercropping (Singh and Singh 2017). It is considered to be one of the highly productive farming systems (Hu et al. 2017), which utilizes the natural resources, such as land, water, and nutrient, efficiently and increases productivity, biodiversity, resilience, and stability of agroecosystem (Ning et al. 2017). Since more than one crop is used and different crops have different adaptability, this system reduces the climate-driven crop failure (Shava et al. 2009). It has been an ancient practice, especially cultivation of pigeon pea with sorghum (Wang et al. 2010). Carbon sequestration (Kumara et al. 2016), nitrogen fixation (Duchene et al. 2017), reduction of requirement of external nitrogen fertilizers (Singh and Singh 2017), increased availability of nitrogen and phosphorous (Latati et al. 2017; Lazali et al. 2016), reduced soil erosion (Forte et al. 2017) are some of the additional benefits of this system.

6.3.3 Crop Rotation

A traditional practice of growing a sequence of crops on a given land area every growing or planting cycle and season (Dury et al. 2012). Some of the benefits due to this system of agriculture include improvement in soil quality (Liu et al. 2016), soil fertility (Pedraza et al. 2015), carbon sequestration (Triberti et al. 2016), increased yield, effective water use, reduced soil erosion (Huang et al. 2003), and nutrient recycling. The use of leguminous crops reduces dependence on external source of nitrogen fertilizers.

6.3.4 Cover Cropping

A traditional practice of cultivating a crop in order to cover the land for reducing the erosion of soil and loss of nutrients (Dabney et al. 2001). The practice of replacing a bare fallow period through cover cropping is a workable way of runoff control and soil erosion prevention (Alvarez et al. 2017). Such crops, which could be both leguminous and non-leguminous (Cooper et al. 2017), are generally harvested before the plantation of the main crop or even alongside the main crops as a living mulch (Robačar et al. 2016). Many beneficial features have been enlisted, namely, improvement in soil microbial biomass, soil health maintenance, water storage, nutrient cycling, weed control, and carbon sequestration (Pinto et al. 2017; Frasier et al. 2016). However, certain disadvantages, such as additional costs, difficulty in tillage, allelopathy, labour, increased disease risk, need a mention (Dabney et al. 2001).

6.3.5 Composting

Composting is an age-old traditional practice (Oudart et al. 2015) of decomposing organic matter by micro-organisms under controlled conditions (Misra et al. 2003) to produce compost, which can be used as an organic manure (Onwosi et al. 2017). Organic manure can be an excellent source of enhancing soil fertility. Waste materials, such as farmyard manure, kitchen waste, sewage-sludge, and crop residues, can be recycled into useful manure to be used in the agricultural fields and fodder lands. In addition to this, it increases soil carbon sequestration, reduces green-house gas emission (Forte et al. 2017) enhances soil aeration, soil microbial diversity, cation exchange capacity, soil moisture, soil organic matter, soil carbon, or nitrogen levels and minimizes soil-erosion and controls pests and diseases (Liu et al. 2013; Zhang et al. 2012; Ge et al. 2008), makes soils more resistant to drought (Lal 2008). In the current scenario, the popularity of organic products is increasing globally and hence organic manure production is gaining popularity too. Composting is a prevalent practice in Asian countries (Yadav et al. 2017). The use of compost produced from

farm yard manure has been a traditional practice in China (Yang et al. 2004), South Africa (Kangalawe et al. 2014), and India (Gopinath et al. 2008). Vermicompost production, as a small-scale venture, is helping rural women folk in India to earn their livelihood and is empowering them in many ways (Hamadani et al. 2020; Baraskar et al. 2018).

6.3.6 Integrated Livestock Farming

One of the oldest practices of integrating livestock farming with agriculture is considered to be the backbone of cultivation in developing countries (Patel et al. 2019). It is an efficient way of resource utilization, wherein a by-product of one venture is utilized as an input of another. One example of integrated dairy farming with agriculture is illustrated in Fig. 6.1, which clearly shows the flow of resources in an integrated manner. The main products from a dairy farm, that is, milk and calves, form a direct source of income to the farmers, whereas the waste product, dung is either sold as such or is recycled to a more valuable vermicompost. The vermicompost, if sold directly, fetches the farmer an additional income or can be applied in the agriculture and fodder lands in place of fertilizers to improve soil fertility as well as production. The main produce from the agricultural land contributes directly to income of the farmer, and the by-products can be utilized in feeding the animals. The fodder produced can be sold, as well as utilized for animal feed production, which can again be sold as well as fed to own animals. The manure can also be used to produce biogas, which can also be utilized at the farm to carry out various operations.

Integrating livestock component to crop sector has a positive impact of agrobiodiversity, food diversity, land resource management, food security, income generation (Singh and Singh 2017). The system also acts as a buffer in times of a crop failure due to natural disasters such as droughts and floods.

6.3.7 Shifting Cultivation

It is also referred to as slash-and-burn cultivation and encompasses a practice of growing crops on a land covered with ashes produced from burning piles of wood obtained by chopping the trees (Muimba-Kankolongo 2018). The burning procedure is carried out just before the onset of the rainy season in order to destroy pests and to fertilize the land with ash. When the soil shows signs of exhaustion, the land is left uncultivated and allowed to grow again into a forest and, till then, the farmer moves to another plot (Alam et al. 2014). The cleared plot is cultivated for 2–3 years and then left fallow for approximately 10–20 years (Brady 1996). The increasing human population pressure demanded decreased fallow period (Thomaz et al. 2014). It has been argued that fallow period less than 10 years may not be sustainable



Fig. 6.1 Illustrated diagram of integrated dairying with crop production showing the utilization of products and by-products efficiently

(Borggaard et al. 2003). The sustainability of this system relies on the length of fallow period. Shorter periods may not allow replenishment of nutrients and longer periods may lead to runoff and soil loss (Thomaz et al. 2014). Further, large amounts of fuel may intensify the fire and cause damage to the top layer of the soil (Thomaz 2009). Other disadvantage of this system includes its implications on the environment in terms of deforestation and air pollution caused due to burning of the forests. Shifting cultivation landscapes (both cultivated and fallows) cover around 280 million hectares worldwide (Heinimann et al. 2017). It has been estimated that there might be a decline in shifting cultivation over the next decades, which may possibly raise issues of livelihood security among people currently depending on shifting cultivation (Heinimann et al. 2017).

6.4 Summary

Traditional farming has been practised by the farmers since ancient times and it has been ever-evolving. Tremendous population expansion, shrinkage of resources, changes in mind-set, and invention of new technologies and tools with passing time have all contributed to this. However, indigenous knowledge passed on by the ancient people across generations is still being used today and has its own merits, especially being in tune with the environment and being less resource intensive. Therefore, such farming practices are gaining attention as the world is looking for new alternatives to our production systems, while facing serious challenges such as climate change, biological magnification, and environmental degradation.

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Chapter 7

Soil Organic Matter and Its Impact on Soil Properties and Nutrient Status



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7.1 Introduction

Soil organic matter plays a dynamic role in the improvement and maintenance of physical, chemical, and biological properties of soil. Agriculture is most concerned about these relationships, including the critical limits of soil organic matter with the key soil properties. There has been direct and indirect impact of soil organic matter on the soil properties, including its physical, chemical, and biological properties (Bezuglova et al. 2019; Sachkova et al. 2019; Orlova et al. 2019; Garratt et al. 2018; Zhang et al. 2017; Bhat et al. 2017b). In the physical properties, the soil organic matter affect soil structure, water retention, available water capacity, thermal conductivity, erodibility, infiltration, soil aggregate formation, soil color, soil

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compaction, soil aeration, saturated and unsaturated hydraulic conductivity (Beck-Broichsitter et al. 2018; Ajayi and Horn 2017; Blanchet et al. 2016; Angin et al. 2013). In the chemical properties, the SOM effect pH, buffering capacity, CEC, base saturation, zeta potential, exchangeable cations, soil fertility, and nutrient release (Garratt et al. 2018; Kwiatkowska-Malina 2018; Sulman et al. 2018). The biological properties include soil microbial population, soil microbial biomass carbon, nitrogen transformation, mycorrhizal population, root length and root growth, dehydrogenase, phosphatase, and urease (Fedoseeva et al. 2019; Orlova et al. 2019; Tikhova et al. 2019; Kallenbach et al. 2019; Wurzbürger and Clemmensen 2018; Hazard and Johnson 2018; Parker et al. 2018).

Soil organic matter is a complex system of substance ranging from metabolic products of microbes, products of secondary synthesis components of organic residues undergoing decomposition, and humic substance. Soil organic matter is the plant and animal residues, microbial biomass, partly decomposed biomass fragments, stabilized organic matter, and soluble organic fraction (Zuber et al. 2015; Balesdent et al. 2000; Qadri and Bhat 2020). The soil organic matter generally describes the non-living product of plant and animal origin, but in broader term, it includes the total soil biomass, including the meso and macrofauna (Jiang et al. 2018; Laossi et al. 2008) and the biomass decomposition product. The soil organic matter is the heterogeneous product resulting from the chemical and microbial transformation of organic debris (Sidhu et al. 2016; Streubel et al. 2011; Liang et al. 2006; Mikutta et al. 2006; Dervash et al. 2020; Mushtaq et al. 2018). The soil organic matter has two important constituents: the non-living part, including the decomposed and un-decomposed products, and the living fraction of soil organic matter which is composed mainly of bacteria (10^9 organisms per gram of soil), fungi (10^7 organisms per gram of soil), actinomycetes (10^8 organisms per gram of soil), protozoa (10^6 organisms per gram of soil), algae (10^3 organisms per gram of soil), and nematodes (50 organisms per gram of soil). In the soil organic matter, these microbial populations play an important role in fermentation, mineralization, and humification process (Yuan et al. 2018; Gougoulis et al. 2014; Ahmad et al. 2007; Tiquia 2005; Alfreider et al. 2002; Zaman et al. 1999; Khanday et al. 2016; Singh et al. 2018a, b). The major constituents of the organic inputs are polysaccharides (hemicelluloses, cellulose) and lignin (Hsu et al. 2018; Liu 2014; Torres 2014; Zhu 2010; Kiem and Kögel-Knabner 2003), the others being biopolymers, such as (polyester, protein, tannins, suberin, cutin, and chlorophyll pigments) (Rui et al. 2016; Liu 2010; Bhat et al. 2017a, b). Soil organic matter serves as a substrate for microbial activity, nutrient source, soil conditioner, and major factor for sustaining agricultural productivity (Oldfield et al. 2018; Singh et al. 2020).

Soil organic matter decomposition is directly related to emission of atmospheric carbon, leading to global climate change. Environmental variables (soil moisture and soil temperature), microbial activity, soil chemical properties, and soil organic matter inputs (e.g., root exudates, plant litter, dead fine roots) usually impact decomposition dynamics (Dar et al. 2013, 2016; Genardzielinski et al. 2018; Yang et al. 2018; Dar and Bhat 2020). Apart from the organic inputs, the most influential are

microbial activity and soil climate. In the soils, organic matter influences its output through priming effect (Fig. 7.1). The priming effect is small period transition in soil organic carbon turnover caused by the external organic matter input. A positive priming effect accelerates the decomposition rate, while as negative priming effect retards decomposition. The decline in the soil organic matter has a deleterious effect on the soil properties (Matos et al. 2019; Johnson et al. 2017; Huo et al. 2017; Kumar et al. 2016; Pausch et al. 2013; Zimmerman et al. 2011). As predicted in (European Environment Agency 2010) EU Soil Thematic Strategy, a decline of SOM contents is considered among eight main threats for soils. About 108–188 Pg C have been lost since the mid-nineteenth century, mostly from terrestrial ecosystem. However, several mitigating practices in agriculture, such as use of crop residues and residue incorporation into soil, maintain and build up soil organic matter. The mineralization and decomposition of soil organic matter is prejudiced by the land management, especially agricultural lands. Soils easily and quickly lose organic matter when natural soils are converted into agricultural soils. About 20–25% of the soil organic carbon is lost into atmosphere during its conversion and the possible reason is the destruction of soil aggregates. The sustainability in agriculture can be achieved now by the supply of exogenous organic matter. The organic matter having rich carbon pool and poor nitrogen content (e.g., straw, brown coal, wood and tree coniferous) act as substrate for microorganisms and relatively stable energy source (Singh et al. 2018a, b; Wei et al. 2017; Gogoi et al. 2017; Xiang et al. 2015) The current paper reviews the importance of soil organic matter in the

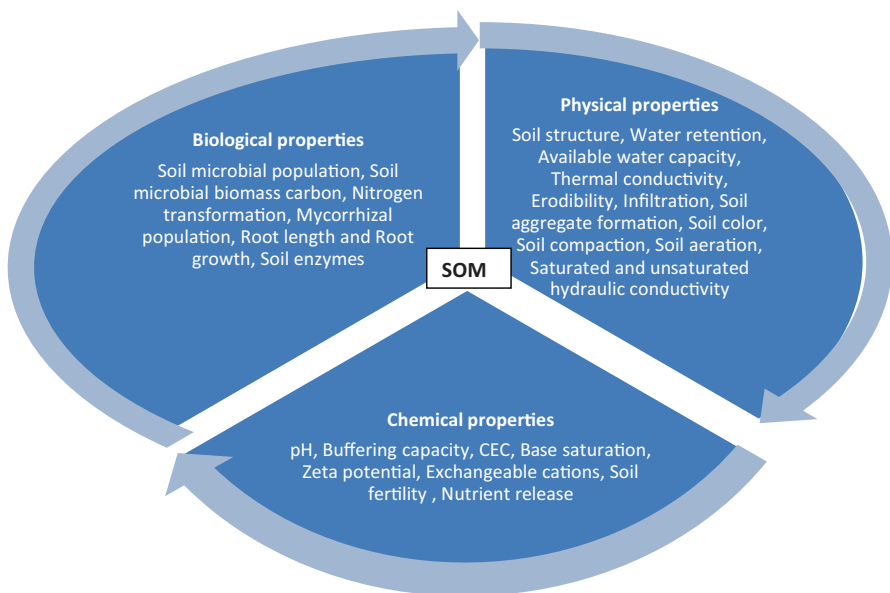


Fig. 7.1 Impact of soil organic matter on soil's physical, chemical, and biological properties

Table 7.1 Various fractions of the soil organic matter and their relative proportion

Fractions	Source	Composition	Amount (%)	Available form
Living organic matter	Plant biomass	Plant litter and roots	1	Active pool, labile soil carbon, decomposable plant materials (low C:N ratio), resistant plant material (high C:N ratio, high lignin)
	Microbial biomass	Bacteria	1–5	
Fungi				
Non-living organic matter	Particulate organic matter	Litter	5–20	Labile soil carbon, active pool, decomposable plant materials (low C:N ratio, low lignin), resistant plant material (high C:N ratio, high lignin)
		Macro-organic material		
		Light fraction		
	Dissolved organic matter		0.1	
	Humus	Non humic	65–80	Slow soil carbon
		Humic		
Inert organic matter	Charcoal and biochar	1–5	Passive soil carbon, inert organic material	

enhancement and amelioration of physical, chemical, biological, and fertility aspects of the soil (Table 7.1).

7.2 Effect of Soil Organic Matter on Soil Physical Properties

7.2.1 Soil Structure and Aggregate Stability

Soil structure stability indicates the resistance of soil to structural arrangement of particles and to different stresses (compaction/trampling, cultivation, and irrigation). Soil structure is the most important property influencing biological, chemical, and physical processes within soil, such as determining water and nutrient movement, air accessibility, seedling emergence, root penetration, resistance to erosion, and soil drainage (Sandin et al. 2017; Toosi et al. 2017; Jozefaciuk et al. 2015; Mehmood et al. 2019). The soil structure is a property which can be greatly influenced and manipulated by organic agriculture management practice. The organic matter has been associated with improved soil structure through the production of organic acids, biodiversity improvement, chelates, and increased earthworm population (Pylak et al. 2019; Bongiornoa et al. 2019; Moos et al. 2016; Kravchenko et al. 2015; Peng et al. 2015; Regelink et al. 2015). The amount of water stable aggregate is connected to macro-aggregate stability and positively related with labile organic carbon. A minimum of 2% soil organic carbon is necessary to maintain soil structure stability (Liu et al. 2013; Celik et al. 2010; Huang et al. 2010). The concept of soil aggregation and soil structure involving different binding agents have been revealed by the work of many researchers. Fine network of roots and

hyphae in soil with high soil organic matter held together large aggregates (200 micron), while as aggregates above this size are held by organic cementing agents. Water stable aggregates of 2–20 micron are bound by living and dead bacterial cells. The idea of aggregate hierarchy reveals that organic matter controls aggregate stability and destruction of large aggregates creating smaller and more stable aggregates (Keller and Håkansson 2010; Moni et al. 2010; Kaiser and Guggenberger 2003; Christensen 2001; Oades 1984). Particulate organic matter acts as a substrate for microbial activity, enhancing the production of microbial bonding material. Fresh and active part of soil organic matter (mono, polysaccharides, exudates, fungal hyphae, and roots) are largely responsible for soil aggregation (De Curtis et al. 2019; Fraç et al. 2018; Plaza-Bonilla et al. 2013; Miao et al. 2009). There has been seen a positive relationship between organic farming systems using FYM, compost, vermicompost, sewage, and sludge with the aggregate stability.

7.2.2 Soil Compaction

Bulk density is an indicator of soil compaction and affects infiltration, soil porosity, plant nutrient availability, available water capacity, and soil microbial activity (Karlen et al. 2019; Nunes et al. 2019; Laiho et al. 2004; Aşkın and Özdemir 2003). The soil compaction is regarded as the most serious problem caused by conventional agriculture and the most difficult type to determine, as it shows no evident marks on the surface (Meurer et al. 2018; Mastro et al. 2007; Håkansson and Lipiec 2000). The effects of soil compaction on soil properties are very complex and the state of soil compactness is evident soil attribute and determined mostly by the bulk density and soil strength (Singh et al. 2014; Hati et al. 2007). The bulk density and soil strength gives the direct comparable value of soil compactness. Soil organic matter retains soil water, thus helping the soil to overcome the problem of soil compaction. The adequate amount of organic matter in the soil stabilizes soil structure and makes it more resistant to soil degradation and also decreases the bulk density and increases soil strength (Das et al. 2018; Gharahi-Gheni et al. 2012; Heuscher et al. 2005; Calhoun et al. 2001; Han et al. 2012). The various mechanisms by which the organic matter influences the soil compaction are as follows: (a) binding of mineral particles to soil; (b) aggregate wet ability reduction; and (c) influencing the strength of soil aggregates, which is a measure of inter-particle bond. There has been a varying result between the soil organic matter and soil compactness and different researchers have reported different behavior for the different types of organic matter (Pravin et al. 2013). These differences seem to be due to different types of organic manure, C/N ratio of manure, and degree of resistance to degradation. Soil compactness is more influenced by readily oxidizable soil organic matter than the total organic matter. The organic farming system increases the organic matter in the soil and thus improves the bulk density of soil. Furthermore, using high quantity of organic manure or wastes decreases the bulk density of the soil due to a dilution effect caused by the incorporation of the added organic material with the denser

mineral fraction of the soil (Almajmaie et al. 2017; Boizard et al. 2017; Guimarães et al. 2017). The plant residues are the common source of organic matter, but the farmers also use animal manures to reduce the soil compaction in the different organic farming systems (Avnimelech et al. 2001; Curtis and Post 1964; Huntington et al. 1989; Bhat et al. 2018a, b). The elasticity of the manure reduces the transmission of stress toward subsoil thus acting as a buffer to subsoil compaction. Incorporation of 50–100 t/ha of cattle manure in the silty clay loam top soils significantly reduces the effects of load 1–2 passes of 48.5 kW tractor (Mosaddeghi et al. 2000). Green or brown manure may not be an efficient source of nutrient for high-yielding environment, but acts as a beneficial practice in improving soil physical properties in compacted soils. Reddy (1991) observed a decrease in bulk density and soil strength of 0.02 Mg/m³ and 11.8 kpa in the sandy loam soil due to the incorporation of 10 t/ha of green leaf manure. Incorporation of various organic matters in subsoils may prove a better alternative for stable retention in tackling soil compaction.

7.2.3 Soil Porosity

The architecture of soil refers to arrangement of soil particles and soil pores. The soil organic matter directly influences plant nutrition, penetration, and seedbed preparation, ease of cultivation, improved bulk density, greater aggregate stability, increased water holding capacity, and enhanced porosity (Xu et al. 2016; Ibrahim et al. 2013; Jack et al. 2011; Jarvis et al. 2007; Malkawi et al. 1999; Haynes and Naidu 1998). The organic carbon is mostly located in these pores between mineral grains as discrete particles or adsorbed on these particles. The soil porosity can influence the organic material stability through its effect on aggregate stability, isolation, and entrapment of decomposers and water and oxygen availability (Li et al. 2014; Masri and Ryan 2006). The changes in the pore size distribution are accompanied by the higher rates of organic matter mineralization at equivalent values of air-filled porosity. Rose (1991) studied that FYM not only changes aggregate stability but also affects the inter- and intramicro porosity. By the application of FYM and compost, both the micro porosity and macro porosity has increased (Pagliai and Vittori Antisari 1993). The increase in micro porosity occurs as a result of increase in elongated macropores of the newly formed aggregates. The effect of organic farming system can indirectly enhance soil porosity through the influence of soil fauna whose burrowing and feeding activity modify porosity (Park and Smucker 2005; McCallum et al. 2004). The various organic farming systems can also enhance the soil porosity by reducing soil crusting, clay dispersion, tillage, and compaction (Fig. 7.2).

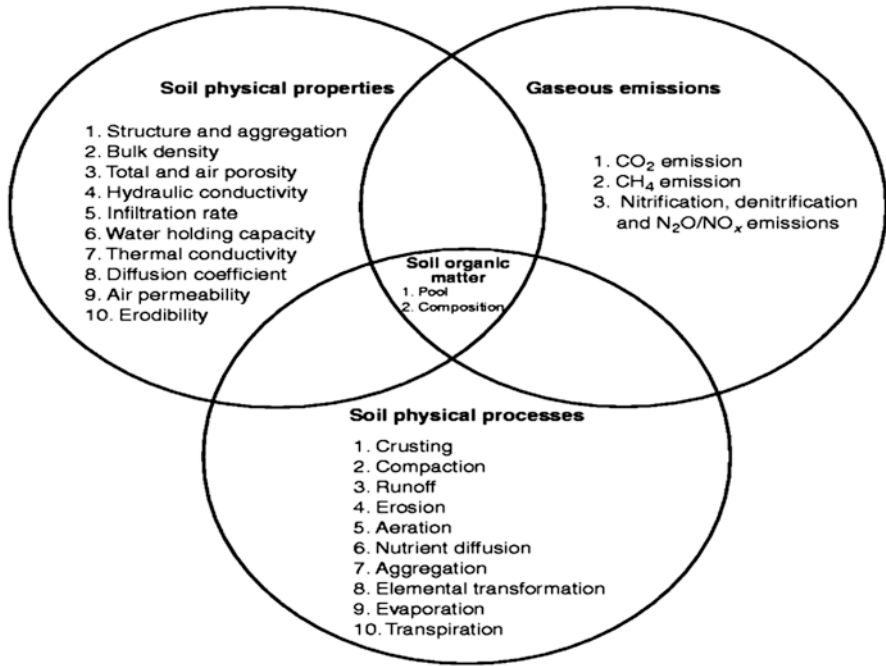


Fig. 7.2 Effect of soil organic matter on the soil's physical properties and processes

7.2.4 Soil Color

A strong relationship exists between soil color and many other important soil properties, including soil fertility mineral composition, soil organic matter soil drainage class, soil moisture, and land suitability (Ben-Dor et al. 2008; Barron and Torrent 1986; Alexander 1971; Brown and O'Neal 1923). The soil color is used to characterize, classify, and differentiate soils. The most convenient way to determine soil color is by the Munsell color chart. The soil color determines the pedogenic process in soil and the most important soil pigmenting agents are organic matter, iron, and manganese (Erskine 2013; He et al. 2003; Ketterings and Bigham 2000). The application of soil organic matter darkens the soil. The concept of Russian chernozem and Mollisol having the high organic matter are mostly defined by relative thick, dark surface horizons. The dark brown soils having high amount of soil organic matter are generally considered as an ideal soil. Within similar soil textural class and landscape, the soil color and soil organic matter has a good linear correlation (Ertlen et al. 2015; Kirillova et al. 2015; Kweon et al. 2013; Li et al. 2012; Ertlen et al. 2010; Gao and Xia 2009). The dark color soil having high amount of the organic matter applied by various organic farming systems holds a large amount of water, absorbs more radiation, and affects heat transfer (Sánchez-Marañón et al. 2011; Ketterings and Bigham 2000). The relationship between burned soil color,

soil fertility, and fire severity found that color and chroma decreased with increasing heat severity due to decrease of soil organic matter and the soils appeared red, while the light heated soils appeared black due to incomplete burning of soil organic matter (Valeeva et al. 2016).

7.2.5 Water-Holding Capacity

An important soil physical characteristic is the capacity of the soil to supply and store water and air for plant growth. Soil's water holding capacity is the ability of the soil to store water. The soil organic matter increases the water holding capacity of the soil. The effect of organic matter on the water holding capacity is generally assumed to be positive (Ankenbauer and Loheide 2017; Basche et al. 2016; Jordán et al. 2010; Franzluebbers 2002; Bauer 1974; Jamison and Kroth 1958). An increase of 50% water content with per gram addition of organic carbon at -10 kpa suction (Emerson and McGarry 2003; Haynes and Naidu 1998).

The soil organic matter can enhance the hydraulic conductivity by improving the aggregate stability and porosity of the soil (Yang et al. 2014; Xu 2014). The organic manures reduce the compactness of soil, therefore improving water penetration. The effect of organic matter was more pronounced in the coarse-textured soil, followed by medium textured soil, and then the fine soils (Haynes and Naidu 1998). Several researchers have reported that, with an increase of soil organic matter, there is an increase of water holding capacity at both field capacity and wilting point (Sohail-Ur-Raza et al. 2015; Wang et al. 2015; Evrendilek et al. 2004; Matsi et al. 2003). The water holding capacity of soils is mainly dependent on the number of pores and specific surface area of soil. Both these pores and the specific surface area are increased by the application of organic matter. The increased water holding capacity is basically the result of an increase in the number of smaller pores at lower tension (Gülser et al. 2015; Hati et al. 2007; Khaleel et al. 1981). At higher tensions, all the pore space in the soil is filled by air and the water is retained mainly due to specific surface area and the thickness of water film on these surfaces.

7.2.6 Soil Thermal Properties

Soil thermal properties are considered a function of soil organic matter and soil carbon pool. Soil organic matter alters the thermal properties of soil because of its black dark nature. The albedo of soil gets reduced by the potential increase of dark color and more heat gets absorbed. The soils with least organic matter have albedo value of 0.6, while soils with 5% organic matter have albedo value of 0.08. At least, a soil organic matter of 2.5–3.0% is considered significant (Bi et al. 2018; Di Sipio and Bertermann 2018; Cai et al. 2017; Hortensia et al. 2018; Dębska et al. 2016).

Table 7.2 Thermal characteristics of the various soil constituents

Soil constituents	Density	Specific heat capacity	Volumetric heat capacity	Thermal conductivity
	kg/m ³	kJ/kg/°C	kJ/m ³ /°C	W/m/°C
Quartz	2.7 × 10 ³	0.8	2 × 10 ³	8.8
Clay minerals	2.7 × 10 ³	0.8	2 × 10 ³	2.9
Water	1.0 × 10 ³	4.2	4.2 × 10 ³	0.6
Air 20 °C	1.2	1	1.2	0.025

The soil organic matter also affects the actual thermal properties of soil, including both its storage and flow of heat (Table 7.2).

The soils with good amount of organic matter have ample germination and higher crop growth because of the favorable temperature. The soil organic matter has substantially different physical characters in view of other soil constituents and with the increase in the soil organic matter the potential change in the soil thermal properties occurs (Bi et al. 2018; Wardani and Purqon 2016; Mondal et al. 2015; Usowicz et al. 2013; Tarnawski et al. 2009). Soil organic matter has the direct effect on the bulk density, which later affects heat conductivity and capacity of the soil. Mostly increase in the soil organic matter decreases the thermal conductivity and the wet soils have higher heat capacity and require lot of heat to raise its temperature. The wet soils are considered to have higher thermal conductivity than the organic and other soils.

7.2.7 Soil Infiltration and Percolation

The soil organic matter influences the infiltration or the admittance of water into a soil, and percolation, or the descendent movement of water, in a soil. The rate of infiltration depends on soil structure, developed soil horizon, soil slope, soil texture, depth of water table, chemical content of water, rate of water applied, and the amount of organic matter. Initially, in any soil, the infiltration rate is higher and then decreases with time (Basche and DeLonge 2019; Korucu et al. 2018; Loecke et al. 2017; Haghazari et al. 2015). The important characteristic of soil affecting the soil infiltration is porosity of soil. The organic matter has a direct impact on the soil porosity and as the soil organic matter increases the porosity of the soil is also enhanced. The rate of infiltration reduces with time due to deflocculation and breakdown of the peds. The soil organic matter enhances soil aggregate stability and thus allows the greater rate of infiltration. Initial infiltration is higher, but the later infiltration is sometimes superior if there are no macropores on the surface and the soils have good aggregate stability and no surface crusting (Zeng et al. 2019; Acharya et al. 2018; Chavarria et al. 2018; Rodrigo-Comino et al. 2018; Diadin et al. 2018;

Balázs et al. 2018). In the tropics and semi tropics, the infiltration rate is more dependent on hydrous oxides and clay minerals because of having mineral origin of aggregates. In the humid and temperate zones, the infiltration depends on the soil organic matter because the stability of aggregates largely depends on the soil organic matter. The rate of infiltration varies from 0.1 to 5 inches per hour and the general infiltration rate varies from 0.3 to 1.0 inch per hour. The large amount of crop residues, mulches, and soil surfactants can regulate good infiltration rate by breaking droplet size of the rain water.

Percolation or downward movement of water is reliable on uninterrupted pore space in soil. For every doubling of pore size diameter, the rate of percolation in the soil increases by four times. The other factors affecting percolation rate are soil texture, soil structure, soil compaction, and amount of organic matter present. The soil temperature, soil moisture, and depth of soil horizons also affect the percolation rate (Issaka et al. 2018; Bullard et al. 2018; Jakab et al. 2017; Di Prima et al. 2017). The percolation of soil is restricted by the insufficient pans, clay pan, fragipans, plow pans, hard pans, and the presence of higher water table (Gómez et al. 2017; López-Vicente et al. 2016). In general, the percolation rate is considered higher in sandy soils, but the light soils having well stable soil aggregates, good soil structure, and high amount of organic matter have higher percolation rates than the fine sands. Mulches, crop residues, compost, FYM, and other organic soil amendments increase the soil macropores, thus increasing the percolation rate in the soil (Hlavčová et al. 2019; Gómez et al. 2018; Ben-Salem et al. 2018; Lucas-Borja et al. 2018; Fortugno et al. 2017).

7.3 Soil Chemical Properties

7.3.1 Buffering Capacity and Soil pH

Buffering capacity of soil is an important aspect as it assures the stability of soil pH. The buffering capacity of soil is the resistance to change in pH when an acid or base is added. At the pH value between 5 and 7.5, soil organic matter and clay acts as a sink for H and OH and the buffering capacity is governed by exchangeable reaction (Yuan et al. 2011; Nelson and Su 2010; Zhang et al. 2008; Herre et al. 2007). The presence of various functional groups (amine carboxylic, alchoal, phenolic, and amide) in soil organic matter allows it to act as a buffer over a wide range of soil pH (Sohi et al. 2010; Larney et al. 2008). The organic rich surface soils have higher buffering capacity than the mineral soils. James and Riha (1986) reported the buffering capacity of 18–36 Cmol_c/kg in the forest soils and 1.5–3.5 Cmol_c/kg in the mineral soils. Bloom (1999) studied that soil organic matter has a buffering capacity of 200 Cmol_c/kg, while buffering capacity of soil organic carbon was reported 300 times in comparison to kaolonite. Cayley et al. (2002) recorded a good relationship between the buffering capacity and soil organic matter and the importance of soil

organic matter to maintain soil pH despite certain acidifying factors. A strong correlation existed between initial soil pH value and the buffering capacity of subsurface horizon, and the highly acidic soils were better buffered than less acidic soils (Curtin and Trolove 2013; Lieb et al. 2011; Bowman et al. 2008; De Vries et al. 1989; Bhat et al. 2018a, b). The soils are poorly buffered between 4.5 and 6.5 and well buffered below 4 and above 7. A number of researchers have studied the relationship between soil organic matter and soil pH. The decomposition of young shoots of trees in Ultisols and Oxisols increased soil pH and decrease in exchangeable aluminum content and the acid neutralization was due to aluminum and proton complexation by the organic anions (Wong et al. 2000; Tian et al. 1992; Hue 1992; Vallis and Jones 1973). The under saturation of aluminum adsorption would result in 3 mol of protons consumed for each mol of aluminum dissolved. The aluminum dissolution by the organic anions would result in proton consumption and the pH increase. The pH increase is not the primary cause for the decrease of soluble and exchangeable aluminum, but also due to adsorption of these substances on soil organic matter (Luo et al. 2015) The soil organic matter and the exchangeable aluminum has a negative correlation at greater depths and the effect of soil organic matter was greater at lower pH values. The relationship of soil organic matter and pH was studied when different type of plant material was incubated in top soil and there occurred increase in pH_{kcl} in a few days and the magnitude of which depends on type of soil organic matter and rate of application. The organic anions (malate, citrate, oxalate) balance the plant derived cations and the oxidation of anions consumes H^+ ion and the release of OH^- ion (Najafi and Jalali 2016). When the decomposed organic matter is added to the soil there is rise in pH due to complexation of proton by organic anion. If the undecomposed organic matter is added, the rise in soil pH is due to decarboxylation of organic anions during microbial decomposition. The decomposition of organic matter will transfer organic nitrogen to ammonia and releases OH^- and results in increase in soil pH (Zhang et al. 2013; Qin et al. 2013; Galloway et al. 2008; Ju et al. 2004). The specific adsorption of soil organic matter onto the aluminum and iron hydroxides results in release of OH^- ion. The long-term effect of organic matter increases soil pH as there occurs accumulation of humic material which complexes with aluminum compounds and decreases their solubility and protects soil from toxicity.

7.3.2 Cation Exchange Capacity

Cation exchange capacity of soil is the measure of exchangeable cations that soil can hold and represents negative charge per unit mass of soil. Soils with high cation exchange capacity is favorable as it holds many plant nutrients and this cation exchange capacity is expressed as centimol of positive charge per kilogram of soil (Soares and Alleoni 2008; Wiseman and Puttmann 2006; Adams and Evans 1979). The soils are having two charges: permanent charge CEC_p and pH-dependent charge CEC_v which depends on pH and the soil organic matter (Bache 1976; Bascomb

1964). The addition of organic matter generally causes increase in CEC_v and this increase is due to the presence of certain functional groups in the soil organic matter. The organic compounds with high molecular weight contribute less to cation exchange capacity compared to low molecular weight compounds (Albuquerque et al. 2014; Asai et al. 2009; Bonfante et al. 2010; Das and Varma 2011; McClellan et al. 2007; Lehmann and Rondon 2006). The contribution of soil organic matter to CEC is in the range 25–90% and the variation is mainly due to presence of functional group in soil organic matter and the soil type. There is a greater increase due to addition of soil organic matter to the cation exchange capacity in the coarse-textured soils than the medium- and fine-textured soils. Due to the addition of soil organic matter to mineral soil, there is greater increase of cation exchange capacity than in the surface soil (Schulz and Glaser 2012; Kammann et al. 2011; Peng et al. 2011). The soil organic matter has a cation exchange capacity of 150–250 $Cmol(p^+) kg^{-1}$ and in the arable calcareous soil with high organic matter, the cation exchange capacity ranges from 230 + _ 47 (Wolf and Snyder 2003). In sandy forest soils, soil organic matter contribution to cation exchange capacity is very high in comparison to Vertisols derived from basalt. Stevenson (1982) worked on several laboratory methods to determine the relationship between soil organic matter and cation exchange capacity. The regression equation was developed for predicting the relationship between soil organic matter and cation exchange capacity (Hallsworth and Wilkinson 1958). The cation exchange capacity of soil organic matter varies and it depends on type of functional group present in soil organic matter and the pH of soil. The measure source of negative charge that contributes to cation exchange capacity is the carboxylic groups. The cation exchange capacity of soil organic matter in acid soils was estimated to be 134 $Cmol(p^+) kg^{-1}$ and in chernozem it ranged upto 297 $Cmol(p^+) kg^{-1}$. The potential sites for cation exchange in soil organic matter are more than the measured as the many sites become unavailable due to the association with polyvalent cations (Table 7.3).

Table 7.3 Cation exchange capacity ($cmol_c kg^{-1}$) of the various soil constituents

Soil constituents	Capacity ($cmol_c kg^{-1}$)
Kaolinite	1–5
Fe and Al oxides and hydroxides	5–40
Illite	20–40
Allophane/imogolite	20–50
Smectite	80–150
Vermiculite	150–200
Soil organic matter	>200

7.3.3 Adsorption and Complexation

Adsorption reaction due to soil organic matter are dependent mostly on pH as well as cation exchange capacity because similar types of organic carbon species are involved in adsorption reaction (Ahmed et al. 2015; Figueroa-diva et al. 2010; Frossard et al. 2002; Jones and Edwards 1998). The presence of functional groups (COOR, NH₂, OH, NHR, CONH₂) are very important for adsorption of ions on humus particles (Wu et al. 2012). The important mechanism for protection of soil organic matter from decomposition is its adsorption with clay particles. The positive relationship between soil organic carbon, clay content and soil surface area illustrates the significance of adsorption of soil organic matter on to the clay particles (Schaumann 2006; Schulten 2002). The interaction of soil organic matter with clay is governed by nature of soil organic matter and type of clay present. The interaction between soil organic matter and positively charged ions is through cation exchange reaction (between positively charged cation and negatively charged carbon group) (Cheng et al. 2014; Schwarz et al. 2012; Ghosh et al. 2000). The complexation of soil organic matter with inorganic material enhances the soil fertility as it increases the availability of soil phosphorus by blocking iron, aluminum, and calcium adsorption sites. Soil organic matter decreases the phosphorus adsorption in oxisols and the greater phosphorus adsorption was observed in cultivated soils (800 mgP/kg) than forest soils (560 mgP/kg) and it is attributed to greater amount of soil organic carbon in the forest soils (Bai et al. 2012). With the exception of few non-crystalline minerals, soil organic matter has the greatest capacity to form bonds with metals and it is associated by a positive association between solubility of metals with soil organic matter content as well as to high amounts of trace metals in organic rich soils compared with the non-organic soils (Sparks 2003). The increased concentration of soil organic matter decreases the concentration of cupric, zinc, and manganese in soil solution and also decreases the extraction of calcium by calcium chloride and acetic acid. The adsorption of copper was much stronger than zinc and manganese in the peat, solid humic acid, and acid washed peat (Klucakova 2012; Barancikova et al. 2003). The organic manures reduce the aluminum toxicity and increases the phosphorus availability and the important organic carbon groups in this complex reaction were low molecular weight aliphatic organic acids and soluble humic molecules as it complexes with the monomeric aluminum. The formation of complexes between polyvalent metal ions and humic substance is due to O-containing functional groups (enolic, phenolic, alcoholic, and carboxylic) as hydroxyl there is also the decreases the sorption of chlorinated aliphatic hydrocarbons and low molecular weight organic acids (fumaric, lactic, oxalic, citric, tartaric, propionic, butyric, acetic and formic acid) which are derived from leaves and from microbial biomass and also forms complex with AL³⁺. Hydroxyl acids form stronger complexes than carbon groups. The presence of these functional groups in soil organic matter does not only determine sorption capacity but also have certain synergistic effects, such as aromaticity, polarity, and hydrophobicity.

7.4 Soil Biological Properties

7.4.1 Soil Organic Matter as a Driver of Biological Activity

The fundamental function of organic farming system is to supply high amounts of organic matter which provides metabolic energy and drives soil biological process. In the organic farming systems, there is basically the transformation of carbon compounds by macro and microorganisms and plants that provide energy and connects above and below surface energy by the formation of a cycle (Rossiter and Bouma 2018; Wade et al. 2018; Roper et al. 2017; Bonfante and Bouma 2015; Tiquia 2005). The plants assimilate carbon from atmosphere and form glucose and other complex plant biomolecules which, upon plant senescence, enter the soil through roots, litter, and root exudates (Zhang 2013; Zuber et al. 2018; Bashir et al. 2016). The plants supply energy to heterotrophs and, to a less extent, to chemotrophs (microbes, fungi, and earthworms) by the formation of recalcitrant organic matter (Adeniji and Babalola 2019). The carbon source acts as a source of energy and as long as net primary production exceeds respiration the organic carbon will accumulate in the soil (Miranda et al. 2019; Oburger and Jones 2018; Conrad et al. 2018; Mahanty et al. 2014; Dijkstra et al. 2013). The different organic farming systems provide various amounts of soil organic matter in soil, which reflects the balance between carbon produced and carbon leached (Gopalakrishnan et al. 2015). This balance occurs due to energy requirement of biota and is governed by certain factors (temperature, clay content, moisture, humidity, and rainfall). The transformation of labile soil organic matter into more complex form, that is, humus stabilizes this soil organic matter and can be used as a source of energy for longer period in an edaphic environment. The energy released from the soil organic matter decomposition is in the form of heat and the heat losses from 1 hectare is nearly equal to heat value of 1 metric ton coal and the highly productive organic matter soil releases heat of about 12 megagram of coal annually. The soil microorganisms play an immense role in the transformation of organic matter as these microbes carry 80–90% of total soil metabolism (Fernández-Gómez et al. 2019; Cui et al. 2019; Lamprecht et al. 2018; Pascual et al. 2018; Shen et al. 2014). The 1–5% of nitrogen and carbon are being preserved in the microbial tissue. A concept of microbial catabolic evenness (CE) was introduced by Degens et al. (2000) to measure soil microbial diversity by short term respiration response of soil over a certain range of organic compounds. They found a direct relationship between microbial catabolic evenness and soil organic pools and reported higher (CE) in pastures followed by agriculture and horticultural crops and the least was reported in the arable soils (Yuan et al. 2014). It was also found that, with the depletion of soil organic matter or carbon stock, there was greater decline in the microbial catabolic evenness.

7.4.2 Soil Organic Matter and Soil Microbial Population

Soil is species rich habitat on earth having diverse and abundant species which help in the formation and development of soil. The soil biodiversity is indicator of soil health, as greater biodiversity means greater soil stability in terms of certain functions, such as maintenance of soil structure, assimilation of organic wastes, and nutrient cycling (Miranda et al. 2019; Oburger and Jones 2018; Conrad et al. 2018; Mahanty et al. 2014; Bashir et al. 2016; Shen et al. 2014; Dijkstra et al. 2013; Wang et al. 2012; Bhatti et al. 2017). Soil organic matter, soil organic carbon, and soil biodiversity are closely related but distinct. Biodiversity means residing of certain organisms, such as bacteria, fungi, actinomycetes, protozoa, worms, vertebrates, and invertebrates (Oburger and Jones 2018; Conrad et al. 2018). All these organisms depend on soil organic matter for their energy, nutrients, and habitat. The topmost soil of earth, where concentration of organic matter and roots are higher, forms the largest habitat for these organisms (Bashir et al. 2016). A vast diversity of the organisms is present in the soil but only limited microorganism has been explored (Table 7.4).

