The Role of Microbes and Microbiomes in Ecosystem Restoration

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Microbes and Microbiomes for Clean and Green Environment

(Volume 1)

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PREFACE

The book "Role of Microbes and Microbiomes in Ecosystem Restoration" focuses on basic to advanced techniques in various roles of microbes and microbiomes in the abatement and restoration of polluted ecosystems, climate change, production of renewable energy sources, and waste management. It covers ecosystem sustainability, the UN decade of ecosystem restoration, efficient utilization of microbes and microbiomes and their role in socio-economic development, and the current status of polluted and degraded ecosystems.

Stepping into an unusual era of concurrent buffer leads to a shifting global climate. At the beginning of the twenty-first century, one of the active concerns in the human ecological background is the destruction of ecology and ecosystems. Human actions have evolved a remarkable power to affect the ecosystem. To address this developing issue, the science of restoration ecology and its applied practices provide a potentially cost-effective, buoyant answer. The notion of restoration has emerged as the dominant subject in the global environmental context. One of the most important goals of the UN Convention on Biological Diversity from 2011 to 2020 is to restore at least 15% of the world's damaged ecosystems. World leaders adopted the "Bonn Challenge" in 2011, which is a global commitment to rehabilitate 150 million hectares of deforested and damaged land by 2020. Most significantly, in 2015, the UN formalized these worldwide pledges by endorsing the 2030 Sustainable Development Goals, one of which focuses on ecological restoration. Microbes are ubiquitous, providing many critical services to the ecosystem, such as sustainable plant productivity and a stable environment for human life. They help to keep atmospheric CO_2 and nitrogen levels stable, which are now reduced due to greenhouse gases and other hazardous pollutants. On a global scale, microbial organisms are extremely strong. Bacteria create approximately 50% of total oxygen, 75% of added nitrogen to the atmosphere, and 92% of nitrogen removal from the environment. As a result, this book covers the potential of bacteria and microbiomes in many ecosystems.

In Chapter 1, Prasad *et al.* provide an overview of the causes of ecosystem destruction, the need for ecosystem restoration, the significance of microbiome in biomining, restoration of farm and degraded land, control of heavy metals, production of renewable energy, crop growth, biofertilizer production, mitigation of greenhouse gases, and waste management. It also encompasses the role of molecular techniques in ecosystem restoration and the challenges involved in adopting microbiomes for ecosystem restoration.

Microbes are the crucial living elements of soils that contribute to the sustainability of ecosystems because of their capacity for stress tolerance, vast effective genetic pool, ability to survive in various conditions, and capacity for catabolism. However, various factors like soil conditions, geographical and climatic factors, and soil stressors (drought, submersion, pollutants, and salinity) may result in distinct microbial composition and characteristics, as well as its mechanism to support ecosystem restoration and defense against all of these stressors. Hence, Pooja *et al.*, in Chapter 2, deliver the vital edaphic (pH, temperature, oxygen, nutrients, and moisture), geographical, climatic (UV radiation, elevated CO_2, temperature, permafrost thaw), and abiotic factors (drought, submergence, salinity, pollutants) involved in the establishment of microbes and microbiome.

In Chapter 3, Sinduja *et al.* discuss the ecological role of microorganisms participating in biogeochemical cycles, hoping to delineate the role of microbes and microbiomes in biogeochemical cycles. Microorganisms play an essential role in moderating the Earth's biogeochemical cycles; nevertheless, despite our fast-increasing ability to investigate highly complex microbial communities and ecosystem processes, they remain unknown. Hence, this chapter covers the strategies for proper management of prevailing natural resources, considerations for management, its role in the biogeochemical cycle, and the influence of beneficial soil microbes, such as plant growth promoting rhizobacteria and cyanobacteria, on natural resource management, with special emphasis on the role of soil enzymes in nutrient cycling.

Bioleaching (microbial leaching) is being studied intensively for metal extraction since it is a cost-effective and environmentally benign technique. Bioleaching with acidophiles involves the production of ferric (Fe III) and sulfuric acid. Cyanogenic microorganisms, in particular, can extract metal(s) by creating hydrogen cyanide. Besides, bioremediation is one of the most effective approaches for reducing environmental contaminants since it restores the damaged site to its original state. Hence, Chapter 4 by Poornima *et al.* provides a baseline on bioleaching, its types, microbes involved in bioleaching, bioleaching pathways, and the role of microbes in the bioremediation of polluted habitats.

In recent years, microbial-assisted bioremediation has emerged as a promising and eco-friendly alternative for HM remediation. This approach utilizes microorganisms to transform, immobilize, or detoxify HMs, making them less harmful and more accessible for removal. Hence, Naik *et al.*, in Chapter 5, highlight the eco-friendly use of microorganisms, their mechanisms that contribute to the bioremediation of HMs, and their potential use in the future.

In Chapter 6, Sajish *et al.* present the basic principle of an MFC and the role of microbes in a microbial fuel cell, genetic engineering, biofilm engineering approaches, and electrode engineering approaches for increasing the overall efficiency of an MFC for its practical implementation. Microbial fuel cell, a type of BES, is a budding technology that exploits the potential of electroactive microorganisms for extracellular electron transfer to generate electricity. Hence, this chapter encompasses the history of MFC, bio-electrochemically active microorganisms, electroactive microbial genera in microbial fuel cells, factors affecting the development of anode biofilm, biofilm engineering, and the recent advances in strain improvement for improved MFC performance.

Energy crises resulting from the depletion of petroleum resources, hikes in the price of fossil fuel, and unpredictable climate change are some of the recent concerns that have provoked serious research on alternative energy sources that will be sustainable. In this regard, biofuels are a straightforward substitute for fossil fuels. Renewable feedstocks are suitable ingredients that sustainably produce biofuels using microbial-based bioconversion processes. Industrially important enzymes are capable of degrading long-chained biopolymers into short-chained monomeric sugars and fermenting them into energy-dense biomolecules. Hence, Chapter 7, authored by Oyelade *et al.*, comprehensively reviews how sustainable bioenergy production through microbes using feedstocks can provide clean and green energy that can consequently facilitate ecosystem restoration. Feedstocks are pivotal to this biotechnological process.

In recent decades, biofertilizers have gained popularity as a viable alternative to unsafe chemical fertilizers in pursuing sustainable agriculture. They have an essential role in enhancing crop output and preserving long-term soil fertility, both of which are critical for fulfilling global food demand. Therefore, Chavada *et al.*, in Chapter 8, deliver the various microbes involved in nitrogen fixing, phosphorus and potassium solubilizing and mobilizing, sulfur oxidizing, and zinc solubilizing. The role of arbuscular fungi and plant growth-promoting rhizobacteria in biofertilizer production is also discussed.

Knowingly or unknowingly, agricultural systems face stress and resource quality degradation and their depletion by the activities of humans. Abiotic stresses, such as nutrient deficiency, water logging, extreme cold, frost, heat, and drought, affect agricultural productivity. Similarly, biotic factors like insects, weeds, herbivores, pathogens, bacteria, viruses, fungi, parasites, algae, and other microbes also limit good-quality products. Thus, Vijayalakshmi *et al.* discuss the application of microbes and microbiomes in biotic and abiotic stress management in Chapter 9. This chapter especially discusses the adaptive mechanisms of salt tolerance in plants, tolerance to abiotic stress, the emerging microbiome in soil biota, and nanomaterials' efficacy on stress.

Microbes play a significant role as either generators or consumers of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) through various processes. Sethupathi *et al.*, in Chapter 10, discuss the role of microbes and microbiomes in the emission of major greenhouse gases like CO_2, CH_4, N_2O, and NH_3. The potential of the microbiome in mitigating these greenhouse gases is also delivered in this chapter.

Given that there is potential for warmth to boost the release of carbon dioxide from dirt to the atmosphere due to better microbial disintegration of dirt raw material, the impact of environmental change on the soil carbon sink remains uncertain. If forecasted climate modification situations are precise, this boost in soil carbon loss might significantly worsen the dirt carbon cycle responses. Therefore, Chapter 11 by Al-Jawhari introduces us to the soil CO2 balance, environmental effects, and the significance of the soil carbon cycle and microbial decomposers, carbon cycle in soil, ocean, and ecosystem restoration under climate change perspective.

The generation of wastewater increases multi-fold because of industries and the overexploitation of freshwater resources. Wastewater treatment is always linked with waste recovery and its optimum utilization, which broadens the amplitude of wastewater treatment, enhancing the quality of the byproducts and as an efficient alternative for non-potable purposes. Microbiomes are crucial in biological wastewater treatment methods such as activated sludge, anaerobic digestion, and bioelectrochemical systems. The microbial population's activity and resilience in the microbiome significantly impact the performance and stability of these activities. Suganthi *et al.* present the biological wastewater treatment, growth and kinetics, and different microbial community types, including bacteria and fungus, actinomycetes, algae, plants, and the range of microbial wastewater treatment in Chapter 12.

Solid waste disposal is a significant issue that worsens daily as more people move into cities. In Chapter 13, Velusamy *et al.* provide the status of solid waste management in India, sources and types of solid wastes, various conventional solid waste management techniques, and the role of microbes in solid waste management through composting and anaerobic digestion.

Microorganisms are pervasive and genuinely make up the “unseen majority” in the marine environment. Although marine isolates have been the subject of laboratory-based culture methods for more than ten years, we still do not completely understand the ecology of marine microorganisms. Thus, in Chapter 14, Poornachandhra *et al.* explore marine microbial diversity, its utilization in bioremediation, and understanding their role in ecosystem sustainability.

Mangroves and wetlands are critical intermediary ecosystems between terrestrial and marine environments. These ecosystems offer a wide range of invaluable ecological and economic services. However, under the influence of natural and anthropogenic threats, mangroves and wetlands face rapid degradation. Hence, Chapter 15 by Haghani *et al.* is dedicated to enlightening us regarding the most critical features of microbial groups, including archaea, bacteria, algae, and fungi in mangroves and wetlands. Moreover, the biochemical transformations brought about by wetlands' microbial groups and the degree of complexity in microbial interactions are explained.

