Chapter 1
Let's Protect Our Earth: Environmental Challenges and Implications

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Abstract Microbial enzymes play a vital role to maintain the soil health and removal of pollutants from the contaminated land. Soil microflora is closely associated to maintain the fertility of the soil. Use of chemical pesticides, fertilizers and other volatile sprays in the agricultural practices threatening the healthy microbial population in the soil. Every single particle of healthy soil is loaded with millions of bacteria which interact with the nutrients available in the surrounding and sustain the nutrient cycle, and this microflora is an essential component of life on earth. The rapid increase in the industrialization and urbanization polluted the water and air heavily which affected the microbial populations and their existence too. Some microbes have been evolved to breakdown the complex toxic pollutants entering the soil into non-harmful components and helping to maintain the soil fertility. Thus, it is urgently needed to identify these microorganisms and enzymes which are involved in restoring the remediation of toxic substances and restoration of microflora required for a normal life.

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1.1 Introduction

In recent years, an increase in population growth and rapid industrialization not only ameliorate the standard of life but also affected the quality of the environment. Due to the release of harmful pollutants into the ecosystem such as plastics, pharmaceutical ingredients, greenhouse gases, pesticides, and synthetic dyestuffs, every part of the earth has severely affected. These pollutants not merely caused the teratogenic, carcinogenic, mutagenic, and toxic effects on humans beings or organisms but also created a serious risk to the environment (Jacob et al. 2018; Liu et al. 2019). The heavy metal ions from the contaminated land enter in the crop products and edible vegetable fruits or in the fish or aquatic organism from contaminated water which ultimately reaches in the human body. The continuous flow of these poisonous substances or metal impurities allows them to accumulate inside the human body and alter the normal microflora. All microbial genera, bacteria, fungi, algae, nematode, and protozoa play a significant role in bioremediations and maintaining soil health. To meet the energy crisis and food demand with the growing population, it is very important to save the agricultural land from contamination and maintain productivity.

These pollutants are broadly dispersed everywhere in the environment because of various human activities and industrial processes (Bilal et al. 2019b; Rasheed et al. 2019). Numerous methods such as filtration, reverse osmosis, incineration, lagooning treatment, landfill deposition, and bioremediation using microbes and their enzymes have been applied to treat harmful pollutants (Bilal et al. 2019a; Kuppusamy et al. 2017). The major advantages of using microbial enzymes for the degradation of environmental pollutants are high efficiency, minimum by-products, no secondary pollution, economic feasibility, and environmentally safe (Garcia-Garcia et al. 2016).

Various bacterial genera were used in bioremediation that can convert pollutant into less toxic compounds: *Pseudomonas* sp., *Achromobacter* sp., *Burkholderia* sp., *Rhodococcus* sp., *Ralstonia* sp., *Alcaligenes* sp., *Sphingomonas* sp., *Dehalococcoides* sp., and *Comamonas* sp. that ultimately reduce pollutants (Lloyd et al. 2003). However, highly diverse and specific microorganisms present in nature efficiently remove the several pollutants. But microbial-based remediation is usually slow, as compared to the daily production of a huge amount of waste which causes the pollutants to accumulate in the environment. Nevertheless, molecular biology allows producing novel strains of the microorganism with desirable features for the bioremediation process, thus considerably improving the degradation capability of pollutants (Zhao et al. 2017). Microbes play a very important role in nutrient cycles by intracellular digestion of complex macromolecules and converting these into smaller units in their metabolic activities. Secondly, the enzyme secreted in the extracellular environment facilitates the conversion of complex macromolecules into micromolecules which can be easily absorbed by other living species.

1.2 Major Environmental Challenges

1.2.1 Global Warming and Climate Change

Global warming is defined as the rise in earth temperature due to the increased level of carbon dioxide (CO_2) and other greenhouse gases (GHG). It is directly connected to the percentage of CO_2 present in the earth's atmosphere. The consequences of global warming are a rise in sea level, acidic oceans, increased air pollution, deviations in the cropping, and disease patterns. CO_2 is considered as the primary GHG that imparts to climate change, produced by the burning of fossil fuels such as coal, natural gas, and oil (He et al. 2018). A biological method like photosynthesis occurring in the plants convert CO_2 and water into organic compounds and maintains the equilibrium by fixing atmospheric CO_2 on the earth (Mondal et al. 2016). In the literature, many examples are already described for CO_2 conversion using microbes and their enzymes. Various algal species such as *Chlorella vulgaris*, *Nannochloropsis* sp., *Scenedesmus quadricauda*, *Chlamydomonas reinhardtii*, and *Nannochloris* sp. have been studied to sequester CO_2 (Eloka-Eboka and Inambao 2017). During photosynthesis, the RuBisCO enzyme in a photosynthetic organism is responsible for converting CO_2 into inorganic carbon. The major limitation of RuBisCO is a low affinity for CO_2 (Pavlik et al. 2017). However, CO_2 removal using biological methods is not suitable for region specific large-scale CO_2 sequestration such as industries outlets and polluted cities.