7.4.3 Soil Enzyme Activity and Soil Organic Matter

Soil enzymes play a key role in organic matter decomposition and its recycling, with their activities being closely related to microbial activity, microbial biomass, soil physical property, and soil organic matter (Oburger and Jones 2018; Mahanty et al. 2014; Bashir et al. 2016; Dijkstra et al. 2013). These enzymes are either intracellular or extracellular, with intracellular being inside the cell in the cytoplasm bound by the cell wall. The extracellular enzymes are permanently immobilized and are being released into the soil on humic and clay colloids through hydrogen bonds, ionic bonds, covalent bonds, and other mechanism. These soil enzymes act as catalyst for decomposition of organic matter and effect agronomic production, environmental quality, and energy transformation (Wade et al. 2018; Rossiter and Bouma

Table 7.4 Number of species of soil flora and fauna and the percent explored from the soil

Group	Known species	Estimated total species	% known
Bacteria	13,000	1,000,000	1
Fungi	18,000–35,000	1,500,000	1–2
Protozoa	1500	200,000	7.5
Nematodes	5000	400,000	1.3
Ants	8800	15,000	58.7
Termites	1600	3000	53.3
Earthworms	3600	No estimate	No estimate
Mites	20,000–30,000	900,000	2.2–3.3
Collembola	6500	24,000	27.1

2018; Roper et al. 2017; Bonfante and Bouma 2015). Soil enzymes are considered best soil detectors because they respond to the soil sooner than physical and chemical parameters.

7.4.4 Soil Organic Matter as Important Nutrient Source

In considering the importance of organic matter as source of nutrients, it is to be mentioned that soil formation is closely related to diverse forms of organic substance on parent material. Soil organic matter provides all the essential nutrients, including primary nutrients such as nitrogen, phosphorus, and sulfur and micronutrients such as iron, manganese, zinc, copper, boron, molybdenum, and chlorine (Nurhidayati et al. 2018; Pravin et al. 2013; Masto et al. 2007; Laiho et al. 2004; Katyal et al. 2001). These nutrients are being made available during mineralization of organic matter during their growing season and the important fraction of soil organic matter fraction which supplies nutrients is particulate organic matter. Ninety percent of soil organic matter is made of carbon, hydrogen, and oxygen, while 50% of remaining elements is made of nitrogen, potassium, and silicon (Gwenzi et al. 2016; Haynes and Naidu 1998; Mahanty et al. 2014; Moco et al. 2009; Reeves 1997; Steller et al. 2008; Stevenson 1994; Von Lutzow et al. 2005). However, the application of fertilizers supplies a major nutrient available to plants, but the organic matter along with the soil microbes, store and cycle large amounts of nutrients required for growth (Liu et al. 2009). Most of the nutrients held in the soil organic matter are not easily assessable to plants and are resistant to decomposition. Only 1–5% of the soil organic matter is decomposed annually and it takes almost a decade for its complete decomposition (Molina-Herrera and Romanya 2015; Haynes and Naidu 1998). Soil organic matter acts as larger reservoir of macronutrients with 90–95% nitrogen and sulfur and 20–75% phosphorus. The 90–95% nitrogen is held in both available and fixed form. The 40–45% of organic nitrogen is quantified and identifiable as amino-sugars and amino acids and the remaining portion consists of unidentifiable structure. The soil Sulfur in organic form is mainly in the form of amino-acids, such as cysteine, cystine, and methionine. Phosphorus is mainly present in ester form and nitrogen is covalently bonded to C–S or C–O–S. The process of net phosphorus mineralization occurs if ratio of C:P is less than 100, whereas ratio of greater than 300 indicate net immobilization. The soil organic matter has impact on phosphorus availability through specific adsorption reaction because the humic fraction shows competitive character on oxide surfaces (Zhao et al. 2019; Liu et al. 2009; ErdalSakin 2012; Gama-Rodrigues et al. 2008; Geeves et al. 1995; Lal 2011; Zhang et al. 2006; Madejón et al. 2003; Powlson et al. 2001). Mineralization and transformation of organic source of phosphorus occur through extracellular hydrolysis or by the oxidation of organic carbon. Decrease in the soil organic carbon pertains to reduced nutrient supply and less than 1% organic matter is considered a threshold value below which there is no nutrient supply. The release of NPS from organic matter depends upon ratio of these elements to carbon. A narrow ratio

of these elements to carbon usually allows fast nutrient release and a wide ratio reduces their availability (Guan et al. 2019; Hu et al. 2019; Ma et al. 2019; Cai et al. 2018). There are various schools of thought pertaining to nutrient status of soil organic matter, including its C:N:P:S with ratio of 100:10:1:1, 155:10:0.68:1.4, and 140:10:1.4:1.4. C:N:S ratio in the agricultural soils vary from other soils due to higher carbon mineralization of carbon and greater fertilizer input.

In view of the macronutrients, soil organic matter forms a number of chelates that make metal nutrient elements available over a wide range of pH. The micronutrient chelation has greater significance because of the nature of these elements to become fixed in high pH soils. The most chelates formed in the soil are of iron, copper, and zinc. In the plant's heme group forms the most common chelates, including chlorophyll and iron porphyrin. Oxalic and malic acids are reported to have high chelating properties. The root exudates and complex organic matter form the chelates that remain available to plants for a longer period. Ketogluconic acid has been reported as a highly chelating agent, but probably there are more chelating agents formed by organic matter (Shi et al. 2018; Du et al. 2018; Zhou et al. 2018; Liu et al. 2017; Hu et al. 2016; Guo et al. 2015; Wiatrowska et al. 2013). The organic matter supplied to high pH soils forms chelates and corrects lime-induced chlorosis. The carbon dioxide favors bicarbonate formation, which decreases iron uptake and translocation within plants.

7.5 Conclusion

The diverse nature of soil organic matter plays a defining role in determining the dimensions of various soil processes and properties, including its physical, chemical, and biological properties. This chapter revealed that interaction of soil organic matter with soil properties is very complex. The soil organic matter is considered as an important soil health indicator, as most of the soil properties depend on it. Almost all the soil properties were strengthened with the increase in the soil organic matter. The increase in soil organic matter had clear impact on the soil structure, water retention, thermal conductivity, available water capacity, zeta potential, exchangeable cations, soil fertility, erodibility, infiltration, soil aggregate formation, soil color, soil compaction, soil aeration, pH, buffering capacity, CEC, base saturation, and microbial population. The soil organic matter is an important factor in nutrient cycling, nutrient supply, especially nitrogen, phosphorus, sulfur, and micronutrients. The soil organic matter was more dominant where clay content was low. The important conclusion was that, with the addition of soil organic matter, we can improve many soil properties simultaneously. The certain issues that need to be readdressed in future are as follows: (a) application of soil organic matter to enhance soil health, (b) integrated nutrient management for sustainable agriculture, (c) carbon sequestration for mitigating climate change, and (d) organic residues management concerning recycling and environmental protection.

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Chapter 8

Sustainable Agricultural Practices



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8.1 Introduction

Organic farming is an all-inclusive food production managing system which endorses and improves agro-ecosystem well-being, overall biodiversity, organic cycles, besides soil biological activity (FAO/WHO 2011). It gives importance to the local managing practices in place and provides inclination to the practice of off-farm ideas, such as agronomic, biological, and mechanical methods. Organic farming helps to yield healthy food and signifies an emergent source of revenue for industrialized and emerging countries. By the nineteenth century, globally, maximum food was organically produced by means of biological fertilizers, that is, manures (White 1970).

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8.2 Current Status

Currently, 31 million hectares of agricultural land is under cultivation through organic farming practices. This is being made possible by more than 600,000 farmers working globally. Also, more than 62 million areas are certified. Countries with most organic land are shown below.

8.2.1 Prerequisite of Organic Farming

1. Together, in developing as well as developed nations, demand for organic agronomy is increasing with an average growth of 20–25% annually.
2. Excessive use of pesticides affect biodiversity, human and animal health, besides creating pollution in the environment.
3. Expensive contributions in established farming.
4. Deteriorating factor efficiency.
5. Scarcity of essential soil micronutrients.
6. Increase in concentration of carbon dioxide and temperature due to pollution created by use of fertilizers.
7. Agricultural production (1.5%) not meeting population growth rate.
8. Risk of poor harvest.

8.2.2 Sources of Plant Nutrients

Aimed at the improvement of yield output, Indian agricultural lands require 30% of the nutrients. This need is fulfilled by diverse organic sources. Crop production can be sustained by conventional riches such as farm animal droppings which provide added nitrogen content than chemical-based nitrogen fertilizers. The evaluation of NPK from natural sources is founded on over-all nutrient. Collective usage of chemical fertilizers plus organic fertilizers ensures better throughput and improved soil quality on an extensive basis (Chhonkar 2002; Dar et al. 2013; Bhat et al. 2017a, b). Besides providing NPK to plants, organic fertilizers deliver elemental nitrogen, bound phosphates, other required micronutrients, as well as decomposed organic remains in a form accessible to plants.

Application of organic nutrients to plants enhances plant growth and development besides increasing the activity of mycorrhizae and other beneficial soil microbes, therefore aiding to relieve the deficit of micronutrients and consequently support high productivity together with soil health (Nambiar et al. 1992; Bhatti et al. 2017; Qadri and Bhat 2020). High economic gains to farmers are obtained from organically produced crops, such as *Oryza sativa*, *Pisium sativum*, and *Allium cepa*, due to their demand in international markets (Kalyan 2005). In FYM (Farm

Yard Manure), nutrient concentrations are usually small and vary based on duration and storage. Because of inconstant nature, production and storage; FYM have N, P, and K contents stretching from 0.01% to 1.9% on dry weight basis.

The fine decomposed FYM contains 0.2% of P_2O_5 , 0.5% N, and 0.5% of K_2O (Inoko 1984; Zhu et al. 1984; Tandon 1992); 112 kg of N, 56 kg P_2O_5 , and 112 kg K_2O ha^{-1} can be added to soil by applying 25 t ha^{-1} of farm yard manure (Gaur 1992). Globally, numerous researchers have revealed countless profits on the use of FYM on soil properties as well as crop productivity (Prabhakar et al. 2010; Dar et al. 2016; Bhat et al. 2017a, b). Usually the farmers make use of harvested crop chaffs as animal feed and bedding; however, chaff is also put to use for trapping urine in order to enhance N cycling. Wet straw collected daily from the sheds is composted to manure which is either applied during the on-going season or is stored for the following season (Timsina and Connor 2001).

8.3 Practices in Organic Farming

Besides dealing with potential difficulties triggered with chemical fertilizers, organic farming improves soil fertility and likewise increases crop productivity.

8.3.1 Composting

Crop residues, animal wastes are properly decomposed for 1–6 months to form compost. Still sometimes, additional months are required depending upon the composting process used, accessibility of labour, management period, and investment.

8.3.2 Vermicomposting

A specialized and modified method of composting, vermicomposting, uses earthworms which consume farm wastes and turn them into high quality compost in 2 months. Besides an organic fertilizer, vermicompost is exercised for production of compost tea, which is used as a prophylactic against numerous pests and diseases. Vermi-wash, a by-product of vermicompost, is served for the same purpose. Proper sieving of compost should be carried out by applying it in fields. Non-indigenous earthworms are used in vermicomposting, which swiftly colonize and dictate the indigenous species. Indigenous earthworm species can also be used by collecting them from fields using collection baits and form heaps. In traditional way, vermicomposting is practiced all around the world and most places in India; however, it is labour insensitive and requires infrastructure which is beneficial for small compost as compared to large fields which find it expensive.

8.4 Biofertilizers

Biofertilizers improve nutrient availability of the soil through better nitrogen fixation. The microorganisms are added to the rhizosphere of the plant in order to enhance their activity by choosing effective varieties and with this their efficacy to absorb nutrients can be improved. These organisms are cultured and added directly to the soil through seeds. Bioinoculants or biofertilizers employ eco-friendly fungal and bacterial strains of *Azotobacter*, *Azospirillum*, *Azolla*, and phosphate solubilizers.

8.4.1 *Rhizobium Inoculants*

Rhizobial nitrogen fixation is beneficial for legume production as by fulfilling nitrogen requirement and by enriching soil for successive crops. Rhizobium inoculation gains greatest benefit of nitrogen in the shortest of time and hence should be considered as superlative green manure. Microbial inoculations of *Azotobacter*, *Azospirillum*, and *Pseudomonas* are still at the field trials though good responses in wheat, rice, sugarcane, and mustard have been observed.

8.4.2 *Phosphate Solubilizing Microorganisms*

Variety of fungi and bacteria having ability to solubilize and transform inorganic phosphate from insoluble sources as well as from organic acids, for example, *Aspergillus*, *Pencillium*, and *Trichoderma*. Besides solubilisation of phosphorus, these microorganisms mineralize lacked up organic phosphate into soluble available forms. In rhizosphere, these microorganisms bring more phosphorus into solution form and provide it to the plant which they can use for their own growth. The efficacy of these microorganisms are subject to availability of sufficient energy source, carbon in the soil, phosphorus concentration, temperature, and moisture.

8.4.3 *Mycorrhiza*

Mycorrhiza is a symbiotic association of plants and fungi. Fungi acquire carbon as provided by the host plant while the plant is profited by fundamental nutrients, such as phosphorus, potassium, ammonium ions, zinc, and copper, which are otherwise difficult to absorb. In the preponderance of cultivated crops, excluding those fitting to *Chenopodiaceae*, *Amaranthaceae*, *Caryophyllaceae*, *Polygonaceae*,

Brassicaceae, *Commelinaceae*, *Juncaceae*, and *Cyperaceae* families, the fungi are discovered to be allied. The fungal species are omnipresent in distribution in plants growing in temperate and tropical regions. Arbuscular mycorrhizal (AM) fungi are generally found in cultivated soils and report for 5–50% of the soil microbial biomass (Olsson et al. 1999; Bhat et al. 2018a, b; Singh et al. 2018). Glomalin, an organic carbon generated by the fungi surpasses soil microbial biomass by a factor of 10–20. Mineral extraction from soil is reflected to be the primary role of mycorrhizae fungi besides other important properties.

8.5 Mulching, Green Manuring, and Cover Cropping

Organic materials are put to use for covering the soil specifically round the plants in order to check water loss and enrichment of nutrients. Mulching is an inexpensive practice aimed at enhancing the number of earthworms, checking soil loss by erosion besides checking weed growth. Green manuring is a customary exercise predominant from the earliest stretches. It is advantageous in dual ways: (I) for the fixation of nitrogen, and (II) assisting in improving the soil quality in terms of its texture and water holding ability. Green manuring of leaves can also be done if ample leguminous plant leaves are accessible. Atmospheric nitrogen fixation can be carried out by growing legumes, clover, and pea at a rate of 20–200 kg N/ha (Adjei-Nsiah et al. 2008; Bhat et al. 2018a, b; Khanday et al. 2016; Singh et al. 2020), chiefly if soil is deficient in mineral nitrogen.

Loss of phosphorus and nitrogen by leaching and run-off can be prevented by cultivating non-leguminous plants, such as oats, barley, rye-grass, sorghum, rape-seed, and mustard, which trap phosphorus plus nitrogen. Many biocide plants such as *Brassica* Species *B. napus*, *B. nigra*, and *B. alba* decrease infestation of some fungi, such as *Rhizoctonia* spp., and nematodes, *Pratylenicus penetrans*, by producing isothiocyanates (Brown et al. 2008; Bhat et al. 2017a, b) due to myrosinase enzyme. Fusion of grasses with legumes provides tolerance to non-leguminous species by enhancing rate of N₂ fixation. Depending upon the temperature requirement, cover crops can be seeded in autumn, late winter, or early spring and typically in summer at the initiation of flowering stage of crops when the C:N ratio is lesser than 20:1.

8.6 Crop Rotation

Sowing different crops together is crop rotation. Mixed cropping of cereals and legumes, for example, mustard and rice, is the example of crop rotation in temperate conditions (Fig. 8.1).



Fig. 8.1 Mustard (*rabi*) crop followed by rice (*Kharief*) crop in Jammu and Kashmir

8.7 Multicropping

Multicropping is the concurrent farming of two or more crops. In Indian farming practice, it is well-known that as numerous as 15 varieties of crops are cultivated at a time, for example, multicropping of tomatoes and onions.

8.8 Intercropping

Intercropping is the cultivation of another crop between two crops, for example, coconut, banana, pineapple, ginger, leguminous fodder, medicinal, or aromatic plants are cultivated by using the mentioned technique. Inter-cropping additionally allows for potential use of available resources plus it helps in controlling pest populations. Amalgamation of fallen leaves and previous crop remains enhances the soil nutrients or compost heap nutrient quality as they develop into additional nutritional resources available to plants.

8.9 Effective Microorganisms

Effective microorganisms are both aerobic and anaerobic and are supportive in diverse ways, such as enhancing of soil nutrients, repelling numerous pests acting as a prophylactic, and in animal feeds.

8.10 Biopesticides

Biopesticides are useful in integrated pest management and organic agriculture practices as they are harmless for mammals and other non-target species, have high target specificity besides being environmentally more compatible, and aim at reducing the use of harmful chemical pesticides, for example, Bt (*Bacillus thuringiensis*) formulations have been helpful in controlling destructive species of Lepidopterae and *Trichoderma*. *Verticillium lechanii* is employed for regulating the whiteflies, aphids, thrips, and other insect populations; *Bauveria bassiana* for controlling thrips plus other harmful insects; *Paecilomyces fumosoroseus* for monitoring different insects primarily beetles, fire ants, and nematodes; *Corynebacterium paurometabolum* to keep nematodes and other pest populations in check; *Metarhizium anisopliae* for checking termites, several destructive coleoptera insect species, leaf hoppers, and aphids.

8.11 Use of Agro-Industry Remains

Waste from agriculture activities, such as weed residues, cotton litters, biogas slurry, hay, and mushroom waste, supply considerable amount of NPK to the soil in addition to various primary and secondary soil-needed micronutrients.

8.11.1 Oil Cakes as Organic Manure

Inedible oil cakes of castor, neem, and karanji (*Pongamia pinnata*) and edible cakes of groundnut, mustard are extensively utilized in India as organic manure because of their good NPK content. Similarly, animal wastes, such as bone meal and fish meal, rich in nutrients are also widely utilized in organic farming.

8.12 Naturally Occurring Mineral Amendments

According to Alimentarius Commission (Codex), some naturally occurring mineral amendments, such as rock phosphate, potassium, sulphate, guano, basic slag, gypsum (calcium sulphate), Epsom salt (magnesium sulphate), calcite lime, dolomite lime are permissible for use (Singh and Dabas 2012).

8.13 Permanent Grass

Permanent grass helps soil in the following ways: addition of organic matter, nitrogen fixation, nutrient entrapping, checking soil erosion, preventing leaching of nitrate, and nutrient runoff as well as enhancing accessibility of essential nutrients, such as potassium, phosphate, and magnesium (Schliemann et al. 1983; Mushtaq et al. 2018). In addition to this, the association of fruit trees and grass species improve the signs of leaf chlorosis because of Fe insufficiency. The quantity of organic material replaced annually by the plant mass is comparatively easy to estimate. Balesdent and Balabane (1996) stated, though the projected above-ground organic matter ($345 \text{ g C m}^{-2} \text{ year}^{-1}$) was better than the below-ground ($152 \text{ g C m}^{-2} \text{ year}^{-1}$) in corn residue, the latter added more to the soil organic matter than the former. Similarly, cultivation of alley grasses decreased the frequency of downy mildew plus powdery mildew in grape (Marangoni et al. 2001).

Nutrient release from grass litter at dissimilar rates varies with the mineral type, for example, K is released approximately following 5 weeks, whereas N, Ca, and P release takes months (Tagliavini et al. 2007). The application of mowed grass as mulch in organic farming is not much suggested in fruits, such as Pome, owing to negative effects following harvest. All the approaches aimed at prevention of excessive nutrient accumulation in soils, like that of K or P, must be implemented. Controlling iron concentrations in fruit trees is a key concern in calcareous soils, therefore inhibition of diseases such as leaf chlorosis needs to be accomplished by proper agronomic practices such as introducing resilient rootstocks and enhancement in organic material concentration in soils. Orchard management using grasses, such as species of *Festuca*, can diminish or inhibit Fe chlorosis by producing phytosiderophores and compounds, such as mugineic acid (Klair et al. 1995; Dervash et al. 2020), that chelate the insoluble iron present in the soil.

8.14 Nutrient Management and Fruit Quality

Currently, in this age of aggressive marketing, consumers are aware of their rights and concerned about the quality of the food products. Therefore, the price of organically farmed fruits, such as apples, almonds, cashew nuts, and walnuts, has increased

by considerable amounts in comparison to traditionally grown fruits. The effect of organic farming on superiority and chiefly on the nutritional significance of fruits is immensely argued. In comparison to traditional farming, organic fruit management has been established to enhance the concentration of phenols, flavanols in apples (Weibel et al. 2000). Overall, reductions in the concentration of secondary metabolic products such as organic acids and polyphenolic compounds, which are deliberated as advantageous to the human health, are obtained from organic cultivation practices (Winter and Davis 2006). The fruits obtained from organic farming enhance the productivity of plant polyphenols which have resistance mechanisms against harmful insecticides and fungicides (Asami et al. 2003; Chassy 2007) with an enhancement in protein content (Brandt and Mølgaard 2001).

8.15 Role of Soil Organic Matter

Organic matter consisting of plant and animal remains is considered to be an essential constituent of the soil. In the breakdown process, many materials are synthesized biologically from the decomposition products. These materials are categorized as humic and non-humic materials. The organic matter in soil is amorphous, hydrophilic, acidic, partially aromatic, and complex in nature. Humic materials comprises three components: (i) humic acid that is alkali soluble and acid insoluble; (ii) fulvic acid, which is the humic part remaining as a solution when the alkaline fraction acidifies; and (iii) humin, the humic fraction that is not extractable by using dilute base or acid. Soil organic matter (SOM) comprises humic substances as the largest component (85–90%), and non-humic substances as the lesser fraction comprising (10–15%) of the total percentage. The importance of SOM stems from the following points:

- Organic matter is an important nutrient reservoir for soil microbes.
- Organic matter is a repository of numerous plant macro- and micronutrients, especially N, P, S.
- Low inorganic nitrogen quantity is available in the soil, therefore much of it is acquired through conversion from organic forms which makes plants reliant on soil organic matter to supplement their nutritional requirement.
- Soil organic matter has a noteworthy function of improving physical characteristics of the soil, such as soil texture, moisture holding capacity, permeability, and soil drainage.
- Soil organic matter aids in improvising the numerous chemical properties of the soil such as accelerating soil cation exchange capacity that is helpful in enhancing the rate of chelation of nutrients, such as calcium, magnesium, zinc, potassium, copper, and iron.
- Soil organic matter supports soil buffering.
- Soil organic matter benefits in increase in nutrient release from the soil, hence aids in nutrient obtainability.

- Plant growth and development are stimulated by the physiological activities of organic matter.
- The organic material available to plants influence different soil processes, resulting in soil formation.

8.16 Organic Matter as Soil Structure Builder and Storehouse of Nutrients

Organic matter content of cultivated soils is intricately associated with soil productivity, tilth, and fertility. In semi-arid soils, organic matter is comparatively less, within a range of 0.5–3% of the total organic matter, but its impact on soil properties has a considerable implication. Organic matter at low concentrations is the prime substance assisting soil accretion and soil structure stability. Good soil structures are helpful in efficient air and water intake helps as well in protecting soils from erosion by means of wind and water. It also endures root structure. Dark coloured humic component of the soil promotes the capacity of the soil for increasing the heat absorption rate which helps the soil to warm up easily in the spring after harsh winter conditions. Therefore, organic matter is the main reservoir for most fundamental plant nutrients in semiarid soils.

The N, P, and S content of the semi-arid soils is 0.12%, 0.05%, and 0.03%, respectively, with 95% of the total N, 40% of the total P, and 90% of the total S being provided from the soil organic matter. Furthermore, the soil organic matter comprises the principal reservoir of plant nutrients, and variability within this reservoir is of foremost importance for storing and cycling of nutrients. In some dry land cropping systems, more than or equal to 50% of the nitrogen is compulsory for the plants and it arrives from SOM mineralization. The decomposition is facilitated by microbes and releasing of nutrients is controlled by numerous other means, for example, wetting of the soil, tillage, adding plant and animal residues. These affect the overall dynamics of soil and result in escalation of the size of microbes and improve nutrient release.

8.17 Role of Organic Nutrient Sources in Enhancing Nutrient-Use Efficiency

Organic substances play a chief function of maintaining soil buffering capacity, its physical and biological properties. An array of factors, such as soil temperature, water content, and chemical composition of the organic matter, affects the release of N from the soil. Monitoring N release from organic materials is dependent on its amount and quality of nutrients, properties of soil, the many environmental as well as management factors (Singh et al. 2001; Mehmood et al. 2019;

Dar and Bhat 2020). The accumulation of organic material in the soil is essential to increase the N mineralization potential. The task in optimization of N intake of organic as well as cover crop-based systems do not wholly depend on development of organic matter pools but also have an impact on the rate and timing of N mineralization.

The rate of N mineralization in soil is subjected to various interactions amongst contributions of manure, cover crops, fertilizers, and organic matter (Horwath et al. 2006). Owing to gradual releasing characteristics of organic N, it often shows slight improvement on crop growth during the initial time of the cultivable year. After application, the uptake of N increases resulting in the enrichment of the overall N content of the soil. This practice proves the durable competence of organic fertilizers. Nevertheless, in terms of mineral fertilizer equivalents, short-term N release from organic sources contrasts between 0% (with some material) to 100% (with urine). Application of green manure and NPK is encouraging throughout the first three seasons, whereas FYM and NPK were superior throughout later three seasons, signifying long-term usage of FYM for improved yields in inter-cropping practices in vertisols (Subbaiah et al. 2006).

Soil fertility shows a remarkable improvement by making use of FYM and poultry in comparison to the initial status. Unifying nutrient enhancement practices, that is, using leaf manure of sababul, neem, and melia with N fertilizer, can significantly enhance nitrogen uptake, agronomic efficiency, and increased yield in sunflower plants (Panneer and Bheemaiah 2005). When residues were incorporated in the groundnut and maize cropping system, it resulted in enhancing the successive maize yield and an increasing N recycling efficiency was observed (Sakonnakhon et al. 2005). In chick-pea, making use of residue as soil manure significantly improved the physical and fertility parameters of the soil. In maize, the nitrogen uptake effectiveness can be improved by incorporating *Glyricidia sepium* pruning 4 weeks before planting. On the residual effect of linseed and rice, the application of *Sesbania rostrata* proved beneficial as compared to paddy straw. The combined action of urea and green manure increase yield and N uptake. In Rice, N content as high as 50% can be attained by using 1 t/ha (dry weight) *Sesbania rostrate* (Singh et al. 1999).

8.18 Conclusion

Organic farming is system sustainable and environmentally sensitive; therefore, it is essential for achieving sustainable development. For improving productivity and fertility of soil, the various techniques of organic farming such as vermicomposting, integrated nutrient management, and nutrient use efficiency, the part of organic matter in improving soil health, are of critical importance and offer a better scope of sustainable agriculture.

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Chapter 9

Values of Composting



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9.1 Introduction

Compost served as a source of nutrients, and it has numerous advantages as well as disadvantages due to its organic nature. Compost is used as a soil conditioner, added essential humus and humic acid in the soil, and is used as a fertilizer and pesticide for cropland. In a simple method of compost formation, the heap of organic matter is formed, and that material changes to humus after a few months. Different types of waste products, e.g., municipal sewage, cattle manure, tree bark, and root waste, are used for compost formation (Gaind 2014). There are three elements of composting: human management, production of internal heat, and the presence of air. Carbon is required for energy production in composting. Heat is produced at the result of oxidation of carbon by microorganisms (Vidović and Runko Luttenberger 2019; Bhat et al. 2018a). Nitrogen is required for the reproduction and growth of the organism for the oxidation of carbon. Oxygen is used in the decomposition process for the oxidation of carbon, and water is also required during decomposition. At the carbon-to-nitrogen ratio of 25:1, the maximum composting occurs (Tilley 2014). In

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hot container composting, the compost is produced quickly because heat remains inside the container (Haug 2018). The carbon-to-nitrogen ratio should be 30–35 for the decomposition of feedstock, and the production of raw material is considered as the initial step of decomposition (Gil et al. 2008; Bhat et al. 2017a, b; Sofi et al. 2017). Aeration, moisture, type of feedstock, and C:N ratio are said to be the main factors that affect the process of composting. If the condition is anaerobic, it would lead to fermentation, and if the C:N ratio is not correct, it will increase the length of the composting process.

The growth of microorganisms will be affected if the moisture is low in the composting process (Füleky and Benedek 2010). The layout of composting process is shown in Fig. 9.1. The windrow method is said to be the most convenient method of composting. In this method, the raw material is set in parallel rows; the piles are allowed to rotate for the increase of oxygen supply. The moisture of piles is also removed by turning the windrows. The windrows are rotated twice a week usually. Another system used for composting of organic matter is called aerated static piles; in this system, the organic matter is placed in perforated pipes, and the material does not move to another place for aeration (Wang and Li 2009; Bhat et al. 2017a, b, 2018a, b; Qadri and Bhat 2020).

The atmospheric nitrogen-fixing bacteria and archaea are the main microorganisms that transform nitrogen during the composting process (Pepe et al. 2013). In the compost formation, fungi are found during the initial and last stage of the process. *Aspergillus*, *Penicillium*, *Mortierella*, and *Acremonium* are the essential genera (Anastasi et al. 2005). In the degradation of a complex organic compound, actinobacteria play a significant role because it can grow in high temperatures. In the fermentation process, available carbon converts into unavailable carbon due to actinobacteria (Shilev et al. 2007). In the initial stage of composting, the breakdown of organic nitrogen occurs into small compounds, and many types of bacteria, fungi, and other microorganisms take part. In the organic matter to be composted, the microorganisms are present, which convert the protein into amino acids by the release of proteases (Vargas-García et al. 2010; Dervash et al. 2020; Khanday et al. 2016).

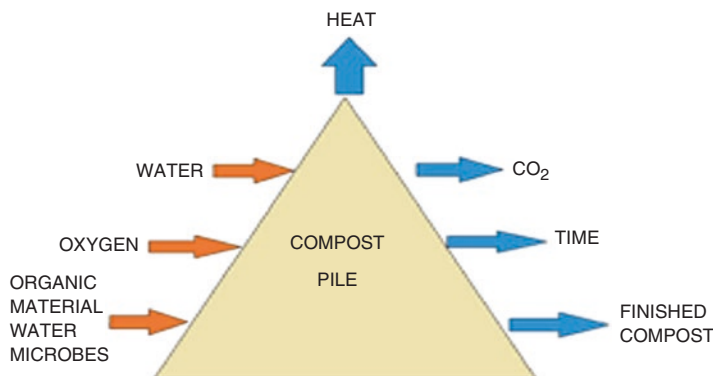


Fig. 9.1 The composting process

9.2 Historical Background and Perspectives

About 6000 years ago, the first pits were built in Sumerian cities; the organic matter of these piles was used to apply in fields for the sake of agriculture. These piles were built outside the houses (Waldron and Nichols 2009). People of India, China, and South America use animal and human residues in agriculture as a source of fertilizer (Howard 1942). Sir Albert Howard was the first person who worked on the management of composting in India, and he also made advancements in modern composting (Howard 1933). Indore process was developed by Sir Howard in collaboration with other persons. Firstly, only animal manures were used in this process, but then human feces and other materials were also used (Brunt 1949). Indore method was improved and named Bangalore process by the Indian Council of Agricultural Research in Bangalore (Diaz and De Bertoldi 2007). In China composting was studied by Scott and some other people, and they used night soil in 1935, but they stopped their studies due to World War II. Later, they mentioned the issue of composting human waste (Scott 1952).

An experiment was performed by Waksman and others from 1926 to 1941 on the formation of compost in the presence of air using vegetables. They studied the effect of different temperatures on degradation and studied the part of microorganisms in the decomposition of organic matter (Stotzky 1965). From 1920 to 1930, Beccari process was adopted by the USA, Florida, and New York. In Scarsdale, a plant was developed, and the awful smell was observed when the doors of the plant were opened. This lousy smell was produced because of anaerobic conditions developed in the plant. The New York Health Department failed to control these issues, so they stopped this facility, unfortunately (University of California 1950).

In the USA, the Frazer process was used in 1949; the organic matter was filed in a digester having an aerobic environment. The organic matter was mixed thoroughly and then moved to screening. The material was sent back to the composting process after screening (Eweson 1953). The composting of biosolids was started in 1973 by the USDA (Willson and Walker 1973). The static pile method of composting was introduced by Beltsville in 1975 (Epstein et al. 1976). Much work had been done on the formation and use of compost in Japan in 1970 and 1980 (Yoshida and Kubota 1979). Composting is being used by gardeners and farmers for many centuries. Composting has been used in agriculture to improve fertility from the time no one knows. The manure from animals and organic materials from vegetables were thrown in piles and placed into pits. These were then decomposed by the microorganisms present in the soil naturally. The time taken by this process ranged from 6 months to a year. The simple techniques to cover it with soil or turning all the materials were utilized. In China, the fecal material of humans, along with that of animal and vegetable manure, is in use for almost 4000 years. This led to an increase in soil fertility that supported a dense human population. A layer of green manures together with river silt, animal waste, and rice straw with superphosphate has been in use in a primitive method called “pit manure.” In this method, a rectangular or a circular pit was dug. The area of this pit was almost 10m². The moisture was conserved by wetting an upper layer of mud. It not only conserved moisture but

assisted in avoiding loss of nutrients and maintenance of temperature. All the material was turned three times per month. The anaerobic conditions were maintained throughout the process (Lopez-Real 1996).

9.3 Types of Compost

The quality and type of compost are one of the essential criteria in its use as an organic amendment and a recycling process for organic waste (Lasaridi et al. 2006). The composts are used extensively in agriculture, mostly due to enormous organic matter and mineral components that are found in manure, municipal waste, sewage sludges, etc. These assist in the reclamation of soil and for better crop production. This is all done after running them through appropriate processes of stabilization (Campitelli and Ceppi 2008).

The following are the types of compost:

- (a) *Aerated static pile composting*
- (b) *Windrow composting*
- (c) *Vermicomposting*
- (d) *In-vessel composting*

9.3.1 Aerated Static Pile Composting

The distinguishing feature of this composting system is the use of a grid for aeration pipes (Fig. 9.2). It is done for forced aeration. The pile is aerated by a fan or blower. This type of composting involves the mixing of dehydrated sludge with wood chips,

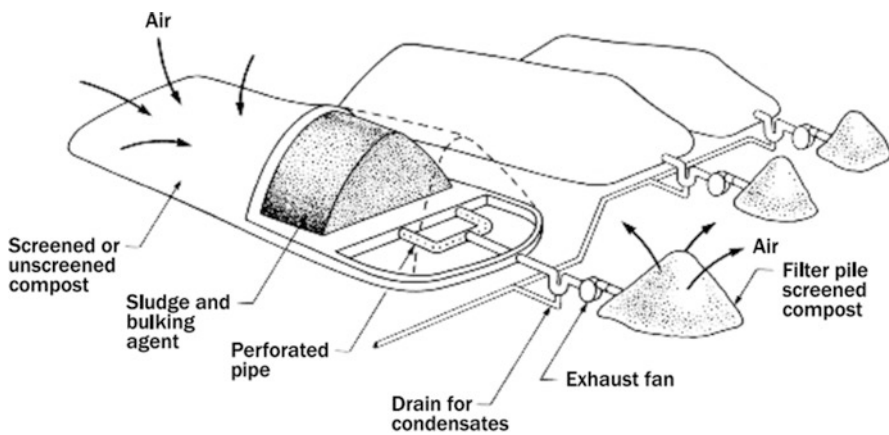


Fig. 9.2 Lay out of an aerated static pile composting

which is used as a bulking agent. The other process is followed by the building of a pile of compost above the grid of aeration pipes, composting, filtering of the compost, curing, and then storage. The following is the procedure of aerated static pile composting. The 100–150 mm plastic pipes are used to make aeration grid. These are then fitted in the 1 ft plenum of wood chips. The wood chips assist in even distribution of air and for absorbance of moisture from the pile. The height of the pile ranges from 6 to 8 ft. The temperature and oxygen are controlled by forced air in aerated static pile composting (Metcalf 2003).

9.3.2 Windrow Composting

In this process, the composting material is thoroughly mixed with a bulking agent. These are then formed in long rows. These parallel rows are called windrows. The height of a windrow ranges from 3 to 6 ft with 6–14 ft width at the base. The height and width depend on the types of equipment used for turning and mixing of the windrows. This type of composting is done on open sites. The rows are continuously turned upside down to expose composting materials properly. The movement of air is eased by it. The moisture is also decreased by it. The most used machine for turning windrows is a front-end loader, which is shown in Fig. 9.3.

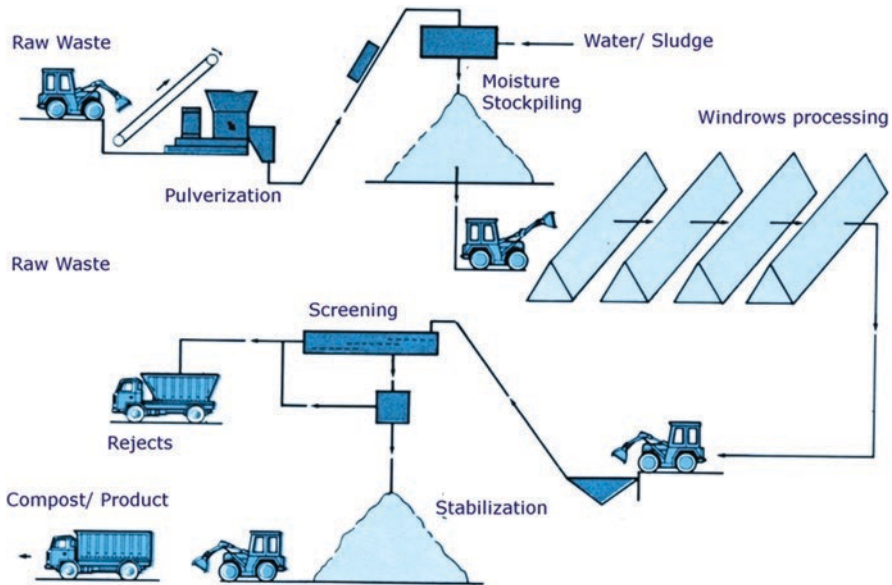


Fig. 9.3 Lay out of an open windrow composting

9.3.3 Vermicomposting

A finely powdered peat-like mature substance which is produced as a result of the non-thermophilic procedure and engaging interaction between earthworms and microbes is known as vermicomposting. This results in stabilization and oxidation of organic matter. The primary process of vermicomposting revolves around the conversion of solid organic waste into vermicompost through the non-thermophilic procedure. This is one of the eco-friendliest technologies that are used as an organic fertilizer. The most found bacteria in vermicomposting are from *Rhizobium*, *Pseudomonas*, *Nitrosomonas*, *Azotobacter*, and *Bacillus*. The nutrition provided to plants by vermicompost is higher than other composts (Joshi et al. 2015) (Fig. 9.4).

9.3.4 In-Vessel Composting

In-vessel composting is done inside a container. A more consistent and stabilized compost can be made by this process in lesser time because environmental conditions like oxygen, airflow, and temperature are controlled. The odors produced as the result of this process are also contained in in-vessel composting. There are two classes of this type, namely, dynamic reactors and agitated reactors. The following schematic diagram (Fig. 9.5) shows this type of composting.

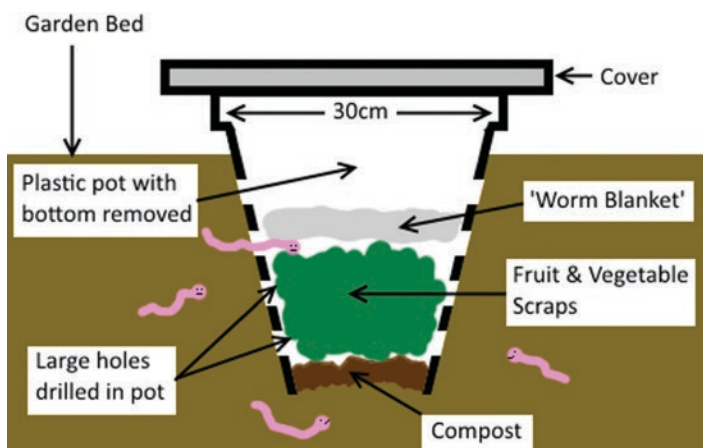


Fig. 9.4 A schematic diagram of a worm tunnel for the production of vermin compost

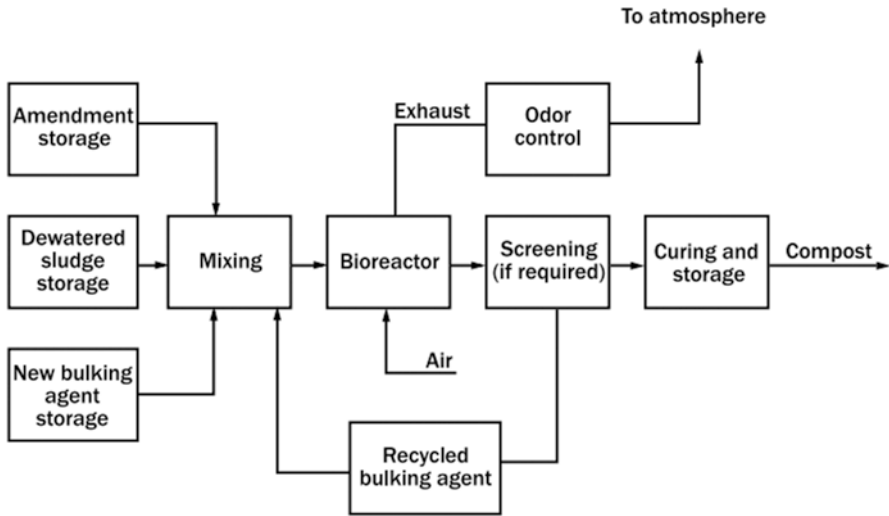


Fig. 9.5 Schematic diagram of in-vessel composting

9.4 Compost: A Viable Tool for Sustainable Agriculture

Compost can be incorporated to the soil, to recover degraded soils; to control plant disease; to increase or maintain soil fertility to reduce negative impacts and production costs of chemical fertilizers, pesticides, and fuel; to sequester carbon into the soil; and to reduce global warming (Dar et al. 2013; Scotti et al. 2016; Sánchez et al. 2017; Vázquez and Soto 2017; Mushtaq et al. 2018). Compost helps to improve microbial activity, organic matter, and bring back dead soil to life. It serves as a mulching material, nursery cultivation substrate, a growing medium, or porous absorbent material that holds moisture and soluble minerals and provides nutrients and support to help plants flourish. Composting can be a well-thought-out C-based system, like agricultural management practices, reforestation, or other waste management industries and an alternative to landfill (Brown et al. 2008; Quirós et al. 2014). Compost possesses little physical similarity to the originated raw material, but its nutrient content differs with the type of materials used for the initial mass preparation. Final compost characterization of generally hinge on initial C:N ratio of used materials (Arbab and Mubarak 2016). Compost provides nutrients to the soil for a long time. Compost acts as a slow nutrient release fertilizer and improves nutrient use efficiency. It takes at least 3 years to establish its full value. As a rule, compost releases 40% of phosphorus, 20% of nitrogen, and 80% potassium in the first year of application. Organic nitrogen released at a constant rate with continuous application of compost from the accumulated humus and increased nitrogen use efficiency of chemical fertilizers over 50% over the years. Phosphorus availability is sometimes much higher than that of inorganic fertilizers (Sinha et al. 2012; Singh

et al. 2020). Compared to the separate addition of biochar or compost, their combined application was more effective to improve soil pH, organic matter, organic carbon, and available potassium (Tang et al. 2020). The on-farm composting process resembles the recent indication and provision of the European Commission on agricultural biomass recycling for production and application of organic-based fertilizer in soil to the management of the organic matter. These approaches are supposed to signify an important influence and valuable chance to advance the circular economy at local as well as regional scale (European Commission 2017). The compost application in agriculture could encounter the European Union country's target objective to cut the organic waste quantity going to landfill sites by 50% by 2050 (European Commission 1999).