Jerome *et al.*, in Chapter 16, articulate the significance of forest microbiomes in ecosystem restoration and sustainability. Generally, forest microorganisms are essential to how plants interact with the soil environment and are necessary to access critically limiting soil resources. This chapter focuses on the ecosystems below and above ground level of a forest microbiome, including the soil microorganisms, their importance, and the diverse interrelationships among soil microorganisms (parasitism, mutualism, commensalism).

Employing field-based monitoring and restoration assessment techniques, surveying microbes or microbial populations is challenging or impossible. In contrast, it is now possible to precisely and quickly describe and quantify these diverse and functional taxonomic groups by sequencing large quantities of environmental DNA or RNA utilizing genomic and, in particular, meta-omic technologies. Hence, Nagendran *et al.*, in Chapter 17, throw light on using meta-omics techniques to monitor and assess the outcomes of ecological restoration projects and to monitor and evaluate interactions between the various organisms that make up these networks, such as metabolic network mapping. An overview of functional gene editing with CRISPR/Cas technology to improve microbial bioremediation is also provided herewith.

Chapter 18 by Satpathy *et al.* provides details on metagenomic approaches like Multi-Locus Sequence Typing (MLST), MOTHUR, Quantitative Insight into Microbial Ecology (QIIME), and PHAge Communities From Contig Spectrum (PHACCS) in the restoration of the temperate and tropical ecosystem.

Soil microorganisms also play a fundamental role in ecosystem functioning and conserving plant diversity. Exploring voluminous beneficial microorganisms and promoting the reestablishing of those beneficial microbes in the soil will preserve Earth's diverse native plant populations. Hence, Prasad *et al.*, in Chapter 19, delve into fundamental and conventional techniques and approaches that can be employed to maintain soil microbial populations. Furthermore, the chapter investigates the possibility of creating protocols for regulatory or commercial objectives, emphasizing the significance of ecological restoration by using bioinoculants or microbial colonies in degraded sites.

In Chapter 20, Shivakumar *et al.* examine the application of molecular methods to ecosystem regeneration. The various available molecular methods and how they have been applied to monitor ecosystem health, identify microbial communities in ecosystems, and comprehend interactions between microbes and plants are discussed. The chapter also discusses the application of molecular methods to the restoration of ecosystems that have been damaged, including the use of plant-microbe interactions to promote plant development in contaminated soils.

The sustainable industrial revolution is the way forward to help humankind to prolong its existence on Earth. John *et al.* enlighten us with the role of the microbiome in a sustainable industrial production system. In Chapter 21, they disclose the energy sector's current status, microbes' role in organic and amino acid production, and the role of microalgae in sustainable agriculture.

The human microbiome plays a vital role in human development, immunity, and nutrition, where beneficial bacteria establish themselves as colonizers rather than destructive invaders. In Chapter 22, Pradyutha *et al.* introduce microbes' role in human and animal health security. The various human and animal diseases and the potential of microbiota, such as probiotics, in disease treatment are also discussed in this chapter.

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Part 1: Introduction to Microbes and Microbiomes in the Ecosystem Restoration and Green Environment

CHAPTER 1

Overview of Microbes and Microbiomes in the Restoration of Terrestrial, Aquatic, and Coastal Ecosystems

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Abstract: Ecosystems consist of biotic and abiotic components, including flora and fauna, along with the conducive environmental factors of a particular place. These are imperative for maintaining the ecosystem's structure and energy flow between trophic levels and providing ecosystem services for the well-being of humans and other living organisms. However, ecosystems are being threatened by human activities, which disrupt the balance of nature. Thus, it impacts billions of people by causing economic loss and threats to the survival of terrestrial, aquatic, and other species. Climate change and increasing pollution also adversely affect the functions of the ecosystem. Microbes and microbiomes are reported to restore terrestrial, aquatic, and coastal ecosystems. The diverse microbes such as bacteria, archaea, algae, fungi, and protozoa help detoxify the polluted ecosystems through various physical, chemical, and biological mechanisms. They also help with the nutrient cycling and mineralization of nutrients from the soil to plants in their available forms. With the focus on ecorestoration, there is a need to take collective action to protect the environment and prevent ecosystem degradation worldwide.

Keywords: Clean environment, Ecosystems, Ecosystem restoration, Green environmenta, Microbes, Microbiomes.

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INTRODUCTION

The ecosystem is the complex of living organisms and non-living things interacting with each other in their physical environment called habitat, and all the interrelationships between organisms occur in a particular space unit. It is divided into (i) terrestrial, *e.g.*, desert, forest, grassland, taiga, and tundra, and (ii) non-terrestrial, *e.g.*, aquatic, marine, and wetlands. Ecosystems play a vital role in balancing the natural phenomenon, structural organization, energy flow, and nutrient cycling and provide various ecosystem services and benefits to human society. However, human activities negatively influence the well-being of 3.2 billion people. These activities cost more than 10% of the annual global gross product by losing biodiversity and vital ecosystem services. Human activities are reported to reduce productivity in 23% of global terrestrial areas [1]. Vegetation cover is decreasing, influencing grasslands, croplands, woodlands, and rangelands, particularly in vulnerable regions. Desertification has severe consequences for 38% of the global population. Wetlands have declined 70% over the previous century [2]. The global forest area has decreased by 100 million hectares since 2000 [3]. Reversing this fact can have substantial advantages. It can help improve the food and water supply, reduce GHG emissions, and mitigate adverse effects related to climate change.

The restoration of ecosystems is vital across global international conventions and agreements to achieve their goals and priorities regarding biodiversity, climate change, desertification, and a sustainable future. Global action is required to restore ecosystems and enhance positive global impact. Investment in ecosystem restoration projects can deliver many advantages to society, including biodiversity conservation [4]. At an international level, restoring the ecosystem degradation is essential to maintain temperatures below 2ºC [5, 6]. IUCN calls for collective action to set the world on a transformational trajectory in the UN Decade on Ecosystem Restoration, enabling the implementation of the Post-2020 Framework [7]. The UN also calls for accelerated and scaled-up ecosystem restoration by 50% to reverse loss in the area by 2030. They focused on spatial integrated planning in all ecosystems to cover 50% of the land, freshwater, and ocean regions by 2030 to reduce pressure on ecosystems and maximize biodiversity and ecosystem services.

Microbes and microbiomes are vital in ecosystem restoration [6, 8]. They contribute to nutrient cycling, decomposition of organic materials, soil fertility maintenance, and crop productivity enhancement [6]. Bacteria, archaea, and fungi exhibit distinct assemblies along vertical and horizontal profiles in reforested ecosystems. The diversity of bacteria and fungi decreases with increasing soil depth while archaeal diversity increases. As reforestation progresses, bacterial communities' vertical and spatial variation declines while archaeal and fungal communities proliferate. The distribution patterns of soil microbes are linked to the soil's physical and biochemical properties and the existence of plant roots. Bacterial and archaeal communities play influential roles in deep and superficial soil layers in multi-nutrient cycling. Soil fungi comprise various dynamic kingdoms of eukaryotes and are vital for maintaining ecosystem processes and functions [6].

Understanding microbial community assembly processes and biogeochemical cycling during ecosystem restoration is critical for optimizing management strategies [9]. Factors such as variation in community assembly processes, measurable microbial community attributes, and linkages to ecosystem function must be considered. By examining microbial succession, insights can be gained into microbial community structures in various ecosystem recoveries. They can help determine the success of ecosystem restoration efforts and maintain ecosystem stability [10]. Native mycorrhizal fungal communities play a significant role in restoring native plants [11]. Understanding microbial community assembly processes, managing microbial contamination risks, and identifying effective bio-indicators of soil health are crucial for optimizing ecosystem restoration efforts and promoting ecosystem stability and resilience. This chapter discusses an overview of microbes and microbiomes' role in ecosystem restoration.

CAUSES OF ECOSYSTEM DESTRUCTION AND THEIR IMPACTS

Ecosystem degradation is defined as a long-term reduction in the structure and functionality of the ecosystem or loss in delivering services and proficiency to benefit people. The significant causes of ecosystem destruction and their impacts are overviewed in the following sub-headings. The essential causes of ecosystem destruction are shown in Fig. (**1**), which is happening at an alarming rate due to various anthropogenic activities such as changes in land use practices, unrestricted natural resources utilization, deforestation, habitat loss, climate change, warming ocean waters, ocean acidity, and pollution.

Resource Exploitation

Resource exploitation, particularly mining and extraction activities, can positively and negatively impact ecosystems, socio-economics, and the environment. Saputra and co-workers [12] investigated the effect of sand mining in Indonesia and reported its promising impact on the socio-economics and environment. They also stated that the river deepens after sand mining, enabling it to hold considerable water and control overflow during the rainy season. Similarly, exploiting mineral resources like coal can impact local communities and

CHAPTER 2

Role of Environmental Factors Influencing Microbes and Microbiomes for Ecosystem Restoration

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Abstract: Ecosystem degradation poses a significant and growing environmental threat. Restoring degraded ecosystems is vital to restoring their ability to provide essential services and benefits. In 2021, the United Nations declared the Decade of Ecosystem Restoration to emphasize the importance of coordinated efforts in this area. Microbes, with their stress tolerance, genetic diversity, adaptation to various conditions, and capacity to break down substances, are crucial for ecosystem sustainability. Their critical functions are vital in restoring ecosystem function and biodiversity. This chapter describes the role of microbes in a microbiome and their interactions, instilling optimism about their potential. It also covers how various factors shape the soil microbiome spatially and temporally. Soil microorganisms such as bacteria, archaea, and fungi are found around, on, and in plant roots, and they play an essential role in responding to abiotic stressors. Factors like soil conditions, geographical and climatic factors, and stressors like drought, pollutants, and salinity can result in distinct microbial compositions and characteristics. This chapter provides an in-depth overview of how these factors can impact soil microbial communities and their role in ecological restoration. This chapter also covers beneficial microbiome-based strategies, including microbial engineering for ecosystem restoration. These strategies are essential and a source of hope for the future.