In a recent study, *Methylobacterium extorquens* formate dehydrogenase was reported for the conversion of CO_2 to formate (Jang et al. 2018). Carbonic anhydrase is an enzyme that is mostly used for conversion of CO_2 to bicarbonate (Sharma et al. 2018). Many bacterial species having CA enzyme are *Aeribacillus pallidus, Lactobacillus delbrueckii, Bacillus* sp., *Pseudomonas fragi, Serratia* sp., have been studied for conversion of CO_2 into calcium carbonate (Bose and Satyanarayana 2017; Li et al. 2015; Sharma et al. 2009; Srivastava et al. 2015; Sundaram and Thakur 2018). CO_2 sequestration using microbes offers a reduction of the major greenhouse gas CO_2 and, hence, ameliorates global warming.

1.2.2 Plastic Pollution on Earth

Synthetic plastics represent the main anthropogenic waste entering and accumulating into the environment. Indeed, plastic pollution is now considered a global environmental threat, together with ozone depletion, ocean acidification, and climate changes (Barboza et al. 2018). Plastic is used in everyday life such as packaging material, clothes, water bottles, and carpets. Plastics such as polyethylene terephthalate (PET), polypropylene, and polyethylene present a serious risk to plant and animals growing in the marine ecosystem. The chemical bonds between the plastic monomer are stronger, so they are resistant to natural degradation.

While microplastics are plastic particles less than five millimeter, are of special concern due to their small size, high surface/volume ratio, long environmental persistence, and their ability to enter into the cells and cause adverse effects (Peixoto et al. 2019). The abiotic degradation of man-made plastic by temperature, oxygen, UV radiation, and physical stress (Gewert et al. 2015) slowly degrades plastic and generates microplastic which can spread into the environment by wind (Urbanek et al. 2018). Due to the agglomerations of plastics in the environment, microorganisms are evolving catabolic pathways and enzymes to partly degrade plastic (Yang et al. 2015).

In the literature biodegradation of polyethylene by different microbial strains such as *B. subtilis, Acinetobacter baumanni, Arthrobacter* sp., *Staphylococcus epidermidis and Flavobacterium* sp. was reported (Restrepo-Flórez et al. 2014; Vimala and Mathew 2016). A newly discovered bacteria *I. sakaiensis* enzyme PETase was reported that uses polyethylene terephthalate (PET) as a major energy and carbon source for growth and converts into nontoxic form (Yoshida et al. 2016). This enzyme thus offers a platform for further modification using directed evolution and protein engineering strategy to boost the efficiency of the enzyme, toward the persistent challenge of highly crystalline polymer degradation (Austin et al. 2018). Thermoset plastics such as polyester polyurethane and aliphatic polyester are simply attacked by microbes because of easily digestible ester and urethane bonds in their structures. Other enzymes secreted by microbes that show biodegradable activity include the esterases, lipases, dehydratases, depolymerases, cutinases, ureases, and proteinases (Dang et al. 2018; Masaki et al. 2005; Sood et al. 2016; Zheng et al. 2005). Nowadays bioplastic made from renewable natural resources has received a lot of attention and can be used to replace the plastic (Mostafa et al. 2018). However, bioplastic has not completely replaced the petroleum-based plastic due to various economic and manufacturing challenges.

1.2.3 Chemical Pesticides as a Pollutant on Earth

Currently, pesticides are applied in agricultural production to halt the growth of pests and associated diseases. The most commonly used pesticides include atrazine, lindane, chlordane, DDT, aldrin, cypermethrin, and heptachlor (Pereira et al. 2015). The rise in the use of pesticides/chemical fertilizers in agriculture practices has led to contamination of water, air, and land and adverse effect on human health

(Craig 2019; Li 2018). Although pesticides play a vital role in agriculture, these are recalcitrant to biodegradation and persist in the ecosystem for many years (Kumar et al. 2018; Nicolopoulou-Stamati et al. 2016).

Various microbial species such as *Arthobacter*, *Aspergillus*, *Chorella*, *Penicillium*, *Pseudomonas*, *and Flavobacterium* have shown a capability to degrade pesticides into a less toxic product by using enzymes (Kumar et al. 2018). Various enzymes have been isolated from microorganism such as diisopropyl fluorophosphatase, parathion hydrolase, phosphotriesterase, esterase, and paraoxonase, to study the pathways involved in the biotransformation of these xenobiotic compounds (Cycoń and Piotrowska-Seget 2016; Lu et al. 2013; Singh and Walker 2006; Zuo et al. 2015). These indigenous microbes have limited degradation efficiency, so at present several bacteria containing pesticide-degrading gene can be used for constructing genetically engineered bacteria (Hong et al. 2010). For environmental sustainability, the development of biological pesticides has become the key to safeguard the health of human and agricultural development.