Compost helps to conserve soil and water, balance the soil pH in a way that diminishes plant stress, control soil runoff and erosion, and improve chemical properties of soil and invigorate degraded soils and ecosystems (Hernandez et al. 2015). It improves availability to plants by nutrient mobilization, soil structure water-holding capacity, and aeration, suppresses soilborne diseases, and promotes microbial life in soil (Tejada et al. 2009; Hadar 2011; Macci et al. 2012). Vermicompost, human compost, and cow compost affected soil chemical properties in the cultivated green bean plot. A significant increase in soil EC, soil pH, and organic carbon of soil and green bean yield under human compost was observed than in other composts for 0–15 cm depth. This proves that the fertilizer value of the sanitary products was higher than that of vermicompost and cow compost. Sanitary products (human waste) can be used as a soil amendment and nutrient source, but it is high in salinity (Uwamahoro et al. 2019).

Application of green manure and compost (0, 10, and 20 t ha⁻¹) alone or in combination with mineral nitrogen (0, 43, and 86 kg N ha⁻¹) recovers soil properties and improves growth and yield of sweet pepper. In this context, compost was better than green manure (Mahgoub 2014). Composted poultry manure application along with some microbial inoculation improved nutrient status of calcareous soil (Iqbal et al. 2016). Additionally, application of wheat straw and cow manure compost at the ratio of 3:1 alone (0, 4, 8, 12, and 16 ton fed⁻¹) or in combination with chemical fertilizers 86 kg N ha⁻¹ and 43 kg P₂O₅ ha⁻¹ for wheat and 86 kg N ha⁻¹ for okra crop had significantly improved soil properties. Nutrient uptake increased significantly by plant and improved the yield and growth for wheat and okra. The combination of chemical fertilizers with compost (16 ton fed⁻¹) gave the highest nutrient content, grain yield, crude protein, potassium, and phosphorus percent (Eltayeb 2018). However, this high amount of compost is not good from an economic point of view. The application of sewage sludge compost is helpful in enlightening soil conditions and the yield of sorghum fodder. The combination of sludge compost with recurrent irrigation can upsurge yield by more than 47% compared to air-dry sludge treatment (Shashoug et al. 2017).

The use of compost is a sustainable approach to mitigate, control, or prevent harmful plant diseases and pests. It affects vascular, foliar, as well as root pathogens. Compost can decrease crop losses by soilborne diseases by disease suppression. On the other hand, it acts as a shelter and food source for the enemies of plant

pathogens and stimulates their proliferation (Hadar 2011). Disease control by compost largely depends on the substrate or soil properties, i.e., biotic and physico-chemical parameters, the composting process used, and raw material composition in the preparation of compost, and on the quality and maturity of the compost (Janvier et al. 2007). Long periods of compost maturation can decrease microbial activity and, therefore, decrease the disease suppression ability (Zmora-Nahum et al. 2008). Bacteria belonging to genera *Enterobacter* spp., *Bacillus* spp., *Streptomyces* spp., *Trichoderma* spp., *Pseudomonas* spp., as well as several *Penicillium* spp., isolates, and other fungi have been recognized as biocontrol agents in compost-amended substrates (Pugliese et al. 2008). Sterilization or heat treatments can drop the suppressive effect of the compost (Pugliese et al. 2011). However, insufficient research is found about the direct effect of compost on pest management. For instance, both indirect and direct links were found between aphids, predators, and compost. Compost-treated plots confirmed lower aphid population numbers pointedly when predators' numbers are suggestively higher. Aphids were also lower significantly than in plots without compost (Bell et al. 2008). The compost teas have an extensive series of microflora like actinomycetes, bacteria, fungi, etc., with variable occurrence and population. Most of the microfloras are potential antagonists against different disease agents such as *Alternaria alternata*. Individually, actinomycetes and anaerobic bacterial isolates were proved more effective than some others (Praveena and Reddy 2013).

9.5 Compost Application in Peri-urban Areas

Peri-urban agriculture is an integral part of the twenty-first-century economy. It is a rural-urban transition zone. Because of the increasing population and spread in urbanized areas, agriculture farms once sited in rural areas are now surrounded by urban areas (Cohen and Reynolds 2015). Peri-urban agriculture is the livestock rearing or crop production for sale or consumption within the city areas. It is well-known that peri-urban agriculture addresses the threefold security global goals (FAO 2008), i.e., (i) sustainable management and use of natural resources, (ii) sustainable increases in food production and availability, and (iii) economic and social progress (Bougnom et al. 2014). Peri-urban agriculture plays a role in the urban social, economic, and ecological systems (Mougeot 2002). Achieving food security requires an increase in production in the cities, along with a sustainable farming system. Peri-urban agriculture requires a large number of inputs, such as plant nutrients. Chemical fertilizers are usually suggested to maintenance farmers, but they are expensive to use. Chemical fertilizers and pesticides are persistent and remain in water and soil. They will cause pollution by accumulation in soils, runoff, and horticultural crops, by the accumulation of organic compounds and heavy metals in aquatic life, by seepage into aquifers, by airborne chemicals, and by direct contact. Therefore, to replace inorganic fertilizers, composting material is used in many areas of the world. Composting seems to be a possible way to handle organic

waste management in the cities and to provide peri-urban agriculture with organic fertilizers (Bougnom et al. 2014; Dar et al. 2016). Compost lessens transportation costs because some of the waste can go into the compost piles as a replacement for landfilling (Eureka Recycling 2001), while composting without an understanding of the adverse effects on neighbors can have damaging effects on communities (Krasa et al. 2017).

The commercial compost contains the raw material from various sources and may have a considerable amount of heavy metals in their composition (Riaz et al. 2017). Seven different composting mixtures from fresh vegetable leaves and fallen tree leaves mixed with maize or grass straw (0%, 10%, 30%, and 50% w/w) are common in peri-urban areas of Harare. The composts with a 30% straw mixture effectively reduced nitrogen losses and had higher potential as a soil amendment in the peri-urban areas of Harare (Mhindu et al. 2013). Raw fecal sludge and wood chips and maize cobs from three peri-urban communities were composted. The results showed that the total N and carbon contents of all materials decreased. At the end of the composting process, the experiment showed lower phosphorus and potassium (K) available concentrations than in the original substrate materials. Maize cob that contained more phosphorus, nitrogen, potassium, and carbon is the most ideal (Appiah-Effaha et al. 2016).

9.6 Compost Versus Environmental and Soil Pollution

Human activities, such as mining, chemical manufacturing, smelting, fertilizer application, fossil fuel combustion, and tanning, are the main reasons of heavy metal, pesticide, harmful chemical, and oil-based hydrocarbon accumulation and pollution in soil (Liu et al. 2019a, b; Tang et al. 2019). Heavy metals and other chemicals are generally not degradable, and their buildup in the soil causes pollution and threatens human health (Liu et al. 2019c; Bhatti et al. 2017). Many countries have been endangered by heavy metal pollution in soil, including China, the USA, Italy, Mexico, etc. (Tang et al. 2019). Many studies have been done on removal or immobilization of heavy metals in soil with numerous inorganic and organic additives (Lu et al. 2017). Compost can lessen exchangeable and mobile metal fraction of contaminated soil and has been used as a highly effective heavy metal removal amendment (Liang et al. 2017; Dar and Bhat 2020). Compost comprises a large number of humic substances, which can form stable organometallic complexes with metal ions in the soil to reduce the mobility of metals (Arif et al. 2018; Gusiatin and Kulikowska 2016). Moreover, compost with low carbon-to-N ratio and a high proportion of humic substances to TOC can more effectively reduce the mobility of heavy metals in soil (Gusiatin and Kulikowska 2016). Conversely, some heavy metals such as Cu may be activated by humic acid (Zeng et al. 2015). The increase in available P by composting decreased heavy metal availability, possibly by complexation and with phosphate precipitation (Ahmad et al. 2012). More phosphate availability increased arsenate availability because of the same chemical

nature (Beesley et al. 2014). Compost increases soil organic matter, and it acts as an essential heavy metal (Cd and Zn) adsorbent because of the presence of many functional groups, such as $-OH$ and $-COOH$. These functional groups can bind metal ions and form stable anti-desorption complexes (Yang et al. 2016). Compost with biochar remediates the heavy metal pollution in soil. Compost and biochar significantly reduced the availability of Zn and Cd but activated Cu and As slightly. Also, soil enzyme, catalase, dehydrogenase, and urease activities were activated by compost (Tang et al. 2020). Combined compost and plant technology can remove 50% of hexavalent chromium in chromium eluted soil (Mangkoedihardjo et al. 2008). The use of vegetal material compost has been encouraged strongly and better explored gradually for the remediation of the contaminated soil. Vegetal materials compost caused vertical transport and rapid mobilization of As and trace metals (Beesley and Dickinson 2010). Green waste composts comprise carboxylate groups (8.8%) and inorganic ash (46.1%) and immobilized Cu soils contaminated by metals (Tsang et al. 2014).

Animal manure compost with more than 50% inorganic fraction (high phosphorous) decreased the amounts of water-soluble lead by over 88% compared to the soil without compost. However, the microbial enzyme activity levels were the same or less than those in the control soil. Animal manure compost with 25% inorganic fraction did not suppress the water-soluble lead existed during the first 30 days, but it improved microbial enzyme activities (Katoh et al. 2016; Mehmood et al. 2019).

Pesticides are used for pest and weed control. However, it affects the soil and air quality as these chemicals can drift to other sites (Arias-Estévez et al. 2008). Pesticides induce detectable changes in structure, functionality, and size of the microbial community, thus changing life dynamics, functions, and biodiversity of soil organisms (Yañez-Ocampo et al. 2011; Chen et al. 2015; Cruz et al. 2015). Composting can stabilize pesticides in soils through microbial degradation and can improve soil quality (Chen et al. 2015). Biochar and compost, two frequent amendments, were used to investigate their combined influence on enzymatic activities and microbial communities in organic-polluted wetlands. Compost application (2% and 10%) enhanced degradation efficiency of sulfamethoxazole by 0.033% and 0.222%, respectively, along with biochar due to the upsurge of biomass and enzymes (Liang et al. 2020). Composts of cow dung, yard manure, corn stalks, corn fermentation by-product, and sawdust have been used to improve the herbicide removal of trifluralin, metolachlor, and atrazine in contaminated soils (Moorman et al. 2001). Compost addition to soil has improved degradation of herbicides, MCPA (4-chloro-2-methylphenoxyacetic acid), and benthocarb (S-4-chlorobenzyl diethylthiocarbamate). Composting of contaminated sawmill soil degraded chlorophenols effectively (Megharaj et al. 2011). Composting pile produced out of straw compost and chlorophenol-contaminated soil degraded more than 90% of the chlorophenols (Chen et al. 2015).

Rumen residue and yard waste alteration accelerate the aromatic and aliphatic fractions of petroleum hydrocarbon degradation in crude oil-contaminated soil as the primary substrate in the composting process. Petroleum hydrocarbon degradation efficiency was 31 times higher in soils added with rumen residue and yard

waste mixture than contaminated soil, which satisfied the quality standard of soil ($6974.58 \text{ mg kg}^{-1}$). The total petroleum hydrocarbon degradation might be completed by *Bacillus* sp. and *Bacillus cereus* as the main bacteria at the end of the composting process (Sari and Trihadiningrum 2019).

Motor oil pollution in the soil is a major environmental issue related to illegal dumping and improper handling of industrial waste. A combined alternative biological treatment that focuses on composting the polluted soil with yard trimmings was done. A 12% degradation of total petroleum hydrocarbons present in motor oil after a 9-week composting process was achieved. An additional 50% decrease in total petroleum hydrocarbons was reached after planting *Lolium perenne* with the highest microbial count, 2.8×10^7 CFU, of bacterial species, *Azotobacter vinelandii*, *Bacillus brevis*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* (Escobar-Alvarado et al. 2015).

Composting decreases environmental problems related to waste management by decreasing waste volumes and by killing potentially dangerous organisms. Composting can effectively recycle valuable organic matter nutrients that are trapped in the environment. Landfills generate emissions, mostly containing methane, a more toxic greenhouse gas than carbon dioxide. Landfilling is considered a significant contributor to the increase in greenhouse gas emission. Developing countries caused about 29% of these emissions in the year 2000, and this is predicted to increase to 64% in 2030 and 76% in 2050 (Monni et al. 2006). Moreover, landfills produce hazardous leachate that can degrade habitats and water quality as well as poison flora and fauna if it enters water sources. The US Composting Council notes that although barriers are often put in place in an attempt to prevent emissions and leachate escaping, if these liners break down, contaminants can be leaked into surface runoff and groundwater. By diverting organic wastes from landfills, the lifespan of municipal landfills can be lengthened, reducing the need to create new landfills. Keeping organics out of waste stream continually can extend the life of municipal landfills. This improves the air quality by reduced emission processing as well as anthropogenic greenhouse gas production (EPA, CalRecycle, Clean Air Council, Global Alliance for Incinerator Alternatives, US Composting Council).

Composting, as an alternative to waste incineration, has huge inferences for civilizing environmental quality. The Environmental Protection Agency and the US Composting Council reported that aerobic composting does not expressively add to an increase in CO_2 emissions. The Global Alliance for Incinerator Alternatives reported that waste incinerators produce more CO_2 emissions compared to coal, oil, or natural gas-fueled power plants. Any emissions from aerobic composting are considered part of the natural carbon cycling. Aerobic compost can also be used as a landfill cover to reduce methane emissions. Aerobic compost as a bio-filter can eliminate 80–90% of volatile organic compounds from gas streams. It sequesters carbon in the ground, acts as a carbon sink, and promotes soil structural stability and fertility (EPA, US Composting Council, the Global Alliance for Incinerator Alternatives).

During composting, microorganisms consume oxygen and decompose organic materials, generating water vapor heat and carbon dioxide. During decomposition,

mineral nutrients such as sulfur, P, and N are released, and a substantial amount of potent greenhouse gasses (i.e., methane and nitrous oxide), ammonia, and NO_x are produced during composting as well. Several strategies have been established to diminish greenhouse gas emissions and N losses from composting (Chowdhury et al. 2014; Wang et al. 2014). A recent study showed the influence of biochar and bean dreg addition during pig manure composting on the emission of N, greenhouse gas, and ammonia. During pig manure composting, the combined application of biochar and bean dregs decreased N loss (24.26%), greenhouse emissions (29.56%), and ammonia emissions (33.71%) (Yang et al. 2020).

The use of straw from vegetable waste composting reduced total N loss by 33% and from manure composting by 27–30% (Vu et al. 2015). High C:N materials, for example, straw, sawdust, and biochar, decreased N₂O emissions by 37–43% and methane emissions by 70–90% from composting (Jiang et al. 2011; Chowdhury et al. 2014; Vu et al. 2015). Aeration is another option for mitigating N losses and greenhouse gas emissions from composting because it reduces the presence of anaerobic hotspots in a composting pile. Anaerobic compost method decreased total N losses by 70% and ammonia emissions by 90% (Sagoo et al. 2007). A decrease of 72–78% N losses was reported by composting (Shah et al. 2012, 2016), and N losses of less than 2% of the initial total N by leachates were reported under relatively higher aeration rates (De Guardia et al. 2010). Nonmixing of cow manure compost produced 3.5 times less N₂O compared to the unturned pile composting method (Ahn et al. 2011). However, in another study, the mixed composting method increased ammonia volatilization and reduced N₂O emissions to the environment (Szanto et al. 2007). The emission of N₂O from soil was increased by sludge or biomass composting (He et al. 2016). Frequent turning of compost pile increased the total N losses by more than double compared to less frequent turning. More aeration increase N loss by ammonia volatilization (Cook et al. 2015). An increase of over 88% in total N was recorded during higher aeration. Methane emission is decreased by composting (Chowdhury et al. 2014).

9.7 Conclusion

Compost is used in agricultural soil as a soil amendment and conditioner. Compost improves the soil structure, organic matter content, and water relations. It helps to remediate the soil from heavy metals, polyaromatic hydrocarbons, pesticide, and herbicide residues by making complexes. Nutrients such as P content are improved by compost that also helps in remediating the soil from heavy metal, especially arsenate, by making complexes. Compost releases many greenhouse gasses to the environment and causes pollution. Different amendments and different types of composting materials helped to reduce the emission of greenhouse gasses. Agriculture farm practices near cities known as peri-urban agriculture recently used compost as a soil amendment, but due to the much civil legislation and neighbor's rights legislation, its use is under restriction in many areas of the world. However, it is used in many peri-urban areas as fertilizers successfully.

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Chapter 10

Introduction to Microbiota and Biofertilizers



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10.1 Introduction

With an annual growth rate of 1.8% per year, the world populace is perpetually pacing up which is expected to grow from the contemporary population of 7.4 billion to 9.6 billion by 2050. It is casted that the total food demand will grow in proportion to world population (United Nations 2013). These days, crop production is coming across challenges due to increase in population, change in climate, and also an increased demand for sustainable output. A large chunk of productive soils are being reserved for other requirements due to increased urbanization which produces high pressure to expand agriculture; most of the fertile soils in favorable environments are being diverted to other uses. The agrochemicals cannot increase the crop output past a certain limit. Besides, because of paucity of knowledge, farmers put in more synthetic fertilizers than actually recommended. The over usage of synthetic chemicals prompts an otherwise effect on health of consumer and further results in reduction of soil fertility as they augment salt substance to the soil (Swapna 2013; Dar et al. 2013; Bhat et al. 2017; Bhatti et al. 2017). Such unsustainable cropping practices have imposed new challenges to agricultural output. Plant pathogens, low temperatures, high temperatures, excess nutrients, deficient nutrients, soil salinity, excess water, and deficient water are among a multitude of biotic and abiotic factors affecting the production of crops. In order to push up the actual output of different crops, it is a dire need to change biotic and abiotic factors so as to put our

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agricultural demands at par, where the traditional farming practices baffle. As of late, harmful impacts of synthetic fertilizers on the environment and human life have moved the attention on environmental-friendly options. The traditional agriculture has a pivotal role with regard to fulfilling the staple needs for a perpetually pacing up human populace, prompting an expanding reliance on the utilization of synthetic fertilizers and pesticides for production enhancement (Santos et al. 2012). Synthetic fertilizers are scientifically made preparations, the utilization of which results in pollution of air, water, and subsequently the nutrient enrichment of water bodies (Youssef and Eissa 2014; Dar et al. 2016). However, the act of utilizing synthetic fertilizers and pesticides quickens acidification of soil (Chun Li et al. 2014; Bhat et al. 2017; Mushtaq et al. 2018; Dar and Bhat 2020); it likewise represents the danger of polluting the environment and groundwater (Mehmood et al. 2019; Dar et al. 2020). Besides, it also debilitates the plant roots consequently making them defenseless to certain pathogenic diseases. Therefore, advancement of biofertilizer-based organic farming is required direly as interest for residue-free and safe food is pacing up tremendously. In this context, the use of microbial inoculants is among the potential methods of realizing this objective (Calvo et al. 2014; Pertot et al. 2016; Singh et al. 2016; Bhat et al. 2018). Because of the proven possibility of microorganisms as biopesticides as well as biofertilizers, a rising demand has been felt to include them as one of the choices replacing chemical products in cultivation methods (Mendes et al. 2013; Mitter et al. 2016; Rashid et al. 2019).

Biofertilizers are appended by the living microbes which inhabit the roots endogenously, thereby boosting the delivery of nutrients to the host crops, thereby enhancing the growth and development of plants, once put into operation with seeds, plant surfaces, or soils (Vessey 2003; Bardi and Malusa 2012; Malusa and Vassilev 2014). The chosen processes of microbes are widely accelerated by biofertilizers which increase the nutrient availability for easy uptake by the plant. By converting the ambient nitrogen into usable forms and solubilizing the inaccessible phosphorus into phosphates, they enhance soil fertility by secreting plant development promoting chemicals in the soil (Mazid and Khan 2015; Dervash et al. 2020). The primary job of biopesticides or biofertilizers is to avert or inhibit the diseases of plants. Under normal conditions rhizosphere and aboveground plant parts are inhabited by protozoa, bacteria, algae, actinomycetes, and fungi. Bacteria constitute 95% of colonizing microorganisms (Glick 2012; Khanday et al. 2016). The development of plant is profoundly influenced by direct and indirect processes carried out by bacteria which inhabit the roots of plants either exogenously or endogenously constituting the plant growth-promoting bacteria or rhizobacteria, thereby making them eligible to be called as biopesticides and biofertilizers (Glick 2012). The soil encompassing plant roots is a principal source of bacterial agents advancing growth and development of plants.

Secretion of chemicals by the roots of plants helps in proliferating soil microbes found in the surroundings of their roots (Walker et al. 2003; Bhat et al. 2018). Stage of development, health, fitness, and plant genotype determine the properties and composition of root exudates. Particular microbiomes have been distinguished for every organ (Vorholt 2012; Philippot et al. 2013; Hardoim et al. 2015) and plant

species (Berg and Smalla 2009) in spite of functional and taxonomic overlap within the plant microbiota (Bai et al. 2015). The fortification of microorganisms by the plant root is certainly not an arbitrary but rather a focused process (Johnston-Monje et al. 2016; Adam et al. 2016) and the attraction of microbes to roots by supplements such as carbohydrates and amino acids in combination with plant-specific secondary metabolites (Moe 2013; Weston and Mathesius 2013). Differences in plant root exudates assume a significant job in the working of both chemoattractants and repellents (Badri and Vivanco 2009). Plant defense signaling assumes an extra role in these processes (Doornbos et al. 2012).

10.2 Types of Biofertilizers

Biofertilizers can be grouped based on their function and nature in the below mentioned groups (Fig. 10.1). These biofertilizers have been upgraded to bring in the processes of mobilization of nutrients which are biologically accessible, thereby enhancing the fertility status of soils enormously and subsequently the yield of crops (Pandey and Singh 2012).

10.2.1 Nitrogen-Fixing Biofertilizer (NFB)

Being copious and omnipresent in atmosphere, nitrogen still turns out to be a limiting nutrient owing to its non-fixation as molecular nitrogen and difficulty in uptake by plants, though some microbes which live in association with roots or in the soil around the roots are able to fix nitrogen significantly. This microbial association prevents nitrogen losses by denitrification and reduces chances of leaching and volatilization that aids effective uptake of fixed nitrogen by plants. These microbes can be:

10.2.1.1 Free-Living

Though it is difficult to evaluate the fixation of nitrogen by free-living bacteria, it has been found to range in between 3 kg N/hectare and 10kgN/hectare in plants like *Medicago sativa*. In addition to slime production which aggregates soil, *Azotobacter chroococcum* is estimated to fix 2–15 milligram of nitrogen per gram of carbon source in cultivable soils. Nevertheless, *Frankia* has been found to fix nitrogen in the root zone from atmosphere in their host as well as non-host plants (Thomas and Singh 2019). Potential of cyanobacteria has been utilized in cultivating paddy in India under ideal conditions producing approximately 20–30 kg N/hectare (Kannaiyan 2002).

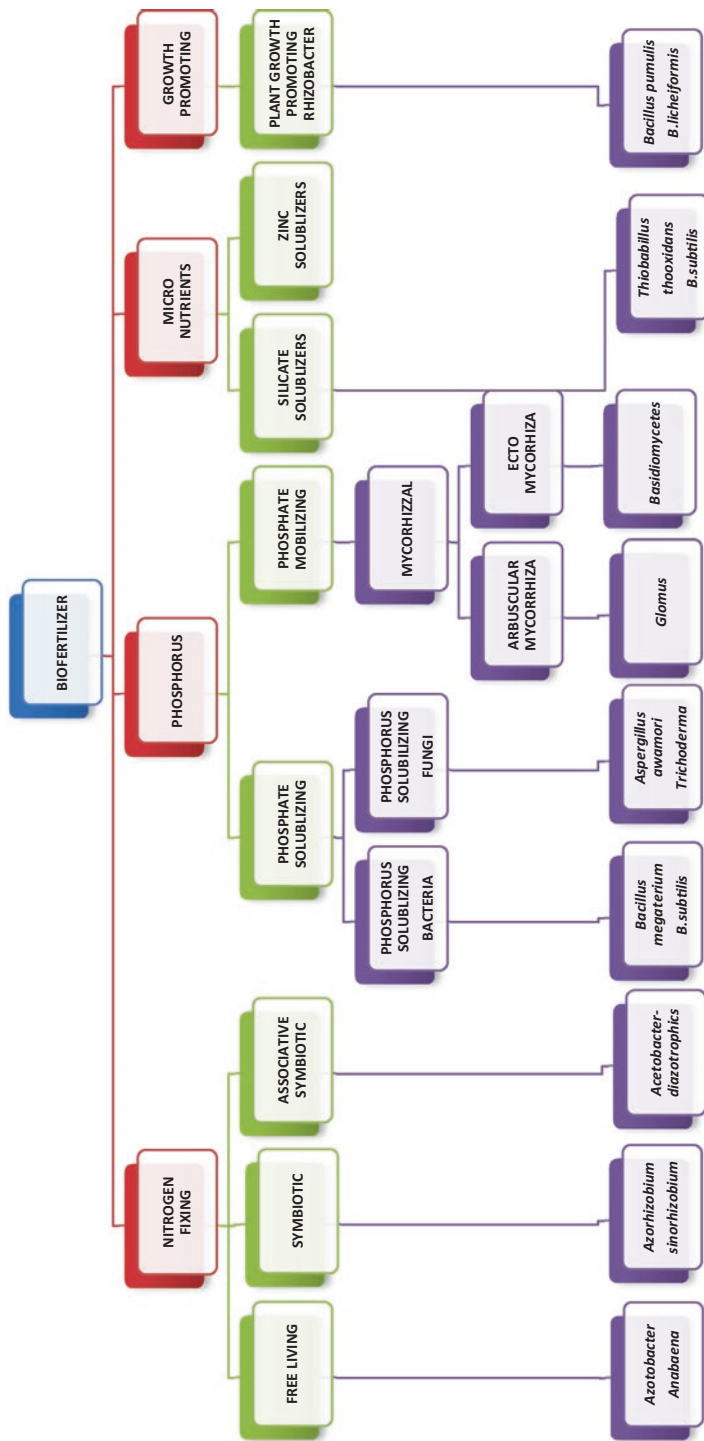


Fig. 10.1 Classification of microbial fertilizers

10.2.1.2 Symbiotic Associations

A significant group of organisms like *Sinorhizobium*, *Allorhizobium*, *Bradyrhizobium*, etc. by forming nodules in the roots of host legume species can show nitrogen fixation efficiency of up to 450 kilograms of nitrogen for each hectare of land (Graham and Vance 2000; Unkovich and Patat 2000; Thomas and Singh 2019). Granular, liquid, and powder preparations in various aseptic carriers of charcoal, peat, perlite, and mineral soil may be used for the rhizobial biofertilizers (Stephens and Rask 2000). *Frankia* being an *actinomycete* also fixes atmospheric nitrogen like *Rhizobia* by forming nodules in the roots of many woody plants (Wall 2000). This bacterium forming mycelium lives in symbiotic association with roots of many nonlegume plants like *Alder*, *Casuarina*, *Myrica*, etc. The inoculation of *Frankia* in perturbed or dry environments is regarded as beneficial (Sprent and Parsons 2000). In addition, *Xanthomonas* and *Mycobacterium* living symbiotically in the internal cavities of the leaves of *Ardisia* fix nitrogen turning such leaves into a pool of nitrogen for the soil (Miller 1990). Another ecologically viable group is the blue-green algae (BGA) of cyanobacteria, some of them contribute to around 36% of the world's nitrogen fixation, such as *Trichodesmium*, *Nostoc*, and *Anabaena*, and have been found to help improve rice crop fertility in many parts of the world (Gallon 2001; Irisarri et al. 2001). BGA is also known as a potential advantageous remedy for dry environments or flood-prone ecosystems (Malam Issa et al. 2001; Sofi et al. 2017). However, inadequately developed application and production of blue-green algae are ought to be regarded as a biofertilizing agent in sustainable agriculture for different environs (Hashem 2001; Thomas and Singh 2019).

10.2.1.3 Associative Symbiotic (Without Endophytic Symbioses)

These nitrifying microorganisms are distantly associated to roots compared to endophytic symbionts. *Acetobacter diazotrophicus* and *Herbaspirillum* are the examples of associative symbiotic nitrogen fixers which are associated with plants like sugarcane, maize and sorghum (Boddey et al. 2000), species of *Bacillus*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas* are associated with rice & maize and species of *Azoarcus* with kallar grass (James 2000); and *Azospirillum* with large host specificities including a broad range of perennial and annual plants. Many studies have reported that fixation of nitrogen and secretion of growth-promoting chemicals by *Azospirillum* has improved the yield and growth in cotton, oak, sugar beet, carrot, pepper, eggplant, and wheat (Thomas and Singh 2019). *Azospirillum* inoculum can be manufactured at low cost with a simple formulation of peat (Broek et al. 2000). *Acetobacter diazotrophicus* biofertilizer has been found to supply almost 70% nitrogenous demand for sugarcane by fixing approximately 150 kilograms of nitrogen for each hectare of land annually. As a result, nitrogen fixation capability makes them the potential choice for biofertilizer application (Thomas and Singh 2019).

10.3 Phosphorus Biofertilizers

10.3.1 Phosphorus-Solubilizing Biofertilizers (PSB)

Though the phosphorus concentration is high in the soils, most of it remains in unavailable states making phosphorus the second most deficient nutrient in the plants after nitrogen. The phosphorus-solubilizing biofertilizers can be grouped in two categories:

10.3.1.1 The Phosphorus-Solubilizing Bacteria (PSB)

Bacteria that solubilize phosphorus such as *Bacillus* and *Pseudomonas* function by solubilizing insoluble phosphate forms within soil, thereby increasing the supply of phosphorus to plant species (Richardson 2001).

10.3.1.2 The Phosphorus-Solubilizing Fungi (PSF)

Penicillium and *Aspergillus* also increase the phosphorus available to plants by mobilizing it out of nonavailable states in the soil. Bacteria and fungi secrete organic acids which bring down pH in their vicinity, causing bound phosphates in the soil to disintegrate (Fig.10.2). The use of *Bacillus megaterium* var. *phosphaticum*, along with the cheaper rock phosphate, was found to increase the quality and yield of sugar cane by 12.6 percent and to reduce the requirement of phosphorus by 25 percent, thus further reducing the costly superphosphate consumption by 50 percent (Sundara et al. 2002).

10.4 Phosphate-Mobilizing Biofertilizers (PMB)

They operate through the scavenging and mobilization of phosphates from soil layers into soil; such biofertilizers are applied to like mycorrhiza. Occasionally, phosphate-solubilizing biofertilizers can behave as phosphate mobilizers too (Chang and Yang 2009). The phosphate-mobilizing biofertilizers have a broad spectrum. Figure 10.3 shows the activation of soil phosphorus and its immobilization by bacteria. The mycorrhizal biofertilizers are discussed in the following section.

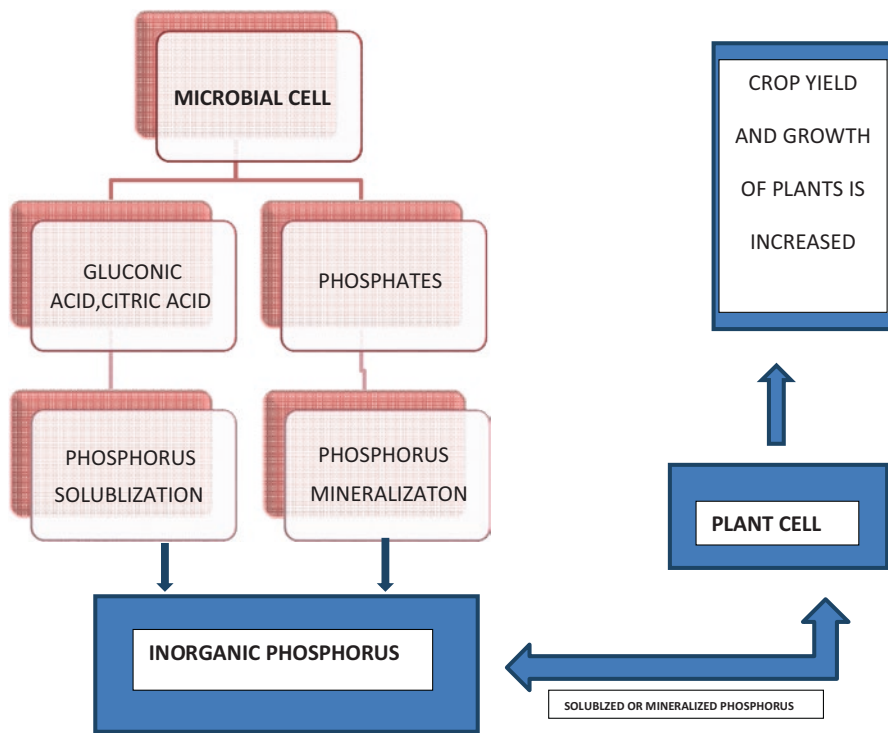


Fig. 10.2 Solubilization of inorganic phosphorus by phosphate-solubilizing bacteria and fungi

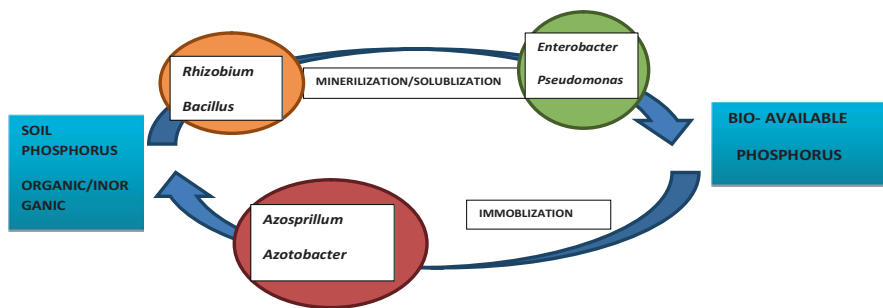


Fig. 10.3 Diagrammatic representation of phosphorus mobilization and immobilization by the soil microbes

10.5 Mycorrhizal Biofertilizers

These biofertilizers are also known as phosphate absorbers that help in mobilizing phosphorus. Here the fungus lives obligately or facultatively in association with almost 80% plants resulting in mutualistic symbiosis where the fungal component

derives phosphates and energy; plant component derives a wide variety of benefits from the fungus it hosts (Thakur and Singh 2018). Fungal mycelium stretches from the host root surfaces to soil and thereby enhances the surfaces of the plant, deriving energy from insoluble phosphorus, other sources such as calcium, copper, zinc, etc. helping in more efficient nutrient access and acquisitions to the plant (Singh and Gira 2017). Besides, such biofertilizers improve and enhance the aeration process in the soil, improve the fertility status of the soil, balance the water dynamics of the plant, augment the sequestering potential for heavy metals, and increase the tolerance of plant species to drought, thereby making them less susceptible to pathogenic diseases and herbivory (Rillig et al. 2002; Thakur and Singh 2018). It means that these fungi have a high potential for use in forestry, soil conservation, or habitat restoration (Thomas and Singh 2019). The two types are as follows:

10.5.1 *Arbuscular Mycorrhizal (AM)*

The fungi such as the *Glomus* are intercellular, nonspecific mandatory symbionts living endogenously bearing vesicles in roots as special structures functioning as an extended root providing water to the crop as well as different microniche nutrients to the soil in addition to the increased phosphorus accessibility and availability helping plant to grow and develop adequately. Obligate association and non-culturability of arbuscular mycorrhiza has made inoculation not been consistent with broad-scale industrial agriculture and may thus necessitate additional research (Ryan and Graham 2002). Nonetheless, adjusted uniform growth of crop has been made possible by inoculating AM proving beneficial for the development of nursery stocks. The source of inoculums determines the colonizing potential of fungi in certain host plants which can vary for agricultural purposes (Klironomos and Hart 2002). The process of inoculating the host plant or cultured root organs with AM is an effective symbiotic way of producing infective propagules, but the high cost involved in its production in addition to prolonged turnover and challenges of excluding some pathogens of roots turns out to be some of the limitations. The most reliable inoculation of AM is done in the form of spores. For some taxa, effective inoculums are applied as fragments of AM colonizing the roots. In addition, a combination of both these methods can be used where the mycelium in soil in combination with pumice, perlite, and vermiculite as carrier substrate can be inoculated (Klironomos and Hart 2002; Gaur and Adholeya 2000; Thomas and Singh 2019; Singh et al. 2020).

10.5.2 Ectomycorrhiza

Basidiomycetes in *Eucalyptus*, peach, and pine penetrate internally in intercellular spaces in the cortex forming a mantle on the surface of root where plant-secreted sugars advance the nutritional availability to the plants. These fungi absorb water, minerals, and inorganic nutrients by increasing the surface area of roots and secrete chemicals which act as antimicrobials providing protection to plants against a broad range of root pathogens. Ectomycorrhizal symbiosis has been found to be important for the growth and nutrient acquisition in tree plantations in particular for larger inocular practices in nurseries or forest areas (Thomas and Singh 2019).

10.5.3 Biofertilizers for Micronutrients

Excluding nitrogen and phosphate, soil microbes are also used as biofertilizers for the supply of different micronutrients as zinc, silicates, etc. Such biofertilizers can be categorized in the following two groups:

10.5.3.1 Zinc Solubilizers

As zinc is found in the crust of the earth in minute concentration, its low occurrence forces the farmers to have an external application of it as the costliest soluble zinc sulfate to resolve its deficiencies in the plants. *Saccharomyces*, *Bacillus subtilis*, and *Thiobacillus thiooxidans* are among the bacteria which solubilize less costly oxides, carbonates, and sulfides of zinc in the soil (Ansori and Gholami 2015).

10.5.3.2 Silicate Solubilizers

These are some microorganisms that hydrolyze aluminum silicates by adding protons and form cationic complexes by secreting organic acids, thereby keeping silicates in dissolved form which benefits plants during metabolism. For example, increased rice growth and the yield of grain were observed by increased silica and soil nutrients using *Bacillus* sp. combining rice paw, rice husk, and black ash with silica residues (Cakmakci et al. 2007).

10.5.3.3 Plant Growth-Promoting Biofertilizer (PGPB)

In addition to a nitrogen-fixing (Fig. 10.4) and phosphorus-solubilizing microbe, some microbes ideal for biofertilizers stimulate growth of plants by synthesizing chemicals which have been found to generate significant amounts of host plant

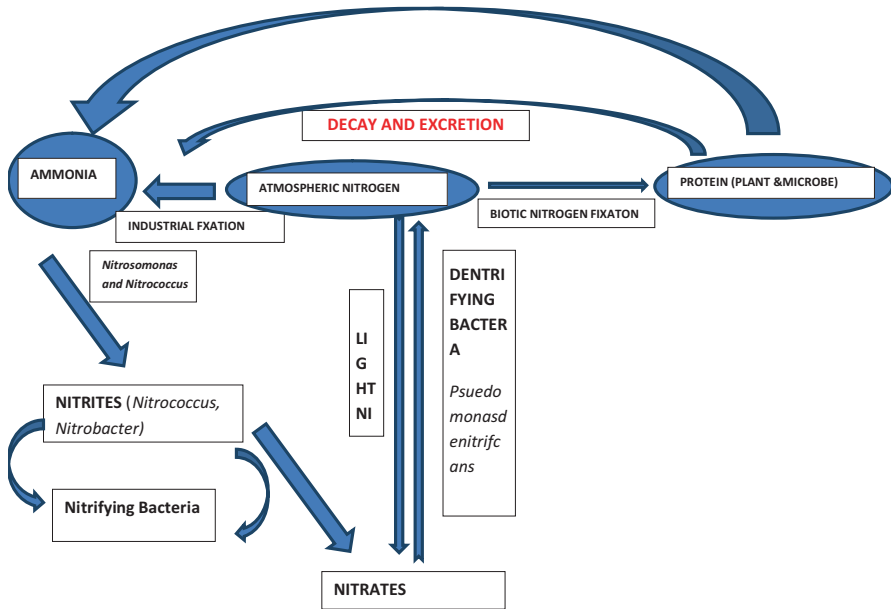


Fig. 10.4 Schematic representation of nitrogen cycle

hormones with significant physiological activity (Gutierrez-Manero et al. 2001; Thomas and Singh 2019), e.g., rhizospheric *Bacillus pumilus* and *Bacillus licheniformis*. However, *Paenibacillus polymyxa* showed a broad range of beneficial property, like fixation of nitrogen, phosphorus solubility, antibiotic production, cytokinase, hydrolytic enzymes, chitinase and other enzymes, and soil porosity enhancement.

In addition, plant hormones were reported for certain *Azospirillum* species (Thomas and Singh 2019). The synthesis of antimicrobial metabolites by rhizobacteria, such as enzymes that have the ability to degrade fungal cells, causing cytolysis, ion leakage, membrane disruption and inhibition of mycelial growth and protein synthesis, are among the antagonistic mechanisms of phytopathogenic microorganisms which helps in improving plant health (Idriss et al. 2007; Lugtenberg and Kamilova 2009). For example, antifungal metabolites such as phenazines, pyrrolnitrin, pyoluteorin, or cyclic viscosinamide lipopeptides can be produced in *Pseudomonas* strains to prevent *Pythium ultimum* sugar beet infections. The *Pseudomonas fluorescens* synthesizes siderophores such as pseudobactin and pyoverdine which bind and take up ferric ions, making them more competitors of iron and thus preventing pathogenic microbes such as *Pythium ultimum*, *Fusarium*, and *Rhizoctonia bataticola* (Hultberg et al. 2000), which prevent them from growing and proliferating (Hultberg et al. 2000). Siderophores like pyoverdine, pyochelin, and salicylic acid are produced by *Pseudomonas aeruginosa*, and resistance is further induced against *Botrytis cinerea* (on tomato and bean) and *Colletotrichum lindemuthianum* (on bean) (Audenaert et al. 2002). Nevertheless, some *Pseudomonas*

species make the *Fusarium solani* mycelia to break with extracellular chitinase and laminase. Biofertilizers also provide protection from certain soil diseases and insect pests and plant diseases, such as the use of antibiotics in *Azotobacter* to limit the spread of soil pathogens such as *Pythium* and *Phytophthora* (Wani et al. 2013).