Keywords: Abiotic stressors, Ecosystem restoration, Environmental factors, Microbes, Microbiome.

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INTRODUCTION

Population growth, industrialization, and urbanization have greatly influenced the environment. As a result of developmental activities, the environment has degraded. The significant impacts of developmental activities include habitat degradation, biodiversity loss, environmental pollution, and global climate change [1]. One of the leading causes of the decline in biodiversity and the integrity of ecosystems is anthropogenic pressure. The ecosystems need to be more resilient to cope with the degradation activities. This necessitates the urgency for ecological restoration. According to the Society for Ecological Restoration Science and Policy Working Group, the process of assisting in recovering an ecosystem that has been degraded, damaged, or destroyed is known as ecological restoration [2]. It entails various tasks like eliminating invasive species, reintroducing native species, and enhancing soil health. In 2021, the United Nations (UN) declared the Decade of Ecosystem Restoration, a significant global initiative in response to the destruction and degradation of ecosystems. This proclamation recognizes the need to rapidly restore damaged ecosystems worldwide to restore their ability to offer necessary services and benefits [3]. The majority of ecological restoration projects aim to stop further changes and restore ecosystems and ecosystem processes to their pre-injured state. The goal of restoration is to bring an ecosystem back to a functional state by reinstating its biodiversity, enhancing nutrient cycling and energy flow, improving the fertility of soil, water regulation, protecting against soil erosion, pest control, maintenance of a variety of pollinator species services, and reduction in CO_2 emissions and its buildup, among other factors. Microbes play an essential role in ecosystem restoration. Table **1** provides an overview of the ecological functions of microorganisms.

Microbiome refers to distinctive microbial communities, including viruses, fungi, bacteria, archaea, protozoa, and other micro-eukaryotes occupying well-defined habitats [11]. Also, these are the rich reservoirs of biodiversity that possess the ability to conserve and restore the functioning of various ecosystems. Microbiomes exist in habitats such as humans, plants, soils, sediments, and livestock animals. Many scientific disciplines depend heavily on microbiomes, including the medical, veterinary, and environmental sciences. A schematic representation of the application of microbiomes in environmental sciences is given in Fig. (**1**).

Microbiomes are not just crucial for developing novel, long-lasting bioeconomy applications, such as industrial biotechnology and waste recycling. They also hold the potential to address urgent social concerns. Applications of the microbiome as pre and probiotic food supplements, biofertilizers, and biocontrol agents are anticipated to significantly aid in eradicating hunger, stopping biodiversity loss, and slowing climate change. This potential gives us hope and inspires us to continue our research and application of microbiomes in various sectors.

Table 1. The major groups of microbes and their ecological roles.

Type of Microbes	Beneficial Roles	Ecological Function	References
Archaea	Nitrification, methanogenesis, *etc*	Regulation of climatic and atmospheric conditions, nutrient cycling, *etc*	[4]
Chemoautotrophic bacteria	Sulfate reduction, iron oxidation, nitrogen fixation, *etc*	Purification of water, cycling of nutrients, and climate control	[5]
Heterotrophic bacteria	Mineralization, oxidation of organic matter, synthesis of polymers, *etc*	Decomposition, water filtration, nutrient cycling, carbon sequestration, and climate control	[6]
Photoautotrophic bacteria	Photosynthesis	Chlorophyll production	[7]
Fungi	Organic matter consumption and mineralization	Soil formation, nutrient cycling, *etc*	[8]
Arbuscular mycorrhizal fungi	Nutrient cycling	Primary product (indirect)	[9]
Protozoans	Mineralization and consumption of other microbes, wastewater management	Mineralization, nutrition cycling, organic matter decomposition, *etc*	[10]
Virus	Cell lysis	Biogeochemical cycling, microbial evolution	[10, 11]

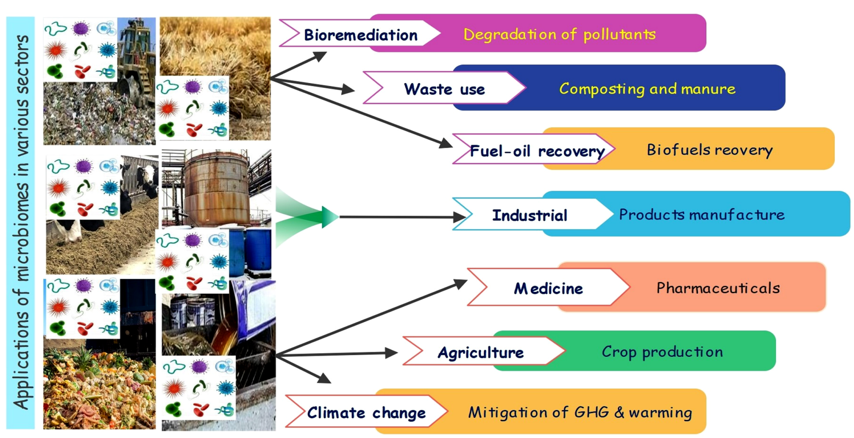

Fig. (1). Application of microbiomes in various sectors.

Part 2: Role of Microbiomes in Restoring Polluted Ecosystem and Environment

CHAPTER 3

Microbial-assisted Bioremediation: A Greener Approach for Restoration of Heavy Metal-contaminated Soil

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Abstract: Heavy metals (HMs) pollution is a major environmental concern, posing serious threats to human health and ecological systems. Anthropogenic activities have increased the levels of HMs in the environment, and their pollution is a major issue. Exposure to high levels of these metals can have harmful effects on human health, and they can also damage soil structure, diminish microbial biodiversity, and inhibit plant growth and development. In addition, traditional remediation methods for HMs contaminated soil are often expensive and negatively impact the environment. In recent years, microbial-assisted bioremediation has emerged as a promising and eco-friendly alternative for HM remediation. This approach utilizes microorganisms to transform, immobilize, or detoxify HMs, making them less harmful and more accessible for removal. This chapter highlights the eco-friendly use of microorganisms, the mechanisms that contribute to the bioremediation of HMs, and their potential use in the future.

Keywords: Bioleaching, Biosorption, Bioaccumulation, Biomineralization, Environment, Heavy metals, Microorganisms, Microbial-assisted bioremediation, Mmicrobial diversity, Plant growth, Soil.

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INTRODUCTION

Heavy metals (HMs) are generally defined as metallic elements and metalloids that have a high atomic weight and density, typically above 5 g/cm^3. From the biological and environmental science perspective, the term 'heavy metals' is often used to describe a group of metals and metalloids that are toxic to plants and animals even at low concentrations because these metals can accumulate in living tissue and interfere with biological processes, leading to a range of HM health problems and environmental damage. HMs are naturally occurring elements found in soil, water, and rocks. However, human activities like industrialization, urbanization, mining, smelting, burning of fossil fuels, and use of pesticides and fertilizers have led to increased levels of these metals in the environment, particularly in soil, water, and air [1, 2]. The HMs of particular concern due to their toxicity and prevalence in the environment include chromium, mercury, arsenic, cadmium, lead, nickel, copper, and zinc [3]. The physicochemical properties of HMs, such as their ubiquity, non-biodegradability, toxicity, accumulation, and persistence, have made their pollution a significant global concern [4]. As a result, there is growing recognition of the link between HM contamination and public health. The toxicity of HMs to living organisms depends on various factors, including the type of metal, the concentration, and the duration of exposure [5]. High levels of these metals can harm human health, such as neurological damage, cancer, and respiratory problems [6]. HMs can damage soil structure, diminish microbial biodiversity, and inhibit plant growth and development, reducing crop production and quality [2]. However, various microbial species and plants have developed unique coping mechanisms to deal with the toxicity of HM in polluted soils [2, 4]. These adaptations can range from changes in their cell structure and physiology to changes in their gene expression and metabolism. These adaptations can be used for bioremediation, which uses microorganisms and plants to remove or detoxify HMs from polluted soils [7]. Physicochemical techniques such as excavation, soil washing, and chemical stabilization can effectively remove or immobilize HMs from contaminated soil. However, these techniques are often expensive, energy-intensive, and generate toxic waste, making them unsuitable for large-scale remediation projects.

Additionally, these methods may not be environmentally friendly or inappropriate for soils with low metal contamination levels. As a result, biological remediation strategies, such as phytoremediation and bioremediation, are becoming increasingly popular due to their cost-effectiveness, sustainability, and ability to restore soil health. Bioremediation technologies based on microorganisms have become increasingly popular in recent years due to their effectiveness in removing environmental pollutants [8]. Microorganisms such as bacteria, fungi, and algae can transform, immobilize, or detoxify HMs through various mechanisms [2, 9].

The process involves various mechanisms, including bioleaching, biosorption, bioaccumulation, and biomineralization [10]. Bioleaching involves using microorganisms to solubilize and mobilize HMs from the environment, making them more accessible for removal. Biosorption involves binding HMs to the surface of microbial cells, which can then be easily removed from the environment. Bioaccumulation involves the accumulation of HMs within microbial cells, usually in specialized compartments such as vacuoles, which can then be easily removed from the environment. Finally, biomineralization involves the conversion of HMs into less toxic forms, such as metal sulfides or metal carbonates, which are then immobilized in the environment. The current chapter highlights the eco-friendly use of microorganisms and their mechanisms that contribute to the bioremediation of HMs.