1.2.4 Pharmaceutical Pollution and Increased Antimicrobial Resistance

The excessive use of antibiotics in animal and human medicine, as well as in agriculture, has not only led to their accumulation in the environment but also developed the broad range of highly antibiotic-resistant microorganisms (Almakki et al. 2019). Most bacteria develop resistance against commonly used antibiotics like methicillin-resistant *S. aureus, erythromycin-resistant Streptococcus, penicillin-resistant Pneumococcus, and tetracycline-resistant Shigella* (*Sengupta* et al. 2013; Ventola 2015). Wastewater effluents from pharmaceutical industry enter into the rivers, rich in antibiotics, steroids, hormones, and analgesic components, which have adversely affected the microbial ecology (Ding and He 2010). The continuous exposure of terrestrial and aquatic microorganisms to pharmaceuticals products has affected the genetic composition of microbial genera and developed the antimicrobial resistant genes.

However, biological methods for converting pharmaceutical pollutant into non-toxic forms are attractive because they are inexpensive and environment-friendly (Zur et al. 2018). Many bacterial and fungal strains such as *Klebsiella, Penicillium, Pseudomonas, Aspergillus, Sphingomonas* sp., *Bacillus, Enterobacter, Aeromonas, and Streptomyces* have been reported for biotransformation of pharmaceutical pollutant (Rana et al. 2017). Biotransformation results in the formation of the end product that is less toxic and more stable than the initial compound.

1.2.5 Heavy Metal Pollution on Earth

Heavy metals are metalloids that have density more than 5 g/cm^3 such as mercury, arsenic, and lead (Tchounwou et al. 2012). Heavy metals occur naturally in the earth's crust and anthropogenic activities such as smelting, mining, petroleum combustion, and burning of coal in power plant, and use of fertilizer increases its existence in the environment (He et al. 2005). In natural systems, heavy metals affect cellular organelles like endoplasmic reticulum, lysosome, cell membrane, nuclei, mitochondria, and various enzymes involved in detoxification, metabolism, and damage repair. Metal ions interact with nuclear proteins and DNA, causing conformational changes and DNA damage that leads to apoptosis or carcinogenesis (Wang and Shi 2001). Due to the persistence of metal in the terrestrial environment, heavy metal pollution poses a risk to animal, plant, and human health (Mishra 2017).

Heavy metal bioremediation by microorganism is emerging as an efficient technique. Different mechanisms used by microorganisms to tolerate the metal toxicity are extrusion, biotransformation, use of enzymes, and synthesis of metallothioneins and biosurfactants (Igiri et al. 2018; Ramasamy et al. 2007). *P. putida* is cadmium-tolerant strain and has the intracellular ability to sequester zinc, copper, and cadmium, by using cysteine-rich low molecular weight proteins (Higham et al. 1986). *Bacillus pumilus*, *Alcaligenes faecalis*, *Brevibacterium iodonium*, and *Pseudomonas aeruginosa* were reported for the removal of cadmium and lead (De et al. 2008). Microbial bioremediation is a cost-effective and eco-friendly technology for the clean-up of heavy metals.

1.3 Restoration of Soil Health Using Microbes

Soil respiration, microbial biomass, enzyme activities, and microbial diversity are the major biological indicators of soil health. Healthy soils are necessary for the integrity of terrestrial ecosystem or to recover from trouble, such as climate change, pest infestation, drought, pollution, and human exploitation including agriculture (Ellert et al. 1997). Moreover, with the continuous increase in the world's population, the demand for food production has increased (Fageria et al. 2008). But nowadays agricultural practices include the use of potentially dangerous chemical fertilizers that affects the soil and human health (Glick 2018). Protection of soil is therefore of high priority, and a thorough understanding of ecosystem processes is a critical factor in assuring that soil remains healthy. These enzymes catalyze many vital reactions necessary for the life processes of soil microorganisms and also help in the stabilization of soil structure. Although microorganisms are the primary source of soil enzymes, plants and animals also contribute to the soil enzyme pool. Soil enzymes respond rapidly to any changes in soil management practices and environmental conditions. Their activities are closely related to the physiochemical and biological properties of the soil. Hence, soil enzymes are used as sensors for soil microbial status, for soil physiochemical conditions, and for the influence of soil treatments or climatic factors on soil fertility. Overall the microbe and enzyme profile in the soil must be stabilized in order to maintain the health of the soil, its fertility and to sustain agricultural growth. At present, the biogeochemical cycles in the agricultural ecosystem have been disturbed as a result of increased pollution and toxic level in the environment. The rate of organic content decomposition becomes lesser due to the extinction of various soil microflora. Therefore, it becomes necessary to find the suitable troubleshoot methods to prevent the increase in soil pollution to restore the decreased fertility of soil using biological approaches.

1.4 Conclusion

The soil microflora and enzyme quantity vary with external factors, physical and chemical conditions. Whichever may be the source of pollution either industries, fertilizers, pesticides, or urbanization and automobiles, each source has seriously damaged the microbial population and enzyme activity of the soil. This is also very complex to determine the exact level of microbial existence and enzyme activity in various geographical areas. Undoubtedly, molecular biology techniques, such as directed evolution and recombinant DNA technology, have revolutionized the speed of enzyme and microbe engineering which could be a milestone to restore the microbial population and enzyme activity in the soil. In this book chapter, we have given an overview of various types of pollutants which are affecting the soil or water bodies on the earth and how various microbes and enzymes are correlated with the existence or increasing level of this pollution.

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