10.6 Microbiota Used in Biofertilizers

Organisms that are mostly used as biofertilizer components include nitrogen fixers, phosphorus solubilizer, and phosphorus mobilizers used solely or in combination with fungi. Majority of the bacteria used in biofertilizers have a close association with roots of plants for example Rhizobacterium which dwell in rhizosphere are having a symbiotic association with the roots of legumes (Khosro and Yousef 2012). Most of the time, bacteria and fungi make insoluble phosphorus accessible to the plants (Gupta 2004). A few soil fungi and many bacteria have the capacity to change insoluble phosphate in soil into dissolvable structures by secreting organic acids. These acids bring down the soil pH and achieve the disintegration of bound forms of phosphate (Gupta 2004). While Cyanobacteria, *Rhizobium* and *Azolla* are crop specific, bioinoculants such as PSB, *Azospirillum*, *Azotobacter* and VAM could be viewed as wide-spectrum biofertilizers (Gupta 2004). VAM being fungi improves the buildup of nutrients in plants by being associated with most of the agricultural crops (Khosro and Yousef 2012; Wani et al. 2013). It has been recommended to stimulate plant by physiological impacts or by diminishing the seriousness of infections brought about by soil pathogens. The following are the microbiota that are commonly found in biofertilizers:

10.6.1 Bacteria

Bacteria are life forms that have just a single cell and, hence, microscopic. There are about 100 million to 1 billion microbes in only a teaspoon of damp, fertile soil. They decompose dead plant material and natural waste. The process of decomposition discharges supplements that different living beings could get to (Abu Bakar et al. 2013). The bacteria do this by converting the nutrients from non-usable to usable structures. This course of action is essential in the phosphorus and nitrogen cycles.

10.6.2 Actinomycetes

They are also soil microorganisms like the bacteria and fungi and have attributes connecting them to the two classes. They are frequently accepted to be the missing evolutionary connection between fungi and bacteria; however they share a greater

number of attributes practically with bacteria than they do with fungi. Actinomycetes contribute to soil its peculiar smell. They have likewise been the source of a few huge helpful medicines (Shukla and Livleen 2015).

10.6.3 Fungi

Fungi bunch themselves into sinewy strings called hyphae; hyphae further branches into mycelium which are 0.8 mm wide but can get up to a few meters. They are useful, yet could likewise be destructive, to soil creatures. Fungi are useful in light of the fact that they can breakdown supplements that different life forms cannot (Nutongkaew 2014). They at that point discharge them into the soil, and different life forms find a good place to use them. Significant development of the plant can occur when fungi live in symbiotic association with plant. This is an advantageous association called mycorrhizal. The fungi help the plant by giving it required nutrients, and these organisms get sugars from the plant. The soil fungi perform some important functions.

- Saprophytic fungi act as decomposers and help in breaking the dead organic matter into carbon dioxide and organic acids and converting some portion into fungal biomass.
- Mycorrhizal fungi act as mutualists by colonizing the roots of the plants. They convert bound forms of phosphorus to soluble forms and nitrogen, water, and micronutrients to the plant they are in association with. Ectomycorrhizae are found in trees where it grows on the surface layer of roots while endomycorrhizae are found in vegetables and grasses where it grows endogenously in the root cell.

10.6.4 Algae

They are living in the vast majority of the soils where dampness and daylight are accessible. Their number ranges from 100 to 10,000 for each gram of soil. They are equipped for photosynthesis, whereby they acquire carbon dioxide from the environment and sunlight from daylight and synthesize their own food. The significant functions of algae in soil are:

- Playing a significant role in the upkeep of soil richness, particularly in tropical soils.
- In the uncultivated soils, it prevents leaching and drainage of nitrates, hence keeping a check on loss of nitrates.
- Adding organic content to soil when they die and accordingly increasing the amount of natural carbon in soil.

- By secreting slime, it helps in binding particles of soil, hence diminishing and forestalling erosion of soil.
- Helping to build the water retention limit of soil for longer timespans.
- It encourages submerged aeration by releasing ample oxygen in the soil environment via photosynthesis.
- By secreting organic acids, it accelerated rock weathering and hence proves valuable in the buildup of soil structure.

10.6.5 Protozoa

These are drab, single-celled animal-like organisms. They are bigger than microscopic organisms, changing from a couple of microns to a couple of millimeters. Their population in arable soil ranges from 10,000 to 100,000 for every gram of soil, and they are abundant in surface soil (Johns 2017). They can endure hostile soil conditions, as they are portrayed by an ensured torpid stage in their life cycle. The major functions, roles, and features of protozoa are:

- Many protozoans get their food from feeding or ingesting soil microorganisms, and in this way they assume a significant job in keeping up microbial/bacterial balance in the soil.
- Some protozoa have been as of late utilized as natural control agents against life forms that cause detrimental maladies in plants.

10.6.6 Viruses

Soil viruses are critical, as they may impact the nature of soil organic networks through both a capacity to move genes from host to host and as a potential reason for microbial mortality. Accordingly, viruses are significant players in worldwide cycles, affecting the turnover and convergence of nutrients and gases. Soils most likely harbor numerous novel viral species that, together, may speak to a huge repository of genetic diversity (Johns 2017). A few specialists accept that researching this generally unexplored diversity of soil viruses can possibly change our comprehension of the virus infections in worldwide biological systems and the advancement of microbial life itself.

10.6.7 *Nematodes*

Nematode worms are normally 50 microns in breadth and one millimeter long. Species responsible for plant infections have gotten a lot of consideration; however far less is known about a great part of the nematode network, which assumes useful jobs in soil. A staggering variety of nematodes have been found to work at several levels of the soil food web. Primary level nematodes feed on the algae and plants, secondary level nematodes feed on fungi and bacteria, and higher level nematodes feed on other nematodes. Free-living nematodes can be separated into four general gatherings dependent on their eating regimen. Bacterial feeders expend microscopic organisms. Contagious feeders feed by puncturing the cell walls of fungi and sucking out the internal contents. Predatory nematodes eat a wide range of nematodes and protozoa. They eat little living beings entirely or append themselves to the cuticle of bigger nematodes, scratching endlessly until the prey's interior body parts can be extricated. Like protozoa, nematodes are significant in mineralizing, or discharging, supplements in plant-accessible forms. At the point when nematodes eat microscopic organisms or parasites, ammonium is discharged on the grounds that fungi and bacteria contain considerably more nitrogen than the nematodes require. Nematodes may likewise be valuable pointers of soil quality due to their colossal diversity and their investment in numerous capacities at various degrees of the soil food web (Johns 2017).

10.7 Functions of Plant Microbiota as Biofertilizers

The individuals from plant microbiome include advantageous, neutral, or pathogenic microorganisms. The following are the significant functions which are performed by plant microbiota as biofertilizers:

- (a) Plant growth-promoting bacteria secrete auxin, gibberellins, and cytokinin affecting growth of plants through endogenous modulation of hormone levels, thereby advancing plant development by either immediate or aberrant mechanisms.
- (b) Nitrogen fixation, phosphate solubilization, indole acetic acid, growth and stress tolerance, and mechanisms involved in improved uptake of nutrients are some important plant growth promotion properties that have been found in soybean roots and wheat harboring *Pantoea* spp., *Paraburkholderia* spp., and *Pseudomonas* spp. (Rascovan et al. 2016).
- (c) *Arthrobacter* spp. and *Bacillus* spp. are some of the plant growth-promoting bacteria that secrete an enzyme, 1-aminocyclopropane-1-carboxylate deaminase, which enhances growth of plant by reducing ethylene level, i.e., the main stress hormone in the plant.
- (d) Some microorganisms can cause infection manifestations through the secretion of phytohormones, phytotoxic mixes, and proteins. *Erwinia amylovora* is a

pathogenic bacterium that causes fire blight disease of ornamentals plants and fruit trees. *Pseudomonas syringae* is a notable plant pathogen having an exceptionally expansive host range including olive, tobacco, green bean, and tomato. *Xylella fastidiosa*, *Xanthomonas* species, and *Ralstonia solanacearum* are likewise connected with numerous significant maladies of banana and potato (Mansfield et al. 2012). The seriousness of plant infection relies upon the combination of numerous components like pathogen populace size, susceptibility of host, plant microbiota, and favorable environment that by and large decide the result of plant-pathogen interaction (Brader et al. 2017).

- (e) Both subterranean and aboveground plant-related microorganisms have been found to improve resistance of host against pathogen disease either through interaction of commensal pathogen or through plant defense modulation (Rudrappa et al. 2008; de Vrieze et al. 2018). Production of siderophores, antibiotics, pathogen inhibiting volatile compounds, and lytic enzymes are some of the activities which protect the plant against disease progression and invasion of pathogen (Hopkins et al. 2017; Berg and Koskella 2018). Specifically, genera like *Paraburkholderia*, *Paenibacillus*, *Pseudomonas*, *Bacillus*, *Enterobacter*, *Pantoea*, *Streptomyces*, and *Burkholderia* have been reported for their role in pathogen suppression (Gomez et al. 2017; Schlatter et al. 2017). The nonstop utilization of agricultural soils can facilitate pathogen pressure and can likewise create malady suppressive soils containing microorganisms interceding suppression of diseases (Santhanam et al. 2015; Duran et al. 2018). *Firmicutes*, *Acidobacteria*, and *Actinobacteria* controlled the attack of *Fusarium* wilt at a continental scale (Trivedi et al. 2017). Soil disease suppressiveness of *Paraburkholderia graminis* PHS1 against fungal root pathogen was accounted to synthesis of cysteine desulfurase and dimethyl sulfoxide reductase (Carrion et al. 2018). *Enterobacter* and *Serratia* were found to be the best possible endospheric community of bacteria for suppression of take-all diseases, i.e., *Gaeumannomyces graminis* (Duran et al. 2018).

10.8 Factors Affecting Plant Microbiota

A number of biotic and abiotic factors, including the abundance of microbial predators (e.g., protists or nematodes) and the amount of carbon available, can impact the total amount of microbial biomass found in soil at any given time. On a global scale, availability of soil moisture is the best indicator of total soil microbial biomass. The wetter ecosystems of the world like tropical rainforests harbor larger quantities of microbial biomass (Serna-Chavez et al. 2013). However, not all microbial taxa are equally abundant in soil. Bacteria and fungi are commonly the predominant microorganisms found in soil; these groups usually have 10²–10⁴ times more biomass than the other significant components of the soil microbiome (protists, archaea, and infections) (Lynch and Neufeld 2015).

There is no typical soil microbiome. The relative abundances of major bacterial and archaeal taxa found in the soil microbiome can vary considerably depending on the soil type. This is true even for the soil samples collected from sites that are just a few centimeters apart (O'Brien 2016). This variation in the composition of the microbiome can be attributed to spatial variability in the soil environment; it also depends on the taxa in question and the type of soil being analyzed. Thus, there is no single biotic or abiotic factor that is reliably the most significant in determining the soil microbial composition. Among abiotic factors, soil pH is usually taken as the best indicator of bacterial and archaeal community composition (Lauber et al. 2009; Griffiths 2011). Yet these pH impacts may not be apparent when soil samples cover a smaller range of pH values and not all taxa react to soil pH changes. Apparently there are many variables which can directly or indirectly affect the spatial structure of microbial soil communities. In addition to soil pH, the most important factors with significant influences on the structure of soil bacterial communities are most likely nitrogen availability (Cederlund 2014), organic carbon content of soil (Sul 2013), temperature (Oliverio et al. 2017), redox status (Ridge and Firestone 2005), and salinity (Fierer 2017). Nature of exudate, morphology of root, and rhizodeposits associated with different genotypes are the factors determining the composition of microbiome harbored by plant species (Hartmann et al. 2009; Ladygina and Hedlund 2010; Chaparro et al. 2014; Reinhold-Hurek et al. 2015). Plant species growing in the similar soil environment recruit significantly different microbial communities in both rhizosphere and root compartments (Hacquard 2016; Samad et al. 2017; Aleklett et al. 2015).

10.9 Mechanism of Action of Various Biofertilizers

The static lifestyle of plants forces them to optimize their well-being within the biotic environment they are a part of. The microbes in soil influence the plant fitness besides making use of the host plants for their proliferation. This has led to a very strong plant-microbe association. The coevolution of microbes and their host plants has produced mechanisms that control the growth and development of both plants as well as microbes (Jones and Dangl 2006; Oldroyd 2013). The plant roots have strong affinity toward a variety of microorganisms. The microbes in soil are regulated by the root exudates (like p-hydroxy acids, quinones, cytokinins, and flavonoids). This is the first step in root colonization where microbes show chemotactic response toward a number of root exudates (Zhengand and Sinclair 1996). The complex interactions that a plant experience are physical, chemical, and biological interactions. These interactions take place in rhizosphere of the plant. It includes root-root and root-microbe interactions.

The rhizospheric microbiome is the major determinant of plant growth and development. It helps the plant in nutrient uptake, gives protection against diseases, and also helps in abiotic stress resistance (Berendsen et al. 2012; Bulgarelli et al. 2013; Dobbey et al. 2018; Sasse et al. 2018). Thus, the interaction between the

microorganisms and their host plants is utmost important for improving plant growth and maintaining appropriate soil conditions. The plant science has already recognized the significance of root exudates in maintaining these biological interactions (Baetz and Martinoia 2014). A few examples are secretion of isoflavones by soybean roots which attract a mutualist (*Bradyrhizobium japonicum*) and a pathogen (*Phytophthora sojae*) (Sasse et al. 2018). The chemotactic response of *Pseudomonas fluorescens* towards the root exudates of tomato plant drives colonization in them (de Weert et al. 2002). There are some endophytic bacteria which show fivefold increase in the chemotaxis in the presence of exudates of rice roots. Due to the diverse functional characters of PGPR such as enzyme synthesis, hormone production, solubilization of nutrients, and effective root colonization for sustainable agriculture, it attains a unique and extraordinary position among the varied microbial communities. The symbiotic relationship between plants and microbes can be seen as an integrated ecological unit called as holobiont (Vandenkoornhuysen et al. 2015). The varying microbial composition of soil is attributed to differences in root exudate chemistry (Bais et al. 2006; Rasmann and Turlings 2016) and in plant nutrient uptake rates (Bell et al. 2015). Knowledge and understanding of ecology, growth-promoting features, mechanism of action, and application of naturally occurring microbial communities are essential to plant growth. There is still wide scope to understand the various types of plant-microbe interactions.

10.10 Types of Biofertilizer Formulation

Biofertilizer is a live, viable microbial cell designed to boost soil fertility. They are formulated to make them viable and at the same time to increase growth of plant, fertility of soil, and yield of crops. The biofertilizer preparation method is carried out through multilevel processes, in which several strains are combined with some additives which protect cells all through the storage cycle (Herrmann and Lesueur 2013). The type of formulation has a significant function in biofertilizer preparation. Besides enhancing the activity of microbes at larger pace in host plant when inoculated, an efficient formulation also proliferates the number of soil microbes (Arora et al. 2010). Better biofertilizer formulations, hence, are needed in order to produce in the market a new, more reliable, safe, and better-quality biofertilizers that meet the needs of farmers (Bashan et al. 2014). Various enviable characteristic properties of good-quality formulation are as follows:

- (i) Good formulation must be allowing nutrient adding, easily adjustable pH, and adequate supply and availability of reasonably low priced raw material (Catroux et al. 2001; Herrmann and Lesueur 2013).
- (ii) It should be environmentally gracious, i.e., biodegradable, non-pollutant, and nontoxic, in line with contemporary environmental issues concerning the usage of chemicals which alter characteristics of soil.

- (iii) Release of bacteria into the soil must be speedy and regulated, and it may be used with regular seeding equipment (Malusa et al. 2012).
- (iv) Biofertilizers should be endured and metabolized under harsh conditions in a high number (Malusa et al. 2012). Most commonly used formulations are tourbillon, oil, granules, and lyophilized powders.

10.10.1 Peat Formulations

Peat consists of decayed plants that have partly collapsed over the years. This allows spectacular range of microbes to grow, which are covered by particles and in the colonies of their cells, as well as creates a nutrient-enriched and protective habitat (Bashan et al. 2014). Foremost advantageous characteristics a peat formulation must contain are its nontoxicity, extreme absorptivity, lofty organic content, enhanced water retentivity, economic affordability, and easy to sterilize. Peat is indeterminate and dynamic matrix of various substances showing variability in its capacity for maintaining growth and survival of cells (Malusa et al. 2012). Survival and growth of inoculated microbes may also get impacted by sterilization of toxic chemicals. It could lead to problems in ensuring clear output and field outcomes (Bashan et al. 2014).

10.10.2 Liquid Formulations

Aqueous (broth crops), mineral and organic, and water-based oils, or polymer-based suspensions, are the basis for fluid formulation. The easy handling and use of liquid biofertilizers, either on seeds or in soil, has become more popular (Herman and Lesueur 2013). Generally they hold high number of cells that contain high concentrations of cells, but the lower levels of formulations can also be applied. In addition, in contrast to solid formulation, liquid formulation enables a manufacturer to add sufficient nutrient quantity, cell protective agents to advance performance (Sahu and Brahma Prakash 2016). In case of a large farm machinery, including air seeders or seed augers, it has been reported that these are non-contamination formulations, have longer shelf life, and improved protection against environmental stress compared to peat formulations. Further, large farm machinery find liquid formulations easy to carry (Bashan et al. 2014). In addition, small-scale biofertilizer farmers, who do not have the capacity to handle peat as carrier, are preferred (Singleton et al. 2002). In most developing countries, however, other restrictions have now prohibited their use. Broth-based biofertilizers lack supporting security and quickly lose seed viability. The addition of certain other ingredients such as gum arabic, saccharose, and glycerol may, however, enhance the continued existence of liquid microbes (John et al. 2011).

10.10.3 Granules

Granules consist of tourbillons or little grains of calcite, marble, and silica, wetted adhesively and mixed by means of powder-specific inoculations. Granules are impregnated or coated by target microorganisms (Bashan et al. 2014). Granulate dimensions vary, but the relation between population density and quality of the end product is straightforward. Better mother culture gives better final product (Herman and Lesueur 2013). The benefits of granules in comparison to peat include the following: less sandy, easy handling, easy applicability. These biofertilizers are placed in a groove next to seed in order to have lateral – root interaction with the seed, however, there is no proximity with the chemicals/pesticides that are toxic to microbes (Bashan et al. 2014). There are however other drawbacks, such as the bulkier type, which makes transportation and storage costly. In order to achieve this desired result, the application rate must also be increased (Herman and Lesueur 2013).

10.10.4 Lyophilized Powders

Dry biofertilizers produced using soil, organic, or inert carrier have been used in certain cases (Bashan et al. 2014).

10.11 Potential Significance of Beneficial Microbiome in Sustainable Agriculture

Suggestions have been put forward that utilization of transfer therapy of customized central microbiome for its benefits in agribusiness can prove to be a prospective methodology for overseeing plant maladies in various crops (Gopal et al. 2013). The metagenomic study gives the individual, the core rhizosphere, and endophytic microbiomes action in *Arabidopsis thaliana* utilizing 454 sequencing (Roche) of 16S rRNA quality amplicons (Hirsh and Mauchline 2012). Rhizospheric microbial networks as alternative replacing synthetic manures turn to be a subject matter of incredible enthusiasm for economical agriculture and biosafety programs.

For each gram of root, 10^{11} microbial cells are present in constricted zone of soil encompassing plant roots commonly recognized as rhizosphere (Egamberdieva et al. 2008) or above 3×10^3 species of prokaryotes that, by and large, advance productivity of plant (Mendes et al. 2013). Microbiome is an aggregate genome found in rhizosphere microbial network surrounding the plant roots and is bigger in comparison to host plant (Bulgarelli et al. 2013), the interactions of which decide the crop well-being in characteristic agrobiological systems by offering various types of services to crop plants, viz., nutrient procurement, water assimilation, cycling of nutrients, control of weeds and pests, and decomposition of organic matter (Berg

et al. 2013). Biofertilizers form additional constituent in soil and cultivation traditions, in other words soil fertility renewal, rotation of crops, maintenance of tillage, organic adjustments, crop residue recycling, control of pathogens by bioagents, and management of pests, whose operations can serve to sustain the productivity of different crops (Sahoo et al. 2013b). The PGPRs found to increase in the soil without the treatment of tillage or minimum tillage include cyanobacteria *Azotobacter*, *Rhizobium*, *Azospirillum*, phosphorous, and K-solubilizing microbes; some PGPRs are the mycorrhizae present in soils facing no tillage or minimum tillage (Dogan et al. 2011; Aziz et al. 2012). In *Helianthus annuus*, significant amounts of nitrogen may be produced by efficient strains of *Azospirillum*, *Azotobacter*, *Phosphobacter* and *Rhizobacter*; also an increase in height of the plant, leaf number, diameter of the stem and dry seed weight has also been observed (Dhanasekar and Dhandapani 2012). Likewise, in rice, physiology and root morphology get enhanced by incorporating *Azotobacter*, *Azospirillum*, and *Rhizobium* (Choudhury and Kennedy 2004). By utilizing beneficial microbes for sustainable crop production, safe, secure, eco-accommodating strategies will hold a significant focal point in the near future (Nina et al. 2014). In general, these microbes include various natural microorganisms which when inoculated in soil ecosystems promote physicochemical properties of soils, crop productivity, soil health, soil biodiversity of microbes, growth of plants, and development (Sahoo et al. 2013a). Microbial crops that are agriculturally useful include rhizobacteria that promote plant growth, cyanobacteria fixing nitrogen, mycorrhiza, bacteria that suppress plant maladies, and endophytes that promote stress tolerance and biodegrade microbes (Singh et al. 2011). As *Azotobacter* has a number of metabolic functions, it performs significant function in the process of nitrogen cycle (Sahoo et al. 2013a). *Azotobacter* has an ability to make thiamine, riboflavin (vitamins), as well as cytokinin, auxins, and gibberellins (plant hormones) (Abd EL-Fattah et al. 2013) in addition to playing a role in nitrogen fixation (Revillas et al. 2000). *Azotobacter chroococcum* increases seed germination and promotes root development, thereby promoting growth of the entire plant (Gholami et al. 2009) by repressing pathogens found in the vicinity of root frameworks in different crops (Mali and Bodhankar 2009). *Azotobacter nigricans*, *Azotobacter vinelandii*, and *Azotobacter paspali* are applied as biofertilizers for coffee, jute, rice, sorghum, coconuts, etc. (Wani et al. 2013).

Azospirillum is another free-living, gram-variable, and flood-prone aerobic bacterium (Sahoo et al. 2014), promoting various plant growth and development aspects (Bhattacharyya and Jha 2012). In both greenhouse and field trials, *Azospirillum* has proven to have beneficial effects on crop and plant growth (Saikia et al. 2013). Different *Azospirillum* species including *A. brasilense*, *A. amazonense*, *A. lipoferum*, *A. halopraeferens* and *A. irakense* have been found to improve productivity of different crops (Sahoo et al. 2014). Interestingly, inoculation of *Azospirillum* can alter the root morphology through the production by siderophore production of crop regulatory substances (Bashan et al. 2004; Sahoo et al. 2014). The lateral roots are increased as well, and root hair formations are enhanced so that more root surface areas are used to absorb enough nutrients (Mehdipour-Moghaddam et al. 2012).

This enhances the plant's water status and helps the nutrient profile of plant growth to grow (Ilyas et al. 2012).

Azospirillum brasilense and *Rhizobium meliloti* plus 2,4-D were co-inoculated, and the grain yield and N, P, and K content of *Triticum aestivum* increased significantly (Askary et al. 2009). For many years, rhizobium has been used as an effective nitrogen fixer. The transformation of atmospheric nitrogen into usable form plays a key role in increasing yields (Sharma et al. 2011). Rhizobium normally enters the root hair, multiplies there, and forms nodules (Nehra et al. 2007). The grain yield of Bengal grams and the lentil (Rashid et al. 2012) by rhizobium inoculants in different places and soil types has been significantly increased. Such wild rice-derived rhizobium isolates have been reported to provide nitrogen to the rice plant for growth and development (Peng et al. 2008). A *Rhizobium meliloti* 1021 species infects nonleguminous plants such as rice to support growth through increased endogenous plant hormone levels as well as photosynthesis to confer plant stress tolerance (Chi et al. 2010). In groundnut, the rhizobium strain IRC-6 has resulted in enhancing several useful characteristics in 50 DAI (days after inoculation) such as increasing the number of color pink nodules, reductase activity in nitrate, and leghemoglobin content (Sharma et al. 2011). Plants like the Mexican bean beetle (Thamer et al. 2011) and the greenhouse whitefly *Trialeurodes vaporariorum* (Menjivar et al. 2012) are shielded against pathogens and herbivores by rhizobial symbiosis.

10.12 Important Uses of Biofertilizers

10.12.1 Biofertilizer Boosts Up Photosynthetic Activity

Enhanced plant growth is shown by higher photosynthesis, as about 90 percent biomass in plant is derived by assimilating carbon dioxide during the process of photosynthesis (Long et al. 2006). Biofertilizer inoculation in particular *Rhizobium* sp., *Bradyrhizobium* species IRBG 271, and *R. leguminosarum* augmented photosynthesis rate for a single leaf in comparison with plant left without inoculation, i.e., control. The IRBG strain showed average increases in photosynthetic activity in all three candidates tested (14 percent) in the plant relative to the control (Peng et al. 2002). Some test strains were reported to significantly enhance plant surface area, gross photosynthetic rate, stomatal conductance, and water quality, demonstrating that rhizobial rice inoculation is able to boost the plant's photosynthetic ability significantly (Mia and Shamsuddin 2010). A few reactive oxygen species are caused by water stress, leading to photosynthetic apparatus damage to the plant (Heidari and Golpayegani 2012). The mixture of *Pseudomonas*, *A. brasilense*, and *Bacillus lentus* was found to increase both antioxidant expression and the content of chlorophyll in stress leaves (Heidari and Golpayegani 2012), as a consequence of which photosynthetic machinery gets fully developed. Therefore, biofertilizer will improve plant's photosynthesis, allowing the plant to grow well even under stress.

10.12.2 PGPR Reduces Contamination of Soil with Pesticides in a Sustainable Way

Plant diseases are regulated or prevented by nematicides, insecticides, herbicides, and fungicides. Pesticide application is imperative to modern agriculture because they control pesticides economically. Continuous undue application of pesticides however is harmful to the environment, posing an impending risk to plant kingdom in addition to human race since it can easily pass into tissue and thus cause biomagnifications (Akhtar et al. 2009; Kumar and Puri 2012). Bioremediation methods attracted substantial consideration in treatment of contamination by pesticides owing to its eco-accommodating nature, economical efficacy, and noticeable decontamination of the environment (Nawaz et al. 2011). Therefore, researching bacterial strains degrading pesticides offers a considerable alternative in mitigating harmful effects of pesticides. Numerous studies have been made so far on the potential role of PGPR in agriculture, horticulture, forestry, and conservation of the environment. Consequently, a set of investigations have been made to study the PGPR role in pesticide bioremediation. Microorganisms such as *Enterobacter*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Serratia*, *Bacillus*, etc. have been found capable of attenuating pesticide toxicity (Shaheen and Sundari 2013). Apart from these species, actinomycetes also have significant potential in transforming and degrading pesticide biologically. For pesticide degradation, the enzymatic lysis is the principal process of microorganisms. Three main enzyme systems associated with the majority of degradation of pesticides are as follows: first-stage hydrolyses; mixed function oxidases (MFO) and esterases; and second-stage glutathione-S-transferases (Ortiz-Hernández et al. 2013). In addition, a varied number of reactions, including hydrolyzing, oxidation, addition of an amino group to a nitro group, dehalogenation, nitro group reduction to an amino group, oxygen substitution of the sulfur, cleavage of ring, and side chain metabolism have also been researched to attenuate pesticidal toxicity (Ramakrishnan et al. 2011). On the basis of various studies, PGPR shows a promising approach to the sustainable reduction of pesticide contamination in soil.

10.12.3 Variation in the PGPR Microbial Population Varies the Type of Amino Acid Secretion in the Plant

Rhizosphere (du Jardin 2015) is the region of ground around the root system, and rhizobacterias are a community of rhizosphere bacteria colonizing the vicinity of roots (Shahaby et al. 2016). Besides providing anchorage to plant, helping in uptake of nutrients and water, roots of plant synthesize and exude a broad range of chemicals including amino acids (Wälker et al. 2003; Westön et al. 2012; Moë 2013). Root exudates are usually referred to as the chemical products that roots secrete into soils. Plant root secretion products serve as chemicals attracting a large number of

heterogeneous microbial communities. Exuding various chemicals alters physico-chemicals of soil and, hence, greatly controls composition of the microbial soil culture in its direct surroundings (Bulgarelli et al. 2013; Huang et al. 2014). There is therefore a reliance on plant species and associated microorganics to the plant for type of amino acids plus the chemical constitution of radically secreted exudates (Kang et al. 2010; Bardgett and Putten 2014). Hence, the kind of amino acid secretion from the plant varies significantly with the variability in the adhering microbial PGPR population.

10.12.4 Role of Biofertilizers in Remediation of Heavy Metal Toxicity

Metals exist naturally in soils, many of which are needed as micronutrients for growth of plants. Nevertheless, unceasing anthropological activities, intensive agriculture, and rapidly growing industries have contributed to many environmental problems through the release of heavy metals, toxic waste, agricultural contaminants, and so on (Shinwari et al. 2015). Inorganic heavy metal pollutants are water soluble and nondegradable and get amassed in the soil (Akhtar et al. 2013). Toxic heavy metals like cadmium, nickel, mercury, arsenic, zinc, and chromium exist in different valence states. Although some metals are required by plants as micronutrients, disproportionately high concentration of heavy metals is harmful to most of the plants. When high levels of heavy metal ions occur, the root system quickly absorbs and relocates to the shoots and leaves, causing stress to metabolism, decreasing growth, or even causing plant mortality (Mehes et al. 2013). In addition, heavy metal concentrations above threshold in soil decrease fertility of soil affecting microbial communities as well (Lenart and Volny 2013).

Attenuating heavy metals in soil is difficult, considering that they are not organically degradable. Only by altering the oxidation state can it be detoxified. Most heavy metals are oxidized and reduced in toxicity (Wuana and Okiemen 2011). So far, various methods like physical, chemical, and biological treatments have been put forth to treat metal pollutants; bioremediation still continues to occupy forefront owing to its easy and economical application when compared to various costly detoxifying methods (Lim et al. 2014). Role of PGPR in bioremediating metal toxicity has been explored by various researchers, and various microorganisms have been identified to decrease or detoxify heavy metal toxicity (Dixit et al. 2015). *Brevibacillus* sp., *Ralstonia metallidurans*, *Achromobacter xylosoxidans*, and *Psycrobacter* sp., are some of the most important PGPRs among the wide range of PGPRs in heavy metal bioremediation (Shinwari et al. 2015). A well-known finding that toxicity is caused by heavy metal induces plant stress which in turn inhibits production of ethylene when found at high level (Hossain et al. 2012). One of the most important defensive mechanisms exercised in PGPR is 1-aminocyclopropane-1-carboxylate synthesis that decreases ethylene production in plants (Singh et al.

2015). Therefore, ACC deaminase production through PGPR provides efficient protection for the host plant against heavy metal toxicity stress response.

Production of microbial siderophores is one more successful mechanism used by the PGPR for reducing toxicity of metals (Radzki et al. 2013). Siderophores contribute in reducing stress of plant by complexing with noxious metals like zinc, copper, lead, copper, and cadmium (Dimpka et al. 2009; Saha et al. 2016). In addition, through the biosorption process, PGPR can also help to reduce metal toxicity either by metabolism-dependent or metabolism-independent methods (Dary et al. 2010).

10.12.5 Microbial Biofertilizers Exert a Significant Nematicidal Activity

El-hadad et al. (2011) have confirmed that three strains of *Bacillus megaterium*, four strains of *Paenibacillus polymyxa*, nitrogen-fixing bacteria, and phosphorus-solubilizing bacteria have been individually inoculated in plants of tomato infested with the root nematode *M. incognita* in potted sandy soil with significant nematicidal action wherein *P. polymyxa* NFB7, *B. circulans* KSB2, and *B. megaterium* PSB2 inoculation demonstrated a high decrease in number of nematodes compared to infested control of the uninoculated nematode.

In contrast to nematode-infested tomato plant that did not get inoculated by biologically fertilized agent, these biofertilizers often increase the length of stem (cm), leaf number, stem dry weight, and root dry weight (g). Inoculation with *Azospirillum* and *Azotobacter* augmented productivity and quality of nematode infested capsicum (Khan et al. 2012). Six Egyptian trade biofertilizers (BFs) have been tested by Ismail and Hasabo (2000) for regulation of blue-green algae, microbien, nitroben, serealin, phosphorine, and rhizobacterin for controlling *M. incognita* in sunflower. The nematode population was significantly reduced by all the biofertilizers studied. Rhizobacterin therapy followed by phosphorine and nitroben drugs was the fastest suppressors in the nematode populations. The use of biofertilizer-like bacteria to prevent parasite nematodic growth in plants has been found to commonly produce many volatile compounds, hydrogen sulfide, fatty acids, enzymes, alcohol, hormones, and phenolic compounds which hamper nematodic growth. Such materials may be nematodically toxic or may alter the rhizosphere climate, indirectly resulting in a decline of nematodes (Youssef and Eissa 2014).

10.12.6 Effect of Biofertilizers on Ecosystem

Though biofertilizers have found extensive agricultural usage in recent decades, colonizing and ecology knowledge are not well illustrated. Moreover, the interaction mechanism between the plants and the resident microbial community remains

a question of people's curiosity. The presence of indigenous microflora in rhizosphere is the main factor determining the biofertilizer's effectiveness in the natural ecosystem. Survival and growth in plants by biofertilizer application can be influenced by this highly competitive culture with different rhizosphere species (Hibbing et al. 2010). Along with this, seed and seedling bacterization or soil modifications might produce change in indigenous microfloral community which will have to be taken into consideration as far as the safety of bacterial introduction in the environment is considered (Dey et al. 2012). Therefore, it becomes imperative to assess nontarget impacts on ecosystem and populations before biofertilizer is released in the environment, and therefore, before changing agricultural practices, a detailed study of the effects of organic fertilizers is certainly important. Finally, impact on biogeochemical cycles, soil texture, soil properties including water retaining capacity, fertility, porosity, and erosion prevention must be taken into account properly (Pereg and Mcmillan 2015). Although there is some study on the effect of biofertilizer on soil rhizosphere and food web nontargets, the magnitude of changes in the rhizosphere and the importance of them on the ecological function of these products remain unreportable due to the introduction of rhizosphere inoculants (Martinez-Viveros et al. 2010). The questions as to which species are selected in the indigenous microflora in biofertilizer competitions and which are beneficial or harmful to the host and resident microbial community are still unanswered and yet unfounded. Attempts to find answers are therefore unavoidable, while further experiments and studies are planned with the inoculation of microorganisms in the soil. The impact of the introduction of biofertilizers into the home communities is stated to be based on various factors, including soil characteristics, application method of biofertilizers, and a different environment (Dey et al. 2012). Nevertheless, there have been very few research carried out to date over a longer period, which is very important to determine the effectiveness of bioinoculants as well as their risk factors. It is, therefore, clear that extensive studies must be performed before bioinoculants are released safely for commercial purposes to study the longer-term impact of bioinoculants on nontarget species. In addition, both a culturally dependent and culturally indigenous method, including genetic and physiological tests, has investigated the impact of biofertilizer on nontarget communities. Evaluation of microbial structures of organisms by both plating and cytochemical methods is a good way of researching the impact of biofertilizer on the resident microflora. The use of the latest technologies is necessary for investigating the impact of biofertilizers on native microflora and soil functions. While DNA is a valid and effective marker on population diversity and potential, recent successful mRNA reports are sufficient to include the experiment with biofertilizer for risk and efficiency assessment studies. In addition, a mRNA-based approach would consider a real functional diversity of biofertilizers at any given level. Hence, high-performance and higher-resolution methods and traditional multidimensional analysis techniques must be used to assess biofertilizers in the efficacy, diversity, and risk assessment studies prior to their release into the ecosystem (Sharma et al. 2012).

10.12.7 Effect of Biofertilizer in Soil Reclamation of Degraded Land Ecosystem

Mining of mineral resources leads to serious soil degradation, changes in microbial ecosystems, and damage to vegetation, which lead to the loss of a large amount of land (Juwarkar and Jambhulkar 2008; Sheoran et al. 2010). Gradual growth in such landscapes can not only threaten the productivity of agro-forest but also disrupt the ecosystems and the ecological balance. It has been reported on several occasions that nutrients are leached during various mining processes due to accelerated erosion rates that destroy soil productivity (Sheoran et al. 2010). Reclamation is the process in such vulnerable circumstances that restores ecological integrity in these disturbed mining areas. The advancement of a functioning indigenous microbial network responsible for the improvement of soil structure helpful for plant and plant nutrient production by a variety of biogeochemical cycles is key to successful recovery of mine spoil dumps (Juwarkar et al. 2001; Juwarkar and Singh 2007; Kumar et al. 2013). A mining site's rehabilitation process is troublesome as such mining sites lose fertility status, productivity, as well as their capacity to support plant and associated microbial networks (Chaubey and Prakash 2014) that have a significant role in balancing ecosystem productivity. Restoration of disturbed land systems may somehow return to their predisturbed state with assistance of tree ranches along with organic amendments, in this manner, improving fertility and productivity. The soil of mining sites becomes extremely acidic due to the practice of mining, which affects plant growth. This is possible by incorporating organic modifications, which not only increase the pH of soil but also improve the quality of the soil and its ability to maintain water and to slowly release fertilizer (Diacono and Montemurro 2010). It can therefore be said that for the soil recovery of the mining sites, it is necessary to keep leguminous plants in the plant community and to have a proper microbial population.

10.13 Benefits and Limitations of Biofertilizers

Biological practices can offer a wide scope of opportunities for the improvement of better agrarian practices because of the benefits and advantages provided for the soil, crops, and the farmers. However, restrictions of these practices are additionally all around examined and perceived, which infers that attainability studies ought to be completed to discover better answers for every specific case in farming exercises. A portion of the advantages and constraints are referenced to feature the need of future research on certain issues.

10.13.1 Benefits of Biological Fertilizers

1. Biological fertilizers can prepare supplements that favor the development of natural processes in soils.
2. Maintenance of plant well-being is upgraded by the expansion of adequate nutrients.
3. Food supply is given, and development of microorganisms and helpful soil worms is prompted.
4. Because of the good structure given to the soil, root development is promoted.
5. The organic matter in soil is higher than ordinary levels.
6. Biological fertilizers promote the improvement of mycorrhizal affiliations, which expands the accessibility of phosphorus (P) in the soil.
7. Biological fertilizers help to dispose of plantar sicknesses and give ceaseless stockpile of micronutrients to the dirt.
8. Biological fertilizers contribute to the maintenance of stable nitrogen (N) and phosphorus (P) concentrations (Chen 2006).

10.13.2 Limitations of Biofertilizers

1. The biofertilizers nutrient composition is highly variable; the expense is highly contrasted with some synthetic fertilizers.
2. Extensive and long-haul application may bring about salt accumulation and heavy metal accumulation that could cause antagonistic impacts on plant development, growth of life forms of the soil, water quality, and human well-being.
3. Large volumes are required for land application because of low nutrient content, in correlation with synthetic fertilizers.
4. Main macronutrients may not be available in adequate amounts for development and improvement of plants.
5. The nutrient release rate is too slow to fulfill crop requirements in a short time, which could lead to nutrient deficiency.

10.14 Future Perspectives of Biofertilizers

The utilization of different biofertilizers as a fundamental part of agricultural practice is the new rising field nowadays. These microbes are as of now being effectively utilized in many nations and are required to develop further with time (Weekley et al. 2012). Consequently, it is sensible to anticipate that later on, the extensive utilization of biofertilizers will offer different effective techniques for by and large advancement of farming field. However, widespread usage of biofertilizers will

require handling few issues with more consideration and necessary actions to solve the issues (Gamalero et al. 2008).

1. Efficient multifunctional biofertilizers need to be produced for different varieties of crops.
2. From lab experiments to commercializing, the use of biofertilizers needs a number of latest techniques for formulation, maintenance, storage, shipping, and application of these microbes.
3. It is important to instruct individuals and farmers about the long-term advantage of utilizing biofertilizers in place of synthetic fertilizers. The unfriendly and hazardous impacts of synthetic fertilizers should also be featured before farmers. The misguided judgment about microbes that they only cause diseases should be cleared to farmers so that they will use biofertilizers on a large scale.
4. Although initial biofertilizers are likely to be non-transformed bacterial strains which have been chosen for certain positive attributes, development of genetically designed strains which are increasingly effective in invigorating plant development is required. In any case, researchers should demonstrate to both people in general and administrative organizations worldwide that genetically designed strains do not present any new perils or hazards.
5. A quality control framework should exist for the creation of inoculants and their application in the field to ensure and explore the advantages of plant-microbe's beneficial interaction. "Biofertilizer Act" and strict guideline should be established for quality control in business sectors and application.
6. The microbial survival of biofertilizers in soil under disturbed conditions needs to be examined. Agronomic, soil, and monetary assessment of biofertilizers should be carried out for the different agricultural outputs.

10.15 Conclusion

Biofertilizers are an essential part of organic farming in modern agricultural practices in terms of best alternative to synthetic fertilizers which pose different environmental hazards. Biofertilizers are capable of fixing atmospheric nitrogen in soil and root nodules and consequently make it available to plants. They help in solubilizing phosphate (from insoluble sources such as aluminum phosphates) into accessible form, moving phosphates from one soil layer to another, synthesizing hormones and antibodies to help in plant growth and resistance against diseases. They also help in the decomposition of organic matter, thus taking part in soil mineralization. This leads to expanded crop yields, increased soil structure (influencing soil particle aggregation for a better water relationship), and induced plant dry spell resilience (increasing leaf water and turgor capacity, keeping up stomatal functioning, and increasing root development). Nevertheless, increased demand and understanding among farmers and growers regarding the use of biofertilizers will definitely pave the way for new businesses to join the manufacturing of biofertilizers, which

additionally needs consolation and backing from governments. Biofertilizer innovation, which is an essential part of sustainable agriculture, must be reasonable for users' social and infrastructural conditions, monetarily plausible and feasible, stable in the long term, suitable to various sections of society. Therefore, it is clear that by proper training of dealers and farmers, comprehension of the significance and monetary suitability of applying biofertilizer innovation must be expanded.