HEAVY METALS: SOURCES AND TOXICITY

HMs are a group of elements with a high density (greater than 5 g/cm^3 or a specific gravity at least five times greater than water) and tend to be toxic to living organisms in high concentrations [2]. HMs can be divided into two main categories: essential and non-essential HMs. Some HMs are necessary for living organisms in small amounts for growth and development; these are called essential HMs, and examples include iron, copper, zinc, and manganese [2, 11]. Exposure to high levels of non-essential HMs, like lead, mercury, cadmium, and arsenic, can harm human health. They are not required for normal biological function and can be toxic in large amounts. High concentrations of these metals can cause damage to the nervous system, liver, and kidneys, increasing the risk of cancer [12]. Natural processes and anthropogenic activities are responsible for the entry of HMs into the soil. However, compared to natural processes, anthropogenic activities are more critical for causing HM pollution [13]. Some soil types have naturally high concentrations of HMs due to the parent materials from which they are formed. These soils are often found in areas with high levels of volcanic activity or mineral deposits, which can contain high levels of HMs such as lead, cadmium, arsenic, and mercury [14]. For instance, San Joaquin Valley in California is where naturally occurring geological vents have elevated selenium levels in the soil [13].

HMs are primarily derived from the parent materials from which the soil was formed. Approximately 95% of the Earth's crust comprises igneous rocks, with the remaining 5% comprising sedimentary rocks [13]. Generally, basaltic igneous rocks are abundant in HMs like cadmium, copper, cobalt, and nickel, whereas shale typically has higher concentrations of lead, copper, zinc, manganese, and cadmium [15]. HMs contained in rocks can enter the soil system through various natural processes such as weathering, erosion, leaching, and volcanic activity

Part 3: Role of Microbiomes in Agriculture Crop Growth and Development

CHAPTER 4

Role of Microbes and Microbiomes in Biofertilizer Production and as Plant Growth Promoters

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Abstract: In 2050, 8.3 billion people will live on Earth, and 70 to 100% more food will be needed. Food and its products are available through agricultural practices. Soil biological systems play an essential role in food production. However, it is a complex process that leads to the stability of crop production and the maintenance of soil health. Healthy food with eco-friendly agriculture practices is required to sustain the soil ecosystem globally. Additionally, the continued depletion of the Earth's natural resources and the increasing use of harmful chemical fertilizers are significant concerns for agriculture's future. Biofertilizers are gaining popularity as a viable alternative to unsafe chemical fertilizers in the pursuit of sustainable agriculture. Biofertilizers have an important role in enhancing crop output and preserving long-term soil fertility, both of which are critical for fulfilling global food demand. Microbes can interact with agricultural plants to improve their resistance, growth, and development. Nitrogen, phosphorus, potassium, zinc, and silica are the fundamental elements needed for crop growth, yet they are normally present in insoluble or complex forms. Certain microbes dissolve them and make them accessible to plants.

Keywords: Biofertilizer, Biological N_2 fixing bacteria, PGPR (Plant Growth Promoting Rhizobacteria), PSB.

INTRODUCTION

Farmers are applying various crop nutrition tactics to assist and fulfill the increasing demand for food caused by the world's population growth. According to FAO predictions, agricultural product consumption will climb to 60% by 2030 [1]. One of the primary issues of the twenty-first century is increasing output while protecting the environment [2]. Fertilizers have been widely utilized to boost crop output from agricultural land. Increased use of chemical fertilizers in agriculture may help a country become self-sufficient in food production, but

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chemicals are harmful to both the environment and living beings. Furthermore, chemical fertilizers are costly, have an impact on the soil, impair its water-holding capacity and fertility, induce nutrient imbalances in the soil, and result in unsustainable levels of water contamination [1]. Biofertilizers, on the other hand, are environmentally benign, cost-effective, non-toxic, and simple to apply; they assist in sustaining agricultural land soil structure and biodiversity. As a result, they are an excellent replacement for chemical fertilizers [3].

In the period 2022-2027, the biofertilizer market is expected to grow 13.81%, according to a new report by IMARC Group (Bio-Fertilizer Market, forecast 2029, CAGR report by the United States biofertilizer market), where the India biofertilizer market is expected to grow 15.89% (CAGR report) [4]. Growth controllers, genetically modified crops (GM crops), and tolerant varieties have been applied to improve crop resistance to salt stress [5 - 7]. These methods have been restricted due to their cost, labor work, and environmental threats. The soil needs several essential minerals to carry out plant growth. Very significant minerals are nitrogen, phosphorus, and potassium. Other 13 minerals as microelements are required for plant development and yield production [8 - 10]. They describe two categories based on their quantitative requirement: 1) macronutrients and 2) micronutrients. Nitrogen and phosphorus are necessary for plant growth and development; potassium is the third essential plant nutrient [11]. After nitrogen and phosphorus, five million tonnes of potash are required every year in the world, but in India, the commercial source is not available for potassic fertilizer production, so it is imported from other countries [12, 13].

Biofertilizers, also known as microbial inoculants, are organic materials formed from plant roots and root zones that include particular microorganisms. They have been demonstrated to increase plant growth and yield by 10-40% [14]. When applied to the seed, plant surface, or soil, these bioinoculants colonize the rhizosphere and the interior of the plant, stimulating plant development. By supplying nutrients to the soil, they not only boost soil fertility and crop yield but also protect the plant against pests and diseases. They have been found to improve root system development, lengthen its life, destroy hazardous elements, boost seedling survival, and shorten the time to bloom [15]. Another advantage is that after 3-4 years of continuous usage of biofertilizers, there is no need for their application since parental inoculum is adequate for development and multiplication [16]. The plant needs 17 critical ingredients for effective growth and development. Nitrogen (N), phosphorus (P), and potassium (K) are three of the most important. Several microorganisms, including phosphate-solubilizing bacteria, moulds, and fungi, and nitrogen-fixing soil bacteria and cyanobacteria, are routinely utilized as biofertilizers [17]. Similarly, phytohormone-generating bacteria are utilized in the manufacture of biofertilizers. They give the plant growth-promoting chemicals such as amino acids, indole acetic acid (IAA), and vitamins, thereby improving soil fertility and plant productivity.

Biofertilizers contain efficient bio-inoculants that improve the nutrient quality of plants and regulate their physiological properties [14, 18]. Biofertilizers are produced by soil-helpful living microorganisms [19]. Synthetic fertilizers can be applied to plants to improve plant growth. In soil systems, chemical fertilizers can cause leaching, volatilization, acidification, and denitrification if not appropriately managed [20 - 23]. Synthetic fertilizers and pesticides have caused several environmental issues (greenhouse effect, ozone layer depletion, and water acidification). The application of bio-fertilizers and biopesticides can overcome these issues. In addition to being natural and beneficial, both are eco-friendly for the user. Community waste and sewage sludge are alternative sources of organic fertilizer. It is an inexpensive and attractive source of nutrients [21, 24]. However, due to the presence of heavy metals and some poisonous chemicals, it cannot be used as an ideal organic fertilizer; heavy metals produce adverse effects on crop growth, so they cannot be an appropriate source of fertilizer [20].

Biofertilizers are grouped into different types on the basis of their functions and mode of action (Fig. **1**). The commonly used biofertilizers are nitrogen fixer (N-fixer), potassium solubilizer (K-solubilizer), phosphorus solubilizer (P-solubilizer), and plant growth promoting rhizobacteria (PGPR). In one gram of fertile soil, up to 10^{10} bacteria can be present, with a live weight of 2000 kg ha^{-1} [25]. Soil bacteria could be cocci (sphere, 0.5 µm), bacilli (rod, 0.5–0.3 µm), or spiral shaped (1–100 µm). The presence of bacteria in the soil depends upon the physical and chemical properties of the soil, organic matter, and phosphorus contents, as well as cultural activities. However, nutrient fixation and plant growth enhancement by bacteria are key components for achieving sustainable agriculture goals in the future. Microbes also facilitate various nutrient cycles in the ecosystem.

The microbial strains used as biofertilizer and their effect on crop yield are given in Table **1**. Burris [26] confirmed that atmospheric nitrogen is fixed biologically in legumes. A French agriculturist suggested that legumes are higher in cereals plants; they can play a vital role in biological nitrogen fixation and may deliver nitrogen to plants. Bacteria, fungi, and blue-green algae are used for biofertilizer applications [19]. They are applied to the rhizosphere of plants to improve their soil ecosystem. Efficient strains are isolated from soil (other environments) and are suitable for soil environments and climate [27 - 29]. These strains are multiplied in the laboratory to produce a suitable mass of isolates and then provide farmers with suitable carriers like peat and lignite powder to have a good shelf life for their farm.

CHAPTER 5

Role of Microbes and Microbiomes in Biotic and Abiotic Stress Management in Agriculture

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Abstract: Agriculture is our sensible recreation and the foremost food source for all animals and human beings. It gives laurels to us, but knowingly or unknowingly, agricultural systems face stress, resource quality degradation, and depletion by human activities. Abiotic stresses, such as nutrient deficiency, water logging, extreme cold, frost, heat, and drought, affect agricultural productivity. Biotic factors like insects, weeds, herbivores, pathogens, bacteria, viruses, fungi, parasites, algae, and other microbes limit good-quality products. Climate change leads to more complications when interpreting how plants and microbes interact to protect themselves from stress. Plants need water, carbon, and nutrients to grow. The extreme conditions mentioned restrict the growth of plants. Although plants can sense and exhibit natural mechanisms during stress conditions, increased non-sustainable agricultural practices and other human activities lead to highly stressful conditions for plant growth and yield. While in stressful situations, fungi play an essential role in energy transfer and uptake of nutrients by releasing the adverse effects of stress on plant growth. Many strategies in bacteria and fungi need to be addressed here, including stress conditions such as cysts and spore formation, cell membrane deformation, production of damage repair enzymes, and chemical synthesis to relieve stress. The mechanism of salt tolerance, symbiotic microbes, xenobiotics, and hazardous tolerance genes induces plant growth in unfavorable conditions. In recent days, technological improvements such as gene modification by genetic engineering have shown the potential to enhance the positive effects on agricultural production and products.

Keywords: Microbiomes, stress management, agriculture, climate change, mitigation.