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Chapter 11

Fungi and Their Potential as Biofertilizers



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11.1 Introduction

Fungi which include about 144,000 known species belong to the kingdom Fungi and include yeasts, nuts, smuts, mildews, mushrooms, and molds. Besides this, there are many other fungus-like organisms that include slime molds and oomycetes which normally are not included in the kingdom Fungi but are often called as fungi. These fungus-like organisms are included in the kingdom Chromista (Park et al. 2005; Pereira et al. 2007; Shenoy et al. 2007). It is a known fact that fungi are among the most widely distributed organisms that are present on earth and also are of great environmental as well as medical importance. Fungi are mainly free-living in soil and water, and also some other form parasitic as well as symbiotic relationships with plants and animals (Dar et al. 2013, 2020; Mehmood et al. 2019). The cells of fungi contain membrane-bound organelles besides well-defined nuclei, that is, they are eukaryotic organisms (Khanday et al. 2016; Rashid et al. 2019; Soares and Barreto 2008; Than et al. 2008a, b). Fungi are different from all other living organisms including animals because of their principal modes of vegetative growth as well as the phenomenon of their nutrient intake. In addition, they were historically included in the kingdom Fungi, but because they lack chlorophyll and the area is also different because of their unique physiological and structural features, they were separated from plants. Fungi are also unique because they grow from the tips

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of their filaments (hyphae) that are responsible for making up the bodies of the organisms (mycelia), and they have the characteristic of digesting the organic matter externally before absorbing that organic matter into their mycelia.

Fungi are ubiquitous with some of them having beneficial effects on plants, while some may also be detrimental (Anderson and Cairney 2004; Bhat et al. 2017a; Ipsilantis and Sylvia 2007). It has been observed that a constant decrease in the yield of crops which occurs as a result of plant disease and for which the causative agent is a pathogen is considered as a negative effect. A few fungi are found to be the main pathogens which are responsible for plant diseases and also are responsible for very high yield losses (Park et al. 2005; Pereira et al. 2007; Shenoy et al. 2007; Soares and Barreto 2008; Than et al. 2008a, b). During the recent years, there have been many ways in order to reduce yield losses which are caused by the fungal disease, which include the application of chemical fungicides that is considered at present the most common method (Rosslénbroich and Stuebler 2000; Sofi et al. 2017; Than et al. 2008a). It has however been seen that chemical fungicides have a negative effect on both the human health and environment (Bhat et al. 2018a; Bhatti et al. 2017; Calhelha et al. 2006; Gavriescua and Chisti 2005; Haggag and Mohamed 2007; Soyong et al. 2005; Voorrips et al. 2004). Development of resistance in plant pathogenic fungi has also been observed because of the application of chemical fungicides over a long period of time (Agrios 2005; Benítez et al. 2004; Kim and Hwang 2007). The chemical fungicides become ineffective when this happens, and in that case the use of other fungicides should be undertaken for effective disease control. A potentially effective alternative method is the use of microorganisms as the potential biological control agents in order to control the plant diseases (Alabouvette et al. 2006; Bhat et al. 2017a, b; Emmert and Handelsman 1999; Kulkarni et al. 2007; Mitchell et al. 2008; Raghukumar 2008; Tejesvi et al. 2007).

During the last 30 years, microorganisms have been characterized as well as tested for being used as biological control agents against the diseases that are caused by soilborne soil pathogens. During the recent past, plant diseases have been controlled by using biological control agents and more importantly antagonistic fungi with more than 90% of their application being formulated using various strains of *Trichoderma* which includes *T. harzianum*, *T. virens*, and *T. viride* (Benítez et al. 2004). A number of species have also been found to be antagonistic against various soil microorganisms which include many species of *Chaetomium*, e.g., *C. globosum*, *C. cupreum*, and *C. cochlioides* (Kanokmedhakul et al. 2002, 2006; Soyong et al. 2001). Besides this, a number of biocontrol agents have been developed and have been used commercially as mycofungicide products (Benítez et al. 2004; Fravel 2005; Kim and Hwang 2004). The USA and France are considered to be the two main biofungicide users, although it has been seen that many other countries are also promoting biocontrol of agents because of bans that has been put on synthetic chemical pesticide residues that they have on agricultural products (Ecobichon 2001; Mushtaq et al. 2018; Ricard and Ricard 1997; Spadaro and Gullino 2005; Wesseling et al. 2005). It has been observed that in the early stages of mycofungicide development, there is a collection of fungal isolates and

that there is screening of these mycofungicides for the purpose of identifying the effective strains that are helpful against target plant pathogens both in the greenhouse and laboratory and also in the field. Along these lines, it has been seen that the most important consideration in their mass production is given to the compatibility of the product with regard to both the formulation and their application techniques (Jenkins et al. 1998; Khetan 2001).

Another and a very effective alternative way in order to increase the crop yield in addition to using chemical fertilizers is the use of biofertilizers. These are the substances that are found to contain living microbes which were when applied to seed or soil or plants were found to help in promoting the growth through the supply of very essential nutrients which include nitrogen, phosphorous, and other important mineral nutrients. Besides this, they are also helpful in promoting increased absorption of essential nutrients in plants (Chen 2006; Hart and Trevors 2005; Vessey 2003). Biofertilizers mainly include substances that are obtained from living organisms and also from microbial sources (Chen 2006; Rola 2000). Biofertilizers are also known to have various other benefits, which mainly include helping plants in increasing their access to nutrients, providing them growth-promoting factors, and besides this they are also very useful in composting and efficient recycling of solid wastes (Das et al. 2007; Gaur and Adholeya 2004). Microbial inoculants are the common name of biofertilizers and are normally produced from cultures of some soil organisms which help in the improvement of soil fertility and also crop productivity such as mycorrhizae (Dervash et al. 2020; Malik et al. 2005; Marin 2006). Mycorrhizae which normally forms mutualistic relationships with roots of plants and help to improve the absorption of nutrients and water. Besides, it helps in controlling plant diseases and also help in the improvement of soil structure (Chandanie et al. 2006; Das et al. 2007; Gaur and Adholeya 2004; Rinaldi et al. 2008; Rola 2000; Zhao et al. 2003). It has also been observed that plants which are colonized by mycorrhizae are found to grow better than those which are colonized without them and also have been found to be beneficial in natural as well as agricultural systems (Adholeya et al. 2005; Marin 2006; Singh et al. 2008; Yeasmin et al. 2007).

11.2 Role of Microbial Technology in Sustainable Agriculture

Biofertilizers are usually microbial inoculants which are normally considered as a preparation that contain live and dormant cells of usually very effective strains of nitrogen-fixing, P-solubilizing, and other cellulolytic microorganisms. When we compare them with chemical fertilizers, they are normally considered as viable microorganisms which usually are not considered to be a source of nutrients but instead help plants in accessing the nutrients that are available in the rhizospheric region. In the recent years, many microorganisms are used as biofertilizers which include nitrogen-fixing soil bacteria, e.g., *Azotobacter* and *Rhizobium*; also nitrogen-fixing cyanobacteria, e.g., *Anabaena*; P-solubilizing bacteria, e.g.,

Pseudomonas sp.; and AM fungi. Besides this, several other phytohormone-producing bacteria and cellulolytic microorganisms are also used as biofertilizers. These formulations of microbes are normally used in order to enhance different microbial process so as to make the nutrients available in a form that can easily be assimilated by plant. Biofertilizers are normally considered as a source of nutrients that are of low cost and are also renewable.

Biofertilizers during the recent past have been seen to have gained momentum because of their advantages such as they help in maintaining the soil health and also bring down the environmental pollution by making use of different chemicals in the agriculture (Dar et al. 2016; Dar and Bhat 2020; Muraleedharan et al. 2010). For proper growth of plants, it is necessary that they should be provided essential nutrients in sufficient quantities, but it has been seen that only a small portion of nutrients are released from the soil every year through various biological and chemical processes. Besides this, it is the type of fertilizer used that determines the crop yield which is basically used to enhance the essential nutrients that are important for normal plant growth and development (Rinaldi et al. 2008; Rola 2000; Zhao et al. 2003). It is therefore natural that the main use of fertilizers is to basically supplement the nutrients that are already present in the soil. In addition to the nutrient supplementation, it has been seen that biofertilizers have various other important benefits which mainly include controlling the soilborne diseases and helping the improvement of soil health which ultimately results in higher yield rates. During the recent past, a number of commercial biofertilizers have been found to be available in diverse formulations, and in this regard various strategies have been employed in order to make sure that there is maximum viability of the microorganisms that is used in such formulations. The various strategies include application of biofertilizers in the liquid form, the necessary optimization of their formulations, and also the use of thermotolerant and genetically modified strains. In the production of biofertilizers, different types of microorganisms and their associations with various crop plants are being exploited, and in this regard, they are grouped in different ways which mainly depend on their nature and function (Das et al. 2007; Gaur and Adholeya 2004).

Mycorrhiza is an association of fungus with the roots of higher plants, and in spite of being a mystery, it has been found to be helpful in serving as a model system that has helped to understand the mechanism that occurs as result of mycorrhizal inhabitation which ultimately leads to the stimulation of growth in the root cells (Malik et al. 2005; Marin 2006). Mycorrhiza has been found to secrete bioactive ligands like Myc factors and Nod factors which are then perceived by host roots in order to trigger the signal transduction pathway which in turn initiates other signal transduction pathways which ultimately have been seen to trigger release of Ca^{2+} in the cytosol through some unknown receptors. It has been found that DM1 proteins play an essential role in helping to maintain the periodic oscillation of calcium ions which occurs inside and outside of the nucleus, while nuclear pore complex and some other proteins like NUP have been found to play a very important role in calcium spiking. Besides this, Ca^{2+} channel proteins have been seen to facilitate this process with the help of various transporters.

11.3 Sustainable Production Through Biotechnological Tools

If we see in a strict sense, biofertilizers are not considered as fertilizers but instead are used for crop plants in order to supply nitrogen to them. The most commonly used biofertilizers among microorganisms are bacteria, fungi, and blue-green algae as these are added to the rhizosphere of plant in order to enhance their activity in the soil (Chen 2006; Hart and Trevors 2005; Vessey 2003). These microorganisms indirectly help the plants by helping them in enhanced nitrogen fixation and also increasing the nutrient availability in the soil, and besides this, it has been seen that they can be applied alone or in combination when it comes to observing their mode of action. Systematic research has made it possible to identify the efficient cultures which are then able to grow in particular soil and climatic conditions.

For the purpose of distributing these cultures to farmers, they are prepared at large scale in the laboratory, and for increasing their shelf life, they are packed in peat and lignite powder. Biofertilizers during the recent past have gained tremendous momentum, and as already known that only small part of the nutrients are released from soil every year through biological and chemical processes, it therefore becomes necessary to make use of the fertilizers in order to supplement the nutrients that are already present in the soil (Bhat et al. 2018a, b; Chen 2006). In accelerating the microbial processes which mainly include controlling soilborne diseases and improving the soil health, microorganisms of biofertilizers have been found to play a very important role. For realizing the ultimate goal of the farmers which is the goal of increasing crop productivity, biofertilizers have been found to provide an economically feasible option as these are of low cost and are also considered to be renewable sources of plant nutrients which overall help in supplementing the chemical fertilizers.

11.4 Fungi and Sustainable Agriculture

Biofertilizers are considered to be eco-friendly as well as inexpensive besides also being an important source of nutrients for plants. They are also important in increasing the soil fertility and playing a very important role in the improvement of soil nutrient status which ultimately leads to increased crop productivity (Adholeya et al. 2005; Marin 2006; Pal and Gardener 2006; Singh et al. 2020). When applied in a natural field system whether alone or in combination, fungal biofertilizers are known to cause a very important beneficial impact on the development of plants besides having impact on its growth as well as yield (Gentili and Jumpponen 2006). Differences in plant roots among different plant groups which include herbs, shrubs, and other terrestrial plants normally grow in natural conditions but have been found to develop various types of mycorrhizal associations when they are grown in conditions that are found to have low bioavailability of necessary essential elements that mainly include phosphorous, zinc, iron, and copper (Zhu et al. 2008).

Among the various types of fungal biofertilizers, P-solubilizing biofertilizers are the most commonly employed as biological agents in order to improve plant growth and also help in its development besides helping in phosphorous uptake in plants. It has been seen that fungi possess a property of phosphate solubilization which mainly contributes to the availability of phosphates that are present in the soil to the plants (Zhu et al. 2008). Seven P-solubilizing fungi have been found from Teff rhizosphere soil which have been found to enhance the phosphate availability to plants, and besides this, it has been found that fungi which belong to *Aspergillus* genera have also been found to be reported from the rhizospheric region of various plants (Gizaw et al. 2017). The most commonly employed fungi that is used for biofertilizer production is *Trichoderma* that is usually found in agricultural soils. The rhizospheres which are inhabited by *Trichoderma* sp. have been seen to interact and also parasitize other fungi, and these have also been recognized because of their ability to enhance the plant productivity by helping the plants to enhance their crop nutrition as well as nutrient acquisition (Chang et al. 1986; Harman 2000; Lindsey and Baker 1967; Vinale et al. 2008; Yedidia et al. 2001). Inoculation of soil with *Trichoderma* sp. and their subsequent utilization as a culture filtrate have been seen to increase plant growth, and as far as its ease of cultivation under laboratory conditions is concerned, it has been seen that this species of fungus can act as model organism not only for its good benefits with regard to plant microbe interaction but also it has been seen that it can act as a good and enhanced tool for increasing the plant productivity (Varma et al. 1999).

As far as the biological properties of the yeast are concerned, its application as biofertilizer has recently gained tremendous interest, and also it is safe to both humans as well as the natural environment (Agamy et al. 2013). Keeping this thing in mind, it has been seen that *Saccharomyces cerevisiae* which is also called as Brewer's yeast has been considerably used as a biofertilizer. The use of yeasts either live or dead has been found to increase the availability of nitrogen and phosphorous to the roots of sugarcane plants. This yeast has been found to be less expensive biological fertilizer because it increases or enhances the plant nutrient status and also the potential of plants and therefore helps in the overall nutrient uptake as well as the growth of plants (Lonhienne et al. 2014). During the recent past, the use of microbially synthesized indole acetic acid which is a type of phytohormone in plant microbe interactions has gained tremendous importance (Singh et al. 2008). Among the bacteria (Idris et al. 2007; Radhakrishnan et al. 2013; Sachdev et al. 2009), fungi and yeasts which are found to be capable of IAA synthesis have been seen to promote plant growth and therefore have been recommended for their use as biofertilizers (Ahmad et al. 2008; El-Tarabily 2004; Sasikala and Ramana 1997; Waqas et al. 2012). It was seen that under laboratory conditions, the synthesis of IAA by yeast was under the control of changes in temperature and pH of the medium (El-Tarabily 2004; Sasikala and Ramana 1997). It has been observed that most of the plant species have mutualistic association with arbuscular mycorrhizal fungi (AMF), and it was seen that complete or partial degeneration of activity of AMF in the soil can cause tremendous changes in the properties of the soil which helped in the overall increase in the agricultural production (Varma et al. 2012). It was

observed that AMF helps in conferring resistance to plants against different types of pathogens and heavy metals besides helping them in combating environmental stress and also facilitating the growth of plants by alleviating the bad effects of disease-causing factors. Above all, the AMF interaction helps the plants by reducing the chances of development of disease that is caused by different phytopathogens (Ene and Mioara 2008; Hildebrandt et al. 2007).

11.5 Ectomycorrhiza as Potential Biofertilizers

It has been observed that there is a history of technique development regarding the incorporation of ectomycorrhizal fungal inoculation into various nursery plantation practices (Mikola 1969, 1970; White 1941; Wilde 1944) and in this regard, the importance of EM symbiosis which was found to be helpful for normal growth of trees and nutrient acquisition which was already proposed by Frank (1885). It was long observed that tree plantations will not be successful until and unless endemic or inoculated EM fungi were available on site, and in this regard the proper selection was largely based on enhancement of tree crop and their application for a very large-scale inoculum practices (Smith and Read 1997). Although various alternatives have been used and surveyed, the most widespread inoculum programs have been developed for *Pisolithus tinctorius*. The advantages for the use of *P. tinctorius* included its wide host range and extensive geographic distribution, as well as its occurrence on sites burdened by recent disturbance, drought, high temperatures, and/or chemical contaminants. *P. tinctorius* inoculum can be produced and applied as vegetative mycelium in a peat vermiculite carrier. The nutrient solution, which is necessary for the vegetative growth of *P. tinctorius* throughout the substrate, will also facilitate the competitive exclusion of other root-colonizing fungi (Smith and Read 1997). Various alternative techniques and formulations for inoculation have been developed (Marx and Kenney 1982; Marx et al. 1984). Although liquid or spore suspension techniques would avoid the problems resulting from bulky solid inoculum production and storage, they often suffer from delayed EM establishment or mycelial fragmentation and shredding.

Inoculation programs with EM fungi have had some success. However, as with AM or bacterial inoculum applications, there seems to be no single fungal species or strain that could be universally applied across different sites and host species. When compared to local strains and species in the north-western USA, the *P. tinctorius* strain that had proven extremely favorable for seedling growth and establishment elsewhere seemed less beneficial (Perry et al. 1987). In many cases, the strains that easily colonize seedlings in the nurseries and are easy to manipulate have only limited positive effects on the performance of the planted seedlings (Jackson et al. 1995; le Tacon et al. 1992; Perry et al. 1987). The limited success of the fungi, which have been selected for the inoculation programs, may be simply due to the ubiquitous presence of endemic mycorrhizal fungi in reforested sites and the competitive exclusion of the nursery-inoculated fungi in the field.

Accordingly, the inoculation with EM fungi may be most important on sites with poor reforestation history or on plantations, which will be established on previously non-forested sites. In the research focusing on the development of the forest nursery inoculation programs, one issue that has received relatively little attention is the impact that imported and possibly invasive EM fungi have on the endemic fungi and their community composition. The inoculated fungi may persist in the root systems for extended periods of time and outcompete less invasive endemic strains and species (de la Bastide et al. 1994). Although no direct evidence for such competitive exclusion currently exists, introductions may homogenize local fungal populations and communities.

11.6 Potential of Mycorrhiza as Biofertilizer

AM fungi are microorganisms which are symbiotic in nature as they are not able to grow on synthetic media without the host plant (Hart and Trevors 2005). It is therefore necessary that these fungal inocula must be produced with the host plants through their association, and it has been observed that there are a number of constraints when it comes to their large-scale commercial production. They are produced on a large scale with the process of pot culture either in greenhouse or through growth chambers (Bagyaraj et al. 2002; Gentili and Jumpponen 2006; Kapoor et al. 2008; Marin 2006; Raja 2006). When it comes to their preparation, it is through the process of multiplication of the selected fungi in the roots of susceptible host plants that are grown in the sterilized soil or substrates (Naqvi and Mukerji 2000). Its spores and hyphae have been seen that they can be isolated from the rhizosphere of the soil and then later mixed with carrier substrates, and when it comes to the inocula of AM fungi, it has been seen that it can be applied as spores as well as fragments of colonized roots (Gentili and Jumpponen 2006). It has been found that the spore inocula are resistant and have been seen to withstand unfavorable environmental conditions and also has been observed that they are able to colonize new root systems more slowly than other preparations. It is because of this reason that both inocula and the spores fragments of colonized roots are combined when it comes to their commercial production (Marin 2006).

For the production of fungal biofertilizers on a commercial level, some important steps are essential, and they include first the selection and then the large-scale production. After this, there is the selection of the carrier and its preparation which is followed by mixing and curing. Finally there is the proper maintenance of desired number of inocula which is then followed ultimately by strong quality control (Malik et al. 2005). It has been kept in mind that the criteria which are being employed for the selection of AM fungi will mainly depend on the local environment and the condition of soil and host plants of that area. For obtaining better results, the AM fungi must be able to colonize the roots rapidly soon after the inoculation and should also be in a position to absorb phosphates from the soil. Besides this, it should also be able to transfer phosphorous to the plants and help in increasing the

plant growth. Finally it should be able to persist in the soil and should also be able to re-establish the mycorrhizal symbiosis during the subsequent seasons besides forming propagules that would remain viable during and also after the production of inocula (Tanu et al. 2012).

During the recent years, commercially producing the mycorrhizal inocula has evolved very quickly, and it has also been found that there are different types of microbial cultures as well as inoculants that are today available in the market and have rapidly increased because of the tremendous advances in the field of biotechnology (Douds et al. 2000; Raja 2006). There are currently more than 30 companies that are present throughout the world that are involved in the marketing of mycorrhizal products, and among these, some have been found to be composed of one or more than one mycorrhizal fungal inoculum. The products are found to be promoters of plant growth and are utilized in mainly agriculture and forestry (Schwartz et al. 2006).

11.7 Some Other Potential Biological Biofertilizers

Recently several fungal biofertilizers have been used in order to increase the growth of plants, and these mainly include *Penicillium* species. It has been seen that these species are mainly P-solubilizing microorganisms which have found to improve phosphorous absorption and also stimulate the growth in plants (Pradhan and Sukla 2005; Wakelin et al. 2004). It has been seen that *Penicillium bilaiae* when applied increases the dry matter, phosphorous uptake besides also increasing the seed yield in *Brassica napus* commonly known as canola and as a result it has been used as a commercial product which is named as Jumpstart. It was then released into the outside market in the form of a wettable powder in 1999 (Burton and Knight 2005; Grant et al. 2002). Besides this *P. radicum* and *P. italicum* have also been found to be P-solubilizing taxa (El-Azouni 2008; Wakelin et al. 2004; Whitelaw et al. 1999), and it has been seen that *P. radicum* when isolated from the rhizosphere of roots of wheat, has shown a good promise with regard to the plant growth promotion (Whitelaw et al. 1999). In the case of *P. italicum* isolated from rhizosphere soil and it's evaluation for the ability to solubilize tricalcium phosphate (TCP) has been found to promote the growth of soybean (El-Azouni 2008).

It has been reported that a number of species of *Aspergillus* are involved in the process of solubilization of inorganic phosphates which include *A. flavus* and *A. niger* (Akintokun et al. 2007). These have been found to solubilize the inorganic phosphates through the process of producing acids which mainly include citric and gluconic acid (Barroso et al. 2006). Also it has been reported that another species of *Aspergillus*, that is, *A. fumigates*, is normally isolated from compost and has been found to be a potassium-releasing fungus (Lian et al. 2008). Apart from this, a product of *Chaetomium* species has been found to be a fungal biofertilizer which mainly includes Ketomium which is mainly produced from *C. globosum* and *C. cupreum* and has been found to be not only a mycofungicide but has also been reported to be

a growth stimulant because a number of plants which include tomato, corn, and pepper when treated with Ketomium have been reported to show a greater plant growth and also high yields as compared to the nontreated plants (Soytong et al. 2001).

11.8 Future of Fungal Biofertilizers

A number of reports are there which suggest the success with regard to the ability of the fungus to control plant diseases and to promote plant growth as biofertilizers. As far as the use of fungi as potential fungicides as well as biofertilizers, it has been found that most of them have been developed during the last two decades. Fungal biofertilizers along with mycofungicides have been found to help in minimizing the use of synthetic chemical fungicides as well as chemical fertilizers, and this has been reported to be very beneficial because these synthetic chemical compounds have probable disastrous effects on both humans and the environment (Calhelha et al. 2006; Haggag and Mohamed 2007). As compared to the chemical compounds, mycofungicides and chemical fertilizers are used on a very large scale, and as far as the development of fungal products is concerned, there has been a very little investment because it has been found that they have very poor effects in the field (Tang et al. 2001). However, it has been seen that there is a comparatively bigger gap between the unpublished research that is carried out in the laboratories and those which are developed for their use in the field. It is therefore necessary that the research should make it mandatory that the fungal products must have a suitable impact when it comes to their field applications and when they should remain in a stable form. Regarding this, the things that should be kept in mind are first making a note of strains of fungi that are used and also making it sure that they are cheap when it comes to their production on a large scale. It should also be kept in mind that they should not have detrimental effects on the environment and above all should be safe to both humans and the environment. To make it possible, there is need for communication between various agencies that mainly include researchers and industry especially during the early stages of the development.

In spite of the fact that there are numerous biocontrol products available, still many problems need to be overcome in order to achieve the successful commercialization of potential biocontrol products. It has been observed that some of the biocontrol agents have a greater efficacy when it comes to their application in the field as compared to their effects in the field (Tang et al. 2001). As far as the control of fungal diseases by biological means with the help of fungal antagonists is concerned, it continues to remain a challenge for further research (Spadaro and Gullino 2005). Keeping this thing in mind, a number of species of fungal antagonists have been developed and then subsequently registered as commercial products, and it has been seen that these products are used on a small scale because of their limited capacity to control plant diseases in the field (Paulitz and Belanger 2001; Tang et al. 2001). When it comes to the use of secondary metabolites, it has been observed that a number of biological control agents are able to produce these

metabolites which have the capacity to control different plant diseases. It is because of this fact that secondary metabolites are developed as mycofungicides but should however be tested and should also be harmless to both humans and the environment. With the advances in the field of molecular genetics, the genetic studies of biocontrol agent strains have proven to be a powerful tool that has helped to increase the effectiveness of biological control activity and also the utilization of genetic potential of fungal antagonists (Haggag and Mohamed 2007; Irtwange 2006; Paterson 2006). Research should be carried out which would find the potential of fungal biofertilizers when it comes to their application in the soil as it has been seen that they help to increase the crop yield and also make the soil quality better (Tanu et al. 2012). When it comes to the advantages of fungal biofertilizers, it has been found that they have a number of advantages both in terms of nutrient supply and crop growth besides also being safe to the environment (Smith and Zhu 2001). It should be kept in mind with regard to the development of new strains of fungi that they should help in the improvement of nutrient uptake and should also be fast growing, and as far as their production on a large scale is concerned, the cost should be low (Marin 2006). The future research on the development of fungal biofertilizers should therefore be on the production of more stable strains with the help of traditional and molecular techniques (Tanu et al. 2012; Marin 2006).

11.9 Future Perspectives

Various fungi provide a battery of extracellular enzymes, which may be utilized for improved crop yields and reduced costs for inorganic fertilizers (Tang et al. 2001). The emphasis should be on the field trials that use multiple inoculations of different organisms, and besides this, it should be kept in mind that the combinations must be of tremendous value when it comes to using different organisms that have different benefits for the purpose of integrating them with the crop plants (Irtwange 2006; Paterson 2006). It has been observed through the integration of various microbial capabilities that have been integrated into combined biofertilizers, and it has been observed that they have tremendous potential when it comes to their yield promoting effect. It is most likely being made possible by approaching the application of biofertilizers besides keeping in mind their applications at a scale which is very much relevant to the current agricultural practices. In this direction, the first step is basically finding the suitable avenues and then at the second stage funding the desired collaboration that is between the research facilities and biotechnological industries. It is basically the large-scale production of inocula that is very much important for achieving the desired goals of research, and it has been observed that the connection that is present between the industries and research makes it possible for the inoculum production that is suitable for field trials (Paulitz and Belanger 2001; Tang et al. 2001). Finally the major emphasis should be on the establishment of international guidelines for the trade and production of inoculum, and in this direction, the end user of inocula should be

protected, and efforts should be made for the safe choice of commercial inocula that is possible only through collaboration among different research facilities and farmers. It is because of such innovation that will allow a positive start for the production of commercially and economically viable biofertilizers which will be ready for marketing directly to the target consumers.

11.10 Conclusion

We can conclude by saying that using the fungi as biofertilizers will lead ultimately to the decrease in the occurrence of plant diseases which will be made possible only through preventing the growth and decreasing the inocula of pathogens. It will also emphasize on increase in the nutrient uptake from the soil and finally producing various economically important bioactive compounds and enzymes which will lead to better plant growth. These all will lead to increase in the crop production. Currently there are a number of commercial fungal biofertilizers that are present throughout the world, and using these offers an economically and environmentally favorable choice when compared to the use of chemical fertilizers. It has however been reported that there are still certain limitations while using these products which mainly is understood by the fact that their rate of success is determined by the environmental factors. Also the difficulties in their applications coupled with their limited shelf life and a very slow action have been a hurdle when compared to the chemicals and as a result can lead to farmers discouraging the use of these biofertilizers. It is because of these reasons that more research need to be done in order to produce more effective products that have both a rapid action and a larger shelf life.

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Chapter 12

Bacillus thuringiensis as a Biofertilizer and Plant Growth Promoter



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12.1 Introduction

Phosphorus (P) is amongst the chief macronutrients for the plants as well as an important growth-limiting nutrient. In fact, it participates in various functions of plants besides being a constituent of ATP molecules and plant DNA structure (Khan et al. 2014). Nevertheless, the solubility of P is low for most arable land in the world, and this problem directly affects crop production and food supply. Moreover, to get greater productivity, it is obligatory to use a large quantity of P fertilizers. Also, about 5–25% of the used P chemical fertilizer is absorbed by crop, while 75–95% is retained as unavailable forms in soil colloids forming Fe, Al, Ca and organic matter complexes (Dar et al. 2013; Stevenson and Cole 1999). On the other hand, the concentration of bioavailable P in many soils is lower. In this regard *Bacillus thuringiensis* has the capacity to turn the insoluble P into soluble form. In this contest the application of phosphate-solubilizing bacteria (PSB) as inoculant results in increased P utilization in plants. In fact, *Bacillus* strains are amongst the utmost used and potent P solubilizers (Rodríguez and Fraga 1999).

In addition, the inoculation with *B. thuringiensis* is an environmentally friendly practice as it increases solubility of P in soil (Dar and Bhat 2020; Delfim et al. 2018). Soil microorganisms (*B. thuringiensis*) execute a prodigious part in soil P cycle. It also participates in processes of mineralization and immobilization besides

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controlling plant diseases plus tolerance stress. The beneficial effect of the *B. thuringiensis* as biofertilizer and plant growth promoter (PGP) in different crops has been demonstrated in laboratory, greenhouse as well as field trials (Armada et al. 2015; Delfim et al. 2018; Khanday et al. 2016; Wang et al. 2014). Inoculation with *B. thuringiensis* in *L. dentata* has increased the root growth by 412% in drought conditions when compared to non-inoculated conditions (Armada et al. 2016; Bhat et al. 2018) and increment in the tissue percentage of nutrients in maize crop (Armada et al. 2015) as well as improved soil Olsen available P in peanut and wheat rhizosphere (Delfim et al. 2018; Wang et al. 2014).

12.2 *Bacillus thuringiensis*

B. thuringiensis is a microorganism principally utilized for its biologically insecticidal properties. It is also used as a biological fertilizer and PGP. *B. thuringiensis* is a Gram-positive, aerobic, spore-forming bacterium. At the end of its vegetative phase, it produces parasporal crystals (Sanahuja et al. 2011). The insecticidal and phosphate-solubilizing properties of *B. thuringiensis* were documented several years earlier when the bacterium was discovered and recognized. Few studies have hinted that *B. thuringiensis* spores were used in ancient Egypt. This bacterial strain was isolated in 1901 by Shigetane Ishiwatari, a Japanese biologist, while studying wilt disease in silk worms, and he named it *Bacillus sotto*. After a period of 10 years, it was again isolated by Ernst Berliner from a diseased Mediterranean flour moth (*Ephestia kuehniella*). It was isolated in the German region of Thuringia hence; it was named as *Bacillus thuringiensis* (Siegel 2001).

B. thuringiensis has the capacity to produce diverse extracellular compounds such as insecticidal, antifungal and organic acids used by crop defence and plant development (Stabb et al. 1994). Nonetheless, the harmless utilization of *B. thuringiensis* globally on several agricultural crops for biocontrol argues strongly that this bacterium can be utilized as biofertilizer and biostimulator (Cherif et al. 2001; Rashid et al. 2019).

Amongst the most usefully reported microorganisms in various researches, bacteria of the type *Bacillus* are popular. In soil, the *Bacillus* represents an extensive group of the microorganism community (Qiao et al. 2014). Numerous studies indicate that the presence of *B. thuringiensis* in the rhizosphere of plants like cereals and legumes imparts positive bearings on crop establishment. It promotes growth, is tolerant to drought stress and increases nutrient absorption and utilization (Armada et al. 2014; Bhat et al. 2017). The synthesis and productions of enzymes, antibiotics or siderophores, organic acids, phytohormones and P solubilization have also been observed (Cherif-Silini et al. 2016; Raddadi et al. 2008). The process of creating diverse biological composts plus the culturing of bacteria in regulated settings (laboratory) is imperative. Moreover, the ability of sporulation by the *Bacillus* and resistance to environmental settings offers them a great lead in persisting in the rhizosphere of the plants. It also extends multiplicity to

their modes of action resulting in extraordinary stability potentials when used as biofertilizers or as PGP (Qiao et al. 2014; Sofi et al. 2017).

The beneficial and effective use of *B. thuringiensis* should not be limited to its function as a biocontrol, and it should be also used to control diseases and pests. *B. thuringiensis* investigations are principally appointed on its biological control properties and application. However, studies showing on the biofertilizer and biostimulation characteristics and application, including its interactions with plants in varied environments, are limited (Azizoglu 2019). *B. thuringiensis* isolated in different rhizosphere plants and soils is shown in Table 12.1. In addition, Cherif-Silinil et al. (2016) isolated 35 strains including *B. thuringiensis* from the wheat rhizosphere; the strain had the potential to mobilize phosphates at the rate of 15.05–16.65 $\mu\text{g mL}^{-1}$. The strains isolated from arid and alkaline soils were extremely effective in the making of compounds like IAA (Indole-3-acetic acid), siderophores and 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) (Cherif-Silinil et al. 2016).

Lyngwi et al. (2016) collected and analysed soils from five different sacred groves of Meghalaya, and bacterial isolation was performed. One reference micro-organism strain was *B. thuringiensis* strain (MTCC 8996); it provided enlarged pieces of the anticipated size for all the PGP genes screened. This presented various PGP genetic characters that exist in these isolates which proposed that these isolates are able to demonstrate characters that have an important impact on biofertilization, biostimulation, bioprotection and biocontrol undertakings. This can be investigated for agro-biotechnological practices.

12.3 Phosphorus Availability in Soil

Soil fraction P exists as organic and inorganic forms. The organic P is signified principally by inositol or phytates and phosphoesters, while the mineral P comes from rock phosphates, calcium phosphates and hydroxyapatites (Jones and Oburger 2011; Rodríguez and Fraga 1999). Many reports are considerate regarding the quick drop of the global P stores by reason of incessant mining of P (Van Vuuren et al. 2010). In several soils, enormous quantity of inorganic and organic P is present in unavailable (fixed or retained) and immobilized form.

Agricultural soils comprise great quantity of unavailable P coming from chemical fertilizers inaccessible after application because the process of fixation and precipitation in soil is largely reliant on pH and soil type (Rodríguez and Fraga 1999; Singh et al. 2020). Thus, at lower pH, aluminium-phosphorus and iron-phosphorus dominate, whereas at higher pH, calcium-phosphorus is predominantly present. In this context, Lindsay (1979) reported that superphosphate encompasses an adequate quantity of calcium to precipitate and quasi-phosphorus as dicalcium phosphate or dicalcium phosphate dihydrate.

P is an essential nutrient for plant development and yield. Consequently, its accessibility is restricted in various agricultural lands and as a result by plants.

Table 12.1 *Bacillus thuringiensis* isolated from rhizosphere/soil of different plants

Isolate code	Host plant	Organic acid	P solubilizing	IAA	ACC	Phosphatase	References
B1	Wheat	Yes	Yes	Yes	No	Not determined	Cherif-Silimil et al. (2016)
B2	Wheat	Yes	Yes	No	No	Not determined	Cherif-Silimil et al. (2016)
B7	Wheat	Yes	Yes	Yes	No	Not determined	Cherif-Silimil et al. (2016)
B10	Wheat	Yes	Yes	Yes	Yes	Not determined	Cherif-Silimil et al. (2016)
BA3	Wheat	Ye	No	Yes	No	Not determined	Cherif-Silimil et al. (2016)
B1	Tea	Yes	Yes	Not determined	Not determined	Not determined	Wang et al. (2014)
Not determined	Autochthonous shrub species	Yes	Yes	Yes	Yes	Not determined	Armada et al. (2016)
GDB-1	<i>Pinus sylvestris</i> L.	Yes	Yes	Yes	Yes	Not determined	Babu et al. (2013)
KR1	<i>Kudzu</i>	Yes	Yes	Yes	Not determined	Not determined	Selvakumar et al. (2008)
Not determined	Not determined	Yes	Yes	Yes	No	Not determined	Lyngwi et al. (2016)
BMG1.7 and HD22	Not determined	Yes	Yes	Yes	Yes	Yes	Raddadi et al. (2008)
KVS25	<i>B. juncea</i>	No	Yes	Yes	Yes	Not determined	Vishwakarma et al. (2018)
2PIM3	Pea	Not determined	Yes	Yes	Not determined	Yes	Freitas et al. (1997)
KTCIGM2	<i>Gigaspora margarita</i> spores	Not determined	Yes	Not determined	Not determined	Not determined	Cruz and Ishii (2012)

Yes solubilize or produce, No no solubilize or produce, IAA indole-3-acetic acid, ACCdeaminase 1-aminocyclopropane-1-carboxylate deaminase

Microorganisms execute a significant role in P cycle by making its mineralization and accessibility easy for the crops. P availability in the soil is regulated by soil pH, soil type and sorption, desorption plus content of organic matter (Dervash et al. 2020; Jones and Oburger 2011).

12.4 Phosphorus-Solubilizing Mechanisms

The main P solubilization mechanisms used by *B. thuringiensis* include production of organic acids, siderophores, changing the pH of medium, acid and alkaline phosphatase production (Cherif-Silini et al. 2016; Freitas et al. 1997; Raddadi et al. 2008; Vishwakarma et al. 2018; Wang et al. 2014).

As per Freitas et al. (1997), *B. thuringiensis* can be represented as that rhizosphere bacterial class of P solubilizing that acidifies the pH of medium. In fact, Wang et al. (2014) showed that dropping of pH coincides with the incremental performance of P mobilizing action. A greater reduction in pH was connected with a greater extent of P solubilization, for instance, in *B. thuringiensis* B1, in which a considerable quantity of P was liberated from AlPO_4 (321.12 mg L^{-1}) which was ensued by a substantial drip in pH. Still, no noteworthy link has been established vis-à-vis pH and soluble P concentration. This is established with several prior findings (Jorquera et al. 2008; Lin et al. 2001).

A wide variety of compounds derived from *B. thuringiensis* can considerably modify the soil pH which results in several nutrients becoming available for uptake by plants. Whereas, only a few studies reported phosphate solubilizing via acidification or alkalization by *B. thuringiensis* (Cherif-Silini et al. 2016; Freitas et al. 1997; Vishwakarma et al. 2018; Wang et al. 2014). The mineralization of organic P in soil is accomplished mostly by phosphatases (acid or alkaline is dependent the soil pH) and phytases. These compounds play an important role in dephosphorylating reactions (Nannipieri et al. 2011) and enzyme hydrolysis. In addition, the quantity and quality of extracellular soil phosphatases come from the microorganism masses (Dodor and Tabatabai 2003). On the other hand, *B. thuringiensis* isolates present the genetic elements for acid phosphatase and siderophore making, along with hydrolysing mineral P (Raddadi et al. 2008).

Cherif-Silini et al. (2016) assessed 35 strains of *Bacillus* including *B. thuringiensis*. These were isolated from the rhizosphere of wheat plants growing in various arid and semi-arid areas of Algeria. These strains were examined for several characteristics including their biofertilization (phosphate solubilization) and conclude that 78% of the strains had the ability to mobilize phosphates on Pikovskaya medium having tricalcium phosphate (Ca_3HPO_4) as the exclusive supplier of P.

Numerous soils, particularly those with high clay percentage, have high adsorption capability of soil P. In addition, it scarcely supplies sufficient P for the development of plants. It is debatable which inorganic P form is efficient for plant growth growing in clayey soil. However, Al-P and Fe-P forms are largely predominated in acid soil and are often treated with soluble P fertilizer for optimal plant

Table 12.2 *Bacillus thuringiensis* grown on insoluble mineral phosphate substrates

<i>Bacillus thuringiensis</i> strains	Substrate					References
	CaHPO ₄	Ca ₃ (PO ₄) ₂	AlPO ₄	Hydroxy-apatite	Rock phosphate	
B1	nd	nd	+	nd	nd	Wang et al. (2014)
BMG1.7	nd	+	nd	nd	nd	Raddadi et al. (2008)
HD22	nd	+	nd	nd	nd	Raddadi et al. (2008)
Bt	nd	+	nd	nd	nd	Armada et al. (2016)
SG1	nd	+	nd	nd	nd	Lyngwi et al. (2016)
SG5	nd	+	nd	nd	nd	Lyngwi et al. (2016)
SG6	nd	+	nd	nd	nd	Lyngwi et al. (2016)
SG11	nd	+	nd	nd	nd	Lyngwi et al. (2016)
SG17	nd	+	nd	nd	nd	Lyngwi et al. (2016)
KVS25	nd	+	nd	nd	nd	Vishwakarma et al. (2018)
nd	nd	+	nd	nd	nd	Dangar (2008)
2P1M3	+	nd	nd	nd	nd	Freitas et al. (1997)

+ solubilize, *nd* not determined or indicated

growth. If *B. thuringiensis* terminate other strains, this can lead to solubilization of the soil fixed P; they also discharge compounds to mobilize the Al and Fe layering which surrounds the soil occluded P (Delfim et al. 2020; Gong et al. 2010). Some studies have reported that *B. thuringiensis* could solubilize the soil AlPO₄, Ca₃HPO₄ and other sources producing organic acid and siderophores, thus resulting in increment of soil accessible P (Armada et al. 2015; Cherif-Silini et al. 2016; Wang et al. 2014).

Some strains of *B. thuringiensis* are able to solubilize phosphate both in solid and liquid medium. But, the P solubilization by *B. thuringiensis* can depend too on the P source or substrate type Table 12.2, the forms and P fractions in soil (Delfim et al. 2020).

Various microorganisms like *B. thuringiensis* suggest a biological saving system proficient of mobilizing the insoluble inorganic P of soil followed by making it accessible to the plants (Lyngwi et al. 2016). The capability of *B. thuringiensis* to turn difficult to get P into a reachable form is a vital characteristic of a vegetable growth promoter bacterium for enhancing crop yield and environmentally sustainable.

12.5 Biological Fertilizer

Biological fertilizers are microorganisms that increment nutrient availability to plants, improving plant nutrition for either favouring nutrient absorption or increasing nutrient solubility in the rhizosphere, thus improving soil health.

The rhizosphere refers to adhered soil area around plant roots. Generally, this region features great microbial population and activity. Rhizosphere is exposed to

varied microorganisms which influence the number of nutrients obtained by way of the root exudates (Bhatti et al. 2017; Bowen and Rovira 1999).

B. thuringiensis enhance the solubility of P that is previously existent in the soil although inaccessible. In a way these reduce the soil P reservoirs, but considering the low utilization efficiency of P fertilizers, *B. thuringiensis* execute vital part in intensifying the efficacy of P, plummeting build-up in soil and circumvent water pollution (Dar et al. 2016, 2020; Mehmood et al. 2019). In addition, *B. thuringiensis* solubilize P portions that are very stable in soil (extracted by conc. HCl and HCl 1 mol L⁻¹) (Delfim et al. 2020).

Biological fertilizer can be a liquid or solid substance that comprises viable (living) microorganisms, increases the efficiency of nutrient intake (PGP) and is applied to seed, plant, soil or other forms (Raddadi et al. 2007; Vessey 2003). According to Azizoglu (2019), several commercial biopesticide *B. thuringiensis* based products, but there are no commercial *B. thuringiensis* based biofertilizers and PGP products on the market. Furthermore, until now, the ability of *B. thuringiensis* as a biofertilizer, biostimulator and PGP has been demonstrated in different researches (Armada et al. 2015, 2016; Bais et al. 2006; Cherif-Silini et al. 2016; Delfim et al. 2018; Jouzani et al. 2017; Mishra et al. 2009; Raddadi et al. 2007, 2008; Wang et al. 2014).

Bacillus spp. is considered to be the supreme noteworthy PSB (Abdallah et al. 2018; Behera et al. 2014). Although P is unavailable in different soils, it is a major limiting element of plant establishment. The crops absorb P at less concentration although phosphate occurs in the soil and plants uptake phosphate in two soluble forms, i.e. monobasic or dibasic ions (Glass 1989; Raddadi et al. 2007). *B. thuringiensis* and *B. subtilis* strains taken from the wheat rhizosphere have high PGP action, including phosphate mobilization and biostimulation properties (Cherif-Silini et al. 2016; Jouzani et al. 2017).

12.6 Plant Growth Promoter

B. thuringiensis impart good effects on plant development as a PGP, and by using biotechnological applications, it can offer valuable products (Jouzani et al. 2017; Mishra et al. 2009). *B. thuringiensis* is beneficial in promoting plant growth and improve mobilization of P, production of IAA (Indole-3-acetic acid), ACC deaminase, phosphate-solubilizing enzyme, siderophores and ethylene. These also possess antimicrobial activity to counter various plant pathogens, and *B. thuringiensis* have been verified in many crops, which are demonstrated in Table 12.3 (Cruz and Ishii 2012; Jouzani et al. 2017; Raddadi et al. 2007; Sharma and Saharan 2016). *Pseudomonas*, *Bradyrhizobium*, *Bacillus*, *Herbaspirillum*, *Rhizobium* and *Azospirillum* are outstanding bacteria as PGP, and they cooperate with plant roots (Ahmad et al. 2008; Halda-Alija 2003; Pindi et al. 2014). In addition, phytohormones have important functions in plant growth and development as regulators and signals connections for bacterias colonize the plant roots, key roles in plant pathology, and microorganisms interactions (Raddadi et al.

Table 12.3 Name of the crops which were inoculated with P-solubilizing *Bacillus thuringiensis*

Plant	References
Wheat	Selvakumar et al. (2008)
Peanut	Wang et al. (2014)
Soybean	Mishra et al. (2009)
<i>Lavandula</i> and <i>Salvia</i>	Armada et al. (2014)
Maize	Armada et al. (2015)
Wheat	Delfim et al. (2018)
Mustard	Vishwakarma et al. (2018)
Lettuce	Gomes et al. (2003)
Soybean	Bais et al. (2006)
<i>Alnus firma</i>	Babu et al. (2013)
Canola	Freitas et al. (1997)

2007; Sergeeva et al. 2002). Nevertheless, IAA (Indole-3-acetic acid) is a phytohormone and is physiologically, biochemically and genetically well-researched plant growth hormone (Chaabouni et al. 2012; Del Pozo et al. 2005; Raddadi et al. 2007). *Bacillus* strains are successful in promoting the growth of wheat and maize crops. This is because of the formation of IAA (Indole-3-acetic acid), ACC deaminase, siderophores and phosphate-solubilizing enzyme (Beneduzi et al. 2008; Pindi et al. 2014; Trivedi and Pandey 2008). Some *B. thuringiensis* strains with PGP abilities act on plant roots and prove beneficial for sustainable crop yield (Armada et al. 2016; Gomes et al. 2003; Jouzani et al. 2017; Mishra et al. 2009; Sharma and Saharan 2016). Gomes et al. (2003) reports that IAA (Indole-3-acetic acid) produced by *B. thuringiensis* strain C25 isolated from *Brassica oleracea* (cabbage) improves the development of *Lactuca sativa* in controlled conditions. Likewise, Mishra et al. (2009) reported that co-inoculation of *Rhizobium leguminosarum*-PR1 with *B. thuringiensis* strain KR1 (IAA-producing) considerably enhanced the growth of pea and lentil plants in comparison to inoculation of *R. leguminosarum*-PR1 only. Besides, the co-inoculation of *Bradyrhizobium japonicum* with *B. thuringiensis* as well stimulated the growth of soybean and incremented root volume, shoot root weight, total biomass and nodule number compared to the rhizobia inoculation and control (Jouzani et al. 2017; Mishra et al. 2009). ACC deaminase and other compounds are produced by *B. thuringiensis*, in this context (Cherif-Silini et al. 2016).

Furthermore, Bais et al. (2006) observed that *B. thuringiensis* when co-inoculated with *B. japonicum* resulted in increased nodulation, growth and yield in soybean crop. ACC is a pioneer amino acid of the naturally occurring ethylene. It is a central hormone for growth and development plus stress tolerance. It controls numerous developmental courses (Raddadi et al. 2007). ACC deaminase activity is noted in various *Bacillus* spp. It is a good stimulant for root elongation during sprouting. ACC deaminase produced by *B. thuringiensis* strain acts as an efficient plant growth regulator. Praça et al. (2012) highlighted that actual colonization of *B. thuringiensis* on seedling root surface. This affects physiology of host plants plus this bacterium can function as a superior PGP. While Sharma and

Saharan (2016) observed that *B. thuringiensis* has the ability to mature in highly concentrated medium of heavy metals. It has also been stated that this strain displayed noteworthy ACC deaminase activity when 100% of *Vigna radiata* seeds germinated in the presence of *B. thuringiensis*. Also, Armada et al. (2016) states that mixture of arbuscular mycorrhizal fungal (AMF) strains plus native *B. thuringiensis* strains enhanced the growth of *Lavandula dentata* in dearth of water situations. Furthermore, the native *B. thuringiensis* strains isolated from the same soil expended in the testing created ACC deaminase. Additionally, *B. thuringiensis* can associate with AMF, favouring P solubilization, ethylene production and other characteristics which resulted in increasing nutrient bioavailability, primarily in P-bounded settings (Cruz and Ishii 2012). These are the benefits of the links between the AMF and bacteria. This can deliver additional benefits precisely in hostile circumstances like drought, diseases, pests and nutrient deficiencies or unavailability.

Wang et al. (2014) found that *B. thuringiensis* increase peanut biomass and yield. In fact, the inoculation increased the number of nodules per plant by 41.7%. Nodule endophytes act as a profound link between *B. thuringiensis* and the plant because they enhance the efficacy of the circulation of nutrients to the plant. In addition, investigators have witnessed that co-inoculation of phosphate-solubilizing bacteria and rhizobia can raise growth and production of legumes. This also amplifies the intake of N and P in plants (Rosas et al. 2006; Vishwakarma et al. 2018). However, Armada et al. (2015) demonstrated that inoculation treatments did not influence shoot and root biomass yield of maize when plants were grown under good water settings, although it considerably impacts the root length. Thus, dual arbuscular mycorrhizal plus *B. thuringiensis* inoculations improved root length by 20%. On the other hand, under drought settings the highest shoot growth was attained in plants inoculated with only *B. thuringiensis*, while in those dually inoculated, there was 30% increase in shoot dry weight.

Recently, Vishwakarma et al. (2018) worked in pot experiments and found that the co-inoculation of *Pseudomonas* and *B. thuringiensis*, respectively, incremented the growth of *Brassica juncea* (mustard) over to the controls. Together *Pseudomonas* sp. and *B. thuringiensis* cause plant growth promotion and increase in disease resistance (Aeron et al. 2011). *Bacillus* sp. boosts heavy metal tolerance in plants (Upadhyay et al. 2017). In this context, studies demonstrate the complex part of bacterial strains in plant growth promotion besides stress improvement. Similarly, plants inoculated with *B. thuringiensis* upsurge nutrient uptake under drought environments and enhance macro- and micronutrient intake and shoot dry weight of maize crop. Inoculation enhances the macro- and micronutrient content that shows the importance of this practice in modern agriculture (Armada et al. 2015). Moreover, Delfim et al. (2018) evaluated P concentrations in wheat plants that were grown in two dissimilar volcanic soils (andisol and ultisol). These plants were inoculated with *B. thuringiensis*, and the positive as well as negative effects of this strain were observed on plant growth and P nutrition for wheat, showing that it is principally dependent on the soil type. In addition,

Freitas et al. (1997) studied more than hundred bacteria isolated from the rhizosphere of field plants and concluded that the utmost efficient inoculant was *B. thuringiensis*. It considerably stemmed in increase of the number plus weight of pods as well as seed yield deprived of P quantity. This displays the possible application of *B. thuringiensis* as inoculant for canola. In addition, the authors suggest that P solubilization is not the main process in charge of positive growth reaction. Although the positive outcome of this biological fertilizer (inoculation) is not to be restricted to solitary nutrient element like P, several nutrients can be solubilized by *B. thuringiensis*. In addition, the inoculation with *B. thuringiensis* has been tested on various crops and demonstrated their agronomical impacts, in different conditions and parts of the world.

12.7 Environmental Impact

Exist a debate amongst researches as well as politicians regarding the negative environmental influence of the excessive and wrong use of chemical fertilizers, and the alternatives or solutions to reduce the environmental impacts, although it is evident that *B. thuringiensis* as biofertilizer and PGP can provide sustainable crop production and immense environmental benefits. Countries like India, China, Argentina, Brazil and the United States are the greatest fervent users of *B. thuringiensis* in agriculture as pesticide, insecticides and disease control agent owing to its entomopathogenic abilities (Sanahuja et al. 2011; Stabb et al. 1994). In fact, in these countries they can easily encourage the study and use of *B. thuringiensis* as a fertilizer or biostimulator and PGP.

One of the principal impacts of *B. thuringiensis* use in agriculture has been the reduction of pesticide sprays plus an associated lessening in the occurrences of poisonings due to chemical exposure. In addition to it, mean yield increases of up to 10% have raised up net revenue for practically 40% of population (Mushtaq et al. 2018; Subramanian and Qaim 2010). In this regard, the usage of *B. thuringiensis* as biofertilizer is an environmental approach that permits for escalating the accessibility of P in soil and its uptake in plants and can diminish the application of mineral fertilizers in agriculture (Delfim et al. 2018).

Intrinsic stress-tolerant bacteria support the plant and soil health besides developing plant growth, nutrient acquisition, stress tolerance and pest control on the adverse environmental conditions. Bulk of the microorganisms form spores or resting stages, thus resisting more than the others (Armada et al. 2014, 2015; Dangar 2008).

In addition, Babu et al. (2013) reported that *B. thuringiensis* GDB-1 inoculated in *Alnus firma* crop and it increased the competence for phytoremediation of soil encompassing mine tailings tainted with diverse heavy metals such as arsenic, lead, zinc, cadmium, copper and nickel. It can reduce the metabolic disturbances and stress produced by high quantities of heavy metals. Additionally, reduction in CO₂ emissions and conservation of soil and water help in the bioremediation of

high concentration of heavy metals plus additional contaminants, biosynthesis of metal nanoparticles and manufacture of diverse novel bio-products (Jouzani et al. 2017).

12.8 Conclusions

Phosphorus (P) is a non-renewable natural resource in soil and is present in mineral and organic form. It is an important nutrient for plant growth but its availability is lower in soil and for plant growth. It is unavailable to plants as it is typically existent as fixed or retained form in the soil. Therefore, it is necessary to find alternatives to increase its solubility. *Bacillus thuringiensis* possesses the capability to solubilize P plus produce different compounds such as siderophores, enzymes, ACC deaminase and IAA (Indole-3-acetic acid) which help crop growth and support negative environmental conditions. In addition, to become available in soil and for plant intake, both P forms should be possible to use, and *B. thuringiensis* play an important role in this process. In addition, multiple positive responses (i.e. P nutrition, tree improvement, biotic and abiotic stress tolerance and other beneficial effects) were associated to the application of *B. thuringiensis* in various crops. *B. thuringiensis* has a great potential to produce biofertilizer or biostimulator. In this case, researches must be done to optimizing and diffusing this biotechnology by commercial application, therefore improving productivity over low cost and aiding economic viability for farmers.

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Chapter 13

Cyanobacteria as Sustainable Microbiome for Agricultural Industries



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13.1 Introduction

Cyanobacteria are a distinct group of prokaryotic organisms, performing oxygenic photosynthesis. Blue-green algae (BGA) comprise about 150 genera and 2000 species and are classified into 5 subsections, i.e., *Chroococcales*, *Nostocales*, *Oscillatoriales*, *Pleurocapsales*, and *Stigonematales* (Boone and Castenholz 2001). Blue-green algae are the most archaic organisms in the plant world and are not actually recognized as “true” algae. They are called as cyanobacteria, as their structure looks much more like bacteria and have a cell wall made up of peptidoglycan. Their body is formed from a single cell, often clustered cells as colonies of various shapes. Generally, cyanobacteria cells consist of distinct sizes, ranging from 1 μm for unicellular forms to over 30 μm for multicellular species. Moreover, it is larger than other bacteria (Singh and Montgomery 2011). They have contrast morphology with three basic morphological forms including unicellular, filamentous without heterocyst, and filamentous forms with heterocyst (Singh and Montgomery 2011). Heterocysts are specialized and differentiated cells that can fix atmospheric dinitrogen (N_2) that are important for their survival under nitrogen-limiting conditions (Kumar et al. 2010a).

The exponential increase in the global population and changing climate pose a significant challenge to crop production. With this increase in population, the demand for food production has also increased. Therefore, the industries have shifted their focus on input of synthetic fertilizers in order to meet the demand. Although these synthetic fertilizers are capable of enhancing the production, they

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exert severe effects on the quality of soil and water and produce negative effects on plants as well (Bhat et al. 2017a; Dervash et al. 2020). Hence, there is a dire need to intensify the agricultural production in a sustainable way and find solutions to this problem. We need to shift our focus on sustainable organic biofertilizers for better food quality and maintenance of soil health. Plants are associated with complex microbiomes, which have an ability to promote plant growth and tolerance to multiple stresses, regulate plant nutrition, and biocontrol phytopathogens. The combination of useful plant microbe and microbiome interactions may correspond to a promising sustainable solution to enhance agricultural production. Cyanobacterial species are well recognized for their positive contribution as sustainable microbiome in agriculture and can also play an active role in bioremediation. Features of cyanobacteria have drawn the attention of researchers, which is recognized as a prosperous source of different bioactive compounds with antiviral, antifungal, antibacterial, and anticancerous properties (Bhat et al. 2017a, b; Malik et al. 2001). They are one of the most extensively used organisms in farming as organic fertilizers. Nitrogen is considered as the second growth-limiting element after water, and competency of this element could be enhanced by using cyanobacterial biofertilizers. These organic biofertilizers provide organic carbon, phosphorous, and nitrogen which are beneficial for enhancing the soil fertility, growth, and development of plants. Certain cyanobacterial species also help in bioremediation of heavy metals and other toxic compounds that refrain soil and water pollution.

13.2 Evolutionary History

Cyanobacteria belong to kingdom *Monera* (Prokaryotes), division *Eubacteria*, and class *Cyanobacteria*. However, their taxonomic classification at higher levels about the order, family, genera, and species is still controversial (Ernst et al. 2006; Hitzfeld et al. 2000). Cyanobacteria exhibit a wide range of morphologies among all bacteria (Table 13.1). Moreover, the conventional taxonomic classifications of blue-green algae focused on morphology and development have been classified into five principal groups (Rippka et al. 1979):

Group I (Chroococcales) – Organisms that consist solitary and colonial unicellular forms, for example, *Gloeocapsa* and *Synechococcus*

Group II (Pleurocapsales) – Organisms that comprise thallus-forming cyanobacteria, unicellular to pseudo-filamentous with cells capable of both multiple and binary fission, for example, *Pleurocapsa* and *Dermocarpella*

Group III (Oscillatoriales) – Organisms that consist of filamentous cyanobacteria and cell differentiation is absent, for example, *Phormidium* and *Microcoleus*

Group IV (Nostocales) – Organisms that comprise filaments, marked by cell differentiation to develop akinetes and heterocysts, for example, *Anabaena* and *Nostoc*

Table 13.1 System of classification of cyanobacteria

Type of cell	Mode of reproduction	G+C %	Recognized genera	Other properties
Unicells or aggregates	Binary fission or budding	35–71	<i>Synechococcus</i> , <i>Synechocystis</i> , <i>Gloeobacter</i> , <i>Gloeotheca</i> , <i>Gloeocapsa</i> , <i>Chamaesiphon</i>	Almost always non-motile
Unicells or aggregates	Multiple fission to baeocytes	38–47	<i>Dermocarpa</i> , <i>Xenococcus</i> , <i>Myxosarcina</i> , <i>Chroococcidiopsis</i>	Usually some baeocytes are motile
Filaments; unbranched trichomes with only vegetative cells	Binary fission in a single plane	40–67	<i>Spirulina</i> , <i>Lyngbya</i> , <i>Oscillatoria</i> , <i>Pseudanabaena</i> , <i>Phormidium</i> , <i>Plectonema</i>	Usually motile
Filaments can form heterocyst; no true branching	Hormogonia formed, binary fission in a single plane	38–47	<i>Anabaena</i> , <i>Nodularia</i> , <i>Nostoc</i> , <i>Cylindrospermum</i> , <i>Scytonema</i> , <i>Calothrix</i> , <i>Tolypothrix</i> , <i>Rivularia</i>	Often motile; may form akinetes
Filaments can form heterocyst; and true branches	Hormogonia, akinetes, hormocysts; binary fission in more than one plane	42–46	<i>Chlorogloeopsis</i> , <i>Fischerella</i> , <i>Mastigocladus</i>	Greatest morphological complexity and differentiation in cyanobacteria

Group V (Stigonematales) – Organisms that consist of cell-differentiating cyanobacteria with more complex multicellular organization, for example, *Stigonema* and *Mastigocladus*

Molecular phylogenies of the cyanobacteria favor few groups but not all these groupings (Giovannoni et al. 1988; Tomitani et al. 2006; Turner et al. 1999). Unsurprisingly, molecular analyses support the characters like cell differentiation or multiple fission of the phylogenetic affinities of taxa, while unicells and simple filaments do not constitute monophyletic groupings.

Phylogenies usually support in placing *Gloeobacter* as the sister group to all other existing cyanobacteria or as a paraphyletic basal grouping (Turner 1997). Other clades that exhibit consistency in molecular phylogenies comprises of unicellular taxa, where *Synechococcus* and *Prochlorococcus* being the dominated strains; Group II, baeocyte forming cyanobacteria; and the sheath forming Group III filaments forms along with some unicellular lineages (e.g., *Lyngbya*/*Phormidium*/*Plectonema*) (Sánchez-Baracaldo et al. 2005; Turner et al. 1999). The phylogeny of Tomitani et al. (2006), based on sequence analysis of 16SrRNA, *hetR*, and *rbcL* genes and with spacious sampling of multicellular taxa, onward supports the monophyly of cyanobacteria that distinguishes akinetes and heterocysts: Group V lineages form a clade nested within a paraphyletic Group IV.

13.3 Cyanobacteria Under Extreme Conditions

Cyanobacteria are commonly known as blue-green algae and often called as false eukaryotic algae. They are usually Gram-negative prokaryotes, achieve photosynthesis, and can fix atmospheric nitrogen into usable forms. They are omnipresent in ponds, lakes, water streams, rivers, and wetlands. They can survive at low pH value of 4–5 (Pfennig 1969, 1974) with optimal pH of 7.5–10 (Fogg 1956), while can also survive at high temperatures of 45–70 °C (Castenholz 1978).

Cyanobacteria can also grow under extreme environmental conditions such as in hot springs, arid deserts, hypersaline water, and cold environments and are also exploited for the refinement of alkaline soils (Rashid et al. 2019; Singh 2014). Their ability to survive under extreme conditions can be utilized to diminish the salt content from affected soils and can also enhance carbon (C), nitrogen (N), and phosphorus (P) content of the soil. It is said that cyanobacteria stimulate water permeability and soil accumulation and are useful in enhancing soil quality and structure of arid or subarid regions. Rogers and Burns (1994) studied that cyanobacterial inoculation boosts the stability of soil aggregates that enhanced WHC (water holding capacity) and aeration of poor structured soils. Such organisms diminish the sodicity and compaction of soils by enhancing organic carbon, WHC, and soil aeration and favor the biodiversity of other useful microflora as well.

13.4 Cyanobacteria as Potential Biofertilizers

The increased demand for food production is achieved by the application of synthetic fertilizers. Excessive utilization of synthetic fertilizers deteriorates the soil and water quality (Dar et al. 2020; Mehmood et al. 2019), makes plants more prone to diseases, and also causes reduction in soil fertility (Aktar et al. 2009; Dong et al. 2012). These chemicals also lead to increase in salinity and improper content of nutrients and reduce water holding capacity of soils (Bhat et al. 2018a; Dar et al. 2013; Savci 2012; Sofi et al. 2017). Moreover, it has also been found that prolonged use of chemical fertilizers causes changes in soil pH and kills the beneficial soil microbes. Meanwhile, the production of synthetic and chemical fertilizers is an expensive process to produce as they need large energy resources (Mahanty et al. 2017). Therefore, to overcome all these adverse effects, organic biofertilizers seem to be considerable natural inducers for sustainable growth and development of plants (Du Jardin 2015).

Cyanobacteria play a vital role in maintaining and boosting soil fertility, thereby increasing growth and yield of crops as a biofertilizer (Bhat et al. 2018a, b; Song et al. 2005). They are a group of microbes, cosmopolitan in distribution that can fix atmospheric nitrogen and can adapt in various types of soil and environment. Cyanobacterial fertilizers are considered as better in comparison to manure from farmyards and chemical fertilizers due to high amount of organic content that maintains the moisture holding capacity and mineral availability in the soils (Aitken

and Senn 1965; Kumar and Bawaja 2018). These fertilizers have longer shelf life, are cost-effective and convenient for use, and enhance aeration, humus formation, and moisture retaining capacity of soils along with increased nutrient uptake. They can also increase the rate of seed germination, growth, and yield of plants. It is also said that these fertilizers improve resistance to diseases, insects, nematodes, pests, and various stresses including drought, frost, salinity, etc. in plants (Dar and Bhat 2020; Kumar et al. 2012c).

Biological systems with the ability of nitrogen fixation offer an ecological and sustainable alternative to chemical fertilizers (Hegde et al. 1999; Vaishampayan et al. 2001). Several cyanobacterial species are bestowed with the specialized cells called heterocysts that are basically modified cells with thick walls. Heterocysts have been recognized as a site of (N_2) fixation, where a conglomerate enzyme nitrogenase catalyzes the process of nitrogen fixation (Singh et al. 2011b). The fixed N_2 may be released by secretion or microbial degradation in the form of free amino acids, polypeptides, ammonia, vitamins, and auxin-like substances after the cell death (Subramanian and Sundaram 1986). N_2 -fixing ability has been reported in cyanobacteria containing heterocysts as well as in several unicellular and filamentous forms that lack heterocyst. About 2×10^2 metric tons (MT) of nitrogen is fixed annually by biological means (Guerrero et al. 1981), and the total N_2 fixation is ~ 90 kg N $ha^{-1}y^{-1}$ (Metting 1988). The nitrogen fixation is carried out by symbiotic and free-living eubacteria, including cyanobacteria where cyanobacteria can fix <10 kg of N $ha^{-1}y^{-1}$; however, dense mats of cyanobacteria can fix ~ 10 – 30 kg of N ha^{-1} annually (Aiyer et al. 1972). Therefore, blue-green algae account for essential naturally available organic fertilizers (Prasanna et al. 2013; Vaishampayan et al. 2001). Some BGA such as *Anabaena*, *Nostoc*, and *Trichodesmium* contribute to about 36% of nitrogen fixation globally and are reported to enhance soil fertility in fields used for cultivation of rice in different parts of the world. Rice cultivation in tropical areas depends mainly on biological N_2 fixation that is usually carried by cyanobacteria (Vaishampayan et al. 2001). In cultivated agriculture systems, ~ 32 Tg of nitrogen is fixed annually by biological means, and cyanobacteria can fix about 20–30 kg of nitrogen ha^{-1} along with organic components (Issa et al. 2014; Singh et al. 2016; Subramanian and Sundaram 1986). They form symbiotic associations with different organisms like fungi, algae, diatoms, bryophytes, pteridophytes, gymnosperms, and angiosperms (Khanday et al. 2016; Rai et al. 2000; Sarma et al. 2016). A list of N_2 -fixing cyanobacteria is presented in Fig. 13.1.

Besides nitrogen fixation, cyanobacteria are also known to mineralize insoluble form of phosphate in soil. Phosphorus (P) is the second essential element for plants and microbes after nitrogen. There is approximately 0.05% phosphorus available in soil, and only a small proportion (0.1%) of this P is available for plant uptake. Most of the aquatic systems have often limited P and N content. Adjustments with algal biofertilizers can help in overcoming this problem that involves biochemical and physiological adaptations. Meanwhile, they can also excrete various substances that can enhance soil fertility and nutrient availability for plants. They excrete certain extracellular phosphatases immediately during P-limited conditions (Healy 1973).

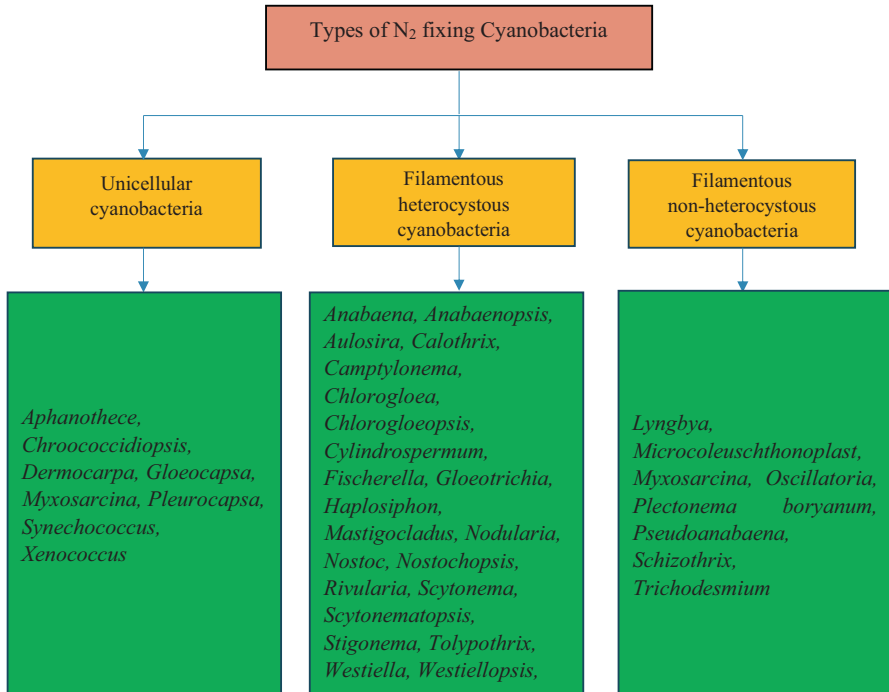


Fig. 13.1 Certain N_2 -fixing cyanobacteria

Cyanobacterial biofertilizers can also excrete some other substance and change the pH of the surroundings, which in turn can make adsorbed P available (Grobbeelaar 1983). Additionally, these biofertilizers can store sufficient P in excess to their basic needs.

Similarly, some cyanobacteria and other algae could produce and release iron-specific chelators, also called siderophores, which have the ability to make iron available to microorganisms and plants (Benderliev 1999). Moreover, they are also reported to enrich other microelements such as Cu, Co, Mn, Zn, etc. in plant parts (Das et al. 1991; Lange 1976).

13.4.1 Merits of Cyanobacterial Fertilizers

- Cyanobacteria enhance the diverse beneficial soil microorganisms, fertility of soil, plant-microbe associations.
- They can also inhibit or suppress several soilborne plant pathogens and parasites and protect the plant from diseases.
- Nutritional status is more balanced in these biofertilizers due to enhanced microbial activities.

- They improve and reclaim the soil nutrient status by increasing the organic matter content of these soils and regulate acidity, alkalinity, salinity, and fertility of soils.
- Enhances the diversity of soil methanotrophs and methane sink strength of these soils in the long term.

13.4.2 *Demerits of Chemical Fertilizers*

- Chemical fertilizers are nonrenewable, inhibit symbiotic N₂ fixation, and disturb plant-microbe associations by suppressing the useful soil microflora.
- Softening of plant tissues that result in increased susceptibility of plants to diseases and pathogens.
- Loss of nutrients from soil through fixation and leaching.
- Suppress the soil methanotroph number and methane sink potential.
- Soil and water deterioration, soil acidification or alkalization, reducing soil fertility, thereby causing damage to ecosystem and its stability.

13.5 Cyanobacteria: As Biocontrol Agents

The approach of sustainability in agriculture is not optimistic without admiring the methods used to suppress the infestation by insects or pests. The use of synthetic chemicals in agriculture systems for the control of insects, pests, fungi, and bacteria is connected with the severe effects on environment and human health. Thus, there is an increasing demand for biological-based alternative products where the role of microbes has been recognized in attaining long-term, durable, eco-friendly, and cost-effective food security. Cyanobacteria and other algae have been suggested as safe and efficient biocontrol agents (Bhatti et al. 2017; Gol'din 2012; Nassar et al. 1999; Schrader et al. 2002) that can be exploited for treating plant pathogens mainly associated with soilborne diseases. There are various species of cyanobacteria that have the ability to produce compounds that exhibit antifungal, insecticidal, nematocidal, cytotoxic, and herbicidal properties (Biondi et al. 2004). However, the role of secondary metabolites formed by these cyanobacteria in the management of phytopathogens and their execution in protection of agricultural yield is receiving a considerable attention (Kulik 1995; Singh et al. 2014). Several bioactive compounds that have the ability to kill or inhibit some useless microflora and fauna are alkaloids, amides, indoles, lipopeptides, and polyketides (Abarzua et al. 1999; Burja et al. 2001). These compounds inhibit the biochemical and physiological activities in the target species. Studies have shown that extracts of *Chlorococcum humicolum* inhibited the growth of *Botrytis cinerea* in strawberry and *Erysiphe polygoni* in tomato seedlings (Kulik 1995). A few cyanobacteria obtained from paddy fields have been reported to possess efficient antifungal

activities against soilborne pathogens (Kim 2006) and also prevent vegetables and flowers from pathogenic fungi (Manjunath et al. 2010; Prasanna et al. 2013). A study revealed that inoculating rice fields with cyanobacterial biofertilizers can reduce the number of mosquitoes (Victor and Reuben 2000). Further, extracts from cyanobacteria have shown mosquito larvicidal properties (Singh et al. 2013). Some have been found useful in preventing root rot disease in cotton and also improve the plant rhizosphere (Babu et al. 2015). Microalgae extracts contain tocopherols, polyphenols, pigments, and oils that possess antimicrobial properties (Dewi et al. 2018).

Furthermore, formulations from cyanobacterial extracts are reported to suppress the growth of saprophytes like *Aspergillus oryzae*, *Chaetomium globosum*, and *Cunninghamella blakesleeana* and phytopathogens such as *Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Kulik 1995). Certain researchers recorded that *Fischerella muscicola* produce a bioactive compound called fischerellin, with antifungal activities against phytopathogens like *Pyricularia oryzae* (rice blast), *Uromyces appendiculatus* (brown rust), *Erysiphe graminis* (powdery mildew), and *Phytophthora infestans*. However, it did not show any significant effect against *Monilinia fructigena* (brown rot) and *Pseudocercospora herpotrichoides* (stem break) (Hagmann and Juttner 1996; Papke et al. 1997).

Extracts of toxins from cyanobacteria can be useful in fighting against leaf roller larvae and moth (Sathiyamoorthy and Shanmugasundaram 1996). Cyanobacterial species like *Nostoc muscorum* has been found effective against soil fungi, known to produce “damping off” disease (De Caire et al. 1990). *Sclerotinia sclerotiorum*, one of the deadly phytopathogens affecting members of family Compositae like lettuce (*Lactuca sativa* L.) and other species of rosette plants causing cottony rot of vegetables and flowers (Tassara et al. 2008), is suppressed by *N. muscorum*. Also, the extracts from *N. muscorum* inhibit the growth of the plant pathogen *Rhizoctonia solani* responsible for causing root and stem rots (Kulik 1995). *Nostoc* sp. is known as a potential producer of cryptophycin and a source of natural pesticides used against the insects, fungi, and nematodes (Biondi et al. 2004; Mushtaq et al. 2018). Furthermore, *N. muscorum* is also known to exhibit antagonistic effects against other fungi like *Aureobasidium*, *Alternaria*, and *Cladosporium* responsible for causing “wood blue stain” (grayish or bluish discoloration of sapwood). Thus, it is evident from the studies that several cyanobacterial strains and different formulations from them can be employed as biocontrol agents in the field of agriculture. However, current information about the biocontrol activities of cyanobacteria explains that only few experiments have been performed in the field conditions, while majority of experiments are performed under lab conditions. Therefore, a pervasive research is needed to determine the feasibility of cyanobacteria as a sustainable tool for biocontrol of several phytopathogens. Some cyanobacterial species exhibiting antagonistic effects against different plant pathogens are depicted in Fig. 13.2.

Extracts from certain cyanobacteria and other microalgae are reported to enhance plant defense enzyme activities, thereby increasing immunity of plants. Application of dry powder from microalgae and inoculation of cyanobacteria were found effec-

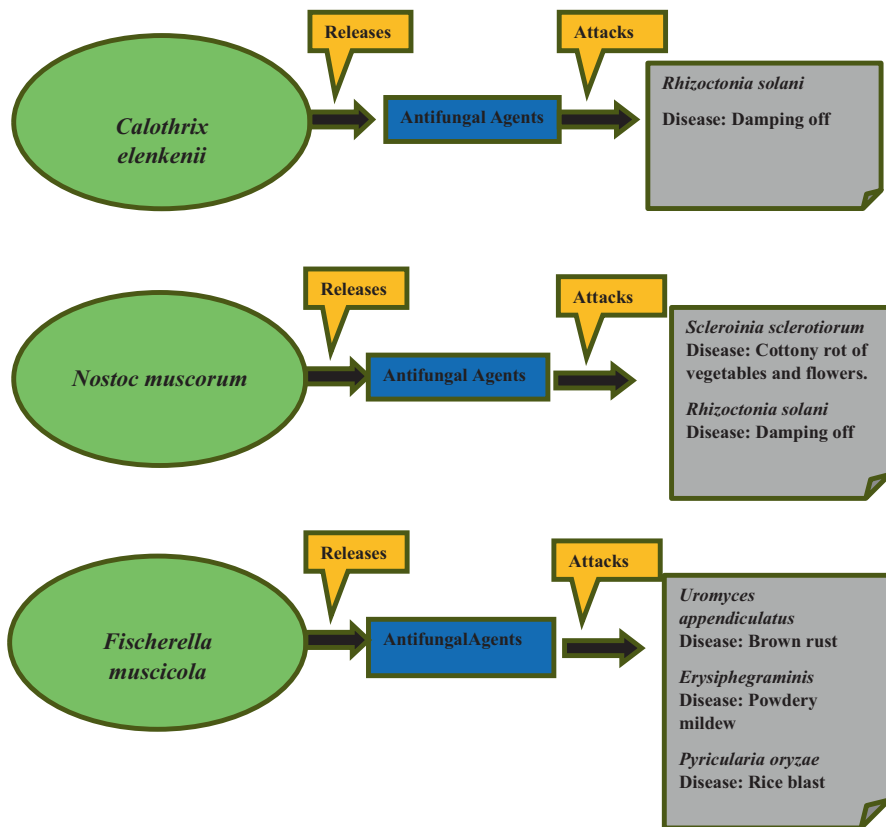


Fig. 13.2 Antagonistic effect of cyanobacterial species against plant pathogens

tive in reducing the gall formation and nematode infestation (Hamouda and El-Ansary 2017; Paracer 1987). In addition to their role as biocidal agents, certain cyanobacteria have been found to degrade organophosphorus and other chlorinated pesticides (Ibrahim et al. 2014; Kuritz 1998; Subramanian et al. 1994).

13.6 Cyanobacteria: As Plant Growth-Promoting Organisms

Besides contributing as biofertilizers, cyanobacteria play an important role in enhancing soil fertility in different ways by producing a combination of plant growth-promoting substances. They secrete extracellular plant growth-promoting chemicals, hormones such as auxin, abscisic acids, cytokinin, or gibberellins (Ahmad and Winter 1968; Marsalek et al. 1992; Rodgers et al. 1979; Singh and Trehan 1973). Studies have shown that some strains of cyanobacteria produce the growth regulators as intracellular metabolites, while others secrete these directly in

the neighboring environment (Abdel-Raouf 2012). These hormones could serve as plant growth-promoting substances in agricultural systems which can activate certain cascades in plant metabolism that ultimately lead to improved growth, yield, and crop quality (Zhao et al. 2005). These hormones can also improve plant tolerance to different biotic and abiotic stresses (Marsalek et al. 1992). Inoculation of various cyanobacterial strains in rice fields showed the presence of indole acetic acid (IAA) and indole butyric acid (IBA) (Li et al. 2018). It was observed that cyanobacterial extracts speed up the seed germination and seedling growth. Also, the crop yield and the quality of protein content were improved in these crops.

They are also reported to produce vitamins, particularly amino acids (Vorontsova et al. 1988), or vitamin B₁₂ and biotin (Grieco and Desrochers 1978; Misra and Kaushik 1989a, b), antibiotics, and toxic substances. During the stressful environmental conditions, plants adjust the hormone production level to fight against various stresses (Peleg and Blumwald 2011).

13.6.1 Auxin (IAA/Indole-3-Acetic Acid)

Auxin is known to enhance plant rooting system, thus promoting the potential of roots to attain improved nutrients (Spaepen et al. 2007). *Anabaena*, *Calothrix*, *Chlorogloeopsis*, *Cylindrospermum*, *Glactothece*, *Nostoc*, *Plectonema*, *Synechocystis*, etc. are some of the cyanobacterial strains that produce auxin (Singh et al. 2016). Further, chromatography analysis of a substance obtained from *Nostoc muscorum* from rice fields exhibited auxinic activity and features like that of indole acetic acid. It has been observed that the synthesis of phytohormones in microalgae depends on the precursors, amino acids, from plants (Zhao 2012). For instance, auxin synthesis in cyanobacteria was observed only with the accumulation of tryptophan (Sergeeva et al. 2002).

13.6.2 Gibberellin

Anabaenopsis and *Cylindrospermum* are some of the gibberellin secreting cyanobacteria. Gibberellin plays a vital role in inhibiting and stimulating germination of seeds; it is needed to break dormancy of seeds. It has been reported that in the absence of exogenous gibberellin, gibberellin-deficient mutants fail to germinate (Gupta and Chakrabarty 2013). Gibberellin-like compound was found in *Phormidium foveolarum* which was active in GA bioassays (Gupta and Agarwal 1973). Increased protein content was observed in pea seeds when inoculated with cyanobacterial strains due to activation of certain metabolic processes caused by gibberellin (Osman et al. 2010).

13.6.3 Cytokinin

Cytokinin acts as a regulator for shoot development and a negative regulator for root development. Cytokinin also promotes cell division, enhances photosynthetic rate, as well as plays a vital role in the recognition of the shoot meristems. In roots, the rate of differentiation of meristematic cell is regulated by cytokinin (Werner et al. 2010). Meanwhile, cyanobacterial strains have been found to exude cytokinin-like substances (Strick et al. 1997). *Anabaena*, *Chlorogloeopsis*, and *Calothrix* are some of the cytokinin-secreting cyanobacteria (Singh et al. 2016). *Anabaena* sp., when inoculated in wheat fields, significantly stimulated shoot length, spike length, lateral root, and weight of grains.

13.6.4 ABA (Abscisic Acid)

It controls various aspects of plant development such as germination, cell division, elongation, embryo maturation, seed dormancy, and floral induction. It also confers the stress tolerance to crops under abiotic stresses (Finkelstein 2013). Blue-green algae have been found to produce abscisic-like substances that can improve growth and yield of crops. *Nostoc muscorum*, *Trichormus variabilis*, and *Synechococcus leopoliensis* are among the few ABA-producing cyanobacteria (Marsalek et al. 1992).

Nitrogen fixation by cyanobacteria is an important factor that maintains soil fertility. They can directly or indirectly benefit plants in one or other ways. Blue-green algae can bind soil particles due to the adhesive properties of mucilage exudates. Thus, they can reduce soil erosion and water loss from soils as well. There are reports which suggest that cyanobacteria may release biologically active compounds into the soil and may significantly enhance plant growth. Marine cyanobacterial species serve as an essential source of vitamin production on a large scale. *Spirulina* is an abundant source of thiamine, riboflavin, vitamin B12, and beta-carotene (Lau et al. 2015). Certain cyanobacteria such as *Nostoc calcicola*, *Anabaena oryzae*, and *Spirulina* reduced the galls in cowpea caused by *Meloidogyne* and enhanced the plant growth (Youssef and Ali 1998). Some reports emphasized that the inoculation of cyanobacteria in rice fields can improve seed germination and root and shoot growth (Misra and Kaushik 1989a, b). It has also been reported that cyanobacterial inoculation in wheat fields improved the dry weight and chlorophyll content of the plants (Obreht et al. 1993). Extracellular chemicals secreted by cyanobacteria and their colonization around wheat plant roots reveal profound effects on growth of plants, although the agronomic efficiency was not estimated (Gantar et al. 1995a, b). The rapid growth of cyanobacterial cells and essential nutritional requirements primarily CO₂, water, and sunlight furnish an extensive opportunity for the commercial application of cyanobacterial strains as plant growth regulators (Ruffng 2011).

Additionally, studies have shown that the treatment of paddy seedlings with the cyanobacterial filtrates of *Anabaena oryzae*, *N. calcicola*, *Microchaete tenera*, or *Cylindrospermum muscicola* had increased both shoot and root length (Mohamed 2001).

13.7 Sustainable Agriculture and Microbiome

The advanced fields of science have provided many sustainable agricultural practices for the improvement of crop productivity. The soil microbiome is responsible for various processes occurring in the environment that are related to the well-being of plants. Several functions are credited to soil microbiome in relation to plants, for example, their ability to provide nutrients (nitrogen fixation and phosphorus solubilization), their role in nutrient uptake, and their ability in plant protection by secreting various bioactive compounds. Microbes associated with plants offer a sustainable solution to overcome the problems connected with soil salinity, fertility, and land deterioration. Soil microbiomes are exceptional as they are directly involved in enhancing soil fertility, plant growth promotion, and lowering different biotic and abiotic stresses (Glick 2010). Cyanobacteria are important organisms that have a significant role in the maintaining soil fertility due to N_2 fixation, making them a suitable natural source for soil fertility enhancement (Song et al. 2005). Blue-green algae have a leading role in sustainable agriculture. Efficient N_2 -fixing cyanobacteria like *Nostoc*, *Anabaena*, *Aulosira*, *Calothrix* sp., etc., have been applied to enhance rice cultivation across the different agroecological regions. The cyanobacterial applications have been practiced in paddy fields since ages, but now their use in various others crops is tested. Cyanobacteria also find its applications as in the production of phytohormones in free-living as well as in symbiotic associations. They perform a vital role in maintaining the structure and fertility of soils (Singh 2014; Singh et al. 2016; Vaishampayan et al. 2001) and in reusing the residues of crops and are involved in processing and preservation of crops (Hanson 1996; Saddler 1993). Activity of microbes in enhancing soil fertility, biocontrol, and bioremediation requires the influx of natural microbial or genetically engineered inoculants. Soil fertility maintenance with inexhaustible resources is the primary demand of sustainable agriculture by mitigating the requirement of chemical fertilizers. Among such resources, transgenic and natural N_2 -fixing microorganisms are the important candidates. The N_2 -fixing microbes can be used as coating on seeds or can be directly inoculated in soil. However, in both the cases, their acclimatization needs to be assured. Certain biosensors have been developed that can be used for manipulation, monitoring, and management of microbial consortia (Burlage and Kuo 1994).