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INTRODUCTION

In every ecosystem, plants are vital to its survival, as are furry animals and microscopic bacteria. Therefore, soil microflora cannot be sustained without a balanced ecosystem. Soil microbes are vital in restoring soil fertility and breaking down agricultural waste, particularly bacteria. They act as an ecological transformation inducer and are apt to live in geochemically extreme environments. Extremophiles can be divided into oligotrophic, acidophilic, alkaliphilic, thermophilic, psychrophilic, and sulfidophilic [1]. Based on oxygen requirements, these are named aerobes, anaerobes, and facultative. In the modern world, plants adapt to grow in all conditions because of recent technological inventions. However, microorganisms are native to the soil; they can change their potential effect with the least changes.

Bacteria are active in the soil near the roots, called rhizosphere. They are categorized as decomposers, mutualistic bacteria, pathogens, and lithotrophs, and they form micro-aggregates, which are very important for water filtration and holding. These live under starvation conditions or stress. Once it turns into a flourishing state, the crop yield and soil fertility improve. Aeromonas, *Streptomycetes,* and Actinomycetes release "geosmin", which provides an earthy smell. *Azotobacter, Azospirillum,* and *Clostridium* also fix nitrogen without the host plant [2].

Rhizobium removes nitrogen from the atmosphere and converts it into ammonia. *Arthrobacteria* sp. is involved in nitrification by turning ammonia into nitrite and nitrate. *Nitrosomonas sp.* convert ammonia to nitrite, and *Nitrobacter spp.* can convert nitrites into nitrates. However, denitrifying bacteria enable the conversion of nitrate to atmospheric nitrogen. Lithotrophs get their energy from other than carbon compounds. Interestingly, the sulphur-oxidizing and sulphur-reducing bacteria are available in the soil bed. Without bacteria, new plant populations struggle to survive. The food chain begins with solar energy, and decomposers act as the vital components at the end. Algae are universal in soils, permanent ice, snowfields, hot springs, and cold deserts. They also produce oxygen for human consumption and other aerobic microbes in our ecosystems [3]. They are the leading producers of organic compounds and play a significant role in the food chain in aquatic and agricultural areas. Mainly, in agriculture, the algae are used as a biofertilizer and soil stabilizer by providing nitrogen and phosphorus. These are grown worldwide for human consumption as dietary supplements. They can yield carbon in the desert and wild lands with minimal freshwater demands and reduce soil erosion.

The biological nitrogen fixation using blue-green algae is termed Algalization [4]. This will help bring the water, micro, and macronutrients of calcium, potassium, and magnesium into the upper layer. This process will maintain the soil structure and property of nutrient uptake up to 30 - 40 kg N/ha/Season. In the world, 90% of marine plants are algae, and 50% of the agricultural microbes almost belong to the algal community. All the molecules of oxygen we inhale come from algae and plants. Cyanobacteria are usually used in rice paddies everywhere in Asia. Marginal soils, such as saline-alkaline and limestone soils are applied [5].

In the emerging microbiome, the marine algae (red and brown algae) are cast off as organic fertilizers on farmlands, capable of reducing atmospheric nitrogen to ammonia *via* heterocysts, and a superior category of seaweeds produce polymers of agars, alginates, tannins, antibiotics, colorants, pigments, defense ingredients for the plant growth. *Tolypothrix tenuis* is grown and added to the rice field for increased yield [6]. In Thailand, *Chlorella* and *Dunaliella* are commercially available for the treatment of wastewater and have been successfully maintained for 55 years for irrigation purposes. The goal of agriculture is to improve existing cultivars and to develop new cultivars.

CLIMATE CHANGE IMPACTS

Climate and agriculture are interconnected; a slight change in climate affects agriculture adversely. The warning of our atmosphere is now impossible to avoid. Climate change indirectly contributes to ocean acidification, shrinking glaciers, and destroying coral reefs and other aquatic plants and animals. Climate change will significantly affect rural communities around the world. According to the Ministry of Agriculture, approximately 40 million hectares of agricultural land in India was severely damaged by hydro-meteorological disasters in 2022. The glaciers will disappear in 80 years, and their cores show a rich chronological history of unique, frozen microbial life reported by the IPCC - International Panel on Climate Change, Fifth Assessment Report 2014 [7]. They also identified the impacts of climate change on biodiversity, ecosystems, and human communities at global and regional levels and examined the vulnerability and ability of the natural world and human societies to adapt to climate change. Microbes have been found in thawed permafrost for more than 400,000 years.

The temperature in the Arctic has risen by 3 to 5°C, according to the IPCC Special Report on Oceans and the Cryosphere [8]. The temperature affects the yield negatively; the opposite applies to the precipitation conditions. Using the probability ratio (PR) concept, we can measure how much of the change in the probability of extreme events is due to a change in the average climate. The biodiversity loss will lead to the maximum percentage of species at high risk of

Part 4: Role of Microbes in Sustainable Waste Management for Protecting our Ecosystem

CHAPTER 6

Role of Microbes and Microbiomes in Wastewater Treatment for Aquatic Ecosystem Restoration

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Abstract: Industrial development improves our life quality. Nevertheless, the industries, such as those producing paper and pharmaceutical products, generate large amounts of industrial wastewater. This wastewater contains various pollutants, which are organic and inorganic. Various physical, chemical, and biological methods have been employed to eliminate the pollutants. Both physical and chemical methods involve more capital and produce secondary contaminants. During wastewater treatment, the wastewater microbiome facilitates the degradation of organic matter, reduction of nutrients, and removal of pathogens and parasites. For the purification of water and the preservation of the ecosystem, microbes in wastewater treatment are crucial. However, little is known about how microbial diversity is controlled and for what reasons. The varied microbial community supports flocculation, heterotrophic respiration, nitrification under aerobic conditions, and denitrification under anaerobic conditions. Although recycled water is reinstated for recreational and agricultural use, biomonitoring is vital for assessing treatment effectiveness. Microorganism-based biological treatment is developing as an effective and environmentally friendly method. This chapter thoroughly introduces biological wastewater treatment, growth and kinetics, and different microbial community types that include bacteria and fungus, actinomycetes, algae, plants, and the range of microbial wastewater treatment, among other topics.

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Keywords: Aerobic process, Biological treatment, Anaerobic process, Bioreactors, Mycoremediation, Microbes, Phytoremediation.

INTRODUCTION

Microbiomes are crucial in biological wastewater treatment methods such as activated sludge, anaerobic digestion, and bioelectrochemical systems. The microbial population's activity and resilience in the microbiome significantly impact the performance and stability of these activities. However, it is challenging to manage these wastewater treatment processes properly, and it is hard to anticipate their efficiency because of the complex nature of the microbiota (microbial type, role, and interactions) [1]. Biological wastewater treatment is regarded as a biological and chemical process that governs the removal and degradation of organic, inorganic, trace, and recalcitrant contaminants in the wastewater [2]. It is also considered an efficient, reliable, and cost-efficient technology for removing pollutants. The diminution of organic matter in the aquatic ecosystem is primarily mediated by microbial respiration and photochemical degradation [3, 4], which strengthens microbes as a feasible candidate for treating wastewater. In recent decades, the increasing occurrence of trace contaminants altogether, with their persistent, bioaccumulative, and toxic nature, threatens the homeostasis of the ecosystem, which has become a critical concern [5]. The diversified pollutants contain personal care products, pharmaceutically active compounds, steroids, antibiotics, chemicals, endocrine disruptors, pesticides, artificial sweeteners, and metabolites [6, 7]. These emerging contaminants do not yet have many regulations regarding their limits.

The generation of wastewater increases multi-fold because of industries and the overexploitation of freshwater resources. Wastewater treatment is always linked with waste recovery and its optimum utilization, which broadens the amplitude of wastewater treatment, enhancing the quality of the byproducts as an efficient alternative for non-potable purposes. Composition, concentration, volume produced, compatibility to treatment, and environmental impact must be considered before selecting the appropriate treatment for the particular wastewater that ropes microbes in since it can detoxify the discrete pollutants. Microorganisms detoxify pollutants through various mechanisms, and the application of microorganisms in treating the wastewater determines the efficiency of pollutant elimination. This section explores various biological treatment processes, possibilities, and the limitations associated with those treatment processes.

WASTEWATER TREATMENT

Even conventional biological wastewater treatment has specific implications, such as high electricity consumption, high-rate filters, and surplus sludge biomass, which lead to their enhancements. The application of microorganisms at various treatment phases significantly influences wastewater treatment efficiency.

Principles of Biological Wastewater Treatment

Samer [8] stated the following principles of biological wastewater treatment.

- The biological system existing in the wastewater treatment system is susceptible to hydraulic loads. Variation greater than 250% is said to be problematic.
- Temperature largely influences the growth of microorganisms. A reduction of 10˚C decreases the rate of biological reactions.
- At doses between 60 and 500 mg L^{-1}, treatment effectiveness is higher. If it is above 500, it can be coupled with an anaerobic process.
- The strength of the wastewater should not be more than 150% or 1000 mg L^{-1} BOD; otherwise, equalization has to be done to ensure the safer strength of wastewater entering the treatment system.
- The carbon: nitrogen: phosphorous ratio in the wastewater should be from 100:20:1 to 100:5:1 for higher treatment efficiency.
- Wastewater should undergo pretreatment

Biological Growth and Kinetics

The biological growth in wastewater treatment plants can be ascribed from the Monod equation [8]: the activity of bacteria and the speed of metabolic processes are influenced by a number of variables. The most crucial criterion is the environment's temperature, pH, dissolved oxygen level within the wastewater, nutritional content, and other contaminants. These beneficial elements are used in fermenters to promote the microbial community's development and reactors' efficiency in wastewater treatment.

$$\mu = \frac{(\lambda S)}{(K_s + S)}$$

Where,

μ stands for the specific growth rate coefficient,

λ for the maximum growth rate coefficient at 0.5 μ_{max},

CHAPTER 7

Role of Microbes and Microbiomes in Solid Waste Management for Ecosystem Restoration

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Abstract: Solid waste disposal is a major issue that is getting worse every day as more people move into cities. Solid waste disposal in India and other developing nations frequently involves open dumping and incineration. This practice increases both health risks and already existing pollution issues. To solve this issue with the least amount of environmental impact possible, it is urgently necessary to employ sustainable techniques. There are many environmentally friendly ways to manage solid waste, including composting, vermicomposting, and anaerobic digestion, with additional benefits like generating byproducts. Among them, biological agents play a significant role in solid waste management. This chapter overviews solid waste management and a long-term solution using biological agents and microbes. The distinctive characteristics of microorganisms can be effectively used to revive the environment. Microorganisms can be used as "miracle cures" for biodegradation and remediation of contaminated sites. Today, microorganisms and nanotechnology are used in nano-bioremediation to clean up radioactive waste effectively. Additionally, using genetically modified organisms (GMOs) in severely polluted areas makes the microorganisms beneficial for human welfare and ecosystem restoration. Numerous environmental phenomena, both natural and man-made, depend on microorganisms for maintenance. They perform beneficial roles that improve and optimize human life. Waste management is one of

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these areas where microorganisms are being used. The proper disposal of the vast amounts of waste that people produce throughout the course of a day presents a significant challenge, one that the government and environmental organizations are constantly looking for new solutions to. Utilizing microorganisms is a crucial component of effectively combating this threat. It discusses the numerous functions of microorganisms in the environment, such as the management of solid waste, and it concludes by highlighting some recent developments in microbiological solid waste management.