The different metabolites produced by microorganisms have the competence to help in the establishment of sustainable agricultural practices, and biocompatible microbial polymers are the perfect example of it that must be used for coating of pesticides, fertilizers, and nutrients. This will aid in targeted delivery of pesticides, fertilizers, and nutrients in agriculture by hindering their application in certain

industry or antisocial activities. Bioremediation of contaminated water and soil is a sustainable approach to convert wasteland into agricultural practices. Transgenic or natural N_2 -fixing microorganisms in mixed cultures are used in different bioremediation practices and wasteland reclamation (Atlas 1995). Microbial interactions along with biogeochemical and ecological balances of affined ecosystems should be recognized for effective bioremediation of terrestrial and aquatic ecosystems (Anderson and Lovley 1997; Tiedje 1997). Among diverse valuable microorganisms, cyanobacteria can fix atmospheric N_2 and CO_2 and produce energy rich biomass containing various metabolites and other substances of economic importance. Therefore, they can be used in energy production, nutrition, and agricultural sectors. Also, advancements in cyanobacterial cultivation, screening, and genetic manipulations have facilitated new manners to use these photosynthetic microorganisms to combat various socioeconomic problems (Sarsekeyeva et al. 2015).

13.8 Cyanobacteria: A Sustainable Tool for Sustainable Agriculture

A serious focus in the last few decades has been put on the safe and eco-friendly approaches by exploiting the beneficial soil microbes in sustainable crop production. Cyanobacteria are known for their significant contribution in regulating the biogeochemical cycles of nitrogen, carbon, and oxygen (De Ruyter and Fromme 2008; Karl et al. 2002). They can fix atmospheric nitrogen in soil and root nodules, solubilize phosphate into usable forms, produce phytohormones and metabolites to uphold root growth, and decompose organic matter for soil mineralization. These features can increase the crop yields, enhance soil structure by aggregation of the soil particles, help in retaining water sources, and induce tolerance to various stresses in plants. They have undergone an array of structural and functional alterations during the evolution that allows their cosmopolitan distribution (Olson 2006). Cyanobacteria enhance the plant growth by improving nutrient uptake, form complex soil matrices, and help in plant defense response against various pathogens and diseases by secreting various metabolites and bioactive compounds. Additionally, they can improve the stress tolerance of plants against salt stress, drought stress, nutrient deficiency, weed infestation, and heavy metal contamination. Cyanobacteria can also help in soil formation processes, as they secrete certain biomolecules and other bioactive compounds. They have gained the attention of agriculturalists not only for their plant growth-promoting characters but also for their role in decomposing organic waste and detoxifying several toxic substances like pesticides and heavy metals (Aislabie and Deslippe 2013; Ma et al. 2016). For example, *Spirulina maxima* is known to acclimatize under high salinity and alkaline conditions (pH 11) that facilitate an advantage of protection from other competitors and grazers (Habib et al. 2008). The cyanobacterial ability of fixing nitrogen aids their survival and growth in the nitrogen-limiting habitats. This cyanobacterial trait makes them economically and agronomically vital as biofertilizers (Singh 2014; Vaishampayan et al. 2001).

Certain cyanobacterial species are known to create symbiotic associations with plants and other organisms. This ability of cyanobacteria could be exploited to develop suitable consortia of microbes for bioremediation of soil and water (Rai et al. 2000; Hamouda et al. 2016). Cyanobacterial exopolysaccharides (EPS) contribute to about 25 percent of the total biomass (Nisha et al. 2007). Cyanobacteria perform the activities in the upper crust of soil and the exopolysaccharide acts as a sticky agent for adhering to soil particles. The EPS can bind soil particles that lead to aggregation of soil particles, accumulation of organic content, and increase in water holding capacity of soil (Malamlssa et al. 2001). Therefore, it supports the growth and survival of other plant growth-promoting microbes. Also, the cyanobacterial growth rectifies the physical and chemical properties of soils. Cyanobacterial EPS and PGPRs may contribute to a positive alteration and reclamation of poor soils (Flaibani et al. 1989; Paul and Nair 2008).

The traditional agriculture practices rely on the input of chemical pesticides and fertilizers and methods like excessive irrigation and intensive tillage. This may lead to overexploitation of natural resources like water and soil, rise on the cost of agricultural production, and environmental pollution (Dar et al. 2016; Kumar et al. 2012a, b, c, d, e). Thus, there is a necessity to accept sustainable agricultural approaches that are eco-friendly and cost-effective and help us to attain long-term food security. An eco-friendly management practice for complex agroecosystems without interrupting the interactions among ecological components such as water, climatic, and edaphic factors including the living components supports the long-term increment for sustainable increase in crop productivity.

Cyanobacterial application (Fig. 13.3) contributes in economic benefits by reducing input costs, N₂-fixation, phosphorus mineralization, nutrient cycling, water storage, refrain pollution, and land deterioration especially through mitigating the utilization of agro-chemicals, nutrient recycling, and restoration of soil fertility (Shukia et al. 2008). Some of the benefits facilitated to the agroecosystem, by utilizing cyanobacteria, are presented below:

- Improved solubilization and motility of nutrients of limited supply
- Biocontrol of plant pathogens and diseases
- Detoxification of xenobiotics and heavy metals; restrict their motility and transport in plants
- Plant growth stimulation with the help of plant growth-promoting attributes
- Reclaiming the chemical and physical conditions of soils

13.9 Role of Cyanobacteria in Bioremediation

Industrial revolution has possibly brought growth in economy and social change globally. These changes have caused a number of environmental issues. Certain types of toxic industrial effluents released from fertilizer factories, oil refineries, dye industries, and other chemical industries may badly affect the environment

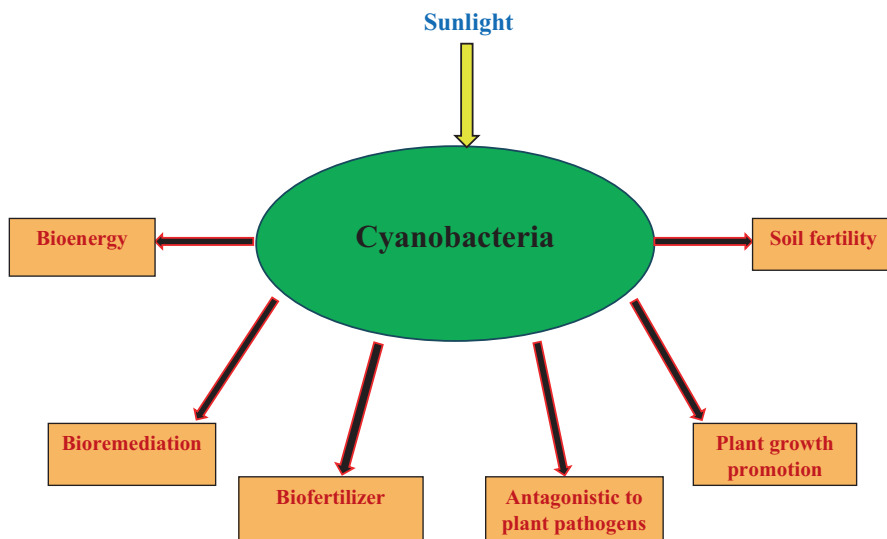


Fig. 13.3 Applications of cyanobacteria

and human health. However, traditional physical and chemical methods are extensively applied for removal of toxic pollutants, but these methods are not found effective and eco-friendly (Mallick 2002; Ruffng 2011). Cyanobacteria can be used as an effective tool for wasteland reclamation and combating various types of noxious pollutants like heavy metals (Rai et al. 1998; Singh et al. 2011b), pesticides (Megharaj et al. 1994), polycyclic aromatic hydrocarbons (Cerniglia et al. 1980a, b; Narro et al. 1992), xenobiotics (Megharaj et al. 1987), crude oil (Al-Hasan et al. 1998, 2001), phenol and catechol (Shashirekha et al. 1997), and radioactive compounds (Acharya et al. 2012) either through their agglomeration or degradation. They play a crucial role in the biodegradation of various industrial discharges including brewery and distilleries, paper and sugar mill, oil refinery, dye, and pharmaceutical industries because of their high potential of metal sorption and multiplication rate. They can be useful also in the treatment of wastewater and agro-industrial effluents (Singh et al. 2016; Vilchez et al. 1997). Certain cyanobacterial species have been observed to show biosorption of various heavy metals including *Spirulina platensis* for Ni, Cu, Cd, Pb, and Zn (Greene et al. 1987); *Nostoc calcicola* against Cu (Verma and Singh 1990); *Oscillatoria angustissima* for Zn and Cu (Ahuja et al. 1999); *Synechococcus* sp. for Ni, Cu, Cd, and Pb (Yee et al. 2004); and *Microcystis* for Cd and Ni (Pradhan and Rai 2000; Rai et al. 1998). *Phormidium bigranulatum*-dominated mats were found efficient in the removal of Cd(II), Pb(II), and Cu(II) from aqueous solutions (Kumar et al. 2012a, c; Kumar and Gaur 2014). Cyanobacteria exhibit a great range of tolerance against pesticides by accumulating them or via degradation mechanism (Ahmad and Venkatraman 1973; Pabbi and Vaishya 1992). Several cyanobacterial genera such as *Anabaena*, *Nodularia*, *Oscillatoria*, *Nostoc*, *Microcystis*, *Synechococcus*, and

Cyanothece possess a great potential for the elimination or biodegradation of lindane residues (i.e., γ -hexachlorocyclohexane) (El-Bestawy et al. 2007; Kuritz and Wolk 1995). Kumar et al. (2010a, b) reported two pesticides 2,4-dichlorophenoxyacetic acid (2,4-D) and paraquat (PQ) have a potent sorbent known as *Oscillatoria* sp.-dominated cyanobacterial mat. Cyanobacterial microflora also increased transformation and biodegradation of some polycyclic aromatic hydrocarbons, organophosphorus compounds, and organic compounds. *Aulosira fertilissima* and *Nostoc* sp. have been found efficient for the biodegradation of organic compounds, and an extensive range of organochlorine and organophosphorus pesticides were biodegraded by *Anabaena* sp., *Nostoc* sp., *Lyngbya* sp., *Synechococcus elongates*, and *Microcystis* sp. (Forlani et al. 2008; Semple et al. 1999). *Synechocystis* sp. has been reported as an efficient tool for the mineralization of anilofos herbicide. Synthetic dyes are not readily degraded; they are mutagenic, toxic, and carcinogenic in nature and cause serious harmful effects to the environment. Several cyanobacterial species like *Oscillatoria rubescens*, *N. linckia*, and *L. lagerteimii* were demonstrated for the elimination of synthetic dyes. *Phormidium* sp. has been reported to biodegrade the extensive range of dyes like Ff sky blue, indigo, and acid red 119 (Dellamatrice et al. 2017). It is reported that certain cyanobacterial species like *Synechococcus* sp., *Oscillatoria salina*, *Aphanocapsa* sp., and *Plectonema terebrans* are helpful in biodegrading crude oil and other surfactants through forming mats in aquatic environments (Cohen 2002; Radwan and Al-Hasan 2000). Cyanobacterial species like *Agmenellum* sp. and *Oscillatoria* sp. were able to oxidize naphthalene into 1-naphthol and n-alkanes (Cerniglia et al. 1979; Cerniglia et al. 1980a). Besides this, the role of cyanobacterial mats in the elimination of several harmful dyes has been also recorded. Kumar et al. (2012b) have investigated that through the batch contact method, an *Oscillatoria* sp.-dominated cyanobacterial mat has efficiency for sorbing methylene blue (MB).

Studies have shown that several cyanobacteria such as *Spirulina laxissima*, *Oscillatoria geminata*, *Nostoc carneum*, and *Nostoc insulare* eliminated radioactive contaminants such as Ra, Cs, Am, and Sr (Pohl and Schimmack 2006), and uranium was also removed by *Anabaena torulosa* (Acharya et al. 2012) from radioactive pollutant contaminated sites. Table 13.2 shows the list of some cyanobacterial species in the elimination of heavy metals in various ecosystems.

The cyanobacterial species have a competence of biodegradation and can be raised through an emergent technology probably known as genetic engineering (Kuritz and Wolk 1995). This can be explored as a maintenance-free and eco-friendly bioremediation tool for befouled ecosystems. Many researches on remediation by genetically modified cyanobacterial species emphasize that it will provide a better future prospectus in the field of bioremediation of various environmental pollutants. The genetically engineered strains of *Anabaena* sp. became paramount in biodegrading both 4-chlorobenzoate and lindane pesticides. These modified *Anabaena* sp. strains were obtained by inserting *Arthrobacter globiformis* operon fcb ABC from plasmid pCH1 (known for biodegradation of halobenzoate) via triparental mating recombinant tool (Kaplan et al. 1994).

Table 13.2 Removal of heavy metals by some cyanobacterial species

Heavy metals	Cyanobacteria
Chromium	<i>N. calcicola</i> , <i>Chroococcus</i> sp.
Cobalt	<i>N. muscorum</i> , <i>Anabaena subcylindrica</i>
Cadmium	<i>Nostoc linckia</i> , <i>N. rivularis</i> , <i>Tolypothrix tenuis</i>
Copper	<i>N. muscorum</i> , <i>A. subcylindrica</i>
Zinc	<i>N. linckia</i> , <i>N. rivularis</i>
Lead	<i>N. muscorum</i> , <i>A. subcylindrica</i> , <i>Gloeocapsa</i> sp.
Manganese	<i>N. muscorum</i> , <i>A. subcylindrica</i>
Mercury	<i>Spirulina platensis</i> , <i>Aphanothece flocculosa</i>

13.10 Conclusion

Due to exponential rise in population, production of food in large quantities has become a problem for agricultural industries. To overcome this problem, industries have become dependent on chemical fertilizers and pesticides that are not only harmful for human consumption but affecting the soil fertility, reducing organic components, affecting the quality of crops, and causing soil and water pollution. Also, the excessive use of chemical fertilizers has caused several environmental problems such as greenhouse effect, ozone layer depletion, and acidification of water. Thus, there is an urgent need to adapt feasible and sustainable agricultural practices for sufficient cost-effective food production for the rising human population on a global scale that will use lesser energy and are eco-friendly. Thus, biofertilizers containing microbes like bacteria, fungi, and cyanobacteria are recommended as possible and sustainable solution for the large-scale agricultural practices which are natural, eco-friendly, and cost-effective and also maintain soil structure as well as biodiversity. In the last few decades, much attention has been paid toward the possibility of using algae as biological tool that can reduce the resultant soil pollution and in addition can also improve both soil structure and plant health. Cyanobacterial biofertilizers could be the perfect substitute to inorganic chemical fertilizers that can play an important role in maintaining the soil integrity and fertility, improving crop production, controlling plant pathogens, and stimulating plant growth. The reason to select cyanobacteria is that it can be maintained at low cost and under simple growth requirements. Plant growth-promoting substances produced by cyanobacteria can improve growth and development of plants, thereby production. They also produce bioactive compounds with anti-algal, antifungal, antibacterial, and antiviral properties and can suppress or inhibit the growth and activity of different plant pathogens. Blue-green algae have photosynthetic ability which when integrated with the other crop plants through genetic engineering can lead to the production of beneficial transgenic varieties. Cyanobacterial exopolysaccharide secretions improve the efficiency of soil by holding the soil particles together, adding carbon, phosphorus, and nitrogen to soil, refraining soil erosion as well. The present chapter emphasized on the role of cyanobacteria in soil fertility, enhancing soil nutri-

tional status and reclamation of soils. All these unprecedented applications of cyanobacterial species suggest their use as a sustainable microbiome in agriculture.

Despite the fact that considerable amount of information has been gathered on the beneficial effects of several strains of cyanobacteria, very little is known about their abundance, periodicity, and succession in the rice field.

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Chapter 14

Intercropping: A Substitute but Identical of Biofertilizers



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14.1 Introduction

The growing human population is expected to reach around nine billion by the mid of the twenty-first century (Gerland et al. 2014). To successfully meet the need of fast-growing population, it is predicted to double the crop production figure of 2005 (Tilman et al. 2011). Although this goal seems pretty unachievable with limited environmental impacts, this challenge can be met through intensification of agriculture. By proper land management practices and increasing yield per capita of some cereal and vegetable crops, researchers have granted human to cope with growing population and reduced the number of un nourished people as compared to the nineteenth century (Pingali 2012). However, current infrastructure of agriculture sector and poor land management practices in underdeveloped and many developing countries are proving quite challenging to ensure the future global food security. The gluttonous desire of increasing yield per capita in short period of time has tremendously increased the continuous cropping and use of chemical fertilizers and pesticides (Gouda et al. 2018; Mushtaq et al. 2018). The use of extensive chemical fertilizers and continuous cropping have caused degradation of agricultural land;

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hence the researchers are now mainly focusing on safer agricultural practices, i.e., biocontrol or diversification of crop species.

Selection of proper cropping system is a key element of good land management practices in sustainable agriculture. Intercropping, which is growing two or more crops simultaneously on same land, has been found effectively curbing crop diseases, increasing nutrient use efficiency, improving soil health, and enhancing overall crop yield (Zhou et al. 2011; Jin et al. 2020; Bhatti et al. 2017). Basically, intercropping is an interspecific interaction in which plants of two or more species interact with each other either to facilitate (positive interaction) or to compete for survival (negative interaction). Some examples of positive interactions are facilitation of neighboring crop plant through nutrient availability, i.e., legume/cereal system (Duchene et al. 2017), nutrient acquisition (Zou et al. 2018; Bhat et al. 2017a, b; Sofi et al. 2017; Shafi et al. 2018), suppression of plant-specific pathogenic microbiota and eventually the diseases (Zhu and Morel 2019), modulation of composition of root exudates (Lv et al. 2020), and warning of possible future danger via release of volatile organic compounds (VOCs) (Khashi u Rahman et al. 2019). On the other hand, the main example of negative interactions in intercropping is allelopathy that can be utilized in sustainable agriculture, i.e., weed management (Bhadoria 2011; Arif et al. 2015; Dervash et al. 2020). In sustainable agriculture, these both kinds of interactions can be used as safer ways to get higher yield per capita.

The mechanism underlying interaction between crop species during intercropping is complex, and its understanding is important for practical development of any sustainable agroecosystem (Fig. 14.1). In aboveground interactions, plants interact with each other in competition or facilitation through physical means and via communication through VOCs. In belowground interactions, plants interact with each other through root exudates, common mycorrhizal network (CMNs), and chemical signals. This chapter will improve our understandings about how intercropping can facilitate sustainable agriculture and the mechanism involved in aboveground and belowground facilitative interspecific interactions during intercropping.

14.2 A Successful Intercropping System

Several factors are considered for a productive and successful intercropping system because the features of intercropping vary with climatic condition, soil type, resource availability, and economic value (Maitra et al. 2020). To assure the proper utilization of available resources and minimize the competition between crops, selection of species is the key as intercropping may not be beneficial or might be harmful if proper species are not chosen. For example, the combination of legumes and cereals is considered for N₂ fixation, and growing maize (*Zea mays* L.) with legume crops, i.e., groundnut (*Arachis hypogaea*), black gram (*Vigna mungo*), and green gram (*Vigna radiata*), assures shade for legume species that are tolerant to shade and for management of cereal components in maize (Manasa et al. 2018). Another important consideration for a successful intercropping system is the matu-

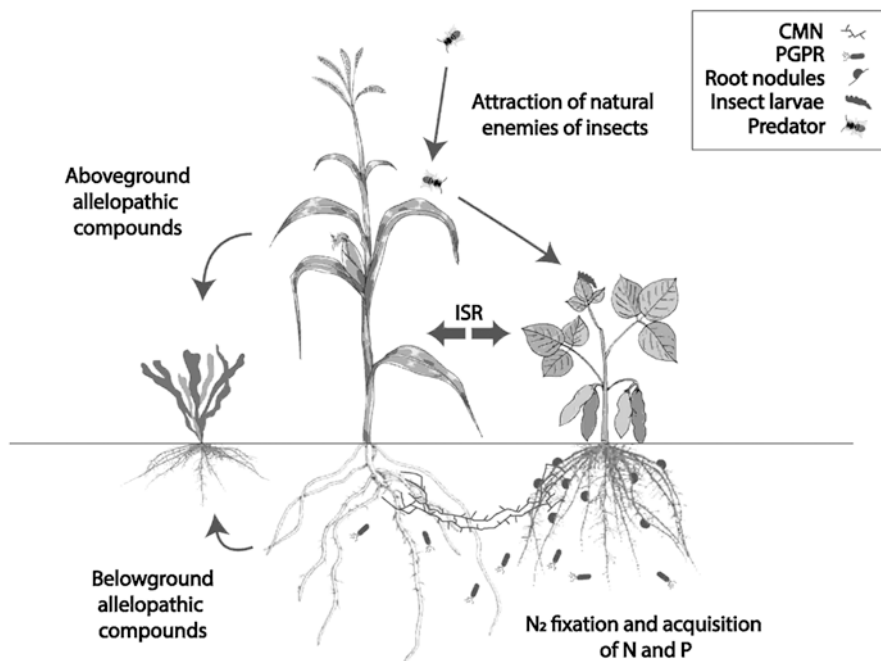


Fig. 14.1 A diagram illustrating possible mechanisms involved in facilitative interspecific interaction between different species during intercropping. Some species such as legumes have natural ability of fixing environmental N_2 and facilitate neighboring species in acquisition of N. Some species interact with each other via common mycorrhizal networks (CMN) and enhance P acquisition as well as induce systematic resistance against disease through recruiting plant growth-promoting bacteria (PGPR). Moreover, volatile compounds secreted from certain species attract natural enemies and facilitate neighboring species to combat insect pests. Similarly, some species possess allelopathic potential which can be manipulated in controlling weeds

rity time of crop species. To minimize the resource competition, crop species selected for intercropping system are usually of different maturity time. When intercropped species have different growing periods, the competition for major resources decreases, and their complementary effects benefit the system in the form of higher yield of both crops. For example, the maize as a base crop is usually planted with legume species as intercrop because most of the legumes are of short duration of life span. In maize/green gram system, green grams reach its reproductive stage after 56–60 days after plantation, while by that time maize only reach its knee-height stage (Maitra et al. 2020). In this way, the demand of stage-based nutrients and water is managed with minimum competition between species. Lithourgidis et al. (2011) suggested that being a long-duration crop, maize can potentially manage its need of major resources after harvest of leguminous crops when intercropped together.

Generally, crop yield depends on crop density and maturity parameters. However, because intercropping is accommodation of two or more crops at same period of

time, several compromises are made during intercropping system. Intercropping is classified into replacement series and additive series (Kour et al. 2014; Singh et al. 2020). In replacement series, all crops are termed as component crops instead of base or intercrop and are sown with equal distribution, without 100% density of particular specie as in sole cropping. For example, the spacing between maize rows is about 60–80 cm in sole cropping, which can be used for other component crop without reducing recommended plant density. However, in additive series, intercrop is accommodated with base crop which is sown in 100% density as monocropping; however, compromises in planting geometry and spacing are made. Overall, it is obvious that some part of population of crops are scarified for another crop in intercropping; however, it is recommended to increase the density of crops to maximize the yield advantages. Time of planting is also crucial to get higher yield and utilization of available resources (Addo-Quaye et al. 2011). In this regard, one crop of long duration is planted as base crop, while short-term duration crops are sown as intercrop so that intercrop can be harvested before the need of resources for base crop tend to increase. In this way, not only the resources are utilized in efficient way, but the higher yield of both crops can be promised.

14.3 Intercropping Facilitating Plant Nutrients

The crop yield is closely related to nutrient acquisition by crop plants. The major role that intercropping plays in a facilitative interspecific interaction is the mobilization of unavailable nutrients and their uptake by plants (Vandermeer 1992; Dar and Bhat 2020). Nitrogen (N) and phosphorus (P) are the main nutrients that are quite essential for plant vegetative growth and productivity. It is now well understood that intercropping can mitigate the stressful condition of plant belowground environment and make N and P available for plant acquisition.

14.3.1 *N₂ Fixation and N Availability*

Although N is abundant on earth, its deficiency in plants due to unavailability causes huge losses in crop production all around the world. Synthetic fertilizers are being used to fulfill plant N requirement; however excess use of fertilizers is also degrading soil stability by unbalancing the N cycle and eutrophication, and the loss of gaseous N₂O has contributed in global warming. To assure long-term solution for this, researchers have been trying to find some biological means, and intercropping leguminous crops is one of them. Addition of legumes in intercropping has been widely practiced due to their natural ability of utilizing atmospheric N₂. In this system the competition between plants for acquisition of N decreases, and it also provides substantial amount of N to base crop after harvest of intercropped legume crop (Bedoussac et al. 2015; Bhat et al. 2018a, b). It has been shown that inter-

cropped legume can store around 40–100 kg/hectare N in plants, and the yield of base crop can be increased up to 30–40% if intercropped with legume (Amossé et al. 2013). It is now widely understood that leguminous crops fix more environmental N_2 when intercropped with a crop with no ability to fix N_2 or have more requirement of N for growth and production. This is because of the natural trait of legumes that absorb unavailable N_2 and transform it to plant-available form only when there is competition between plants for acquisition of N in low N available environment. The main legumes cultivated for the purpose of N_2 fixation are clovers (*Trifolium* spp.), lupin (*Lupinus angustifolius* L.), soybean, pea (*Pisum sativum* L.), pigeon pea (*Cajanus cajan* L.), cowpea (*Vigna unguiculata* L.), faba bean (*Vicia faba* spp. minor L.), *Crotalaria spectabilis* Roth., alfalfa (*Medicago sativa*), and fenugreek (*Trigonella foenum-graecum* L.) (Xue et al. 2016).

The process of N_2 fixation is governed by diazotrophic prokaryotic microorganisms belonging to archaeobacteria and eubacteria. A well-organized mutualistic relationship is formed between host and species for N_2 fixation process (Garg 2009). Generally, these bacteria are free-living and fix N in symbiotic process with plants; however, there are some cyanobacteria with the ability to fix N independently. The process of symbiotic N fixation takes place in specialized structures on legume roots, called nodules, where species from many genera of *Rhizobiaceae* fix inorganic N to available form. In addition to root surface, *Azorhizobium caulinodans* can form nodules on stem of legume crops (Xue et al. 2016). The N_2 fixation is catalyzed by nitrogenase which is an anaerobic enzyme carrying complex metalloclusters on its active sites. The metallocluster usually contains iron as the main element along with molybdenum or vanadium or alone (Curatti et al. 2006). The nodules contain a specific oxygen diffusion barrier and synthesize oxygen carrier protein to protect the catalyzing enzyme, because nitrogenase is inhibited if exposed to oxygen. Moreover, the *nif* genes associated with N_2 fixation process have been well categorized now because of ecological and economic importance of this process.

14.4 P Availability and Acquisition

P is an important element in plant life cycle and is being consumed so quickly. The deficiency of P is the major limiting factor in crop growth and yield as P availability controls the process of N_2 fixation (Isaac et al. 2012). The natural P already available in the soil is already insufficient for plants, and its demand is rising nearly twice as the human population growth. To fulfill the basic requirement of P to plant, P is applied in the form of fertilizers, which are being excessively used in order to maximize the crop yield. According to Syers et al. (2008), only 15–30% of applied P fertilizer is taken up by crop plants in the year of its application because of its slow diffusion and high fixation in soil. P limitation is usually overcome by the application of Pi fertilizers, but such P is basically from a nonrenewable resource (Vaccari 2009), which is expected to become scarce in the near future.

The availability of P in the soil can be increased by different biological processes and land management practices such as intercropping. To the date, many intercropping systems have been reported that can be practiced for utilization of unavailable P in the soil and to improve the P uptake by plant. Among those, intercropping of cereals with legumes have been studied the most because legume crops fix more N_2 when intercropped with those species that are unable to fix N_2 or sole cropping (Dar et al. 2013; Jensen et al. 2020; Bhat et al. 2018a, b). During nodulation/ N_2 fixation, leguminous plants release H^+ and take more cations than anions. The released H^+ ions are specifically important in dissolving soil unavailable P (Tang et al. 1997). In addition, increased N_2 fixation by legumes also increases rate of proton excretion that also plays an important role in P mobilization in rhizosphere (Li et al. 2003). In legume/cereal intercropping system, maize with faba bean (*Vicia faba*), white lupin (*Lupinus albus*), and soybean (*Glycine max*); wheat with faba bean and soybean; and in other combinations, wheat/maize and Alfred stonecrop (*Sedum alfredii* Hance)/upland kangkong (*Ipomoea aquatica* Forsk.) are widely practiced in different parts of the world.

In alkaline soils where pH is the main limiting factor of crop production, P availability can be increased by introducing legumes with cereals via the effects of change in pH and release of organic compounds through root exudation (Zhang et al. 2016). Researchers have found two main processes controlling the process of P availability in rhizosphere that both are regulated by acidification and Ca uptake by plant. These processes are (i) the desorption of P from metal oxyhydroxides and clay minerals which is endorsed by synergetic effects of Ca^{2+} desorption of PO_4^{3-} desorption and (ii) P sorbing on surfaces and dissolution of Ca-P minerals (Messaudi et al. 2020). In intercropping, the roots of the plants interact exclusively with the soil microbiota which further affect plant nutrition uptake efficiency either directly by mobilizing plant-unavailable nutrients and uptake or indirectly through root growth promotion (Richardson et al. 2009). Studies have shown that many microorganisms can effectively dissolve insoluble inorganic P and mineralized organic P during intercropping. For example, the intercropped wheat increases abundance of *Pseudomonas* and *Bacillus* spp. community in cucumber (*Cucumis sativus*) rhizosphere (Jin et al. 2020), and most of the species of these microbes have the ability to solubilize and mineralize phosphate and also enhance the absorption of P by plant roots (Wang et al. 2007). *Trichoderma* spp. is another microbial community which is greatly affected by intercropping and also has the ability to dissolve the fixed phosphate existing in the soil, so as to improve the soil fertility and plant growth (Kapri and Tewari 2010).

14.5 Pest Management

14.5.1 Weed Management

Intercropping play an important role in controlling weeds through utilization of land space, resource-based competition, canopy development, root growth, and allelopathy (Joyful and Pieterse 2019). For example, the intercropping oats (*Avena sativa* L.) and

some varieties of ryegrass and rye (*Secale cereale* L.) with maize has determinantal effects on emergence of weed (Bezuidenhout et al. 2012). Several intercrops secrete allelochemicals through root exudation which suppress germination and early seedling growth of some weeds. The biomass canopy or remaining crop residues of intercrop may also affect germination of photoblastic weed seeds by interfering with phytochrome-mediated germination. Another effect of intercrop biomass on weed germination is through conserving soil water moisture or creating a waterlogged environment to suppress weed germination. According to Teasdale et al. (2007), when intercrop of short duration is harvested, the remaining residues lower the soil temperature and eventually suppress weed germination. Although, intercropping legume crops with cereals or other crops facilitates base crops through availability and acquisition of nutrients, the mechanism of weed suppressiveness via allelopathy in legume/cereal system is still poorly understood. However, the weed suppressiveness effects of legumes in greenhouse and laboratory tests have been reported (Rueda-Ayala et al. 2015).

Allelopathy, a phenomenon of suppressing growth of neighboring plant through belowground or aboveground secretion of allelochemicals, is a potential biological way of controlling weeds. Moreover, the herbicidal effects on weeds decrease gradually when herbicides are frequently used; hence intercropping allelopathic crops not only controls the weeds as biological measure but also maintains herbicidal effects (Jabran et al. 2010). Many crops have been reported with efficiency of allelopathy that can be used in intercropping combating weeds. Rye is the most effective allelopathic crop which releases more than 15 important allelochemicals such as benzoxazinones [2(3H)-benzoxazolinone (BOA) and 2,4-(dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA)] (Schulz et al. 2013). Sorghum (*Sorghum bicolor* L.) is also an important allelopathic crop that can be used in curbing weed; however its efficiency varies with environmental conditions, growth stage, and cultivar. The most important allelochemicals screened from sorghum are cyanogenic glycoside (dhuririn), *p*-benzoquinone (sorgoleone), and phenolics (Weston et al. 2013). Among those, sorgoleone is the allelochemical of high potential released in root exudates of sorghum. Other examples of potential allelopathic crops that may be cultivated as component crop in intercropping include Brassicaceae family and sunflower (*Helianthus annuus* L.). The phenolic compounds and benzoxazinoids secreted from maize plants can effectively reduce germination of weeds especially at early maize growth stage (Jabran 2017). Glucosinolate is an allelochemical of Brassicaceae family which, after release, decomposes into several biological compounds. These compounds inhibit growth of neighboring plants/weed when they are taken up from rhizosphere (Pattersen et al. 2001). Several other crops with allelopathic efficiency have been recently reviewed by Pannacci et al. (2017) and can be manipulated in intercropping against weeds.

14.6 Insect Management

Insects trace their host plant through the emission of specific volatiles by plants. However, if the diversity of plant species increases, the simultaneous emission of volatiles from more than two plants may hinder the location of host plant. In this

regard, intercropping is the best biological way to minimize loss in crop production due to insect attack. Moreover, the non-host plant can emit certain volatile organic compounds (VOCs) that are repellent to some insects. For example, the treatment of extracts of garlic (*Allium sativum* L), tansy (*Tanacetum vulgare*) L., and patchouli (*Pogostemon cablin* Blance) was found to be potential repellents to moth (Landolt et al. 1999). Onion (*Allium cepa* L.) can also be intercropped with base crops as it releases some sulfur containing organic compounds (thiols) that can repel several insects. Garlic has been widely accepted as an intercrop because of the ability of release of certain volatiles. Intercropping garlic with cabbage or other vegetable crops can significantly reduce the insect attack incidence and improve crop yield (Debra and Misheck 2014). Apart from vegetables, when garlic was intercropped with a cereal (i.e., wheat), the incidence of grain aphid (*Sitobion avenae*) significantly reduced, and wheat yield was increased (Zhou et al. 2011). Moreover, citrus-emitted volatiles disulfide and trisulfides (dimethyl trisulfide) inhibit the attack of Asian citrus psyllid (*Diaphorina citri*) (Mann et al. 2011).

Another mechanism involved in intercropping controlling insect incidence is that the VOCs released by non-host plant attract natural enemies of the pest insect. For example, the intercropping wheat with a non-host plant molasses grass corn has significantly increased the abundance of parasite *Cotesia sesamiae* and decreased the infestation of stem borer (Khan et al. 1997). In this study, molasses grass (*Melinis minutiflora*) emitted VOCs to attract the natural enemy of stem borer that reduced the level of its infestation. Similarly, intercropping some aromatic flowering plants like basil (*Ocimum basilicum* L.), French marigold (*Tagetes patula* L.), and ageratum (*Ageratum houstonianum*) in apple orchards hindered the *Aphis citricola* attack by shifting predator-prey abundances (Song et al. 2013). Similarly, Xu et al. (2018) found that releasing of E- β -farnesene and methyl salicylate in wheat/pea system significantly reduced the attack of aphids and promoted the abundance of its predators, i.e., ladybeetle (*Coccinella septempunctata*) and lacewings (*Chrysoperla carnea*). Flowering species are mostly intercropped with base crop because aromatic and colorful plants attract several insects foraging for nectar and pollen, while the architecture of flower provides the support in the form of shelter to combat insect pests (Walton and Isaac 2011). For example, the wasps and ladybeetle are attracted to colors and are the predators of several insect pests. Accordingly, results were found in kale (*Brassica oleracea*)/Apiaceae intercropping system where the abundance of natural predator *Lipaphis erysimi* increased in intercropping as compared to monocropped kale (Silva et al. 2016).

14.7 Soilborne Pathogen and Other Diseases

Intercropping provides diverse exosystemic effects and disease control is one of them. Different field experiments have proved that crop yield of intercropped species was mostly higher as compared to monocropping which was closely related to reduction in disease incidence (Bouws and Finckh 2008; Fajinmi and Odebo

2010; Lv et al. 2020). It was observed that intercropping oat with barley (*Hordeum vulgare*) significantly reduced the overall leaf spot caused by *Drechslera avenae* and rye with wheat reduced the leaf spot damage caused by *Rhynchosporium secalis* (Boudreau 2013). Similarly, wheat leaf rust caused by *Puccinia triticina* and strip rust caused by *Puccinia striiformis* were reduced by intercropping rye (Peng et al. 2006). Cereal/legume is a widely adopted system in many parts of the world due to diverse characteristics of leguminous crops such as N₂ fixation and pathogen management. A 5-year continuous experiment revealed that intercropped faba bean can decline powdery mildew of wheat by 26–49% (Chen et al. 2007). In addition, when barley was intercropped with lupin, the brown spot of lupin caused by *Pleiochaeta setosa* decreased extensively by 78–87% (Hauggaard-Nielsen et al. 2008). Similarly, the disease severity of *Ascochyta* blight in oats reduced up to 70% when intercropped with triticale and 82% when intercropped with faba bean (Fernández-Aparicio et al. 2010). In Nigeria, a series of different combinations of rows of sesame (*Sesamum indicum*) with maize revealed that the *Cercospora* leaf spot and *Alternaria* leaf blight of sesame was significantly reduced (Enikuomihin et al. 2011). Moreover, reduction in maize bushy stunt mycoplasma, maize rayado fino virus, and corn stunt Spiroplasma in intercropping system with beans has been reported (Castro et al. 1992; Shiu and Wu 2010).

Cassava mosaic disease (CMD) has caused severe economic loss and devastating famine mostly in African continents (Legg and Fauquet 2004). Intercropping cassava with different agronomic crops such as maize, beans, sweet potato (*Ipomoea batatas*), and sorghum has been proved to curb CMD and reduce its incidence to 29.5% as compared to monocropping (39.5%) (Night et al. 2011). Potato (*Solanum tuberosum*), another important agronomic crop, reduced the incidence of late blight caused by *Phytophthora infestans* during mixed cropping with faba bean or with cereal or grass-clover as boarder crops (Bouws and Finckh 2008). As compared to agronomic crop, less intensification has been given to horticultural crops in terms of disease control by increasing species diversity. However, there are some dominant examples that some lethal soilborne diseases can be arrested efficiently in intercropping of fruits or vegetables. Tomato is usually intercropped with onion and cucumber to reduce the disease incidence of *Verticillium dahliae* (Li et al. 2018) and tomato leaf curl, respectively, which was reduced up to 80% in intercropping as compared to monocropping (Mabvakure et al. 2016). In addition, interplanting marigold in tomato (*Lycopersicon esculentum* L.) rows has been found effective in controlling nematode infestations such as root-knot nematode (*Meloidogyne incognita*) (Tibugari et al. 2012). In chili pepper, reduction of *Phytophthora* blight (Zu et al. 2008) and *Pepper vein mottle virus* (Fajinmi and Odebode 2010) has been reported when intercropped with maize as compared to sole cropping. Other prominent examples of potential intercropping system may include watermelon (*Citrullus lanatus*)/rice (*Oryza sativa*) to curb *Fusarium* wilt (Su et al. 2008), cucumber/chili pepper (*Capsicum annuum*) to control cucumber root-knot nematode *M. incognita* (Dong et al. 2012), plantains/cassava (*Manihot esculenta*) to reduce black sigatoka of plantains caused by *Mycosphaerella fijiensis* (Emebiri and Obiefuna 1992), banana/marigold to sustain two nematode species *Radopholus similis* and

Helicotylenchus multicinctus (McIntyre et al. 2001), and grapes (*Vitis vinifera* L.) alfalfa (*Medicago sativa*) or white clover to control downy mildew (Ji et al. 2011).

The reduction of disease in intercropping could be because of inhibition of pathogen due to allelopathic effects, induction of plant systematic resistance against pathogen, or by control of insect attack due to non-host plants of component crop. Several plants secrete allelochemical with strong antimicrobial potential through root exudation or volatilization (Massalha et al. 2017; Li et al. 2020a, b; Tan et al. 2020). These allelochemicals play a critical role in depressing some deadly diseases of neighboring species. For example, release of some phenolic compounds in root exudates of non-host plant can inhibit spore germination of *Verticillium dahliae*, *Fusarium* spp., and *Cylindrocladium parasiticum* (Gao et al. 2014; Zhu and Morel 2019). Furthermore, some compounds with nematocidal potential, i.e., thiopurines and thiophenes in root exudates of *Asteraceae* spp., can reduce population of pathogenic nematodes of neighboring plants (Tsay et al. 2004). Similarly, crown daisy (*Chrysanthemum coronarium*) secretes lauric acid that first attracts nematode *Meloidogyne incognita* through chemotaxis which is carried out because of the presence of a neuromodulator peptide in nematode genes and then induces nematode death (Dong et al. 2014). In maize/pepper (*Piper nigrum*) intercropping system, the non-host plant maize attracts *Phytophthora capsici*, a soilborne pathogen of pepper, and then releases antimicrobial compounds [(2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H))] to kill the pathogen, thus protecting pepper plant (Yang et al. 2014; Bhat et al. 2017a, b). The strategy is called “attract and kill,” which is well-studied in the recent decade. Another mechanism underlying intercropping control plant diseases is activation of immune system of neighboring plant either through release of some chemical compounds to affect the expression of markers of immune systems or by perturbation of the environment (Zhu and Morel 2019). For example, the activity of phenylalanine ammonia lyase (PAL), a key biosynthetic catalyst, in watermelon was significantly higher upon infection when intercropped with wheat as compared to sole cropping (Xu et al. 2015; Dar et al. 2016; Mehmood et al. 2019).

14.8 Intercropping and Soil Sickness

Soil sickness is the rise of unfavorable and negative conditions in the soil for plant growth and development induced by the plant itself. It is a negative plant-soil feedback dates to the start of agriculture (Huang et al. 2013). However, persistent works started in the early twentieth century when researchers first studied phytotoxins in root exudates and plant litter and their effects of ion imbalance in the soil (Schreiner and Reed 1907). Since then, many soilborne pathogens in different agronomic and horticultural crops have been screened out in result of soil sickness.

When same crop is continuously cultivated on same piece of land, it starts degrading soil properties with every passing cropping season. The level of crop-specific phenolic compounds released from root exudates and crop residues

increases, and, after a specific period of time, it starts affecting crop plant growth through imbalance in soil nutrition, shift in microbial communities, and increase in disease incidence; the whole phenomenon is called “autotoxicity” (Khasi u Rahman et al. 2019). According to Ogwenyo and Yu (2006), the three main reasons of soil sickness are increase in soilborne pests, degradation in soil physiochemical properties, and autotoxicity. Several studies conducted on exogenously applied phenolic compounds have found that these phenolic compounds, at higher concentration, reduce abundance of plant beneficial microbiota, induce soilborne disease and negatively affect plant growth (Liu et al. 2017; Zhou et al. 2018; Wang et al. 2018; u Rahman et al. 2020). The main autotoxic compounds released through root exudations include phenols, naphthoquinones, aliphatic aldehydes, simple organic acids, coumarins, alkaloids, cinnamic and benzoic acids, long-chain fatty acids, lactones, cyanohydrins, purines, steroids, terpenoids, tannins, and others (Huang et al. 2013).