Keywords: Contamination, Environment, Microbes, Solid waste management.

INTRODUCTION

India is facing a challenge in waste management due to the tremendous quantity of waste products produced due to urbanization and industrialization. The commercial and industrial sectors benefit the country's GDP while it questions waste disposal and management. Around 377 million people in 7,935 towns and cities generate 62 million tonnes of municipal solid waste (MSW) per year. Out of this amount, only 43 million tonnes (MT) is collected. Out of which, only 11.9 MT is treated, and the untreated 31 MT is dumped in landfill sites [1]. One of the essential services provided by municipal authorities to keep the nation clean is solid waste management (SWM). Appropriate waste segregation at the source is a prerequisite for effective waste management. Depending on the type of waste materials, the segregated waste should be taken further for recycling or resource recovery. The final residue should be appropriately managed and disposed of in sanitary landfills [2]. After proper treatment and disposal of other waste products that cannot be recycled or reused, sanitary landfills are the only remaining option for disposing of municipal solid waste. This waste management option is severely constrained by the cost of transportation [3]. The Environment Protection Act of 1986 established all statutory guidelines for the disposal of generated waste and other management standards as waste generation increased day by day.

Special wastes are subject to different regulations and compliances in areas like the type of authorization, record-keeping, and disposal methods, among others. Although the legal protections are sufficient, all municipalities indiscriminately dump their waste at locations on the edge or inside cities. Therefore, according to experts, India's waste management and disposal system is ineffective [3]. Microorganisms have solved numerous issues that humanity has faced in maintaining environmental quality. They have been successfully applied in several fields, including treating municipal and industrial waste, genetic engineering, environmental protection, and human and animal health. Using microorganisms, feasible and affordable responses that were previously impossible to achieve using chemical or physical engineering techniques are now possible [4]. Microbial technologies have also been successfully used to address a variety of environmental problems, particularly those related to waste management [5].

SOLID WASTE MANAGEMENT SCENARIO IN INDIA

The waste generation rate has exponentially increased in the last ten years due to the accelerated economy and rapid population growth. In urban India, 68.8 million tonnes of municipal solid waste are produced annually, or about 1,88,500 tonnes per day [6]. However, only 24% of this massive waste is processed correctly, treated, and disposed of. Even though there are scientifically sound techniques such as composting, incineration, and landfilling, open disposal is a popular method since it is the least expensive and easy to adopt [7]. Open dumping is followed by waste landfilling as the primary method of waste treatment and disposal, but the need for larger areas restricts solid waste disposal, particularly in larger cities [8]. Approximately the combined area of the three most populous Indian cities, 1,400 sq. km. of land, will be needed for landfilling solid waste in India by 2047.

Furthermore, the high amounts of hazardous secondary pollutants they generate, such as smells, leachate, and greenhouse gases, restrict the use of landfills for waste treatment. This circumstance points to the need for a practical procedure, such as MSW pretreatment, which is carried out to homogenize the trash and make it easier for waste to be treated using particular biological technologies before being dumped. Despite having access to a multitude of knowledge and resources, municipalities and local governments are nevertheless under considerable pressure to develop a waste treatment technology that is both inexpensive and sustainable. The most comprehensive process that can be used to manage these wastes is composting, especially in the case of Indian genera, where 50–60% of the MSW (C/N ratio 23) collected is biodegradable. Additionally, composting has several environmental advantages over landfilling organic waste. In addition to decreased greenhouse gas emissions from landfills, soil properties like texture, porosity, organic matter, and NPK availability for agricultural applications have improved. In order to improve the waste recycling system, we must comprehend the technical details of this natural, wet waste recycling process and the effects of any operational changes.

ROLE OF MICROORGANISMS IN THE ENVIRONMENT

To transform environmental natural resources into more palatable forms for consumption, there are several anthropogenic activities, including chemical synthesis. Man also causes pollution issues because of the production of goods. The better way to manage the waste generated and discarded in the environment is

Part 5: Traditional and Advanced Techniques for Ecosystem Restoration

CHAPTER 8

Current State and Future Prospects of Microbial Genomics in Ecosystem Restoration

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Abstract: Ecosystem degradation through human actions is a global phenomenon. The international society has established goals to stop and reverse these trends, and the restoration industry faces the vital but difficult challenge of putting these goals into practice. Microbial communities are integral to all ecosystems because they perform critical roles like nutrient cycling and other geochemical processes. They are the indicators of the success of ecological restoration, including plantation forests, post-mining areas, oil and gas activities, invasive species management, and soil stabilization. Since the last 2 decades, advancements in microbial genomics have allowed researchers to focus on microbial ecology and dynamics of environmentally balanced vis-a-vis damaged ecosystems. Advancements have significantly improved our capacity to define diversity in microbial ecology and its putative functions in meta-omics methods brought about by developments in high-throughput sequencing (HTS) and bioinformatics. These tools may boost the likelihood that damaged ecosystems will be restored. The current article focuses on using meta-omics techniques to monitor and assess the outcomes of ecological restoration projects and to monitor and evaluate interactions between the various organisms that make up these networks, such as metabolic network mapping. We provide an overview of functional gene editing with the CRISPR/Cas technology to improve microbial bioremediation. The existing understanding will be strengthened by creating more efficient bioinformatics and analysis processes.

Keywords: CRISPR-Cas, Ecosystem restoration, Gene editing, Microbial genomics, Metagenomics.

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INTRODUCTION

The process of recovering a degraded ecosystem in order to restore biodiversity and ecological functioning of species equivalent to that of a reference environment is ecological restoration [1 - 3]. Restoration is a tool of global significance to stop biodiversity loss on a global scale [4, 5], and restoration of degraded natural ecosystems has gained recognition [2, 3, 6]. A large portion of the restoration work is predicated on the idea that all animal and microbial species would develop organically when plant species colonize the area, leading to proper ecosystem functioning [7]. However, the significance of the soil microbial community (SMC) to the plant community's expansion, diversity, and abundance cannot be overstated. Due to its diverse impacts on various species, the SMC substantially affects plant performance [8]. It regulates the variety and quantity of plant species [9, 10]. Microbial communities have returned in some restoration projects [11] but not in others, where decades have passed, and microbial community development and nutrient cycling have not occurred [10, 12].

The effectiveness of restoration initiatives must, therefore, be assessed, and without monitoring, it is impossible to determine if restoration efforts have been made or not [13]. Researchers and practitioners can improve future restoration results by evaluating the success of restoration and comparing it to the efficacy of different remediation processes and adjustments [1, 6, 14]. This is crucial for optimizing the restoration program's effectiveness, achieving the anticipated results, and getting the most out of the financial investment. Monitoring offers the chance for adaptive management by showing whether additional interventions may be required to meet goals [6]. Adaptive management can aid in reducing monitoring periods by highlighting situations where it is doubtful that completion conditions will be reached and additional remediation work is thought to be required. As a result, sensitive indications are required to track the degree of restoration [15]. Microbes are essential for constructing soil composition, nutrient cycling, growth and development of plants, the emergence of biodiversity, and ecosystem operations, making them key drivers of ecological restoration [10, 16]. A stable ecosystem is different from a degraded environment in that it has a variety of microbial populations. Thus, these SMCs can serve as reliable indicators to determine how much ecological restoration has been accomplished [10].

In order to count, visualize, and sequence bacterial DNA, microbiologists are increasingly skipping the cultivation process altogether [17]. Several reasons justify this shift in the use of techniques for assessing microbes and microbial communities. First, recent technological developments, cost, and scalability have made DNA sequencing a viable option for biological monitoring, especially for hard-to-survey populations like microorganisms [14]. Second, with these technologies, it is possible to quickly, accurately, non-destructively, and reliably provide biodiversity data on a range of organisms in an ecosystem. Third, SMCs are dynamic, and little is known about the ecological services they can provide. This intertwine module can be clarified by meta-omics approach to understanding how complex bacteria function in the ecosystem [18]. In this chapter, we delve into the significance of microbial genomics and meta-omics-based tools in ecological restoration

META-OMICS FOR TRACKING AND RESTORATION

Assessing and Tracking Cryptic and Functional Ecosystem Components

Employing field-based monitoring and restoration assessment techniques, surveying microbes or microbial populations is challenging or impossible. In contrast, it is now possible to precisely and quickly describe and quantify these diverse and functional taxonomic groups by sequencing large quantities of environmental DNA or RNA utilizing genomic and, in particular, meta-omic technologies. Eukaryotes like fungi can be tracked using meta-omic methods [19]. However, it is most beneficial for prokaryotes monitoring. On average, 5% of taxa were amenable to the culture-dependent techniques employed to classify prokaryotes. The use of meta-omic has shown that Earth's microbiota is much more diverse than previously believed and that only around 1% of it is culturable. Metabarcoding, metagenomics, metatranscriptomics, and metaproteomics are the meta-omics methods that are most frequently employed.

Metabarcoding

It is a technique that identifies multiple taxa by amplifying DNA barcodes from bulk samples like water or soil environmental DNA (eDNA) using universal primers followed by DNA sequencing and bioinformatics analysis. In a metabarcoding process, DNA is extracted from materials (such as soil, aquatic systems, *etc*.) and then amplified using polymerase chain reaction (PCR). After the amplified product is sequenced and identified using a reference database, sequencing data can be evaluated using a taxonomy-independent methodology to evaluate changes in community profiles. Metabarcoding-based systems can more quickly and affordably generate comprehensive information than traditional monitoring techniques, depending on the monitoring goal [20]. Furthermore, it enables data collection on populations that would otherwise be impossible to see, such as soil microorganisms. This strategy potential has been observed in varied systematic groups, including plants, invertebrates, and vertebrates, as well as in a wide range of diverse environmental contexts, including the tropics, the boreal

CHAPTER 9

Role of Metagenomics and Microbial Diversity in the Restoration of Tropic and Temperate Ecosystems

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Abstract: The geographical area where all the abiotic and biotic factors interact with each other to make the bubble of life is known as the ecosystem. While many natural and artificial calamities occur to destroy the ecosystem, microbial diversity plays a vital role in maintaining and functioning it. The microbes constitute one-third of the earth's biomass and are composed of enormous genetic diversity from extremely hot (thermophilic) and moderate (mesophilic) to extreme cold (psychrophilic) climatic conditions. Therefore, the principal objective of microbiome research is to elucidate the relationship between microbial diversity and its function in maintaining or restoring the ecosystem. Recent advances in microbial ecology and metagenomic approaches have enabled detailed assessment of the highly complex communities, allowing the establishment of the link between diversity and the function performed by microbes. In this chapter, we will explore some advanced bioinformatic tools for metagenomic studies that can provide quantitative insights into the functional ecology of microbial communities. The detailed study will help us understand the complex microbial diversity in tropical and temperate ecosystems and their functional aspects in ecosystem restoration.

Keywords: Ecosystem restoration, *In silico* approaches, Microbial diversity, Metagenomics, Mesophilic, Psychrophilic, Thermophilic, Tropical ecosystem, Temperate ecosystem.

INTRODUCTION

For ages, nature has always provided us with all the necessities for carrying all forms of life in a wieldy and subtle way. Among all the life forms that live and interact in the ecosystem, humans are known to explore and exploit the ecosystem to meet the rising demands for resources and environmental services. Over the

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past 50 years, humankind has changed the ecosystem in a more hasty and extensive way than any dated time in human history [1]. The result of these changes, no doubt contributed to substantial net gains in economic development and the well-being of humans. However, it is achieved at the rising price of degrading the ecosystem services, exacerbating poverty in some groups of society, and, most importantly, increasing the risk of nonlinear changes, leading to the irreversible loss in the momentum and diversity of life on Earth [1]. These inevitable consequences impact the biotic and biophysical conditions of the ecosystem. They will lead to the competing demand for finite space and ecosystem services, ultimately resulting in a dearth of infinite resources, confining the recourse to abandon degraded areas, and shifting toward the exploitation of non-degraded ones [1 - 4].

It is quantified in many theories that the ecosystem had the capacity of self-replenishment from the perturbations, but keeping in mind the present scenario, it always becomes questionable whether the ecosystem is able to recover from the present perturbation. And if so, how long will it take? The self-replenishment theory always states that the ecosystem could gradually be replenished from the perturbation at a rate directly proportionate to the rate at which the perturbation is abated [5]. According to the current human impact, it can be easily speculated that such recovery will take centuries [6]. But, of course, while replenishing the ecosystem, the demands of humankind can neither be neglected nor can be kept to a halt.

So, conservation efforts must create a portfolio of imminent opportunities that keep an equilibrium between environmental protection and environmental services for the future generation of the burgeoning human population. One such approach is to combine natural and modern techniques. Microbes are known from eternities for upholding and reinstating the ecosystem and are found in all forms of the environment while managing their survival along with maintaining the ecosystem. Combining the contemporary science techniques like metagenomic or *in silico* approaches to clear our understating of microbial diversity and protagonist in ecosystem restoration in a broader view will provide great assistance in more progressive, innovative, and prompt repossession of bionetwork to achieve our goal of "meeting the needs of the present and future without compromising our mother nature".

DAMAGING AGENTS OF THE ECOSYSTEM

How is the ecosystem damaged? It is one of the most underrated questions of the era that needs serious attention. The pace of ecosystem damage from anthropogenic and natural impacts is rapidly leading to a biodiversity crisis. The ecosystem services underpinning human livelihood and the effect of destructed ecosystems on human livelihood are constantly being discussed and highlighted internationally. The Intergovernmental Panel for Climate Change Fifth Assessment Report (2014) indicated that the adaptation capacity of the ecosystem is far more limited than that of humans in response to destruction and damage [7]. But our ecosystem is not just limited to humans; our discussion should also not be concise to humankind only. The primary causes of ecosystem damage that are most discussed and well-known are anthropogenic processes and natural hazards. The anthropogenic process is a premeditated human bustle that is non-malicious but may have a negative impact on society and may trigger or catalyze other hazardous or destructive processes [8]. The well-identified examples of such activities are groundwater abstraction, surface mining, vegetation amputation, chemical detonations, infrastructure loading for urbanization, *etc*.

On the other hand, natural hazards are phenomena that occur naturally and may have a negative or deteriorative effect on the ecosystem or biodiversity. Such as earthquakes, landslides, volcanic eruptions, droughts, floods, subsidence, tropical storms, wildfires, *etc* [8]. Natural hazards are a phenomenon that cannot be controlled or prevented. However, nowadays, the major concern is the "interaction," *i.e.*, the effect of one phenomenon on other phenomena (either anthropogenic or natural) [8] (Fig. **1**).

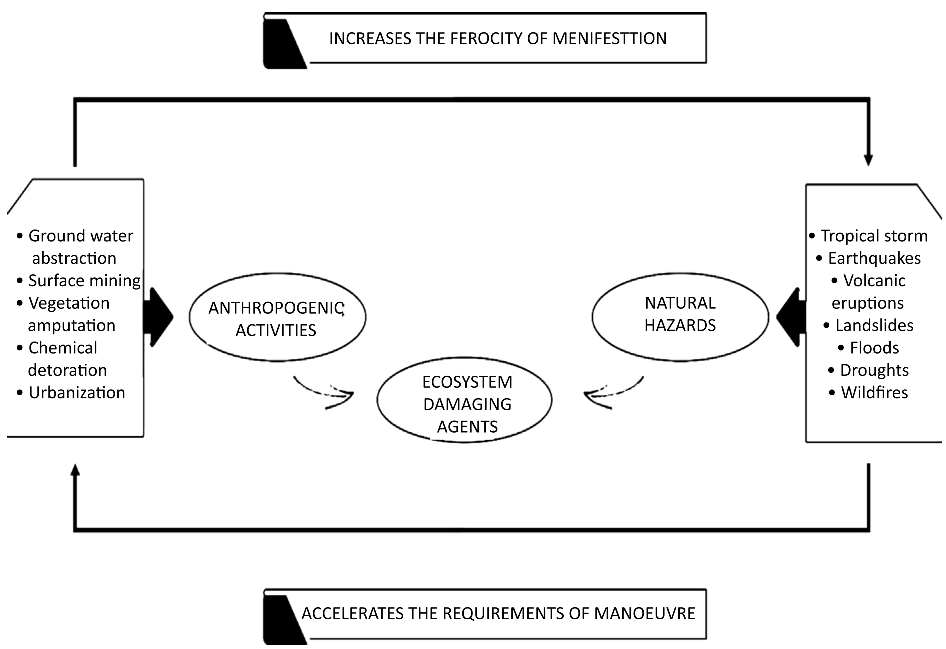

Fig. (1). >Systematic diagram showing the "interaction" of anthropogenic activities and natural hazards.

CHAPTER 10

Basic and Traditional Microbial Techniques in Ecosystem Restoration

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Abstract: The onset of the anthropogenic destruction of ecosystems is one of the ongoing problems that can threaten the existence of organisms, including humans. The emerging problem can be effectively addressed through restoration ecology, a nature-based solution that promises to be cost-effective. Microorganisms, including bacteria, fungi, and viruses, are omnipresent and provide numerous benefits to the ecosystem, such as sustainable plant productivity, enriched soil nutrients, increased soil carbon pool, decomposition, and a stable environment for human life. Soil microorganisms also play a fundamental role in ecosystem functioning and conserving plant diversity. Exploring voluminous beneficial microorganisms and promoting the reestablishment of these beneficial microbes in the soil will preserve Earth's diverse native plant populations, which, in turn, will help in improving soil and be a vital player in enhancing ecosystem primary productivity, food chain, and locking away atmospheric carbon into its plant body and soil. Microbial restoration can be achieved by basic and traditional methods, *i.e.*, (i) by treating the soil with organic matter-rich manure harvested from bio piles, (ii) composting, (iii) graze manuring, (iv) natural manuring, and (v) plant-assisted microbial restoration technique. Regenerative/carbon farming can also be practiced in parallel to enhance the restoration rate and protect beneficial microbial life in the soil. However, the increasing use of microbial inoculants is also raising several queries about their effectiveness and their impacts on autochthonous soil microorganisms, which should be cautiously considered before introducing bioinoculants for restoration. Even if bioinoculants restore the microbial community,

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they have the following shortcomings: (i) prolonged persistence of microbial colonies and detection in soil; (ii) the monitoring of the impact of the introduced bioinoculants on native soil microbial communities, which needs to be monitored examined periodically. This chapter delves into fundamental and conventional techniques and approaches that can be employed to maintain soil microbial populations. Furthermore, the chapter investigates the possibility of creating protocols for regulatory or commercial objectives, emphasizing the significance of ecological restoration by using bioinoculants or microbial colonies in degraded sites.