Soil microbial communities are important element of any agroecosystem, and shift in their composition may lead to strong changes in soil biochemical processes. Some microorganisms facilitate crop plant growth by mineral availability or by inhibiting pathogenic organisms. For example, the common mycorrhizal networks (CMN) formed by arbuscular mycorrhizal fungi (AMF) link plants for signal transport and also stimulate P acquisition. Similarly, some microbial species are producers of plant growth-promoting substances, i.e., cytokinin and indole-3-acetic acid (Khara and Arora 2010; Khanday et al. 2016). Studies have found that increase in plant-specific diversity tend to increase in plant beneficial bacterial and overall microbial abundance and diversity, while continuous sole cropping results in decrease in soil microbial diversity because of accumulation of autotoxins (Larkin 2003).

Intercropping is a practical way of increasing plant species diversity on same land that could decrease level of accumulated autotoxin and alter soil microbial community composition to facilitate crop plants. In maize/sorghum system, benzoxazinoids and strigolactones secreted by sorghum facilitate AMF and attract competitive colonizer *Pseudomonas putida* with plant beneficial characteristics (Neal et al. 2012). Intercropping marigold with Brassicaceae could be an efficient way to reclaim soil sickness by controlling some soilborne diseases and soil nematodes (Cohen et al. 2005). Similarly, Chinese chive (*Allium tuberosum*)-released compounds can inhibit bacterial wilt in many crop species. Overall, intercropping is an efficient and recommended method to recover soil sickness because increase in diversity of crop species does not allow autotoxins to accumulate, facilitates nutrient acquisition, alters soil microbial community composition, and lowers the soilborne disease incidence.

14.9 Conclusion

Intercropping is a cropping system with diverse features that can be utilized to increase crop yield in an eco-friendly way. Among several intercropping systems, cereal/legume is a prominent crop combination to increase soil N and P availability

and acquisition by plants. Moreover, selection of allelopathic species could be used to control weeds without use of chemical-based herbicides. The insect pests and several soilborne diseases can also be combat with intercropping by choosing certain agronomic or horticultural crops with respective potentials. In addition, soil sickness is a major issue because of the trend of continuous monocropping in different parts of the world, which can be solved by introduction of different crop species on same area of land. With so many features, we recommend intercropping to be adopted for the development of sustainable agriculture and eco-friendly agroecosystem especially in soil sickness areas.

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Chapter 15

Application of Phyllosphere Microbiota as Biofertilizers



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15.1 Introduction

In the current scenario of rapid population growth, agriculture plays a critical role to meet the food demands of human population. Time-to-time revolutions in agriculture, particularly the third revolution commonly referred to as the “Green Revolution”, resulted in significant crop production throughout the world. However, it encouraged the increased use of synthetic fertilizers that proved disastrous for our environment. In this regard, efforts have been made to produce fertilizers that ensure nutrient-rich foods along with maintenance of high-quality environment. In this course, the use of biofertilizers seems to be attractive, environmental-friendly, promising, and productive to fulfil the ever increasing demand of valuable natural products.

Biofertilizers are natural fertilizers consisting of living microbial inoculants of different species of bacteria, fungi, and algae, either alone or in combination, with the potential to mobilize essential nutrients in their most usable form through various bio-physiological processes. Plants harbour millions of the microbes, colonizing different plant parts and microbial load in each part having ample specific metabolic activities and physiological tasks linked to plant growth and fitness.

Phyllosphere being the set of photosynthetic leaves represents a most prevalent microbial niche influencing the plant survival and growth through the astonishing microbial interactions with their host plant (Vorholt 2012; Mendes et al. 2013; Bai et al. 2015; Peay et al. 2016; Khanday et al. 2016). The interaction between phyllosphere and its associated microbiota is not unidirectional. The host plant provides hospitable niche to microbial associates, and these microbes in turn alter plant’s well-being in favourable or detrimental ways. There is growing evidence that microbes present in phyllosphere contribute to plant health by not only lifting host

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plant's nutritional status but also providing pathogen defence and resistance to many against abiotic stresses (Arnold et al. 2003; Mendes et al. 2011; Vandenkoornhuyse et al. 2015; Busby et al. 2017; Bhat et al. 2018a, b; Mushtaq et al. 2018; Dar and Bhat 2020). Amidst current climate change, population explosion, as well as challenges in sustainable agriculture, these potential agricultural capabilities of phyllosphere microbiota could be utilized for development of biofertilizers to improve agricultural production.

15.2 An Overview of Plant Microbiota in Agriculture

Plant microbiota are the microbial component of the plant holobiont, associated with different plant surfaces and internal tissues of specific plant organs, viz. rhizosphere (Philippot et al. 2013), endosphere (Hardoim et al. 2008; Andreote et al. 2014; Berg et al. 2014), and phyllosphere (Vorholt 2012). As human microbiome is essential for well-being of every person (Quiza et al. 2015; Bhatti et al. 2017), likewise plant microbiome is critical for plant phenotype and health. From past more than a century now, microbial communities residing in various plant modules have shown the ability to contribute to indispensable plant-specific functions such as seed germination (Bragina et al. 2012; Truyens et al. 2015), nutrient supply (Tkacz and Poole 2015), protection of host from pathogens (Berendsen et al. 2012; Bulgarelli et al. 2013; Bhat et al. 2018a, b; Dervash et al. 2020), adaptation to various environmental challenges (Vandenkoornhuyse et al. 2015), and production of phytohormones and other bioactive metabolites (Zabetakis et al. 1999; Verginer et al. 2010; Shigenaga and Argueso 2016; Sofi et al. 2017). In regard to their agricultural importance, many beneficial plant-microbe interactions are well examined and explored. For example, numerous studies have reported that inoculation of rhizobia having potential to fix atmospheric nitrogen significantly increased the grain yields in several crop plants like lentil (Rashid et al. 2012), alfalfa and sugar beet (Ramachandran et al. 2011), groundnut (Sharma et al. 2011), and soybean (Grossman et al. 2011). Identically, *Pseudomonas putida* has been shown to enhance the rate of germination and promote growth of seedlings of cotton plants under alkaline and saline conditions (Yao et al. 2010). Weller et al. (2012) reported induction of systemic resistance against *Pseudomonas syringae* by *Pseudomonas fluorescens* in *Arabidopsis thaliana*. Similarly, *Piriformospora indica* (root endophyte) have also been shown to enhance plant growth, resistance against pathogens, and tolerance to various stresses (Delgado-Baquerizo et al. 2016). Likewise, there are ample studies showing the enormous agronomic impacts of plant-associated microorganisms.

Most of research has focused on use of single microbial inoculum with specific function. However, microbial consortia have more plant growth-promoting capability in comparison to a single microbe. For instance, the combined strains of *Rhizobium* and *Bacillus* were shown to improve root architecture and augment nodule formation in leguminous plants like bean, pigeon pea, and soybean (Checcucci et al. 2018). Berendsen et al. (2018) also showed microbial consortia containing

Xanthomonas sp., *Stenotrophomonas* sp., and *Microbacterium* sp. conferred better plant growth as compared to single inoculants in *Arabidopsis thaliana*. Similar results of different microbial combinations were also shown in grape vine, potato, tomato, and maize (Rolli et al. 2015; Molina-Romero et al. 2017; Berg and Koskella 2018; De Vrieze et al. 2018).

Moreover, different plant genotypes under varied environmental conditions behave differently with regard to microbial interaction (Da Costa et al. 2014; Thijs et al. 2014; Syranidou et al. 2016; Santos-Medellín et al. 2017; Bhat et al. 2017a, b). Therefore, it is highly crucial to consider the source, type, and habitat of microbial candidate chosen for inoculation under field conditions.

15.3 A Brief Outlook into Phyllosphere Microbial Diversity

The phyllosphere that is often defined as the aerial or the aboveground floral or vegetative parts, with leaves as the dominant part representing about 109 km² (Vorholt 2012), is recognized as a hospitable environment for colonization and continuity of microorganisms. Phyllosphere harbours diverse taxonomic groups of algae, bacteria, filamentous fungi, virus, and, less frequently, nematodes and protozoa (Andrews and Harris 2000; Hirano and Upper 2000; Lindow and Brandl 2003). Of the microbial load, bacteria are found to be most dominant colonizers, often found on an average of 10⁶ to 10⁷ cells/cm² of leaf tissue (Beattie and Lindow 1995; Hirano and Upper 2000; Andrews and Harris 2000; Lindow and Brandl 2003; Singh et al. 2020). The overall microbial richness of phyllosphere is quite high (Jumpponen and Jones 2009), but compared to rhizospheric bacterial communities, phyllosphere-associated communities are less diverse (Delmotte et al. 2009). Research conducted so far has shown proteobacteria, bacteroidetes, firmicutes, and actinobacteria as most prime bacterial lineages of phyllosphere (Ruinen 1965; Corpe and Rheem 1989; Furnkranz et al. 2008; Redford et al. 2010; Innerebner et al. 2011; Atamna-Ismaeel et al. 2012; Vorholt 2012; Watanabe et al. 2016). As far as fungi are concerned, the population of filamentous fungi can average between 10² and 10⁸ CFU/g of leaf, whereas population of yeast can range between 10 and 10¹⁰ CFU/g of leaf (Thompson et al. 1993; Inacio et al. 2002). Mostly, fungal taxa belonging to the phylum Ascomycota and Basidiomycota are found as major groups among the other important fungi on the leaf surfaces (Last 1955; Dickinson 1976; Shafi et al. 2018). However, distribution and functions of other microbial communities of phyllosphere have not been yet fully investigated.

The phyllosphere microbiota primarily colonize through air, soil, water, or from seeds (Vorholt 2012; Dar et al. 2013, 2016; Mehmood et al. 2019). Representing plant-environment interface, this microbial habitat is dynamic in nature, due to rapid fluctuations in environmental variables like radiation levels, daily temperature, humidity, and nutrient availability (Lindow and Brandl 2003; Vorholt 2012; Bhat et al. 2017a, b). Besides these oscillating factors, the microbial community composition is also structured by constant factors like geography and type of plant

species (Whipps et al. 2008; Redford and Fierer 2009; Redford et al. 2010). Consistent with this, Ding and Melcher (2016) demonstrated change in the composition, diversity, and dynamics of endophytic bacteria of tall grass, in accordance with change in collection time and location. Similar, variation in temperate tree leaf bacterial community structure on temporal and spatial scales was also shown by Laforest-Lapointe et al. (2016). Despite differences in such factors, individual plants in the same habitat have been shown to share some prevalent taxa known as “core” microbiome. Kembel and Mueller (2014) showed phyllosphere microbial community on leaves of Neotropical forest trees varies as a function of host plant traits. However, some bacterial clades including α , β , and γ proteobacteria, actinobacteria, and sphingobacteria were consistent in the samples, suggesting that these bacteria act as “core” of phyllosphere microbiota on these Neotropical trees. Similar constant bacterial “core” consisting of *Arthrobacter*, *Bacillus*, *Massilia*, *Pantoea*, and *Pseudomonas* was also distinct in laboratory- and field-grown Roman lettuce plants irrespective of variation in time, space, and environment (Rastogi et al. 2012).

15.4 Role of Phyllospheric Microbiota

The use of advanced culture-independent molecular techniques has defined phyllosphere as the earth’s largest environmental surface area of microbial habitation (Lindow and Brandl 2003; Vorholt 2012; Peñuelas and Terradas 2014). The inhabitant complex microbial consortia can have positive (mutualistic), neutral (commensal), or negative (pathogenic) influences on their host plant, and through their complex plant-microbial interactions can contribute greatly to plant growth and robustness (Vorholt 2012; Bulgarelli et al. 2013; Brader et al. 2017) and consequently to ecosystem productivity. Recent studies have shown phyllosphere-associated microorganisms as important characters in multiple ecophysiological processes such as plant signalling (Shiojiri et al. 2006), bioremediation of environmental pollutants (Sgueros 1955; Sandhu et al. 2007; Nakamiya et al. 2009; Yutthammo et al. 2010; Nadalig et al. 2014), carbon sequestration (Bringel and Couee 2015), climate regulation (Otte et al. 2004; Peñuelas and Staudt 2009; Schäfer et al. 2010), and in global carbon, nitrogen, and other nutrient cycles (Furnkranz et al. 2008; Knief et al. 2012). Besides, it has also proved as best suited model for theoretical ecological studies (Finkel et al. 2012; Meyer and Leveau 2012).

15.4.1 Agro-based Functions

The current omic techniques (metagenomics, proteomics, metabolomics) helped not only in revealing the enormous microbial diversity residing in the phyllosphere but also in identifying and understanding the mechanisms and behaviour of microbe-microbe and plant-microbe interactions that helped in recognition of the expected

benefits of phyllosphere microbiome in plant science in general and agriculture in particular. There are immense evidences for phyllosphere microflora interaction in crop plants affecting fitness, quality, and output (Fig. 15.1). Among the multitude of functions performed by microbes in phyllosphere, one of the key roles is plant nutrition acquisition through various processes like fixation of atmospheric nitrogen (Bentley and Carpenter 1984; Giri and Pati 2004), solubilization of phosphorous (P) (Mwajita et al. 2013; Batool et al. 2016; Thapa et al. 2017), and siderophore production (biofortification of Fe mediated by microbes in different crops) (Scavino and Pedraza 2013; Fu et al. 2016; Thapa et al. 2018). The other important phyllosphere microbe-mediated functions include enhanced plant growth through production of plant growth regulators [like IAA (Spaeppe et al. 2007), cytokines (Holland 2011), abscisic acid (Cao et al. 2011)], biological control of phytopathogens (Elad 1996; Zipfel et al. 2004; Innerebner et al. 2011) through many ways like non-pathogenic microbe-mediated induction of immune response of plant, competitive exclusion of pathogen by non-pathogenic microorganisms, or antibiotic production. Besides this, phyllosphere microorganisms have been also found to promote desiccation tolerance to host plants against environmental stressors (Lindow et al. 1982a, b;

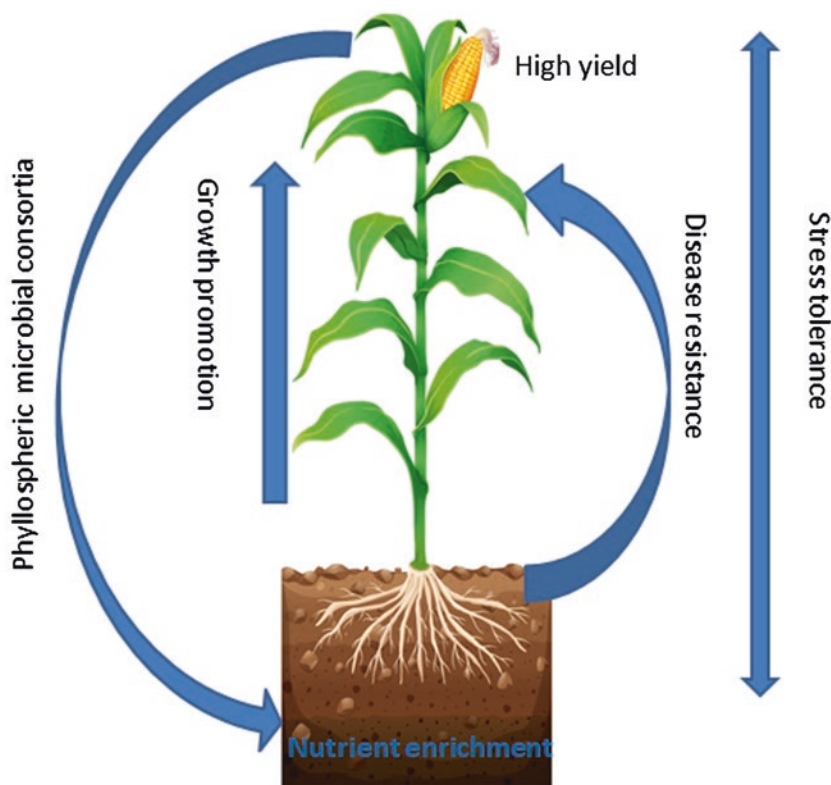


Fig. 15.1 Agro-based roles of phyllosphere microbiota

Wisniewski et al. 1997; Attard et al. 2012; Hubbard et al. 2014; del Rocío Mora-Ruiz et al. 2015), through production of various active metabolites and through bioremediation of harmful chemical compounds (Moulas et al. 2013; Sandhu et al. 2007; Waight et al. 2007; Yang et al. 2011; Ning et al. 2012; Xie et al. 2014).

Rice (*Oryza sativa* L.) is a major staple food crop in many developing countries, nourishing about half of the world's human population with 20% of direct intake of calories (Zeigler and Barclay 2008). Although many new cultivars of rice have been developed, it is facing decrease in yield due to several biotic and abiotic factors. Thus, for achieving sustainable rice yield, the amalgamation of beneficial plant microbes into agricultural productions is trending over the past few years. In this concern, a large number of studies have confirmed the influence of isolated colonizers of rice phyllosphere on yield, growth promotion, and pathogen inhibition (Maliti et al. 2005; Pedraza et al. 2009; Shamima Akter 2015, Thapa et al. 2017). Similarly, in other widely grown economical crop corn (*Zea mays* L.), the yield is limited by leaf disease called southern corn leaf blight (SCLB), caused by fungus *Bipolaris maydis*. Recent studies have shown the correlation between the increased severity of disease and decreased epiphytes of corn leaf (Manching et al. 2014). In other study, Marques et al. (2010) determined growth-promoting ability of six bacterial isolates on maize plant, and all of them have been reported to produce indole acetic acid (IAA), ammonia, and hydrogen cyanide (HCN), in both in vitro and greenhouse experiments.

Similarly, in a wide range of important crops like sugarcane, tomato, pigeon pea, mustard, potato, radish, etc., the beneficial phyllosphere microbes with multifold functional traits related to plant growth promotion and development have been documented, characterized, and well tested in laboratory. However, their field application is still lacking.

Various potential agro-based functions of the different microbial types isolated from phyllosphere of different plant sources are discussed in Table 15.1. This information can be used for development of useful bioproducts for agricultural purposes.

15.5 Conclusions and Future Prospects

Universal organic food production must be increased to meet the demand of rising consumers globally. Many countries have developed policies to reduce dependency on conventional chemical fertilizers owing to their hazardous effects on environmental health. In this context, phyllosphere microorganisms with multifunctional properties assume special significance. The phyllosphere represents the rich biohome of diverse molecular and chemical compounds in nature and often influences broad aspects of plant biology. The exploration of microbial diversity and their likely key roles on phyllosphere are acknowledged widely. There is a plethora of studies showing almost all the important crop functions including growth, production, utilization of essential nutrients, and immunity against various diseases are contributed by these microbial communities through many diverse

Table 15.1 Potential agro-based functions of phyllosphere microbiota associated with diverse host plants

S. No.	Plant source	Microorganism	Function	Reference
1.	Alfalfa (<i>Medicago sativa</i>)	<i>Methylobacterium</i> sp.	Increased IAA production	Omer et al. (2004)
2.	Apple (<i>Malus pumila</i>)	<i>Pseudomonas fluorescens</i>	Competitive exclusion of <i>Pseudomonas syringae</i> in apple and pear	Lindow et al. (1996), Stockwell and Stack (2007)
3.	Aquatic plants	<i>Pseudomonas aeruginosa</i>	Stress tolerance	Costerton et al. (1994)
		Bacteria	Arsenite oxidation	Xie et al. (2014)
4.	Bean (<i>Phaseolus vulgaris</i>)	<i>Methylobacterium</i> sp.	Enhanced plant growth promotion by increased activities of antioxidant enzymes – catalase (CAT) and superoxide dismutase (SOD)	El-Gawad et al. (2015)
5.	Chaparral (<i>Larrea divaricata</i>)	Ammonifiers Nitrifiers	Nitrogen fixation	Abril et al. (2005)
6.	Cotton (<i>Gossypium herbaceum</i>)	<i>Methylobacterium</i> sp.	Enhanced plant growth promotion by increased activities of antioxidant enzymes – catalase (CAT) and superoxide dismutase (SOD)	El-Gawad et al. (2015)
7.	Datura (<i>Datura innoxia</i>)	<i>Methylobacterium</i> sp.	Enhanced plant growth promotion by increased activities of antioxidant enzymes – catalase (CAT) and superoxide dismutase (SOD)	El-Gawad et al. (2015)
8.	Grape vine (<i>Vitis vinifera</i>)	<i>Bacillus</i> sp. <i>Staphylococcus</i> sp.	Antagonistic activity against pathogen <i>Botrytis cinerea</i>	Vionnet et al. (2018)
9.	Groundnut (<i>Arachis hypogaea</i>)	<i>Methylobacterium</i> sp.	Enhanced growth, seedling vigour and yield and induced immunity against rot pathogens <i>Aspergillus niger</i> and <i>Sclerotium rolfsii</i>	Madhaiyan et al. (2006)

(continued)

Table 15.1 (continued)

S. No.	Plant source	Microorganism	Function	Reference
10.	Mesquite (<i>Prosopis flexuosa</i>)	Ammonifiers Nitrifiers	Nitrogen fixation	Abril et al. (2005)
11.	Mistol (<i>Ziziphus mistol</i>)	Ammonifiers Nitrifiers	Nitrogen fixation	Abril et al. (2005)
12.	Mouse-ear cress (<i>Arabidopsis thaliana</i>)	<i>Burkholderia phytofirmans</i>	Enhanced photosynthetic rate, sugar uptake, root enlargement, and induced cold stress tolerance through production of proline and phenolics	Barka et al. (2006)
		<i>Sphingomonas</i> strains	Exclusion of pathogen <i>Pseudomonas syringae</i>	Innerebner et al. (2011)
13.	Mustard (<i>Brassica nigra</i>)	<i>Methylobacterium</i> sp.	Increased germination percentage and enhanced seedling growth	Meena et al. (2012)
		<i>Methylobacterium extorquens</i>	Promoted plant growth via production of indole acetic acid (IAA) and also enhanced seed germination and SVI	Pattnaik et al. (2017a)
14.	Peach (<i>Prunus persica</i>)	<i>Methylobacterium extorquens</i>	Promoted plant growth via production of indole acetic acid (IAA) and also enhanced seed germination and SVI	Pattnaik et al. (2017b)
15.	Pigeon pea (<i>Cajanus cajan</i>)	<i>Methylobacterium</i> sp.	Increased germination percentage and enhanced seedling growth	Meena et al. (2012)
16.	Potato (<i>Solanum tuberosum</i>)	<i>Methylobacterium</i> sp.	Increased germination percentage and enhanced seedling growth	Meena et al. (2012)
17.	Quebracho blanco (<i>Aspidosperma quebracho-blanco</i>)	Ammonifiers Nitrifiers	Nitrogen fixation	Abril et al. (2005)

(continued)

Table 15.1 (continued)

S. No.	Plant source	Microorganism	Function	Reference
18.	Radish (<i>Raphanus sativus</i>)	<i>Methylobacterium</i> sp.	Increased germination percentage and enhanced seedling growth	Meena et al. (2012)
19.	Rice (<i>Oryza sativa</i>)	Methylotrophic bacteria	Growth promotion	Maliti et al. (2005)
		<i>Azospirillum brasilense</i>	Improved yield	Pedraza et al. (2009)
		Fungal antagonists	Controlled sheath blight incidence by inhibiting the growth of <i>Rhizoctonia solani</i>	Shamima Akter (2015)
20.	Red clover (<i>Trifolium pratense</i>)	<i>Methylobacterium</i> sp.	Growth promotion via increased production of IAA	Omer et al. (2004)
21.	Snap broad bean (<i>Vicia faba</i>)	<i>Methylobacterium</i> spp.	Enhanced plant growth promotion by increased activities of antioxidant enzymes – catalase (CAT) and superoxide dismutase (SOD)	El-Gawad et al. (2015)
22.	Spruce trees	Chemolithoautotrophic ammonia oxidizing (CAO) and nitrite oxidizing (CNO) bacteria	Nitrogen fixation	Papen et al. (2002)
23.	Spinach (<i>Spinacia oleracea</i>)	<i>Bacillus</i> sp. <i>Pseudomonas</i> sp.	Inhibition of pathogen <i>Escherichia coli</i>	Lopez-Velasco et al. (2012)
24.	Strawberry (<i>Fragaria ananassa</i>)	<i>Methylobacterium zatmanii</i>	Promoted plant growth via production of indole acetic acid (IAA) and also enhanced seed germination and SVI	Pattnaik et al. (2017b)
25.	Sugarcane (<i>Saccharum officinarum</i>)	<i>Methylobacterium extorquens</i>	Accelerate germination, growth, and yield	Madhaiyan et al. (2005)
		<i>Methylobacterium</i> sp.	Increased germination percentage and enhanced seedling growth	Meena et al. (2012)
		<i>Pantoea</i> genus	Nitrogen fixation	Loiret et al. (2004)
26.	Tiny duckweed (<i>Wolffia australiana</i>)	Bacteria	Arsenic oxidation	Xie et al. (2014)

(continued)

Table 15.1 (continued)

S. No.	Plant source	Microorganism	Function	Reference
27.	Tobacco (<i>Nicotiana tabacum</i>)	<i>Achromobacter</i> sp. <i>Achromobacter</i> sp. <i>Alcaligenes</i> sp. <i>Bacillus</i> sp. <i>Enterobacter</i> sp. <i>Ochrobactrum</i> sp. <i>Pseudochrobactrum</i> sp. <i>Pseudomonas</i> sp. <i>Stenotrophomonas</i> sp.	Pathogen exclusion of <i>Pseudomonas syringae</i> pv. <i>tabaci</i>	Qin et al. (2019)
28.	Tomato (<i>Lycopersicon esculentum</i>)	<i>Sphingomonas</i> sp.	Growth promotion	Enya et al. (2007)
		<i>Stenotrophomonas</i> sp. <i>Pseudomonas</i> sp.	Promoted defence response and accelerated systemic resistance	Bringel and Couee (2015)
		<i>Bacillus</i> sp.	Suppression of <i>Botrytis cinerea</i> plant pathogen	Kefi et al. (2015)
		<i>Methylobacterium</i> sp.	Promoted plant growth nutrient uptake, seed germination, and SVI	Senthilkumar and Krishnamoorthy (2017)
29.	Spiny hackberry (<i>Celtis pallida</i>)	Ammonifiers, nitrifiers	Nitrogen fixation	Abril et al. (2005)
30	White clover (<i>Trifolium repens</i>)	<i>Methylobacterium</i> sp.	Increased production of growth hormone IAA	Omer et al. (2004)

mechanisms. Still, their utilization as biofertilizers in field conditions has not been wholly harnessed until now. The possible reasons could be the knowledge gap among the ecologists and agriculturalists regarding protocols of biofertilizer application, nonavailability of biofertilizers in markets, lack of awareness at cultivator level regarding usage and profitability, and absence of a supportive regulatory and policy framework. Thus, the success related to application of phyllosphere microbiota as biofertilizers requires integrated approach of microbiologists, agricultural advisors, manufacturers, farmers, and law-makers, to accustom plant growers regarding these innovative and eco-friendly products and promote their widespread usage.

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Chapter 16

Biofertilizers: A Viable Tool for Future Organic Agriculture



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16.1 Introduction

The substances that have living microbes and reside in the area surrounding roots, i.e. rhizosphere, and are involved in enhancing the supply of nutrients to plants are known as biofertilizers. They include everything ranging from manures to those of plant extracts. The species of bacteria living in the rhizosphere of plant roots are together known as plant growth-promoting bacteria abbreviated as PGPR. This PGPR serves as the source of many biofertilizers. The biofertilizers are mainly derived from phosphate solubilizers, mycorrhizae, and nitrogen fixer groups of PGPR. Biofertilizers are one of the best ways to provide nutrients to plants without damaging the environment or health of soils and humans. As the biofertilizers are living formulations of beneficial microbes, they can be used to amend soil, root, or seed equally by making the availability of these microbes. This improves the health of the soil. The biofertilizers have living cells that make nutrients available, which

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are not available generally to the plant (Ismail et al. 2013). Many microbes that increase plant growth are involved in regulating many processes of soil. These processes include decomposition of the OM and the availability of different plant nutrients like potassium, iron, phosphorus, magnesium, and nitrogen, enhancing the growth of plants (Dar et al. 2013; Lalitha 2017). The steps involving the production of biofertilizers are the following:

- The first step is the separation of microbes from the soil.
- Screening of microorganisms in the lab for plant growth.
- Greenhouse screening of microorganisms in pots filled with soils to enhance growth in them.
- The screening in the field of those the most effective microorganisms in cropped soil.
- Inoculum refining.
- Production of biofertilizers.

The biofertilizers are prepared by selection of most efficient bacterial strains that are passed by various steps as mentioned above (Bhattacharjee and Dey 2014). Organic farming has gained much attention throughout the world because it is environment-friendly and enables the growth of healthy and safe food free from agrochemicals. It is tough to grow crops without synthetic chemicals for the growing population of the world. But there exist many opportunities where organic agriculture can be promoted. Biofertilizers play a crucial role in organic farming. The microbes have a basic role in fixation of the atmospheric N, which is very crucial in enhancing the fertility of the soil. These microbes also convert insoluble phosphates to soluble, making them available for plants. Biofertilizers have shown a great impact on the mobilization of nutrients in the soil (Venkateshwarlu 2008).

16.2 Biofertilizer Scope

The use of synthetic fertilizers has caused a lot of problems. Pesticides and fertilizers have contaminated water, air, and soil. Their misuse has killed crop-friendly worms and destroyed a beneficial population of microbes in the soil. The prices of fertilizers are increasing day by day because of the depletion of fossil fuels. This is affecting the input costs. The fertility and productivity of the soil are also decreasing due to the increasing gap between the supply and removal of nutrients. Biofertilizers have great potential to cope with this situation. The selection of P-solubilizing and nitrogen-fixing strains according to the environment of the soil is critical to understand. The symbiotic relation between microbes and plants in different agroclimatic conditions is also a great interest for many researchers. Biofertilizers, in combination with fertilizers, are the best option for achieving the goal of maximum yield. A lot of work is being done on formulating new types of biofertilizers. The powdered and liquid formulations are its best examples (Mahdi et al. 2010; Mushtaq et al. 2018; Shafi et al. 2018).

16.3 Organic Agriculture History

Sir Albert Howard is considered the first known person who gave the concept of organic agriculture. He published a book *An Agricultural Testament* in 1943 in which he gave the basic idea of organic farming. He stated that organic waste can be recycled and gave the process like present-day composting. The first person to use term organic for farming was Walter Northbourne. He explained his concept of making a farm a whole organic system in his book *Look to the Land*. Howard may be called a polarizing personality. The era from 1940 to 1978 might be known as the era of polarization of agriculture into organic and non-organic. The farmers of the inorganic approach ignored organic farming, but all the universities and research institutes continued to conduct researches on organic farming. Organic farming was recognized at the national level in the USA from 1979 to 1990. The USDA and universities did a lot of work during this period on organic farming. In the beginning, the standards proposed by the USDA allowed the usage of food irradiation, sewage sludge, and GMOs. But due to the public pressure, they were taken back. The USDA gave the certification of organic foods in 2002 (Heckman 2006).

16.4 Why Organic Farming?

Apart from the adverse impacts of chemical fertilizers on soil and environment, the fertilizer factories are one of the primary sources of air, water, and land pollution. The groundwater of the areas where these factories are situated is reported to be contaminated (Zakharova et al. 2002; Mehmood et al. 2019).

16.4.1 Environmental Benefits

The pesticides being used by farmers across the world are damaging environment in many ways. These pesticides are one of the significant threats to the environment. Their misuse or overuse is very dangerous to the environment as well as the plant population of that area (Kumar and Puri 2012; Dar et al. 2016; Dar and Bhat 2020; Dervash et al. 2020). It is very crucial in this decade of the twenty-first century to put forward environment-friendly alternatives to control the pests. The microbes can be used for degrading these pesticides. There are some bacteria that can reduce the toxicity caused by pesticides. These bacteria are from genera *Klebsiella*, *Azospirillum*, *Pseudomonas*, *Bacillus*, *Gordonia*, *Azotobacter*, *Serratia*, and *Enterobacter* (Shaheen and Sundari 2013). The enzymes have a key role in lysis. These are mostly produced by the microorganisms. Hydrolyses and esterase are one of the most vital among them. The glutathione S-transferases and oxidases have a mixed function in the degradation of the pesticides (Ortiz-Hernández et al. 2013; Sofi et al. 2017; Bhat et al. 2018a; Singh et al. 2020).

Tuomisto et al. (2012) state that organic farming had far less negative influence on the environment in comparison to conventional farming. The way farmers manage their farms is also significant in finding the impacts of conventional and organic farming on the environment. No single farming system can achieve the target yield. An optimal farming system involves the combination of both farming systems. The adoption of this optimal system depends upon the prices of that area. Some systems are designed to meet the specific targets, while others aim to work under sensitive environments. A policy is needed to design the optimal farming system.

16.4.2 Economic Benefit and Profitability

Organic farming systems are more cost-effective than traditional farming systems. Four factors are involved in improved income. The first factor involves the direct links of the farmers with consumers. Many farmers growing their commodities organically provide home delivery for their customers. This eliminates the role of a middleman. Second is that operational expenses of organic farming are one third less in terms of pesticides, chemicals, and energy. There are low input costs in growing cereals organically as low as 50–60%. The input expenses of horticultural and dairy products are 10–20% and 20–25%, respectively. The third factor is the premium price for organic foods in the market. Farmers get an excellent price of their organic produce. The fourth one is that organic farms are very resilient to the changing weather conditions in comparison to conventional farms. The adoption of organic farming improves the financial condition of the rural community as a whole. The on-farm sales of organic farmers are also higher as compared to conventional farmers. Their dependence on off-farm sales is low, and they mostly make money through direct marketing. The wages at the organic farms are much higher as compared to traditional farms that improve the economic condition of local tenants. Some disruptions are faced in the beginning when a farm shifts from conventional to organic farming. These disruptions require extensive research (MacRae et al. 2007).

16.4.3 Health Benefits

Over the last few decades, organic farming and organic foods have gained much popularity all over the world. The key difference between organic and traditional food products lies in its technique of growth. The certifications of organic foods vary among different regions of the world. The organic foods are featured by their strict constraint against synthetic fertilizers and pesticides. The phenolic compounds are produced in the plants by not using pesticides on them (Simonne et al. 2016; Bhatti et al. 2017).

The concentration of vitamin C is found much higher in foods obtained from organic plants as compared to inorganic foods. The organically produced yellow plums, carrots, sweet peppers, and tomatoes have more carotenoids than conventionally grown vegetables. More essential amino acids are present in organic foods (Popa et al. 2019; Bhat et al. 2017; Khanday et al. 2016). The milk produced organically has more amounts of long-chain fatty acids, conjugated linoleic acid, long-chain fatty acids, and alpha-linolenic acid. The organic bovine milk also has more desired fatty acids having higher levels of iron and alpha-tocopherol (Średnicka-Tober et al. 2016).

16.4.4 Organic Foods and Market

The demand for organic food is increasing around the globe. The revenues of organic drinks and food have increased in all regions of the world, but the significant growth in revenues is seen in North America and Europe. A shortage of supply is also seen in many sectors of organic products. These mostly include organic milk and yoghurt, organic meat, organic fruits, and some beverages. The demand for organic foods comes from the most developed countries. G7 countries account for a major import of organic foods. The Asian countries must develop internal markets and must have a check on exports of organic foods ([Organic Monitor](#)).

16.4.5 Organic Food for Sustainable Agriculture and Soil Fertility Management

Sustainable agriculture is not a specific methodology. It is a broad term. It includes many technologies and practices. Its recognition all over the world shows that traditional agriculture is not enough to meet the challenges of food security of the world. Traditional farming is facing the issue of low yield and high expenses of inputs. Monoculture farming has led to decreased soil fertility, destruction of beneficial microbes and insect populations, and vitality of the soil. The extra utilization of pesticides and fertilizers, the high energy consumption for tillage, and high costs of irrigation are one of the major concerns for agriculture.

Organic farming has a key role in sustainable agriculture. The traditional agriculture is exhausting natural resources at a very rapid pace. It is crucial that farmers must understand the ways of conserving their lands by using biofertilizers and other amendments. It is observed that the soil's beneficial microbes are not damaged in any agroecological zones when no pesticide is used. The low agricultural yields are linked to many biological, environmental, and physiological factors. These factors are improved by organic farming. The crops grown organically have shown good resistance against diseases. The selection of agricultural practices and technologies must be selected by keeping the concept of sustainable agriculture in mind. Organic

foods are vital for food security and sustainable agriculture. Sustainable agriculture has a close link with improved soil fertility and productivity. Microbes have a crucial role in it. The plants can get nutrients efficiently from the soils having PGPR. The nutrient cycles become more efficient as microorganisms are involved in mineralization, decomposition, and accessibility of nutrients. The microbes are involved in the degradation of SOM. That is why they directly influence the WHC of the soil, soil acidity and toxicity, CEC, and P, N, and S reserves (Singh et al. 2011).

The farm either organic or traditional may be called sustainable when it produces high-quality food in adequate amounts, is economically viable, and participates in the welfare of the farmer community. The yield of organic foods is low than traditional foods. The most closing yield of organic farming with traditional farming is of soybean, rice, and corn. But the quality of organic foods is much higher (Reganold and Wachter 2016).

16.5 Causes of Low Adoption of Organic Farming

The importance of organic agriculture is being realized by farmers, policymakers, intellectuals, practitioners, academicians, and sensitive citizens. Despite the realization of the environmental, health, social, financial, or personal benefits linked with organic agriculture, the adoption of organic agriculture is restricted due to many challenges. The understanding of the factors that stand as barriers in the adoption of organic farming is important. In this section, all the possible difficulties faced by the organic respondents which hamper the extent of adoption of organic farming practices were grouped into five major categories, viz. infrastructural, economic, technological, socio-psychological, and educational constraints (Jangid et al. 2012; Bhat et al. 2018a). There are certain factors responsible for the adoption of organic agriculture in this era and are shown in Fig. 16.1 with respect to region.

16.6 Organic Agriculture and Biofertilizers

Natural farming/agriculture is characterized as “a creation framework that supports the strength of soils, biological systems and individuals. It depends on natural procedures, biodiversity and cycles adjusted to neighbourhood conditions, as opposed to the utilization of contributions with unfriendly impacts. Natural Horticulture joins convention, advancement, and science to profit the common condition and advance reasonable connections and a decent personal satisfaction for all included” (IFOAM). The Global Alliance for Natural Farming Development’s (IFOAM) meaning of natural agribusiness depends on the standard of well-being, the rule of biology, the guideline of decency, and the rule of care. Natural cultivating is one of the manageable horticultural frameworks and depends less on costly imports, for example, concoction manures and pesticides (Ramesh et al. 2005). Scofield (1986)



Fig. 16.1 Factors with respect to area that hinder in organic agriculture adoption

underscored that natural cultivating does not just confine to the usage of living materials yet weights on the concept of completeness, inferring the precise co-appointment of parts in a single entirety. The point of natural cultivating is to make incorporated, others conscious, earth and financially practical creation frameworks, which amplify dependence on ranch determined sustainable assets and the

Groups	Example	References
<i>N₂-fixing biofertilizers</i>		Vessey (2003)
Free living	Azotobacter, Beijerinckia, Clostridium,	
Symbiotic	Rhizobium, Frankia, Anabaena azollae	
Associative symbiotic	Azospirillum	
<i>P-solubilizing bio-fertilizers</i>		Khan et al. (2009)
Bacteria	Bacillus megaterium var. phosphaticum,	
Fungi	Penicillium spp., Aspergillus awamori	
<i>P mobilizing bio-fertilizers</i>		Jha et al. (2012)
Arbuscular mycorrhiza	Glomus spp., Gigaspora spp., Acaulospora spp.,	
Ectomycorrhiza	Laccaria spp., Pisolithus spp., Boletus spp.,	
Ericoid mycorrhiza	Peizizella	
Orchid mycorrhiza	Rhizoctonia solani	
<i>Bio-fertilizer and micronutrients</i>		Singh et al., (2016)
Silicates and zinc solubilizers	Bacillus spp. Thiobacillus thiooxidans	
<i>Plant growth-promoting rhizobacteria</i>		Mehrvarz et al. (2008)
Pseudomonas	Pseudomonas fluorescens	Bhattacharjee and Dey (2014)

Fig. 16.2 Groups of biofertilizers based on their nature and function

administration of environmental and organic procedures and connections, in order to give adequate degrees of yield, animals and human sustenance, insurance from irritations and infection, and a proper come back to the human and different assets (Lampkin 1994; Bhat et al. 2017).

Bio-composts are being basic segment of natural cultivating. Biofertilizers are generally used to quicken those microbial procedures which expand the accessibility of supplements that can be handily acclimatized by the plants. They enhance soil richness by fixation of the environmental N and solubilizing insoluble phosphates (Mazid and Khan 2015). These biofertilizers have been raised for the collection of the normally available natural arrangement of supplement assembly which colossally forms soil richness and crop yield (Pandey and Singh 2012). These days biofertilizers are more by and by for crop creation; different sorts of biofertilizers are accessible in the market (Fig. 16.2).

16.7 Potential Role of Biofertilizers in Agriculture

The consolidation of biofertilizers (N fixers) assumes a significant job in enhancing soil ripeness and yield crediting characters, and in this manner, the last yield has been accounted for by numerous labourers. Furthermore, their implementation in soil enhances soil biota and limits the exclusive utilization of concoction composts. Under calm conditions, immunization of Rhizobium improved number of cases plant-1, number of seed case 1 and 1000-seed weight (g) and in this way yield over

the control. In rice under swamp conditions, the use of BGA+ *Azospirillum* is fundamentally helpful in improving LAI and all yield ascribing angles. The effectiveness of phosphate manures is low (15–20%) because of its obsession in acidic and antacid soils, and shockingly both soil types are prevailing in India bookkeeping over 34% causticity influenced and in excess of 7,000,000 hectares of profitable land saltiness/basic influenced. Hence, the vaccinations with PSB and other valuable microbial inoculants in these soils become obligatory to re-establish and keep up the compelling microbial populaces for solubilization of synthetically fixed phosphorus and accessibility of other large scale and micronutrients to reap great practical yield of different harvests (Mishra et al. 2013).

16.8 Advantages of Biofertilizers

Biofertilizers are an inexhaustible wellspring of supplements, support soil well-being, and increment the grain yields by 10–40%. Use as supplement concoction composts and supplant 25–30% substance manures, break down plant build-ups, and balance out C:N proportion of soil, improve surface, structure and water holding limit of the dirt, animates plant development by emitting development hormones and has no unfavourable impact on plant development and soil richness, solubilize and prepare supplements. Biofertilizers are an eco-accommodating, non-toxic, and financially savvy technique (Kawalekar 2013).

16.9 Limitations of Biofertilizers

Non-accessibility of proper and proficient strains of microbes. Absence of appropriate transporter, because of which timeframe of realistic usability is short, is another limitation. Showcasing of bio-manure is not simple as the item contains living beings, regular interest and creation of bio-composts, shortage and feasibility of VAM inoculum during capacity, and transportation is the serious issue. Absence of consciousness of ranchers and insufficient and unpractised staff are the other limitations (Kawalekar 2013).

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