Keywords: Ecosystem restoration, Ecosystem functioning, Microorganisms, Microbial colonies.

INTRODUCTION

Land degradation has become a global environmental issue, affecting both natural and human systems. Restoration of degraded land is now indispensable since land degradation adversely impacts soil productivity, resulting in a decline in soil organic matter, soil microbial diversity, and water quality, which consequently affects ecosystemic functions and processes [1]. At the same time, microorganisms play a pivotal role in the mineralization, oxidation, reduction, and immobilization of both organic and mineral materials within the soil matrix. Basic and traditional microbial techniques are now envisaged as a viable solution in ecosystem restoration involving microorganisms to improve soil health and the functioning of degraded ecosystems [2]. Microbes, including bacteria and fungi, are crucial components of healthy, productive ecosystems, playing a pivotal role in soil formation, nutrient cycling, and plant growth. Introducing specific beneficial microorganisms into degraded soils can help improve soil quality, increase nutrient availability, and enhance plant growth [3]. Basic and traditional microbial techniques, such as composting, bio piles, graze manure-based restoration, inoculation with beneficial symbiotic bacteria, regenerative agriculture/restoration, and mycorrhizal inoculation, have been practiced by civilizations for centuries to improve soil fertility [4]. These techniques are largely feasible, low-cost, low-input, and environmentally friendly, altogether making them ideal for use in areas with limited resources or where chemical inputs are undesirable. The current chapter aids in understanding the ecological principles underlying basic and traditional microbial techniques, their conceivable applications in ecosystem restoration, and their importance for endorsing more active, sustainable restoration practices.

By increasing soil qualities, microorganisms can play a key role in recovering degraded lands and facilitating ecosystem restoration. Studies suggest that microorganisms can lessen soil degradation and increase soil characteristics, including fertility [5]. This will encourage plant growth, subsequent ecosystem restoration, and new organic matter produced by bacteria. Collaboration between the domains of microbiology and soil, which has not been very common up to this point, is necessary for such a restoration plan to understand soil microbial dynamics. We accept "ecological restoration" as the most inclusive and enduring phrase to describe the science and practice of repairing harm, deterioration, and destruction at all scales, from a single organism to entire landscapes. Soil microbial communities are an essential part of ecosystems and play crucial roles in ecological processes related to the cycling of carbon (C), nitrogen (N), and phosphorus (P) [6]. Evaluating ecosystem restoration requires an investigation of microbial diversity. The impacts of biochar on microbial biomass and microbial communities, including bacteria, archaea, and fungi, have been the subject of numerous studies. Ecological functions can only be partially understood by studying microbial communities alone.

The ecosystem services delivered by soil, the living skin of Earth, are vital for life: The majority of our antibiotics come from bacteria that live in soil, which also serves as a filter and water storage system, a habitat for a wide variety of creatures, and a medium for plants and heterotrophs to develop in. Climate change, population growth, and land degradation, such as carbon loss, biodiversity decrease, and erosion, present a growing set of challenges for humanity [7]. For example, soil hydrological function is decreased by land degradation, which also affects various other ecosystem services. Changes in hydraulic operation, infiltration and soil moisture storage, carbon cycling, biological activity, movement of nutrients and pollutants, and plant development all contribute to these effects [8]. As soil ecosystem degradation has a growing impact on biodiversity, food production, climate regulation, and human livelihoods, it is essential to understand its effects and take steps to reverse them.

Most soil life consists of microorganisms that control nutrient cycling, break down organic matter, control soil-borne plant diseases, define soil structure, and increase plant productivity [9]. Soil microorganisms facilitate the alteration of the soil environment. On the other hand, research to date has concentrated chiefly on how microbial communities adapt to environmental change, and microbial community structure and diversity can also be employed as indicators of soil health. It was proposed in an article on restoration ecology written 13 years ago that microbial populations, when adequately maintained to support the restoration process and system health, serve as markers of change and support the regeneration of degraded ecosystems. Since then, many researchers have considered the possibility of using microorganisms as ecosystem regulators, particularly to increase crop productivity and engineer dryland restoration. These studies show that microorganisms have the power to change soil functions fundamentally.

CHAPTER 11

Molecular Techniques in Ecosystem Restoration

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Abstract: A damaged ecosystem must be rebuilt to its original form, or a new ecosystem must be created in a degraded area. Ecosystem restoration is a complex procedure. Researchers can now investigate the structure and function of ecosystems at the molecular level thanks to the development of molecular techniques as a potent tool for ecosystem restoration. This chapter examines the application of molecular methods to ecosystem regeneration. The various available molecular methods and how they have been applied to monitor ecosystem health, identify microbial communities in ecosystems, and comprehend interactions between microbes and plants are discussed. The chapter also examines the application of molecular methods to the restoration of ecosystems that have been damaged, including the use of plant-microbe interactions to promote plant development in contaminated soils. The chapter emphasizes the significance of molecular methods in ecosystem restoration and their potential to offer a more precise and thorough comprehension of ecosystem processes. The conclusion highlights the importance of ongoing investigation into the use of molecular methods for ecosystem restoration, especially in creating novel methods and their incorporation with existing restoration techniques. In the end, applying molecular methods can help develop practices for ecological restoration that are more efficient and long-lasting.

Keywords: Bio-sensors, Environmental DNA, Heavy metal pollution check, Metagenomics, Ocean acidification, 16s rRNA sequencing.

INTRODUCTION

Ecosystem restoration is the process of repairing and restoring damaged or degraded ecosystems to their original or functional state. It entails various activities, including habitat restoration, reforestation, soil restoration, and water restoration. Because of growing concerns about climate change, biodiversity loss,

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and natural resource depletion, ecosystem restoration has become increasingly important in recent years. Molecular techniques have transformed the field of ecosystem restoration by allowing scientists and practitioners to study and comprehend the underlying mechanisms of ecosystem functioning and design effective ecosystem restoration strategies.

This chapter provides an overview of molecular techniques used in ecosystem restoration, such as DNA-, RNA-, and protein-based techniques. The chapter begins by discussing the significance of molecular techniques in ecosystem restoration and the problems that these techniques can help to solve. It then details the various molecular techniques used in ecosystem restoration, their advantages and disadvantages, and their applications in various contexts.

IMPORTANCE OF MOLECULAR TECHNIQUES

For various reasons, molecular techniques have become an indispensable tool for ecosystem restoration. For starters, molecular techniques allow scientists to investigate and comprehend the underlying mechanisms of ecosystem function. Identifying the key species and processes that are critical to ecosystem functioning, as well as the interactions between these species and processes, is part of this. Scientists can design more effective ecosystem restoration strategies and predict the outcomes of restoration activities if they understand the underlying mechanisms of ecosystem functioning.

Second, molecular techniques can assist in addressing some of the challenges associated with ecosystem restoration. Human activities, for instance, exploit a large number of ecosystems, altering their biodiversity and composition. When creating reintroduction plans for native species that were once part of the ecosystem, molecular techniques can be used to identify these species. The identification of genes and traits essential to the survival of native species can also be aided by molecular techniques, as can the selection of individuals with these traits for reintroduction. Monitoring the success of ecosystem restoration efforts can be aided by molecular techniques. By observing changes in species abundance and diversity as well as changes in ecosystem processes, scientists can evaluate the efficacy of ecosystem restoration efforts and make necessary adjustments.

VARIOUS MOLECULAR APPROACHES TO ECOSYSTEM RESTORATION

Identification of species is critical in ecosystem restoration, especially when dealing with threatened or endangered species. Molecular techniques such as DNA barcoding and sequencing have become valuable tools for identifying species and detecting cryptic or rare species that are difficult to identify using traditional methods [1]. These techniques have also been used to investigate population genetic diversity and the origins and evolutionary history of species. Another crucial area of study in ecosystem restoration is population genetics. Microsatellites and single nucleotide polymorphisms (SNPs) are examples of molecular markers that have been used to study population genetic structure and monitor individual movement both within and between populations [2]. These markers can also be used to estimate population sizes, gene flow, inbreeding, and genetic drift.

Conservation biology is a branch of biology that seeks to protect and conserve ecosystem biodiversity. Molecular techniques have been critical in this field, particularly in identifying and tracking endangered species and understanding the genetic and ecological factors influencing species survival. Microsatellites and mitochondrial DNA have been used to determine the source populations for translocation programs and monitor their effectiveness. The interactions of species with their environment have also been studied using molecular methods. For instance, transcriptomics and proteomics have been used to study how species react to environmental stressors like pollution and climate change. By shedding light on the molecular processes underlying species' responses to environmental stressors, these techniques can aid in the identification of potential conservation targets.

Overall, molecular methods have emerged as a crucial tool for restoring ecosystems, providing scientists with a potent arsenal of instruments for investigating the genetic diversity, population dynamics, and ecological interactions of species in an ecosystem. This chapter will examine the various molecular approaches that have been used in ecosystem restoration, as well as their advantages, drawbacks, and potential applications in the future.

PCR-based Approaches

Most molecular-based studies begin with DNA extraction from a particular organism, followed by the amplification of particular DNA segments using the polymerase chain reaction (PCR). The fact that only minute amounts of DNA are needed makes PCR worthwhile [3]. This is especially helpful when scientists need numerous samples or cannot obtain large amounts of tissue, as in population genetic studies. DNA was taken from people in various populations and used in a PCR-based genetic diversity survey. This kind of investigation can provide insights into the historical processes that led to variations in the genetic makeup of populations dispersed across a variety of environments and geographies. A schematic representation of the polymerase chain reaction is shown in Fig. (**1**).

SUBJECT INDEX

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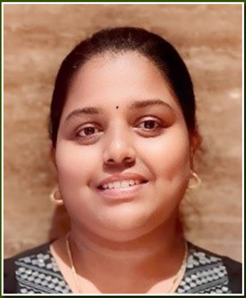

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Sathya Velusamy

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